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MAGYAR ÁLLATORVOSOK LAPJA

XVIII. KÖZÉP-EURÓPAI BUIATRIKUS KONGRESSZUS

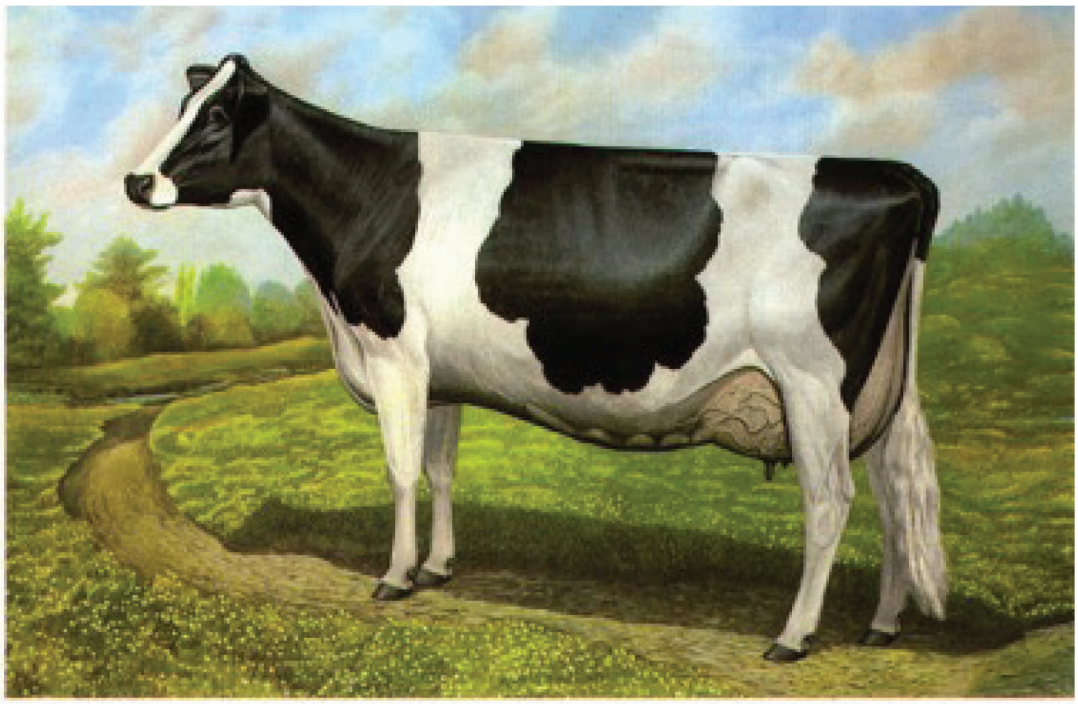
XVIII MIDDLE-EUROPEAN BUIATRICS CONGRESS

EGER & PARK HOTEL,

EGER, 2018. MÁJUS 30. – JÚNIUS 2. / MAY 30 – JUNE 2, 2018

A MAGYAR BUIATRIKUS TÁRSASÁG
XXVIII. NEMZETKÖZI KONGRESSZUSA

XXVIII INTERNATIONAL CONGRESS
OF THE HUNGARIAN ASSOCIATION FOR BUIATRICS



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XVIII MIDDLE-EUROPEAN BUIATRICS CONGRESS

XVIII. KÖZÉP-EURÓPAI BUIATRIKUS KONGRESSZUS

XXVIII INTERNATIONAL CONGRESS OF THE HUNGARIAN ASSOCIATION FOR BUIATRICS

A MAGYAR BUIATRIKUS TÁRSASÁG XXVIII. NEMZETKÖZI KONGRESSZUSA

EGER, HUNGARY

MAY 30 - JUNE 2, 2018

2018. MÁJUS 30. – JÚNIUS 2.

PROCEEDINGS /

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Hungarian Association for Buiatrics / Magyar Buiatrikusok Társasága

EGER, 2018

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Foreword

The Hungarian Association for Buiatrics together with the Austrian and German Association for Buiatrics has played a prominent role in launching the Middle-European Buiatrics Conferences in Middle-Europe. The main aim of the Hungarian initiative was to build up a fruitful communication and collaboration among the Middle-European countries which was missing during the former system. By this way the preliminary and the 1st Middle-European Buiatrics Congress were organized in Siófok and Balatonfüred in 1998 and 1999, respectively. From this time on, Middle-European Buiatrics congresses were organised in Croatia (2003, 2011), Czech Republic (2001, 2010, 2016), Hungary (2004, 2008), Poland (2005, 2014), Romania (2007), Serbia (2013), Slovakia (2000, 2009, 2017) and Slovenia (2006, 2015), respectively. In 2002 and 2012, the World Buiatrics Congresses were held in Hanover and Lisbon, respectively, therefore in those years no separate Middle-European Buiatrics Congress was organized. We still have the task to convince the missing Middle-European countries like Albania, Bosnia-Hercegovina, Bulgaria, Estonia, Kosovo, Latvia, Lithuania, Montenegro and Macedonia to join us.

It is a great honour for us that in this year in Eger, in this great historical town, we can organize the XVIII Middle-European Buiatrics Congress, which is also the XXVIII International Congress of the Hungarian Association for Buiatrics. A number of foreign experts have also joined us for this Congress from Argentina, Austria, China, Czech Republic, Croatia, Denmark, Germany, Italy, Poland, Qatar, Romania, Slovakia, Slovenia, Turkey, Ukraine and USA.

The programme of the congress will aim at updating the scientific knowledge and professional skills of veterinary surgeons and agricultural engineers in bovine, ovine and caprine practice to enable them to achieve an ever-increasing qualification and help them get more familiar with each other.

The legitimacy of antibiotics in modern medicine and veterinary science is unquestionable. Many life-threatening infections can be cured with them, but today they are often unreasonably used. As a consequence, antibiotic resistance and cross resistance are becoming increasingly frequent. The United Nations World Health Organization (WHO) was the first to address the "Appropriate Use of Antibiotics" in 2015, stressing that the spread of super bacteria is enhanced by the fact that the use of antibiotics is not in accordance with their intended purpose all over the world.

In order to be able to keep the animal husbandry competitive it is very important to solve the antimicrobial problem and to decrease the production costs. The main goal of our conference is to discuss the possibilities of correct antimicrobial usage and lowering the production cost. Special emphasis is given to those facts that profitable production is only possible with healthy animals. Discover the new scientific results for your practice! In addition to the scientific programme social programmes will be organised for the participants. The exhibition area will present products manufactured by various companies. The organisers will spare no efforts in offering you successful and useful days in Hungary.

Előszó

A Magyar Buiatrikusok Társasága az osztrák és a német buiatrikus társasággal karöltve a Közép-Európai Buiatrikus Kongresszusok elindításában kezdeményező szerepet játszott. A magyar kezdeményezés legfőbb célja a közép-európai országok közötti gyümölcsöző kommunikáció és együttműködés megteremtése volt. Így 1998-ban és 1999-ben Siófokon és Balatonfüreden rendeztük meg az előzetes és az I. Közép-Európai Buiatrikus Kongresszust. Ettől kezdve a közép-európai buiatrikus kongresszusokat Csehországban (2001, 2010, 2016), Horvátországban (2003, 2011), Magyarországon (2004, 2008), Lengyelországban (2005, 2014), Romániában (2007), Szerbiában (2013), Szlovákiában (2000, 2009, 2017) és Szlovéniában (2006, 2015) rendezték meg. Minthogy a Buiatrikus Világkongresszust 2002-ben Hannoverben és 2012-ben Lisszabonban rendezték meg, ezért ezen években külön Közép-Európai Buiatrikus Kongresszus nem lett megszervezve. Fontos további feladatunknak tartjuk, hogy meggyőzzük a hiányzó közép-európai országokat, mint Albániát, Bosznia-Hercegovinát, Bulgáriát, Észtországot, Koszovót, Lettországot, Litvániát, Montenegrót és Macedóniát, hogy csatlakozzanak hozzánk.

A mostani kongresszusra számos külföldi szakember is jelezte részvételét Argentínából, Ausztriából, Cseh Köztársaságból, Dániából, Egyesült Államokból, Horvátországból, Katarból, Kínából, Lengyelországból, Németországból, Olaszországból, Romániából, Szlovák Köztársaságból, Szlovéniából, Törökországból és Ukrajnából.

A kongresszus célja a szarvasmarha- és kiskérődző-egészségügy és termelési menedzsment legújabb kutatási eredményeinek és gyakorlati tapasztalatainak megismertetése és

közkinccsé tétele a hazai és a résztvevő külföldi szakemberek számára. A konferencián különösen nagy hangsúlyt kívánunk fektetni a termelést és a termelés gazdaságosságát befolyásoló ismeretek megvitatására.

Az antibiotikumok létjogosultsága a modern orvos- és állatorvos-tudományban kétségbevonhatatlan. Számos életveszélyes fertőzést lehet gyógyítani velük, napjainkban azonban mégis gyakorta indokolatlanul használják őket. Ennek következtében egyre sűrűbben esik szó az antibiotikum rezisztenciáról és keresztrezisztenciáról. Az ENSZ Egészségügyi Világszervezete (WHO) 2015-ben először rendezte meg "Az antibiotikumok megfelelő alkalmazásáért" elnevezésű világhetet, ahol hangsúlyozták, hogy az un. szuperbaktériumok terjedését fokozza, hogy az antibiotikumok használata nem azok rendeltetésének megfelelően történik.

Ahhoz, hogy gazdaságaink megőrizhessék versenyképességüket a termelési költségeket kell ésszerűen csökkenteni. A XVIII. Közép-Európai Buiatrikus Kongresszus, ill. a Magyar Buiatrikus Társaság XXVIII. Nemzetközi Kongresszusa ezért az antibiotikum kezelésekkel kapcsolatos probléma megoldását és a termelési költségek csökkentésének lehetőségeit kívánja feltárni, még pedig annak hangsúlyozásával, hogy termelni csak egészséges állatokkal lehet. Ismerje meg Ön is a tudomány által feltárt lehetőségeket!

SZENCI Ottó
President / Elnök

BRYDL Endre
Secretary / Titkár

XVIII Közép-Európai Buiatrikus Kongresszusnak & a Magyar Buiatrikus Társaság XXVIII. Nemzetközi Kongresszusának Programja

*

Programme of XVIII Middle-European Buiatrics Congress & the XXVIII International Congress of the Hungarian Association for Buiatrics

2018. május 30. * May 30, 2018

17.00 – 21.00 Regisztráció * Registration – Eger & Park Hotel, Eger

19.00 Nyitófogadás * Welcome party

2018. május 31. * May 31, 2018

8.00 Regisztráció * Registration

9.00 Megnyitó * Opening **Szenci Ottó elnök / president**

Brydl Endre titkár / secretary

Üléselnök * Chairmen
SZENCI Ottó & BRYDL Endre

9.15	Baumgartner Walter & Khol Johannes Lorenz Austria	Johne´s disease is of considerable importance in cattle *Johne-betegség jelentős fontosságú szarvasmarhákban*
9.45	Donald Niles USA	Evolving Johne's control. A new strategy for defeating an old disease – *Johne beteség kontrolljának fejlődése. Új stratégia egy régi betegség legyőzésére*

10.15	Bognár Barbara & Jurkovich Viktor	A paratuberculosis terjedésében szerepet játszó fontosabb környezeti és tartástechnológiai kockázati tényezők hazai tehenészetekben – előzetes eredmények *Major risk factors for environmental and housing technology involved in the spread of paratuberculosis in Hungarian dairy herds – preliminary results*

10.30 Kávészünet * Coffee break

Poszter szekció * Poster session

Üléselnök * Chairmen
KOFLER Johann & JURKOVICH Viktor

11.00	Mudron Pavel Slovakia	Heat stress and claw health in cattle *A hőstressz és a csülök egészségi állapota szarvasmarhákban*
11.15	Kofler Johann Austria	Surgical treatment of septic arthritis in proximal joints in cattle *Szarvasmarhák proximális ízületeiben előforduló szeptikus ízületi gyulladás sebészeti kezelése*
11.30	Osová Adriana & Hund Alexandra Slovakia	Claw amputation in practice – Challenges of a freestall barn *Csülökamputáció a gyakorlatban – Egy kötetlen tartású istállló kihívásai*
11.45	Führer et al. Austria	Prevalence of thin soles in dairy cows housed on mastic asphalt floors – *Masztix aszfalton tartott tejhasznú tehenek vékony talpúságának előfordulása*
12.00	Weck et al. Germany	Concentrations of cortisol during hoof treatment under pain control in meat Merino sheep – *Kortizol koncentráció alakulása fájdalomcsillapítással végzett csülökezelés során hústipúsú Merinó juhokban*
12.15	Ebert et al. Germany	Development of a system analysis for problem-centered data acquisition of animal- and housing-related risk factors for bovine hoof disorders on dairy farms – *Rendszerelemzés kidolgozása csülökbetegséggel terhelt tehenészeti gazdaságokban a tehén és a tartásához kapcsolódó kockázati tényezők értékelése céljából*

12.30 Ebéd * Lunch

Üléselnök * Chairmen
BAUMGARTNER Walter & KÖNYVES László

14.00	Wittek et al. Austria	Effects of different dosages of propylene glycol in cows according to calving – *Az elléskor adagolt különböző propilénglikol dózisok hatása tehenekben*
14.15	Lénárt et al.	A bendőfolyadék pH és a drencselés kapcsolatának vizsgálata az ellés körüli időszakban – *The effect of drenching on the changes of rumen fluid pH in dairy cows around parturition*
14.30	Lénárt et al.	The influence of pre-partum rumination on post-partum metabolic parameters in dairy cows – *Az ellés előtti kérődzés hatása a metabolikus paraméterekre az ellés körüli időszakban tejelő tehenekben*
14.45	Khol et al. Austria	The capability of blood gas analysis *ante partum* for the prediction of hypocalcaemia (milk fever) in cows *Ellés előtti vérgáz-analízis lehetősége hipokalcémiás tehenek (ellési bénulás) előrejelzésére*
15.00	Könyves et al.	Hipokalcémia monitoring. Helyszíni gyorsvizsgálatok tejhasznú tehénállományokban – *Hypocalcemia monitoring. On-farm quick testing in dairy herds*
15.15	Illek et al. Czech Republic	Subclinical hypocalcaemia and related diseases of the transition dairy cows – *Szubklinikai hypocalcaemia és kapcsolódó megbetegedések a tejelő tehenekben az ellés körüli időszakban*

15.30 Kávészünet * Coffee break

Poszter szekció * Poster session

Üléselnök * Chairmen
WITTEK Thomas & BAJCSY Árpád Csaba

16.00	Bajcsy et al.	Effect of a prepartal treatment with Vitamine D on early postpartum uterine contractility and blood calcium concentrations in dairy cows – *Ellés előtt adott D-vitamin kezelés hatása az ellést követő méhkontraktilitás és a vér kalcium-koncentrációjának alakulására tejhasznú tehenekben*

16.15	Bakony et al.	A hőstressz hatása tejelő tehenek egyes anyagforgalmi mutatóira és az anyagcserezavarok előfordulási gyakoriságára – retrospektív vizsgálat *The effect of heat stress on some metabolic parameters and prevalence of metabolic disorders in lactating cows – a retrospective study*
16.30	Đuričić et al. & Samardžija Marko Croatia	Influence of dietary clinoptilolite supplementation on milk fat to protein ratio and β-hydroxybutyrate serum levels during transition period in dairy cows – *A klinoptilolit (CPL) takarmány-kiegészítő hatása tejelő tehenek a tej zsír:fehérje arányára és a szérum β-hidroxibutirát-szintjére az ellés körüli időszakban*
16.45	Snedec et al. Germany	Verification of an on-farm ketone measurement device for the detection of beta-hydroxy butyrate concentration in blood in dairy cattle – *Egy hordozható keton meghatározó készülék ellenőrzése a vér béta-hidroxi-butirát koncentrációjának megállapítására tejelő szarvasmarhákban*
17.00	Hejel Péter & Könyves László	Egy lehetséges módszer az oxidatív stressz tejelő állományokban történő in-vivo meghatározására: Előzetes eredmények *A possible method for in vivo determination of the oxidative stress in dairy herds: Preliminary results*
17.15	Leonhardt et al. Germany	Haptoglobin concentration in various inflammatory diseases in German Holstein cows under field conditions *Haptoglobin koncentráció különböző gyulladásos betegségekben gyakorlati körülmények között németországi Holstein tehenekben*
17.30	Jurkovich et al.	A borjak ad libitum tejitatásának hatása – szakirodalmi összefoglaló – *The effects of ad libitum milk feeding on calves – review of literature*
17.45	Papp Attila	Miért pont az ELISA? Rövid betekintés a szerodiagnosztikai vizsgálatok kulisszái mögé *Why ELISA? In-depth look into serological diagnostics*

19.00 Kulturális program * Cultural programme

Vacsora * Dinner

2018. június 1. * June 1, 2018

08.00 Regisztráció * Registration

Üléselnök * Chairmen
MUDRON Pavel & KOVÁCS Péter

09.00	Audrius Furmonavičius Denmark	Danish guidelines for the use of antibiotics for the treatment of mastitis in dairy cattle – *Dán iránymutatás a tőgygyulladások kezelésére használt antibiotikumokra vonatkozóan tejhasznú tehenészetekben*
09.30	Schauer et al. Austria	Occurrence of oxacillin/methicillin resistant Staphylococci (ORS) in Austrian ruminants and New World camelids *Oxacillin/meticillin rezisztens Staphylococcus-fajok (ORS) előfordulása osztrák kérődzőkben és újvilági tevefélékben*
09.45	Albert et al.	A hazai tejelő szarvasmarha állományokban előforduló meticillin-rezisztens Staphylococcus aureus törzsek jellemzése – kitekintés és újabb eredmények – *Methicillin-resistant Staphylococcus aureus strains isolated from Hungarian dairy herds in the past 15 years – an outlook and further results*
10.00	Ózsvári et al.	Financial evaluation of vaccination against Staphylococcus and E. coli mastitis on a commercial Hungarian dairy farm *Staphylococcusok és E. coli által okozott tőgygyulladás elleni vakcinás védekezés gazdasági elemzése egy hazai nagy létszámú Holstein-fríz tehenészetben*
10.15	Wu et al. China	Emergence of enterococcal mastitis and its clinical implications *Enterokokkális masztitisz megjelenése és annak klinikai következményei*

10.30 Kávészünet * Coffee break

Poszter szekció * Poster session

Üléselnök * Chairmen
SOBIECH Przemysław & ÓZSVÁRI László

11.00	Kovács et al.	A mintavétel szerepe egyes tőgypathogén kórokozók kimutathatóságában – *The effect of sampling procedures on the identifications of some mastitis pathogens*

11.15	Mikó Józsefné et al. & Kocsisné Gráff Myrtill	A tőgybimbót érő káros fizikai hatások vizsgálata nagyüzemi tehenészetekben – *The examination of teat-end condition on large scale dairy farms (Literature review)*
11.30	Hisira et al. Slovakia	Assessment of udder health in dairy cows with claw diseases by using of mastitis scoring system – *Tőgyegészségügy értékelése masztitisz pontozási rendszer segítségével csülökbetegségekben beteg tejelő tehenekben*
11.45	Kovács et al.	Cardiac responses to milking in dairy cows milked in conventional milking systems with stationary and rotary milking stalls – *Tejelő tehenek szívműködési értékeinek változása hagyományos halszálkás, parallel és karusszel fejési rendszerekben*
12.00	Kiss et al.	Using selection and assisted reproductive technologies to deal with mastitis on a dairy farm – *A szelekciós és támogatott reprodukciós technológiák alkalmazása a tőgygyulladás kezelésére egy tejhasznú tehenészetben*
12.15	Bittner et al. Germany	Teat structure in Brazilian dairy buffalos assessed using ultrasonography – *Brazíliai tejhasznú bivaly tőgybimbó szerkezetének megállapítása ultrahangvizsgálattal*
12.30	**Ebéd * Lunch**	

Üléselnök * Chairmen
ILLEK Jozef & VINCZE Boglárka

14.00	Donald E. Niles USA	Replacement heifer rearing – *Tenyészüszők felnevelése*
14.30	Vincze et al.	A magzati jóllét vizsgálatának lehetőségei ultrahangkészülékkel szarvasmarhában – *Ultrasonographic assessment methods of fetal well-being in cattle*
14.45	Kézér et al.	Heart rate variability, respiratory rate and rectal temperature in heat stressed dairy calves with or without supplemental shade *Árnyékolás és elmaradásának hatása hőstressznek kitett tejhasznú borjak szívritmus-változékonyságára, légzési frekvenciájára és rektális hőmérsékletére*
15.00	Staněk et al. Fleischer et al. Czech Republic	Microbial contamination of colostrum from Czech dairy herds *Cseh tejhasznú tehenészetekből származó kolosztrum mikrobiális szennyezettsége*

15.15	Urban-Chmiel et al. Poland	The experimental phage therapies of diarrhoea in newborn calves – *Kísérleti fágterápia hasmenéses újszülött borjakban*

15.30 Kávészünet * Coffee break

Üléselnök * Chairmen
GENTILE Arcangelo & KOVÁCS Levente

16.00	Kofler et al. Austria & Slovakia	Limb fractures in 32 small ruminants – Treatment and outcome *Végtagtörés 32 kiskérődzőben – Gyógykezelés és annak kimenetele*
16.15	Wysocka Dominica Poland	Effectiveness of mineral supplementation in sheep *Ásványi anyag kiegészítés hatékonysága juhokban*
16.30	Cebulska Karolina Poland	Effect of β-hydroxy β-methylbutyrate acid (HMB) for growth and health traits in young goats *β-hidroxi-β-metil-vajsav (HMB) hatása a fiatal kecskék növekedésére és egészségügyi állapotára*
16.45	Ural et al. Turkey	Calves- and lamb-level association between serum 25-hydroxyvitamin D concentrations and cryptosporidiosis *Szérum 25-hidroxi-D-vitamin-koncentráció és a cryptosporidiosis közötti kapcsolat borjakban és bárányokban*
17.00	Kővágó et al.	A borjak újszülöttkori pancytopeniája néven ismert kórkép és annak hazai előfordulása – *Occurrence of an unusual form of bovine neonatal pancytopenia in a Hungarian herd*
17.15	Lambacher et al. Austria	Comparative study on correlations between coproscopic examination and organ diagnosis for trematodes and lungworms in slaughtered lambs – *Vágóhidi bárányok koproszkópos és szervdiagnosztikai vizsgálati eredményeinek összehasonlítása trematodák és tüdőférgek tekintetében*
17.30	Gentile et al. Italy	CLINICAL ANATOMY: a glance in the abdomen of the cattle *Klinikai anatómia: pillantás a szarvasmarha hasüregébe*

2018. június 1. * June 1, 2018
Eger konferencia terem / Eger Conference Room

Workshop: Does the current veterinary medical education meet the future requirements of cattle practice?
only in English

Üléselnök * Chairmen
DOLL Klaus & SZENCI Ottó

11.00	Kronenberger Maria & Doll Klaus Germany	Does the current veterinary medical education meet the future requirements of cattle practice? – *A jelenlegi állatorvosi képzés megfelel-e a szarvasmarha-gyakorlat jövőbeni követelményeinek?*
11.20	Wittek et al. Austria	A survey on teaching of veterinary students on ruminant medicine in veterinary schools in Europe *Felmérés az állatorvostan-hallgatók kérődző gyógyászati oktatásáról az európai állatorvosi egytemeken*
11.30	Bajcsy et al. Germany	Veterinary medical education for cattle at the University of Veterinary Medicine Hannover – *A szarvasmarha-egészségügy oktatása a Hannoveri Állatorvosi Főiskolán*
11.35	Mudron, Pável Slovakia	The Clinic of Ruminants in Kosice (Slovakia) – teaching of the ruminant medicine – *Kérődzők Klinkája Kassán (Szlovákia) – a kérődző-egészségügy oktatása*
11.40	Ózsvári László	Graduate and post-graduate bovine health education at the University of Veterinary Medicine, Budapest – *Szarvasmarha-egészségügy graduális és posztgraduális oktatása a budapesti Állatorvostudományi Egyetemen*
11.45	Gentile Arcangelo Italy	Is it not the time to split the veterinary education in two tracks: companion and farm animals? – *Nem itt van az ideje, hogy az állatorvosi képzést ketté válasszuk - társállatokra és haszonállatokra?*
11.50	Samardzija, Marko Croatia	Ruminant veterinary medical education in Croatia: Current status – *A kérődző állatorvosi oktatás Horvátországban: jelenlegi állapot*

11.55	Sobiech, Przemek Poland	Practical education of ruminants disease on Veterinary Faculty in Olsztyn, Poland – *A kérődzők betegségeinek gyakorlati oktatása az Olsztyn-i Állatorvosi Karon, Lengyelország*
12.00	Illek, Josef Czech Republic	Ruminant medical education in Brno, Czech Republic *A kérődző egészségügyi oktatás Brno-ben, Csehország*

12.05 Discussion * Megbeszélés

12.30 Ebéd - Lunch

20.00 Gála vacsora * GALA dinner**

2018. június 2. * June 2, 2018

8.00 Regisztráció * Registration

Üléselnök * Chairmen
SAMARDŽIJA Marko & HORVÁTH András

09.00	Szenci et al.	Effect of post-parturient metabolic disorders on reproductive performance in dairy cows – *Az ellés utáni anyagforgalmi zavarok hatása a tejelő tehenek szaporodásbiológiai teljesítményére*
09.30	Szelényi et al.	Effect and consequences of the changing length of the dry period in a dairy farm – *A szárazonállás hosszának megváltozása és következményei egy tehenészetben*
09.45	Čech et al. Czech Republic	The use of transvaginal ultrasound guided access to ovary in dairy cattle – *A petefészek transzvaginális ultrahang vezérelt vizsgálata tejhasznú szarvasmarhákban*
10.00	Wöckel Adriana & Sobiraj Axel Germany	Field trials for the assessment and optimization of the selected insemination day in dairy cows using a quantitative on-farm milk progesterone test – *A termékenyítések optimális időpontjának meghatározása egy kvantitatív, helyszíni tej progeszteron teszt segítségével tejhasznú tehenekben*

10.15	Fodor et al.	Relationships of management practices and reproductive parameters in Holstein cows on large commercial dairy farms *Menedzsment gyakorlat és a szaporodási mutatók közötti összefüggések holstein-fríz tehenekben egy tehenészeti telepen*

10.30 Kávészünet * Coffee break

Üléselnök * Chairmen
KAREN Aly & SZELÉNYI Zoltán

11.00	Yazlik et al. Turkey	The evaluation of neutrophil function, superoxide dismutase activity and metabolic profile ın cows with retained placenta *Magzatburok-visszatartással rendelkező tehenek neutrofil funkciójának, szuperoxid-diszmutáz aktivitásának és a metabolikus profiljának értékelése*
11.15	Kurt et al. Turkey	The average culling rate and major causes of infertility of professional dairy herds in Turkey *Professzionális tejelő állományok átlag selejtezési aránya és a meddőség fő okai Törökországban*
11.30	Kovac et al. Slovakia	Some variables of milk fat profile in different rearing system of dairy cows – *Különböző tartási körülmények között tartott tejhasznú tehenek tejzsír profiljának egyes változói*
11.45	Akin et al. & Gurdal Emre Turkey	Field trial of the organic treatment of bovine digital dermatitis lesions with bovine colostrum – *A szarvasmarha digitális dermatitisz elváltozások kolosztrummal való organikus kezelésének gyakorlati kipróbálása*
12.00	Karen Aly Qatar	Factors affecting embryonic death after embryo transfer in dromedary camels – *Embrió átültetést követő embrionális veszteségeket befolyásoló tényezők dromedári tevékben*
12.15	Kozat Süleyman & Özkan Cumali Turkey	Intercellular adhesion molecule-1 (ICAM-1) and C-reactive protein (C-RP) levels in calves infected naturally with Mycoplasma bovis – *Intercelluláris adhéziós molekula-1 (ICAM-1) és C-reaktív fehérje (C-RP) szintje a Mycoplasma bovis-val természetesen fertőződött borjakban*

12.30 Ebéd * Lunch

Kongresszus zárása * Closing the congress

POSZTER BEMUTATÓK * POSTER PRESENTATIONS

Klinikai Megbetegedések * Clinical Diseases

Bognár et al.	A paratuberculosis, mint lehetséges közegészségügyi veszélyforrás *Paratuberculosis, as a possible public health risk*
Nagy et al. Slovakia	Serum proteins in dairy cows suffering from clinical signs of paratuberculosis – *Paratuberkulózis klinikai tüneteit mutató tejelő tehenek szérumfehérje értékei*
Filipek Jaroslav & Illek Jozef Czech Republic	Isotachophoresis – powerful tool in the large animal laboratory *Izotachoforézis – hatékony eszköz a nagyállat laboratóriumban*
Ózsvári et al.	The financial evaluation of BVD vaccination on a commercial Hungarian dairy farm – *A BVD elleni vakcinás védekezés gazdasági elemzése egy hazai nagy létszámú holstein-fríz tehenészetben*
Capak et al. Croatia	Radiographic study of hind limb digits in Simmental stud bulls *Hátúlsó végtag csülkeinek radiológiai vizsgálata Simmentáli bikákban*
Fleischer et al. Czech Republic	Two years of the national collection of health disorders in Czech dairy cows *A cseh tejelő tehenek egészségügyi rendellenességeinek értékelése az utóbbi két év adatai alapján*

Anyagforgalmi Rendellenességek * Metabolic Disorders

Slivinska et al. Ukraine	Concentration of citric acid and osteocalcin in blood serum for osteodystrophy of cows – *A vérszérum citromsav és az oszteokalcin koncentrációja tehenek osteodystrophiája esetén*
Jezek et al. Slovenia	Changes in milk composition indicating metabolic disorders in dairy cows *A tej összetételének változásai a tejelő tehenek anyagcserezavarait jelzik*
Yazlik et al. Turkey	The evaluation of neutrophil function, superoxide dismutase activity and metabolic profile ın cows with retained placenta – *Neutrofil funkció, szuperoxid-diszmutáz aktivitás és a magzatburok-visszatartással rendelkező tehenek metabolikus profiljának értékelése*
Lychuk et al. Ukraine	Plasma lipid spectrum of cows suffering from ketosis *Ketózisos tehenek plazma lipid spektruma*

Vlizlo et al. Ukraine	The diagnosis of metabolic acidosis in cows suffering from ketosis using the urine study – *Metabolikus acidózis diagnózisa ketózisban szenvedő tehenekben a vizelet vizsgálatával*
Vlizlo et al. Ukraine	Hormonal regulation of energy metabolism in ketotic cows *Az energia metabolizmus hormonális szabályozása ketotikus tehenekben*
Vudmaska et al. Ukraine	Comparison of monensin and hop cones effects on rumen fermentation and blood parameters in transition dairy cows – *A monensin és a komlókomponensek hatásainak összehasonlítása az elléskörüli időszakban lévő tejelő tehenek bendő fermentációjára és vérparamétereire*
Mihajlovičová Xenia & Mudroň Pavol Slovakia	Effects of a different body condition in dry period on energetic profile in dairy cows – *Különböző kondiciók hatása a tejhasznú tehenek szárazon állás alatti energia profiljára*
Mudron Pavol Slovakia	Effects of glucose infusion on blood potassium in cattle *Glükóz infúzió hatása a vér kálium szintre szarvasmarhákban*
Píšťková et al. Czech Republic	Antioxidant status and concentration levels of malondialdehyde (MDA) in dairy cows during periparturient period – *Az antioxidáns statusz és a malondialdehid (MDA) koncentráció a tejelő tehenekben a peripartalis időszaka alatt*
Sakhniuk et al. Ukraine	Distribution, etiology, course and diagnosis specificity of polymorbid internal pathology in cows – *Polimorbid megbetegedések megoszlása, etiológiája, perspektívája és diagnózis-specifitása tehenekben*
Slivinska et al. Ukraine	Prophylaxis of trace minerals deficiencies (Co, Cu, Zn) in dairy cows *Nyomelemhiány (Co, Cu, Zn) megelőzése tejelő tehenekben*
Fedoruk et al. Ukraine	Indices of blood and milk of cows at action of nickel citrate in the first two months of lactation – *Nikkel-citrát hatása a laktáció első két hónapjában a tehenek egyes vér és tej paramétereire*
Erdogan et al. Turkey	On-site measurement of lactate levels during transition period in dairy cows *A laktátszintek helyszíni meghatározása a tejhasznú tehenekben az ellés körüli időszakban*

Reprodukció * Reproduction

Dolnik et al. Slovakia	Evaluation of the sperm fertilization status by flow cytometry *A spermium fertilitási státuszának értékelése áramlási citometriával*

Kuzmina et al. Ukraine	Correlation between of antioxidant defence enzymes activity and malate-aspartate shuttle in ejaculates – *Az antioxidáns védekezési enzim aktivitás és malát-aszpartát transzfer korrelációja az ejakulátumokban*
Novotniné et al.	A szaporodásbiológiai mutatókat javítandó asszisztált reprodukciós technika bevezetésének eredményei Holstein-fríz tejelő szarvasmarha telepen – *The results of introducing assisted reproduction techniques to improve reproductive performances in Holstein-Friesian dairy cow farm*
Béri Béla Brassó Dóra Lili	A keresztezés hatása a tejhasznosítású tehenek termékenységére és állategészségügyi állapotára – *The influence of crossbreeding on prolificacy and health condition of dairy cows*
Fodor et al. Hungary & Austria	Associations of management practices and reproductive performance in Holstein-Friesian replacement heifers – *Holstein-fríz tenyészüszők szaporodásbiológiai menedzsmentje és mutatói közötti összefüggések elemzése*
Ózsvári et al.	Evaluation of the reproductive performance of large Hungarian dairy farms – *A nagy létszámú magyarországi tejelő tehenészetek szaporodási eredményeinek értékelése*
Madoz et al. Argentina	Changes in the endometrial expression of growth factors associated with the estrus cycle in grazing dairy cows – *A legelő tejhasznú tehenek nemi ciklusával kapcsolatos növekedési faktorok endometrialis expressziójának változása*
Madoz et al. Argentina	Effect of milk production on endometrial gene expression in grazing dairy cows – *A tejtermelés hatása a legelő tejhasznú tehenek endometriális génexpressziójára*
Górriz-Martín et al. Germany	Preliminary results of the use of a calving detector in Holstein-Friesian heifers and cows – Ellés kezdetét jelző készülék alkalmazása során nyert előzetes eredmények Holstein-fríz üszőkön és teheneken

Tőgyegészségügy * Udder Health

Baumgartner et al. Austria & Czech Republic	Susceptibility and resistance of selected pathogens of the mammary gland of cattle from Austria and Czech Republic in 2017 – *Ausztriai és csehországi szarvasmarha tőgypatogén kórokozóinak érzékenysége és rezisztenciája 2017-ben*
Kovács Péter & Siegwalt Georges Hungary & Austria	Susceptibility of Gram positive udder pathogens to penicillin and to combinations of cephalexin-kanamycin and penicillin-framycetin in Hungarian dairy herds – *Gram-pozitív tőgypatogén kórokozók érzékenysége penicillinre, ill. cefalexin-kanamicin és penicillin-framycetin kombinációjára magyar tejelő állományokban*

Pitoiu et al. & Siegwalt Georges Austria & Poland & Romania	Susceptibility of major udder pathogens to penicillin and to combinations of cephalexin-kanamycin and penicillin-framycetin in Poland and in Romania – *Főbb tőgypatogén kórokozók érzékenysége penicillinre, ill. cephalexin-kanamicin és penicillin-framycetin kombinációjára Lengyelországban és Romániában*
Klinkon et al. Slovenia	Influence of diseases on milk yield and composition in a herd of Holstein-Friesian cows – *Megbetegedések hatása a tejtermelésre és a tejösszetételre egy Holstein-fríz állományban*

Borjak * Calves

Kozat Süleyman Turkey	The importance of colostrum management in newborn calves *Kolosztrum menedzsment fontossága újszülött borjakban*
Ural Alic Deniz & Ural Kerem Turkey	Effects of short term clinoptilolite supplementation on weight gain in Holstein calves – *Rövid távú klinoptilolit kiegészítés hatása a Holstein borjak súlygyarapodására*
Erdogan et al. Turkey	Alternative phytotherapeutic treatment approach in calves with pneumonia: Pelargonium sidoides (Preliminary results) *Tüdőgyulladásos borjak alternatív fitoterápiás kezelése: Pelargonium sidoides (Előzetes eredmények)*
Scherf et al. Germany	The use of thermography for monitoring the heat development during hot iron dehorning of German Holstein calves – *A borjak forró vassal történő szarvtalanitásakor keletkezett hő ellenőrzése termográffal*

Kiskérődzők * Small Ruminants

Jury et al. Austria	Influence of Alpine pasturing in the epidemiology of Toxoplasma gondii and Neospora caninum in sheep in Austria – *Az alpesi legelő hatása a Toxoplasma gondii és a Neospora caninum epidemiológiájára ausztriai juhokban*
Kadasi et al. Slovakia	Effect of corpus luteum and age of sheep on the embryo survival *A corpus luteum és a kor hatása a juh embriók túlélésére*
Klein et al. Slovakia	Effect of development, quality and GnRH agonist administration on embryo survival in sheep – *Fejlődés, minőség, valamint a GnRH agonista adás hatása a juh embrió túlélésére*

Tóthová et al. Slovakia	Serum protein electrophoretic pattern and acute phase protein concentrations in sheep after induced articular cartilage defect *A szérum fehérje elektroforetikus profilja és az akut fázisú fehérje koncentrációja juhokban indukált ízületi porcelváltozást követően*
Czoller et al.	A vértelen úton történő herélés hatásának vizsgálata a kosbárányok súlygyarapodására, viselkedésére és húsminőségére: Előzetes közlemény *Effect of non-invasive castration (banding or elastor) method on weight gain, behaviour and meat quality of ram lambs: Preliminary notice*
Ayan et al. Turkey	Serum 25-hydroxyvitamin D levels in association with Giardiasis in lambs *Szérum 25-hidroxi-D-vitamin szintje Giardiasis esetén bárányokban*
Sharan et al. Ukraine	Quality of spermatozoa after addition of nanocitrates microelements in diluted ram ejaculates – *A spermiumok minősége nanocitrát-mikroelemek hozzáadását követően hígított kos ejakulátumokban*
Mudron Pavel Slovakia	Study of tetracycline administration effects on ruminal activity in sheep with foot rot – *Tetraciklin adás hatásának vizsgálata a bendő aktivitásra büdös sántaság esetén juhokban*
Pascal et al. Romania	The influence of technological factors on the lactogen potential at goats *Technológiai tényezők hatása a kecskék tejtermelésére*
Ural Alic Deniz & Akin Ibrahim Turkey	The investigations with Pearson correlations of relationships among some testicular and body measurements of Alpine male goats *Az alpesi hímivarú kecskék egyes testicularis és testméretei közötti kapcsolat vizsgálata Pearson-korrelációval*
Šimková Ivana & Indrová Eva Czech Republic	Acute ruminal acidosis in goats: A case study *Akut bendő-acidózis kecskékben: Esettanulmány*
Kazatelová et al. Czech Republic	The effect of maternal age on the quality of colostrum and changes of hematological and biochemical parameters in neonatal goats after colostrum intake – *Az anyai életkor hatása a kolosztrum minőségére, a hematológiai és a biokémiai paraméterek változásaira újszülött kecskékben kolosztrum felvétele után*
Snarska – Sobiech – Chrostowska Poland	Cytological evaluation of erythroblastic cell line in bone marrow smears of young fallow deer (Dama dama) *Erythroblastic sejtvonal citológiai értékelése a fiatal dámvad csontvelő kenetjeiben (Dama dama)*

Tóth et al.	Effects of some biotic and abiotic factors on gastrointestinal parasite infections of sheep – *Egyes biotikus és abiotikus tényezők hatása a juhok belső parazita fertőzésére*
Pálfyné Vass et al.	Az Intézményközi Kiskérődző Biotechnológiai Kutatócsoport (IKBK) megalakulása és eddigi eredményei – *Foundation and results of the Interinstitutional Small Ruminant Biotechnology Research Group*

Johne´s disease is of considerable importance in cattle

Johne-betegség jelentős fontosságú szarvasmarhákban

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Abstract

Johne´s disease (JD) is caused by Mycobacterium avium subsp. paratuberculosis (MAP) and can lead to considerable economic losses in affected cattle herds. These losses can be divided into direct costs, such as culling of infected animals and increased costs for the purchase of replacement cattle and indirect losses, such as reduced milk yield or reduced carcass weight. Additionally, costs for veterinarians, laboratory tests and changes of the farm management in the course of control programmes have to be considered. These losses of several hundred to thousand Euros per affected animal are often not recognized or not linked to JD, as the disease has a chronic and subclinical course. Therefore, the implementation of targeted and practical control programmes to prevent or mitigate economic losses due to MAP-infections in cattle should be aspired.

Introduction

Paratuberculosis, also called Johne´s disease (JD) is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) and can lead to considerable economic losses in affected cattle herds. The disease is characterized by a chronic granulomatous enteritis with a long subclinical incubation period. Depending on the progress of the infection and the clinical picture, JD in cattle is divided into 4 stages: Silent infection, subclinical disease, clinical disease and the final stadium, also called progressed clinical disease (Fecteau & Whitlock, 2010). Animals showing characteristic symptoms of JD, such as emaciation and chronic diarrhoea are only the “tip of the iceberg” of MAP-infections within a herd. For every animal with clinical JD up to 25 animals have to be considered subclinically infected with MAP in the herd (Whitlock & Buergelt, 1996). Clinical JD usually occurs in animals

with a minimum age of 2 years, with the first clinical signs often linked to stress such as calving. JD is considered untreatable and leads to the death of infected animals. The herd prevalence of JD is about 50% in most countries with a considerable dairy industry (Barkema et al., 2010). In Austria a prevalence of about 20% serological positive cattle herds has been reported (Khol et al., 2007). Many different countries have launched control programmes for JD in cattle, either voluntarily or compulsory (Khol & Baumgartner, 2102). Since 2006, clinical JD is considered a notifiable disease in Austria, leading to culling of affected animals, which is financially compensated by the state (Khol et al., 2007).

Clinical JD can be diagnosed based on the history and clinical examination and verified by laboratory testing. Unfortunately, current laboratory test for direct detection of MAP such as bacteriological culture or PCR (polymerase chain reaction) as well as indirect methods to detect specific antibodies by ELISA (enzyme linked immunosorbent assay) show a low sensitivity in early stages of the infection (Nielsen, 2008).

As JD is untreatable, many different programmes for the control or eradication of MAP in cattle have been established. Most of these programmes are based on a test and cull strategy, combined with hygienic measures to prevent new infections.

The aim of this paper is to highlight economic losses caused by JD in cattle, both in single animals as well as on the herd level.

Economic impact of Johne´s disease in cattle

Economic losses due to MAP infections can be substantial in affected herds. These losses can be divided into direct costs, such as culling of infected animals and increased costs for the purchase of replacement cattle and indirect losses such as reduced milk yield or reduced carcass weight (Ott et al., 1999).

Direct and indirect cost caused by decreased productivity

Due to the chronic progress of JD in cattle the disease often is not recognized and estimation of the financial losses, especially indirect losses, remains difficult.

Cows subclinically infected with MAP show a reduction of milk yield of 19% as compared to their previous (uninfected) lactation. In another study, a mean loss of 200 US-Dollars per year for a cow infected with JD in farms with a high incidence of MAP-infections has been reported. The loss to the entire milk industry of the United States of America due to JD was calculated to be 200-250 Million US-Dollars annually (Ott et al., 1999).

Carcass weight also is reduced in cattle with JD. It has been shown that ELISA-positive dairy cows show a reduction of the carcass weight and thereby of the avail of 10-17% at slaughter, compared to negative animals. In case MAP was also detected by faecal culture, this loss increased to 15-31%, with an additional loss of 20-31% in animals with symptoms of enteritis (Kudahl & Nielsen, 2009).

Furthermore, an increase of the calving interval of 28 days has been found in cows with a MAP-positive ELISA result (Johnson-Ifearulundu et al., 2000).

In another study the total annual loss per dairy cow with clinical JD was calculated to be 1.940 Euros and 461 Euros per cow with a subclinical MAP-infection (Dufour et al., 2004). In beef cattle, the loss was estimated to be 1.905 Euros per animal showing clinical JD in the same study (Dufour et al., 2004).

Costs of veterinarians, diagnosis and abatement

In addition to the costs mentioned above, costs for veterinarians, laboratory tests and changes of the farm management in the course of control programmes have to be considered.

Pillars et al. (2009) calculated average costs of 30 US-Dollars per cow and year for a control programme for JD in cattle. Other calculations showed that a “test and cull” programme should be considered economically justified if the intra herd prevalence of MAP-infections is at least 5%. Because of the substantial impact of control programmes for JD, both on economic and management aspects, detailed definition of the goals of such programmes is essential, as eradication of MAP cannot be achieved in most cases. Due to the long incubation period and the high tenacity of MAP in the environment, as well as the limited diagnostic performance of laboratory tests, a minimum duration of 5 years has to be calculated for control programmes. Although eradication of MAP from the herd cannot be achieved in most cases, a substantial reduction of new infections and clinical cases of JD, leading also to a reduction of economic losses can be reached in most herds.

Discussion

Johne´s disease in cattle can cause substantial economic losses in affected herds, both because of clinical and subclinical MAP-infections. Reduced milk yield and fertility, as well as a reduction of the carcass weight at slaughter can cause financial losses of several hundred Euros per infected animal. These losses are often not recognized or not linked to JD, as the disease has a chronic and subclinical course. Therefore, the implementation of targeted and practical control programmes to prevent

or mitigate economic losses due to MAP-infections in cattle is necessary. Such programmes additionally contribute to the prevention of new MAP-infections within and between herds.

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Evolving Johne's control. A new strategy for defeating an old disease

Johne beteség kontrolljának fejlődése. Új stratégia egy régi betegség legyőzésére

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Abstract

Johne's disease control can be very frustrating on a modern dairy farm. While the traditional ELISA testing method along with culling positive cows can reduce the percentage of the herd that is infected, it is not well suited to eradicating the disease from the herd. By using a combination of an ELISA screening test with a reduced cut point, as well as a fecal PCR confirmatory test we have achieved a much higher sensitivity and specificity in our testing program. This allows us to find and cull the cows shedding the vast majority of the MAP organisms, without also culling an unsustainable number of false test positive clean cows. The program can be further improved by using PCR technology to test facilities and equipment that comes in contact with the susceptible population of young animals. With these enhancements in testing and control we feel the eradication of *Mycobacterium avium* subspecies *paratuberculosis* has become an achievable goal for a dairy herd.

For years, dairy farms in the United States have struggled to eliminate *Mycobacterium avium* subspecies *paratuberculosis*. Historically, if our goal is eradication, we have met with limited success.

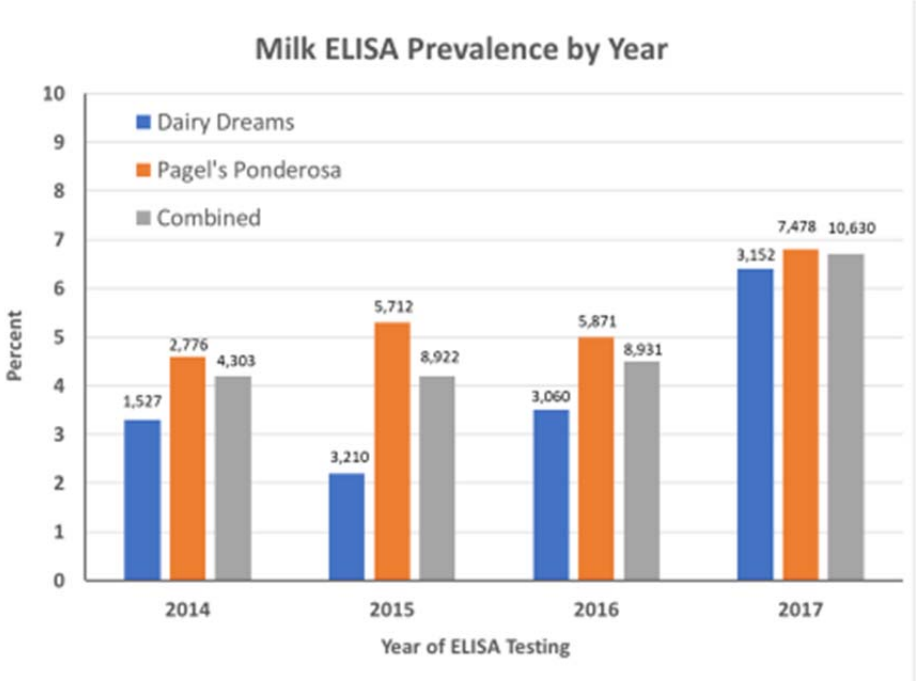
Traditional control programs have emphasized several practices. Control efforts have emphasized environmental sanitation measures focused, in particular, on the maternity and calf raising programs. This is due to the likelihood of transmission occurring between infected adult animals and susceptible young animals. In addition to environmental sanitation measures, herds that have wished to take a more active approach to control have implemented

testing protocols, with a focus of identifying infected animals and segregating them in some manner from more susceptible animals. These programs include testing systems such as milk or blood ELISA testing on a selected population of the herd, generally fresh cows or cows due to be dried up. ELISA positive cows are either culled or managed in such a way as to reduce the likelihood of transmission.

The two herds that I represent have utilized such a program for over ten (10) years. Our two herds include Dairy Dreams, a 2,850 cow Holstein herd that has been a closed herd for ten (10) years, and Pagel's Ponderosa, a 5,500 cow Holstein/Jersey crossbred herd that has been closed for five (5) years. Both herds have been running milk ELISA testing on all fresh cows. The cows that test positive (cut point .3) have been either culled or removed from the breeding herd. Along with the milk testing, we have put exceptional emphasis on sanitation in the maternity and calf facilities.

Unfortunately, it has long been apparent that while we have moved the infection rate down to about 5% of the lactating herd, we have been frustrated in our effort to lower it any further. Of particular concern, was the fact that we had a significant number of primiparous animals showing up as positive. Also of concern, was an actual uptick in the new infection rate. We were stuck. Eradication seemed to be an impossible goal. This lead to actively seeking a more effective control program than traditionally practiced (Figure 1).

Figure 1. Milk ELISA prevalence by year



Through discussions with several Johne's researchers, we learned that a fundamental problem we had was the technical limitations of our testing system. This is explained with simple epidemiological principles. We were using milk ELISA testing for identification of infected individuals. The literature shows that the sensitivity of milk ELISA for Johne's detection with a cut point of 0.3 is 30%. This means that of the population of infected animals, our test will find 30% (true positives) and miss 70% (false negatives). We also saw that our testing system had a specificity of 98%. This means that of the population of non-infected animals our test will correctly identify 98% (true negatives) and miss 2% (false negatives).

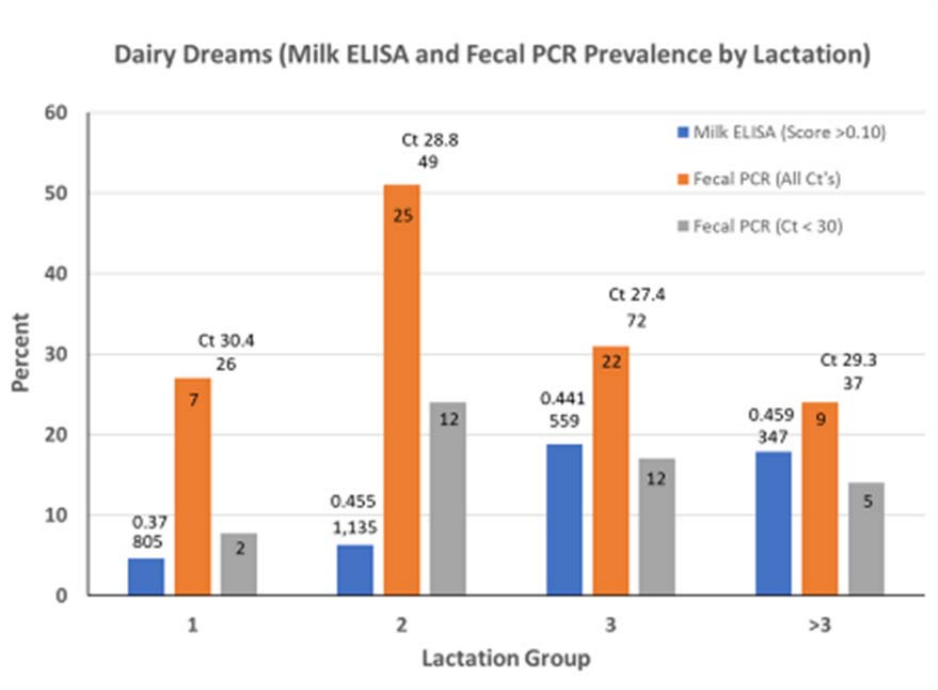
Table 1. Results of the milk ELISA test with a test sensitivity of 30%

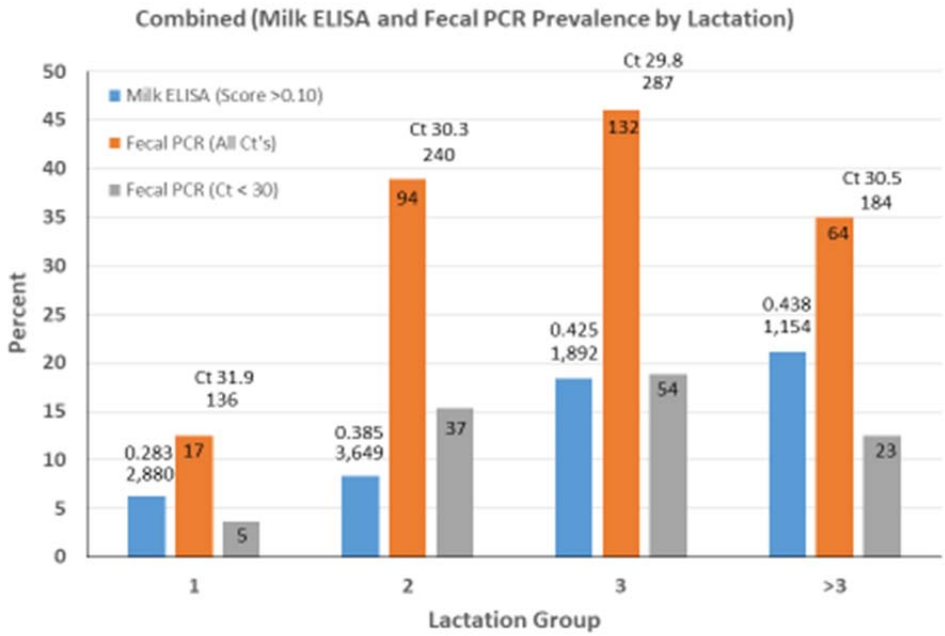
Test Neg	**True Neg**	**True Pos**	
	931	**35**	**966**
Test Pos	**19**	**15**	**34**
	950	**50**	

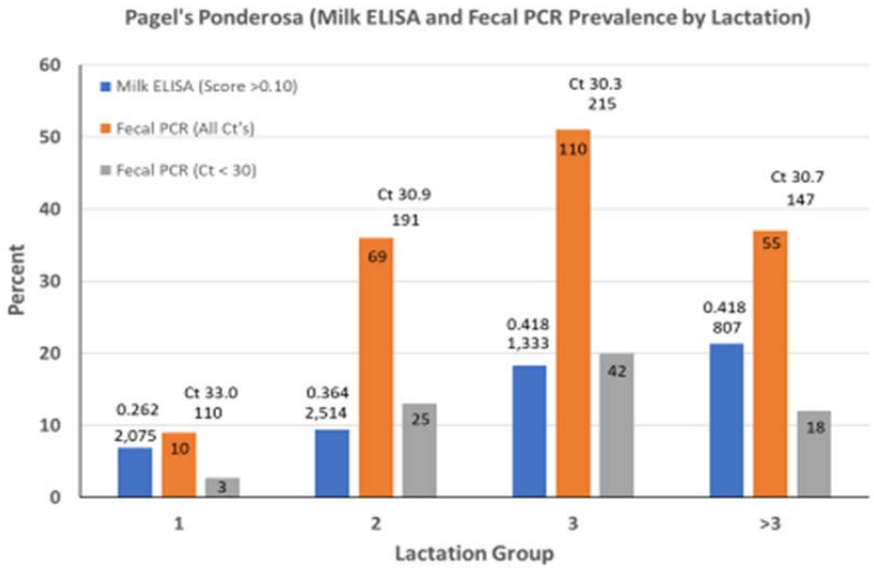
If we assume an actual disease incidence of 5% in a 1,000-cow herd, we will have fifty (50) true positives and 950 true negatives. With a test sensitivity of 30% and fifty (50) true positives, our testing will show 15 true positives and will miss 35 true positives (false negatives). With a specificity of 98%, in a population of 950 true negatives, we will have 931 true negatives that are test negative and 19 true negatives that are test positive (false positives). As a result, of the 34 test positive animals that are culled, more than half were needlessly culled. Of the 966 animals that test negative, 35 are actually infected and should have been culled. This testing scheme essentially locks in a 5% infection rate.

In order to get past this limitation, we needed to go back to basic epidemiological principles. We needed a better more sensitive screening test and also a better, more specific confirmatory test. A screening test should capture almost all positives and needs to be low cost since a large population is being screened. A confirmatory test should identify the true positives in the screened population, and cost is not as critical due to the smaller population being tested. Our solution to this was to utilize the milk ELISA test as our screening test, but with the cut point lowered to 0.1. This way we would increase the sensitivity greatly, which is what we are looking for in a screening test, while at the same time, lowering our specificity which we did not mind in a screening test. The cost of the milk ELISA is $1.25/cow (approximately 1 euro/cow)! We also then included a PCR fecal test of manure samples of the screened ELISA positive cows. This test is nearly 100% specific, meaning that virtually all test positives are truly shedding Johne's bacteria in their feces (Figures 2, 3, and 4).

Figures 2, 3, and 4. The results of milk ELISA and fecal PCR in Dairy Dreams and Pagel's Ponderosa

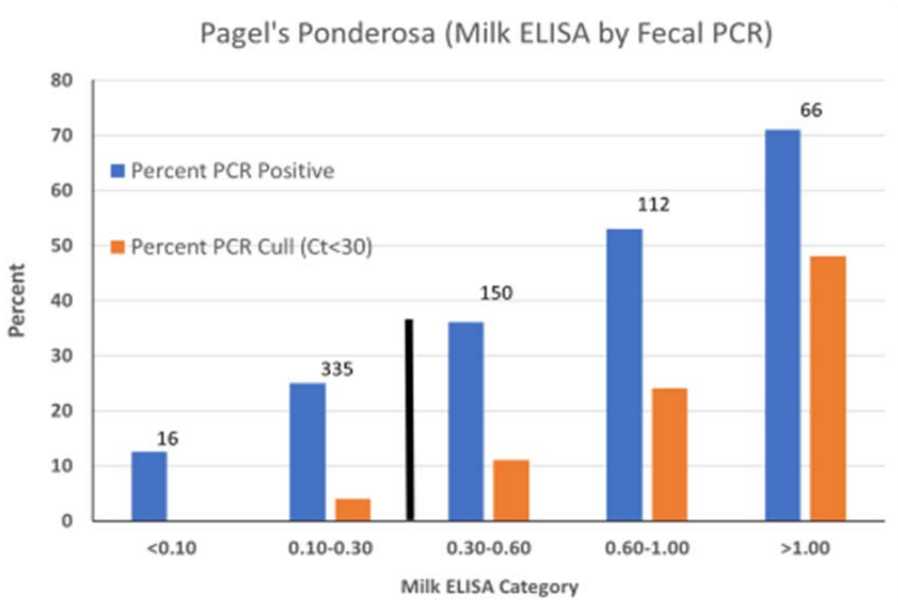


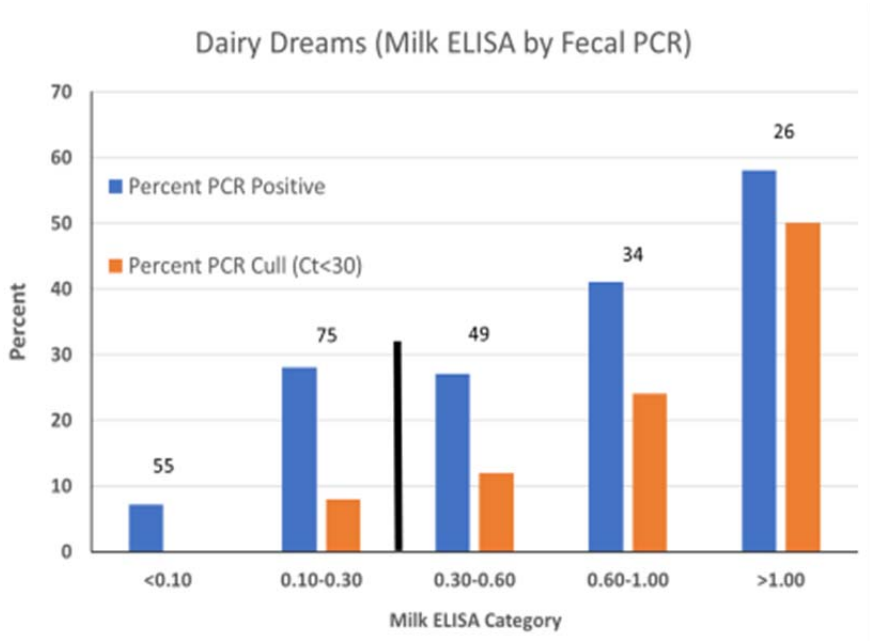


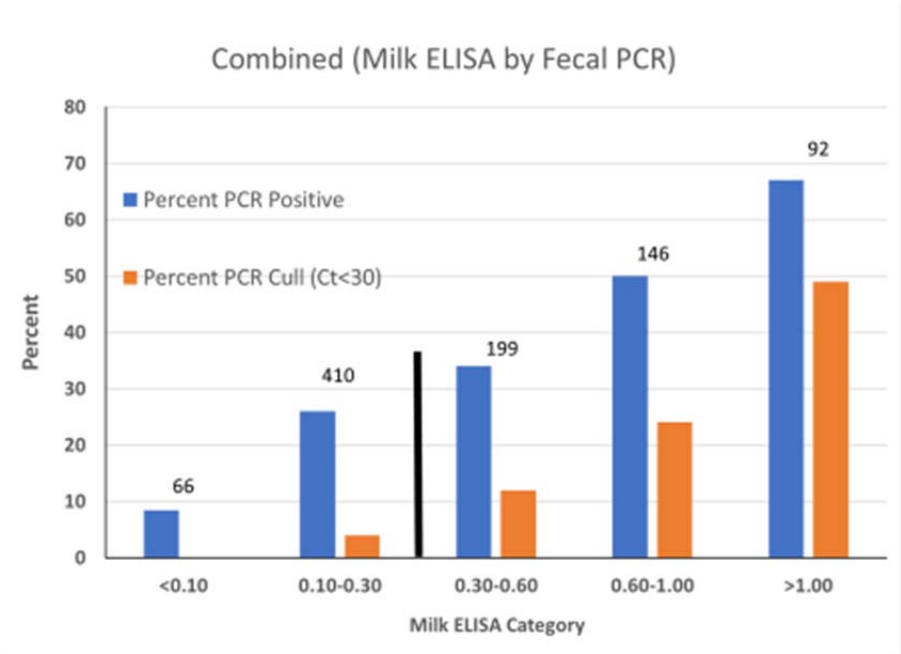


The cost of this test is $25/cow or (30.82 euro/cow). This combination matches a very sensitive screen with a very specific confirmation. We can now be confident that we are catching almost all of our infected cows and are only culling truly infected cows. One further advantage of the fecal PCR confirmatory test is that we can use its CT (cycle time) score to give us a semiquantitative answer. We are able to distinguish between heavy shedders (low CT: <30) and light shedders (high CT: >30) (Figures 5, 6, and 7).

Figures 5, 6, and 7. The results of milk ELISA tests controlled by fecal PCR in Dairy Dreams and Pagel's Ponderosa







Our action taken now is to cull all cows confirmed with fecal PCR CT<30 and to keep, but not rebreed cows confirmed with a fecal PCR CT>30. With this combination, we are finding the majority of our positive cows, but are not incorrectly culling false positive cows.

To this new program, we have also added a second component. We are utilizing PCR testing of environmental samples to target locations on the farms that could lead to transmission of MAP to naïve animals. We are sampling the same sites on the dairy, quarterly, in order to monitor changes occurring in the animals' environment. There have been several key findings, so far, in the environmental testing.

1. There are major differences between our two dairies, despite management – which we believed to be very similar.
2. Dairy Dreams has a very high number of positive environmental samples in the weaned calf area, while Pagel's Ponderosa has very few positive samples.
3. Both herds use digested manure as a bedding source for calves. Environmental PCRs are much stronger positive (lower CT score) for Dairy Dreams vs Pagel's Ponderosa.

We have now developed a theory to explain these differences in environmental PCRs between the two herds. Dairy Dreams uses manure biosolids from the adult cow dairy for heifer bedding. Pagel's Ponderosa uses manure biosolids form only the heifer facility for heifer bedding. The source material at Dairy Dreams, including adult cows has a much higher contamination rate than that at Pagel's Ponderosa. That is reflected in the CT scores of the biosolids at the two facilities.

As a result of these findings, several measures have been put in place.

1. Dairy Dreams calves are now bedded on straw.
2. We are investigating means of reducing MAP in bedding biosolids. One way to do this would be to segregate heifer manure from adult cow manure to be used as heifer bedding material.
3. Commence culturing of manure biosolids to see if MAP organisms are "alive" or "dead".

Conclusion

After stalling out in Johne's control progress using a conventional testing procedure, we have resumed progress by devising a very sensitive screen test and pairing it with a very specific confirmatory test. This allows us to identify and deal with cows that are shedding MAP in their feces. This aggressive testing program can be further augmented by testing the dairies' environment to find areas of likely transmission. By combining these two programs, we are hoping to have Johne's eradication in our herds within reach

A paratuberculosis terjedésében szerepet játszó fontosabb környezeti és tartástechnológiai kockázati tényezők hazai tehenészetekben – előzetes eredmények

Major risk factors for environmental and housing technology involved in the spread of paratuberculosis in Hungarian dairy herds – preliminary results

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Összefoglalás

A szerzők hazai tejtermelő tehenészetekben vizsgálták a paratuberculosis fertőzöttség terjedésének környezeti és tartástechnológiai kockázati tényezőit 2017 szeptemberétől kezdődően, előre összeállított kérdéssor segítségével. A termelésre vonatkozó adatokat a telepeken használt számítógépes telepirányítási rendszer segítségével gyűjtötték. A telep állat-egészségügyi státuszát a telepi állatorvos segítségével mérték fel, a tartástechnológiai adatokat telepszemle során rögzítették. A helyszíni vizsgálatok kiterjedtek a telepi tartástechnológiára, különös tekintettel a borjak tartására, az elletői körülményekre, a borjúnevelés technológiájára. Eddig 15 hazai tejtermelő tehenészetben végezték el a telepszemlét. A telepek átlagos fejt tehén létszáma 641±34 állat, az ELISA vizsgálattal kimutatható paratuberculosis fertőzöttség (látszólagos prevalencia) 4,8±1,3 % (min. 2,3; max. 8,5%). Az előzetes eredményekből kitűnik, hogy a nagyobb mértékű ELISA pozitivitás egyértelműen összefügg a hiányos elletői protokollal, a föcstejitatás ellenőrzésének elmaradásával, az ellető nem megfelelő kialakításával, valamint a borjak körül tapasztalható rosszabb higiéniai körülményekkel. A paratuberculosis elleni védekezési programok hiánya szintén nagyobb mértékű fertőzöttséggel jár együtt. Az előzetes eredményekből az is látszik, hogy a paratuberculosis előfordulásának csökkentése irányába tett menedzsment

lépések segítenek háttérbe szorítani egyéb borjúkori fertőző betegségeket is.

Abstract

The authors have been investigating the environmental and maintenance risk factors of the spread of paratuberculosis infection in Hungarian dairy herds, since September 2017, using a pre-assembled question line. Production data were collected using the computerized herd-management system, the animal health status of the herd was assessed by the herd vet, several details about the animal keeping technology, with special regard to calf housing and calving place, were recorded during the farm visit.

Fifteen dairy herds have been visited until the abstract submission deadline. The average number of lactating cows was 641 ± 34 animals on the farms, the apparent prevalence of paratuberculosis infection detected by ELISA method was 4.8 ± 1.3% (min 2.3, max. 8.5%). From the preliminary results it is apparent that higher ELISA positivity is clearly related to the incomplete calving protocol and bad hygiene conditions around the calves. The absence of paratuberculosis control programs also increases the risk for the disease. The preliminary results support that the management steps and protocols taken to reduce the prevalence of paratuberculosis help to reduce the occurrence of other infectious calf diseases.

Key words: paratuberculosis, management, farming technology

Bevezetés

A paratuberculosis napjainkban a kérődzők egyik legnagyobb gazdasági kárt okozó betegsége. Kórokozója egy sav és alkohol álló baktérium a *Mycobaktérium avium subsp. paratuberculosis* (MAP). A betegség jellemző klinikai tünetei a vízszerű, bűzös hasmenés, lesoványodás és a fehérjevesztés következtében testszerte kialakuló ödémák. A betegség lappangási ideje igen hosszú 2-10 év is lehet (Chiodini et al., 1984). Nagyüzemei tehenészetekben az állatok klinikai tüneteket ritkán mutatnak, mivel sok esetben még a szubklinikai fázis alatt selejtezésre kerülnek egyéb okok, mint szaporásbiológiai, tőgyegészségügyi, lábvég problémák, illetve csökkent tejtermelés (Hasnova & Pavlik, 2006) miatt. A betegség kimutatására használatos diagnosztikai módszerek a legtöbb esetben nem megfelelő érzékenységűek (Donat et al., 2014), így a mentesítés felé tett intézkedések során kulcsszerepet jelentenek a telepen történő menedzsment változtatások (McCaughan et al., 1989). A fertőzi lánc megszakítása a legeredményesebben borjúkorban történhet, mivel az állatok nagyjából fél éves korukig a fogékonyak a fertőződésre, idősebb állatok fertőződésére alig van esély (Garry, 2011). A fertőződés történhet szájon át, a baktériumot ürítő állat bélsarával, szennyezett takarmánnyal, ivóvízzel, illetve föcstejjel is (Sweeney, 1996;

Manning et al., 2010). Méhen belüli fertőződés is lehetséges, ám ennek esélye csak a klinikai tüneteket mutató állatok esetében számottevő (Whittington & Windsor, 2009). A telepi menedzsment legfontosabb feladata a borjak fertőzésmentes felnevelésének megoldása, amiben elsőrendű szerepe van a megfelelő elletői higiéniának, az ellenőrzött föcstejitatásnak és a borjak egyedi ketrecbe kerülése körülményeinek (Carter, 2012). Sajnos a menedzsment ajánlások nagyon összetettek és nehéz megmondani, hogy melyik változtatás vezet a legjobb eredményre a paratuberculosis visszaszorításra tett intézkedésekben.

Munkánk célja felmérni a hazai telepeken alkalmazott elletői és borjúnevelési technológiákat és ezeket összevetni a telepi paratuberculosis fertőzöttség mértékével. A felmérés értékelésekor igyekszünk egy iránymutatást adni azon menedzsment változtatások felé, melyek segíthetnek a paratuberculosis fertőzöttség visszaszorításában, és ezáltal egyéb borjúkori megbetegedések megelőzésében is.

Anyag és módszer

Vizsgálatainkat 2017. szeptember és 2018. február között végeztük az ország teljes területén 15 nagyüzemi Holstein fríz tehenészetben (9 nyugat-, 2 közép- és 4 kelet magyarországi tehenészet) előre elkészített kérdőív segítségével. A kérdőívet telepi szemle során a telepet ellátó állatorvossal és a telepvezetővel töltöttük ki. A számszerű adatokat a telepirányítási szoftver segítségével nyertük, majd az adatokat Excel táblázatban rögzítettük. A telepszemle során az elletőre és a borjúnevelésre vonatkozóan 32 kérdést tettünk fel. A telep fertőzöttségének látszólagos prevalenciáját az utolsó állományszintű vérvétel során ELISA módszerrel pozitívnak bizonyult állatokból számítottuk. A kérdések főként az elletői tartástechnológiára, az alkalmazott elletési protokollokra terjedtek ki, beleértve a borjú-tehén kapcsolatot (szophat-e a borjú, felnyalhatja -e a tehén) a föcstejitatás rendjét (beleértve a a föcstej minőség és a borjú immunglobulin ellátottság ellenőrzését) és a trágyaeltávolítás módját,. A borjúneveléssel kapcsolatban rákérdeztünk, az egyedi ketrecekben előforduló leggyakoribb betegségekre és egy évre visszamenően a megbetegedések és az elhullások arányára. Külön tárgyaltuk a paratuberculosis fertőzött tehenekre alkalmazott protokollokat, amennyiben a telep alkalmazott ilyet.

Eredmények és megbeszélés

A vizsgált tehenészetek fejt tehénlétszáma átlagosan 641±34 állat, a paratuberculosis látszólagos prevalenciája 4,5±1,3 % (min. 2,3; max. 8,5%).

A paratuberculosis elleni védekezés leghatékonyabb módja a borjak fertőzésmentes környezetben történő felnevelése. A megkérdezett tehenészetek közül 4-ben alkalmaztak külön protokollt a paratuberculosissal fertőzött (ELISA vizsgálattal pozitív)

tehenek esetében, ami minden esetben elkülönített elletést, és az állat föcstejének megsemmisítését jelentette. Ezen telepek látszólagos prevalenciája 3 % alatt van. Azokban a tehenészetekben, ahol nincs leírt elletői protokoll a PTBC fertőzöttség mértéke 2%kal magasabb. Meglepő módon 6 tehenészetben a borjút minden esetben felnyalhatja az anyja, arról, hogy szophat-e nem tudtak egyértelmű választ adni. Az ellenőrzött föcstejitatás elmaradása esetén 1,2%-al magasabb a látszólagos prevalencia. Eredményeinkből látszik, hogy a megfelelő elletői protokoll elősegíti a betegség visszaszorítását. A leírt elletői protokoll véleményünk szerint nagy szerepet játszik az előírások betartatásában, megléte a mentesítési programokban elengedhetetlen. A trágyaletávolítás módjára vonatkozóan eddigi eredményink alapján nincs meghatározó különbség a telepek között.

A megkérdezett telepek mindegyikében az egyedi ketrecekben a hasmenést jelölték meg a legnagyobb állategészségügyi problémaként. Az elmúlt 1 évben a borjak 12%-a betegedett meg, ebből az elhullás mértéke átlagosan 3% volt. A paratuberculosissal súlyosan fertőzött telepek (látszólagos prevelancia >4 %) esetében az egyedi ketrecekben történő megbetegedés 15%, az elhullás mértéke 4% volt. Mivel a baktérium fertőzött bélsárral is terjed, így kiemelkedően fontos a borjak körüli higiénia. A fertőzési lánc megszakítása érdekében ügyelni kell a borjak körül használatos eszközök fertőtlenítésére, a bélsárral való szennyezettség elkerülésére. A higiéniai szabályok betartásával csökkenthető egyéb bakteriális fertőzések, mint például az Escherichia coli okozta hasmenés előfordulásának gyakorisága. Az általános higiénia javításával nem csupán a PTBC fertőzöttség mértékének csökkenése a jelentős, sokkal hamarabb lesz látható változás a borjak általános egészségi állapotában. Míg a paratuberculosis prevalencia jelentős csökkentéséhez minimum 5 évre van szükség (Kudahl et al., 2008) a menedzsment változtatásokkal az általános borjú egészségügy területén sokkal gyorsabb eredményekre számíthatunk.

Eredményeink összhangban vannak a nemzetközi szakirodalommal, mely szerint a mentesítés során nem elég csupán a diagnosztikai tesztek eredményeire hagyatkozni (Jubb & Galvi, 2000) és selejtezéssel csökkenteni a prevalenciát. A megalapozott mentesítési programok egyaránt figyelembe veszik a laboratóriumi eredményeket és a menedzsment ajánlásokat. Sikeres gyérítés csak egy megalapozott, a kitűzött protokollokat betartó folyamat során lehetséges (Kudahl et al., 2008).

Köszönetnyilvánítás

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Heat stress and claw health in cattle

A hőstressz és a csülök egészségi állapota szarvasmarhákban

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Abstract

The aim of this study was to investigate the seasonal thermal effect on claw health in dairy cows. The observation was performed on HF dairy farm in Eastern Slovakia. The clinical signs of the heat stress were recognised in several animals during the afternoon milking on July 8, 2015. The claw examination done three month after the heat stress was compared with two examinations: two months prior to the heat stress; ten months after. The examinations were focused on the prevalence of the sole ulcer. Statistical analysis was performed by running a chi-squared test. The THI on July 8, 2015 was 88. We found 18.6, 28.1, and 12.9 % cows with sole ulcer in the spring, autumn, and spring of the following year, respectively ($P<0.05$). In conclusion, results of our observation show that the heat stress in dairy cows can be associated with increase in prevalence of the sole ulcer.

Key words: sole ulcer, heat stress, dairy cows

Introduction

Digital dermatitis, sole ulcers, and white line disease are the most frequently reported foot lesions related to lameness of dairy cows (Green et al., 2002). Lameness is a severe welfare problem in cattle and has a detrimental effect on longevity, productivity, and reproductive performance (Garbarino et al., 2004). Consequently, it is among disorders causing the largest economic losses in the dairy industry (Ettema & Ostergaard, 2006). Regardless of whether foot lesions cause lameness, they have a high impact on animal welfare and farm economics. Foot disorders that result in lameness are estimated to cost $95 on average, whereas foot disorders that do not cause lameness cost $18 per case on average (Bruijnis et al., 2010).These lesions are

painful and, in the case of sole ulcers and white line disease, milk losses were estimated to be, respectively, 574 and 369 kg/cow per 305-d lactation in 30 herds (Amory et al., 2008); furthermore, in cattle treated for digital dermatitis, milk yields increased in the months after treatment compared with the months before treatment (Amory et al., 2008). Sole ulcers and white line fissures also have been associated with impaired fertility, and sole ulcers have been associated with an increased occurrence of milk fever (Sogstad et al., 2006). Sole ulcers, which are one of the most severe pathologies causing lameness, are noninfectious in nature and occur when claw horn formation is disrupted. The underlying tissue then becomes inflamed and sometimes exposed (Murray et al., 1996). Cows are particularly vulnerable around the time of parturition; changes in hormone levels cause increased vascular permeability, increasing the risk of oedema and ischemia in the hoof (Knott et al., 2007), while concurrent weakening of connective tissue causes the pedal bone to drop and compress the corium, further disrupting claw horn formation (Thoefner et al., 2005).

Heat stress is defined as the sum of external forces acting on an animal that causes an increase in body temperature and evokes a physiological response (Dikmen & Hansen, 2009). The temperature-humidity index (**THI**) has been commonly used to estimate the effect of heat stress on production and reproduction. There is general agreement that significant effects are observed at a mean daily THI of around 72 (West et al., 2003). Documented physiological heat stress coping strategies used by dairy cows include increased respiration rate, panting, and sweating, and reduced milk yield and reproductive performance. Behavioural coping strategies include modified drinking and feed intake (e.g., increased water intake and shifting feeding times to cooler periods during the day), increased standing time and shade seeking, and decreased activity and movement (Schütz et al., 2008).The duration of elevated temperatures has an inverse relationship with feed intake. Therefore, it is not surprising that heat stress is considered a major risk factor for lameness, but whether this association is a consequence of increased standing times or due to alterations in nutrient metabolism caused by a decrease in DMI is not known (Cook et al., 2007). Claw horn lesions, such as sole ulcer, are believed to develop from increased pedal bone mobility induced by changes in the corium at calving (Lischer et al., 2002) and potentially from nutritional insults such as subacute ruminal acidosis (Stone, 2004)

In this paper, the risk of heat stress associated with the most common claw diseases is considered in dairy cows.

Experimental methods

The observation was performed on the middle-scale HF dairy farm in Eastern Slovakia. All dairy cows were milked twice

daily (06:00 and 14:30 h) and had free access to water and were fed total mixed rations (corn silage, corn, soybeans, mineral and vitamin, according to their lactation stage. No specific measures to protect the animals against the heat stress were taken on the farm. The clinical signs of the heat stress (increased respiration rate, panting, and sweating) were recognised in several animals during the afternoon milking on July 8, 2015. The claw condition of dairy cows was controlled on the farm three month after the heat stress event (October 2015) and was compared with results of the claw health controls performed two months prior to the heat stress (May 2015) and ten months after it (May 2016). All observations were focused on the prevalence of the sole ulcer. The weather data of air temperature and relative humidity in the region on the day of the heat stress were obtained from the Slovak Hydrometeorological Institute to calculate temperature-humidity index **(THI)**. The equation used to calculate THI was: THI=(1,8xT+32)–(0,55-0,0055xRH)x (1,8xT-26), where T is the outdoor ambient temperature in °C, and RH is relative humidity expressed in %.

Statistical analysis was performed by running a chi-squared test using the statistical software StatSoft, version 8.0. P-values < 0.05 were considered significant.

Results

Comparisons of the three claw health controls revealed a higher prevalence (P<0.05) of sole ulcers in the period three months after the heat stress event (October 2015). The data are demonstrated in Table 1. The local outdoor ambient temperature and humidity on July 8, 2015 were 35.6 °C and 61.3 %, respectively. The calculation of THI resulted in the value of 88.

Table 1. Prevalence of sole ulcers in dairy cows challenged by the heat stress

	May 2015	October 2015	May 2014
Cows total	210	209	212
Sole ulcer (n)	39a	59b	27a
Sole ulcer (%)	18.6	28.2	12.7

[a, b]prevalence differs (P<0.05)

Discussion

Claw lesions are commonly categorized according to their aetiology into infectious and non-infectious lesions (Potterton et al., 2012). Infectious lesions include digital dermatitis, interdigital dermatitis, heel horn erosion, and foot rot, whereas the most common non-infectious lesions are sole ulcer, toe ulcer, sole haemorrhage, and white line disease. Non-infectious lesions affect the claw horn, and their occurrence is associated with metabolic and hormonal events around calving that weaken the foot suspensory apparatus (Tarlton et al., 2002), low BCS (Green et al., 2014), toe overgrowth, exposure to hard flooring (Somers et al., 2003), and thickness of the digital cushion (Bicalho et al., 2009). The prevalence of sole ulcers can differ according to the parity of the cows. Thus, the prevalence of sole ulcers was 4.2 and 27.8 % for parity 1 and parity >1, respectively (Bicalho et al., 2009). In the large scale study on 156 dairy farms in Canada a prevalence of sole ulcer was 6.4 % which was the second most prevalent foot lesion after digital dermatitis (21.8 %) in the study (Solano et al., 2016).

Sole ulcer prevalence in the present study varied between 10 and 20 % in the sessions which were no related to the heat stress event. The higher number of the sole ulcer detected three month after the heat stress in our study indicate a causative effect of the organ and metabolic changes triggered by body reaction to high ambient temperature and humidity. It has been suggested that sole ulcers are a consequence of subclinical laminitis (Vermunt, 2007). Laminitis is an important predisposing cause of claw disorders in cattle. Inflammation of the corium results in the activation of tissue matrix metallo-proteinases (MMPs) that weaken the collagen fibre bundles that make up the suspensory apparatus within the claw. Coincident with this is the release of horn growth and necrosis factors that contribute to the inflammatory process and accelerate claw horn growth (Mulling & Lischer, 2002). The vascular disturbances associated with laminitis preclude the normal diffusion of nutrients and oxygen into the living-cell layers of the epidermis destined to become claw horn. This interrupts the normal differentiation of these cells and leads to the formation of weaker or softer claw horn. Some have suggested that “claw horn disruption” may be a better term for the condition of laminitis, because it more accurately reflects the nature of the anatomical and physiological lesions associated with laminitis (Räber et al., 2004). The pathogenesis of laminitis is believed to be associated with a disturbance in the micro-circulation of blood within the corium which leads to weakening of the suspensory apparatus within the claw and thus permits downward displacement and rotation of the third phalanx (P3). The result is compression of the corium and supporting

tissues that lie between P3 and the sole which predisposes to the development of sole ulcers (Lischer et al., 2002). Rumen acidosis is considered to be a major predisposing cause of laminitis and presumably mediates its destructive effects through various vasoactive substances (endotoxins, lactate, and possibly histamine) that are released into the blood stream in coincidence with the development of rumen acidosis. The subclinical form of rumen acidosis (SARA) is more common than the acute form of this disease. It is generally observed when recently calved cows are introduced to a diet considerably different than that fed during the dry period. Alternatively, it may occur when lactating cows are fed diets low in effective fibre. Major clinical manifestations would include variable feed intake, depressed fat test, poor body condition despite sufficient energy intake, mild to moderate diarrhoea, and occasional cases of epistaxis (nose-bleed) or haemoptysis (the expectoration of blood from the mouth). Conditions such as laminitis or undefined lameness, abomasal disorders, and liver abscesses are generally secondary observations (Garrett et al., 1999). SARA can be triggered not just by increased carbohydrate intake but also by heat stress. The primary avenues for heat loss during periods of hot weather are sweating and panting. In severe heat, panting progresses to open-mouth breathing characterized by a lower respiratory rate and greater tidal volume. The result is respiratory alkalosis as a result of the increased loss of carbon dioxide. The cow compensates by increasing urinary output of bicarbonate (HCO_3). Simultaneously, the salivary HCO_3 pool for rumen buffering is decreased by the loss of saliva from drooling in severely stressed cows. The end result is rumen acidosis because of reduced rumen buffering and an overall reduction in total buffering. The effect of ambient air temperature on rumen pH was evaluated in lactating Holstein cows fed either a high roughage or high concentrate diet in both a cool and a hot environment. Rumen pH was lower in cows exposed to the higher temperatures and those fed the higher concentrate diets (Niles et al., 1998).

The environmental conditions driving heat stress in dairy cattle are presented using the temperature-humidity index (THI), a calculated index that incorporates the effects of environmental temperature with relative humidity (De Rensis et al., 2015). The authors defined THI <68 to be outside the thermal danger zone for cows. Mild signs of heat stress are observed at THI of 68 to 74, and a THI ≥75 will cause drastic decreases in production performance. The THI value is usually the main determinant for management decisions related to heat stress as most meteorological stations close to farms provide this data. Therefore, THI of 88 observed in the present study speaks clearly for a severe stressful event in the examined animals.

In conclusion, results of our observation show that the heat stress in dairy cows can be associated with increase in prevalence of the sole ulcer. The findings may be useful in stressing a need to take measures to prevent the heat stress in dairy cows.

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Surgical treatment of septic arthritis in proximal joints in cattle

Szarvasmarhák proximális ízületeiben előforduló szeptikus ízületi gyulladás sebészeti kezelése

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Introduction

Septic arthritis is a common cause of severe lameness in cattle, most commonly the distal interphalangeal joint is involved (Starke et al., 2007; Heppelmann et al., 2009; Kofler, 2017a). The prevalence of septic arthritis of proximal joints (fetlock, carpal, tarsal, stifle etc.) in cattle is much lower, however the associated prognosis is more guarded and the treatment regime is much more challenging and expensive (Munroe & Cauvin, 1994; Adegboye et al., 1996; Kofler, 1996, 1997, 2017b; Hirsbrunner & Steiner, 1998; Nuss, 2000, 2003; Desrochers & Francoz, 2014; Mulon et al., 2016). Septic arthritis is caused by direct inoculation of bacteria into the joint (primary infection) or from spread of periarticular infection (secondary infection) or by haematogenous spread (Trent & Plumb, 1991; Desrochers & Francoz, 2014; Mulon et al.. 2016).

Diagnosis of septic arthritis of proximal joints in cattle

Up to now, the diagnosis of septic arthritis has been based on careful clinical examination, radiography, arthrocentesis and macroscopic, cytological and micro-biological examination of the synovial sample (Rhode et al., 2000; Shearer et al., 2012; Kofler et al., 2014, 2016; Kofler, 2018a,b). However, during the last 20 years, ultrasonography has proved to be of paramount importance for soft tissue differentiation in bovine orthopedics, adding important information to the clinical findings (Kofler, 1996, 1997, 2009; Gonçalves et al., 2014; Kofler et al., 2014, 2016; Borges et al., 2015). Therefore, today, the application of diagnostic ultrasound is a standard procedure for investigation of all cases associated with swelling of a joint region allowing to identify with certainty the

incriminated joint structures, periarticular/articular soft tissues or bone structures (Munroe & Cauvin, 1994; Kofler, 1996, 1997; 2009; Grubelnik et al., 2002; Nuss, 2003; Kofler & Martinek, 2004; Kofler & Altenbrunner-Martinek, 2008; Starke et al., 2008; Kofler et al., 2014, 2016; Altenbrunner-Martinek et al., 2017). In contrast to radiography, the presence of synovial effusion can be detected reliably even in very early stages of an arthritis by ultrasonography (Kofler, 1996, 2009). If an ultrasonographic unit is available, even linear rectal probes are very suitable for investigation of swollen joint regions. The diseased synovial cavity appears mildly to severely distended, and the thin echoic capsule is distinctly displaced from the articular surface. The echogenicity of the inflammatory effusion ranges from anechoic to echoic depending on its nature (serous, serofibrinous, fibrinous, purulent) (Kofler, 1996, 1997, 2009). Liquid content can be identified based on flow-phenomena, which are characterized by small and large hypoechoic to echoic particles or clots that are seen floating in anechoic fluid (Kofler & Hittmair, 2006). In long-standing cases of sepsis, the precipitated gelatinous masses of fibrin impair or prevent aspiration of fluid, and these semi-solid masses appear hypoechoic to echoic and show no flow-phenomena (Kofler, 1996, 1997; Kofler, 2009; Kofler et al., 2014) Definitive information about the nature of effusion is provided by puncture of the synovial cavity (Rhode et al., 2000; Shearer et al., 2012; Kofler, 2018b).

An ultrasonographic inspection of the region of interest should always be carried out before puncture (Kofler, 2009; Kofler et al., 2014, 2016). The position and size of the distended joint cavity and the location of liquid effusion within as well as its distance and direction from a set point on the skin surface can be determined accurately. After assessing the direction and depth of the structure to be punctured the needle may be inserted with or without (free hand) ultrasonographic guidance (Kofler, 2009; Kofler et al., 2014, 2016). Macroscopic evaluation of the aspirated synovial fluid allows an immediate classification of the inflammatory exudate as serous, sero-fibrinous, fibrinous or purulent, depending on the gross macroscopic appearance (Rhode et al., 2000; Nuss, 2000; Kofler, 2018b). A mild to severely turbid synovial fluid is always a certain indication for the presence of an infection (Rhode et al., 2000; Nuss, 2000; Kofler, 2018b). The exudate in cases of septic arthritis in cattle can be highly fibrinous and render the aspiration of synovial fluid impossible (Kofler, 1996). Radiography reveals increased width of the intraarticular space and subsequently osteolysis, but it does not allow localization and exact determination of the character of the soft tissue swelling (Bargai et al., 1989; Verschooten et al., 2000; Steiner et al., 2010; Kofler et al., 2014, 2016).

Differential diagnosis to septic arthritis

Aseptic arthritis (osteochondrosis), osteomyelitis of the physis, of the epiphyseal growth plate or the metaphysis, periarticular abscessation, periarticular tenosynovitis or bursitis, joint luxation, and epiphysitis (in fattening bulls) (Kofler, 2014a).

Prognosis

The prognosis is depending on the primary disease (penetrating wound, secondary infection, haematogenous spread), the number of involved joints (mono- or polyarthritis), the type of effusion (serous, fibrinous or purulent), the involved joint, the presence of a concurrent osteomyelitis and the presence of concurrent disorders of other organs systems (Nuss, 2000; Desrochers & Francoz, 2014). Therefore, in all cases of septic arthritis of (proximal) joints a thorough physical and orthopedic examination have to be carried out (Nuss, 2000; Kofler, 2014a), that should be complemented by an ultrasonographic investigation prior to make a final decision for treatment or for culling (Kofler et al., 2014, 2016; Desrochers & Francoz, 2014; Mulon et al., 2016).

Treatment of septic arthritis of proximal joints in cattle

An early detection of septic arthritis, in particular of proximal bovine joints, is important for starting an adequate treatment as early as possible and for a favourable outcome (Nuss, 2000; Desrochers & Francoz, 2014; Mulon et al., 2016). The application of diagnostic ultrasound and the subsequent arthrocentesis of an involved joint allow to classify the type of effusion (serous, serofibrinous, fibrinous, purulent), and decision-making based on these important findings for culling of the animal due to a poor prognosis or for treatment by joint lavage, or arthrotomy/arthroscopy or as ultima ratio by joint resection (for purulent arthritis of carpal and fetlock joints only) (Starke et al., 2009; Desrochers & Francoz, 2014; Mulon et al., 2016).

However, in any case of treatment of a septic arthritis of proximal bovine joints, in addition to the topical therapy a long-term systemic antibiotic and anti-inflammatory treatment has to be combined (Trent & Plumb, 1991; Jackson et al., 1996; Nuss, 2000; Anderson & Muir, 2005; Starke et al., 2008; Shearer et al., 2013; Desrochers & Francoz, 2014; Mulon et al., 2016).

Joint lavage and arthrotomy can be carried out in the sedated animal (xylazine: 0.05 mg/kg BW) restraint in lateral recumbency on a surgical table (Rizk et al., 2012). For these surgical interventions on the fetlock, carpal and tarsal joints the application of a regional intravenous anesthesia in combination with an intravenous regional limb perfusion (IVRLP) of water soluble antibiotics is highly suitable (Steiner et al.,

1990; Stanek, 1994; Navarre et al., 1999; Shearer et al., 2013). For the lavage and arthrotomy of the stifle or for lavage of the coxofemoral joint an epidural anesthesia is recommended using procaine-hydrochloride or lidocaine 0.4 ml/kg (8 mg/kg) BW or xylazine 0.1 mg/kg BW, diluted with 0.9% saline to a final volume of 0.4 ml/kg BW (Meyer et al., 2007). However, alternatively in calves a general anesthesia can be administered using xylazine (0.1 mg/kg BW) and ketamine (2 mg/kg BW) intravenously (Meyer et al., 2007).

Depending on the stage of septic joint inflammation (serous, serofibrinous, fibrinous, purulent) one of the following treatment methods is the first choice (Nuss, 2000; Desrochers & Francoz, 2014; Kofler, 2017b):

1. **Exclusive systemic antibiotic treatment:** is recommended only for those cases of haematogenous septic arthritis when it can be started within the first 2 to 4 days of joint infection. If there is no obvious improvement after 36 – 48 hours after starting systemic administration of broad-sprectrum antibiotics, then a topical treatment of the joint should be immediately performed. The antibiotic treatment is then specified following performance of an antibiogram and a resistogram. Chemotherapeutics with a proved penetration into synovial cavities and bones are penicillin, ampicillin, cephalosporins, aminogycosides, oxytetracycline, fluorquinolones, and sulfonamides (Trent & Plumb, 1991; Nuss, 2000; Desrochers & Francoz, 2014). However, the duration of application is very important, so for a septic arthritis and/or osteomyelitis caused by haematogenous spread the agent has to be administered for at least 2 weeks, in some cases for up to 4 weeks (Desrochers & Francoz, 2014; Mulon et al., 2016). The systemic antibiosis can be combined with the application of intravenous regional limb perfusion (IVRLP) of the same antibiotic (Steiner et al., 1990; Stanek, 1994; Navarre et al., 1999; Shearer et al., 2013).
2. **Joint lavage and systemic antibiotic treatment**: The application of a joint lavage using at least two cannulas with a lumen diameter of 1.6 to 2 mm is promising in cases of septic serous and septic serofibrinous arthritis with only small fibrin clots. Depending on the joint and the size of the animal about 1000–4000 ml of 0.9% saline solution should be used (Jackson et al., 1996; Meier, 1997; Desrochers & Francoz, 2014; Mulon et al., 2016). Depending on the duration of the joint infection, the joint lavage have to be applied repeatedly. Macroscopic evaluation of the synovial effusion before the next joint lavage, cytology of the synovial sample and the clinical findings such as

improvement of weightbearing and decrease of joint swelling are suitable parameters for monitoring the healing process (Rhode et al., 2000; Francoz et al., 2007; Nuss, 2000; Kofler, 2017b). By joint lavage, and even more effectively by arthrotomy and arthroscopy, a decompression and drainage of the inflamed joint, a removal of inflammatory breakdown products, and a debridement of fibrin can be obtained. Following joint lavage, antibiotics, such as ceftiofur or penicillin, can be injected into the joint (Steiner et al., 1999; Desrochers & Francoz, 2014).

3. **Arthroscopy, joint lavage and systemic antibiotic treatment:** The application of arthroscopy in cattle is restricted on those rather early stages of septic arthritis showing a serous or serofibrinous effusion. However, this technique is associated with high equipment requirements and the application of a general anesthesia or an epidural anesthesia, allowing the safe application of the instruments (Munroe & Cauvin, 1994; Steiner et al., 1999; Nuss, 2000).

4. **Arthrotomy, joint lavage and systemic antibiotic treatment:** The correct approaches for arthrotomy/arthoscopy of each joint correspond to those localisations that are used for arthrotomy (dorsal/cranial and/or plantar/caudal joint pouches) (Steiner et al., 1999; Nuss, 2000; Kofler & Martinek, 2005; Kofler, 2018b). Arthrotomy should be undertaken in cases of septic arthritis with high synovial clotted fibrin and/or purulent effusion, and in cases of arthritis attributable to a penetrating puncture or laceration wound (Hirsbrunner & Steiner, 1998; Nuss, 2000). In addition, gentamicin-impregnated collagen sponges can be implanted into the joint (Desrochers & Francoz, 2014).

5. **Joint resection and arthrodesis, joint lavage and systemic antibiotic treatment:** This surgical method is recognized as *„ultima ratio“* in cases of long-term purulent arthritis associated with subchondral bone infection. This technique is frequently applied for the treatment of purulent arthritis of the distal and proximal interphalangeal joint (Starke et al., 2007; Heppelmann et al., 2009; Burgstaller & Kofler, 2015; Kofler, 2017a). However, in proximal bovine joints this radical surgical technique can be applied only for the metacarpophalangeal/metatarsophalangeal and the carpal joints using a local intravenous retrograde anesthesia in combination with sedation or a general anesthesia (Geishauser, 1996, 1997; Van Huffel et al., 1998; Riley et al., 1998; Steiner et al., 1999; Kofler & Peterbauer, 2014). This surgical intervention starts with a transverse incision through the skin and joint

capsule on the dorsal/cranial aspect of the fetlock joint, the intercarpal- or the antebrachiocarpal joint, even the medial and lateral collateral ligaments are completely transected. Thereafter, the joint can be completely opened in a flexed position, all the infected tissues (capsule, cartilage, subchondral bone) have to be carefully removed using a curette followed by intensive joint lavage. The skin is sutured and the limb is immobilized by application of a full limb cast (Geishauser, 1996, 1997; Van Huffel et al., 1998; Riley et al., 1998; Steiner et al., 1999; Kofler & Peterbauer, 2014).

6. **Removal of a circumscribed osteomyelitic area localized in the physis/metaphysis:** The intention is to create a decompression of the infected bone area, a debridement of the infected bone tissue, a lavage of inflammatory products and drainage of the cavity. The exact localization of such an osteomyelitic lesion is obtained by radiography using at least two orthogonal projections (Firth et al., 1997; Verschooten et al., 2000; Kofler et al., 2014). A skin incision is made over the identified area, and the bone surface is surgically visualized. If necessary, a hole is drilled into the osteomyelitic lesion, and the infected bone tissue is completely removed using a curette. Finally the lesion is irrigated using 0.9% saline solution, and drainage is applied. In addition, a systemic antibiotic treatment is administered for 2 to 4 weeks (Trent & Plumb, 1991; Steiner et al., 1999; Nuss, 2000; Francoz et al., 2007; Starke et al., 2008; Desrochers & Francoz, 2014).
7. **Antiphlogistic treatment using NSAID´s:** In addition to all the above mentioned surgical techniques, anti-inflammatory drugs are administered for 3 days using NSAID´s (e.g. flunixin-meglumine, ketoprofen, carprofen, meloxicam) for inhibition of the inflammatory mediators, for reduction of the inflammatory swelling, and for improvement of the feed intake (Anderson & Muir, 2005; Shearer et al., 2013; Desrochers & Francoz, 2014; Mulon et al., 2016).

Prophylaxis

Sufficient and early application of colostrum in neonates within the first six hours of life, improvement of the postnatal management of calves to avoid lung- and gastrointestinal infections, check-up of the concentration of immunoglobulins in blood, and correct disinfection of the umbilicus are essential prophylactic measures (Desrochers & Francoz, 2014).

Conclusion

Accurate orthopaedic examination, ultrasonographic and radiographic imaging

and arthrocentesis are common diagnostic tools for septic arthritis (Shearer et al., 2012; Kofler et al., 2014, 2016; Kofler, 2017a,b, 2018b). Based on these important findings and after classification of the type of present effusion (serous, serofibrinous, fibrinous, purulent) by macroscopic evaluation of the aspirated synovial fluid, a well-founded decision-making is possible or for treatment or for culling of the animal due to poor prognosis (Nuss, 2000; Desrochers & Francoz, 2014; Mulon et al., 2016). The treatment strategy must include long-term antibiotics, anti-inflammatory drugs, and depending on the stage and duration of joint infection, joint lavage, arthroscopy or arthrotomy (Nuss, 2000; Desrochers & Francoz, 2014; Mulon et al., 2016; Kofler, 2017b). The knowledge of communication and boundaries for commonly affected joints is essential to perform joint lavage and arthrotomy (Jackson et al., 1996; Meier, 1997; Nuss, 2000; Desrochers & Francoz, 2014). Arthrodesis of the fetlock and the carpal joint is the "ultima ratio" when no treatments were efficient or because of the chronicity of the joint infection, however the joint function will never be restored (Desrochers & Francoz, 2014; Kofler & Peterbauer, 2014). An early detection of septic arthritis of proximal bovine joints and an early application of the adequate treatment are essential for a favourable outcome (Nuss, 2000; Desrochers & Francoz, 2014; Mulon et al., 2016; Kofler, 2017b).

Figure 1a, b, c. Examples of **septic polyarthritis in calves:**
(A): severe distension of the left carpal, left stifle joint;
(B): severe distension of the caudo-lateral and caudo-medial recesses of the tarsocrural joint;
(C): severe swelling of the left carpal region in a calf with septic arthritis of all thre carpal joints: arthrocentesis of the antebrachiocarpal joint with discharge of a highly turbid synovial fluid.

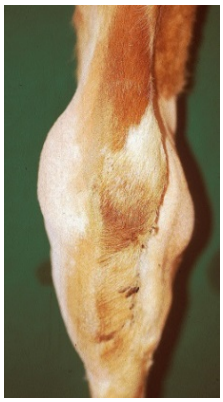

Figure 2a, b, c, d. Simmental calf (4 weeks old) with a **serofibrinous, septic polyarthritis of the left carpal, both tarsal** and both stifle joints: Sonograms of the carpal joint showing a hypoechoic effusion in the antebrachiocarpal- (ABC), intercarpal joint (IC), tarsocrural and stifle joints. GK: joint capsule; Rec: the joint recess is highly distended; Rad: radius; Ocr: Os carpi radiale; RK: trochlear ridge of talus; FRK: trochlear ridge of the distal femur; Ti: tibia. At the distal lateral trochlear ridge of the femur a subchondral bone lesion can be identified (osteomyelitis: OM). Arthrocentesis of the tarsocrual joint revealed a highly turbid synovial fluid, that showed complete coagulation 15 minutes later (septic fibrinous arthritis).

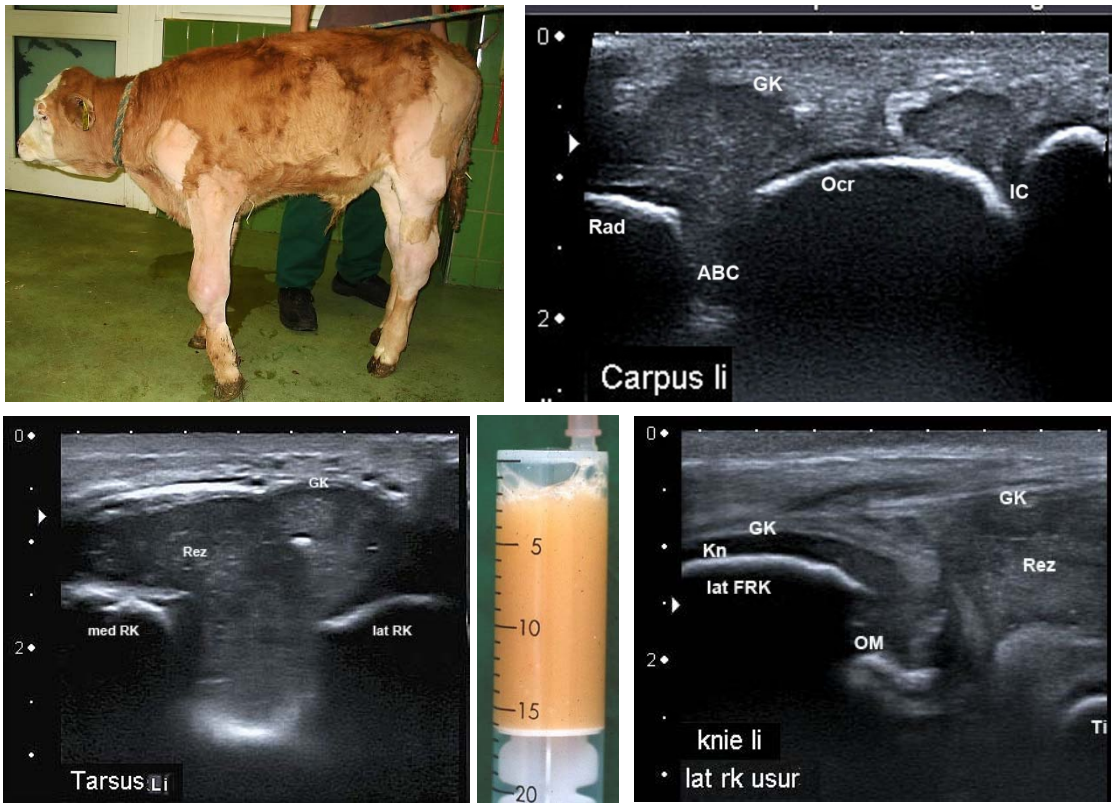

Figure 3. Joint lavage of a metatarsophalangeal joint in a Simmental calf with a septic serofibrinous arthritis caused by a large penetrating wound (on the lateral aspect) 5 days before; the treatment was carried out after application of a local retrograde intravenous anesthesia (the arrow shows the elastic tourniquet) and sedation of the calf; the wound was carefully debrided and a plantar arthrotomy approach was created for joint lavage applying 2500 ml of 0.9% saline solution with added 0.1% povidine solution.

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Claw amputation in practice – Challenges of a freestall barn

Csülökamputáció a gyakorlatban – Egy kötetlen tartású istállló kihívásai

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Abstract

This report of four cases of claw amputationaddresses these challenges of claw amputation and the aftercare in practice and shows how they can be successfully overcome. In all four cows the clinical findings were complicated claw horn lesions affecting deeper structures. The surgical procedures were performed after regional intravenous anesthesia. Perioperatively, the cows received Oxytetracycline and Ketoprofen for 3 days. Bandage changes were performed after three and five to seven days there after. Three of the four cows treated were stillalive 146, 228 and 163 days after the surgery, respectively, and performing well.

Key words: dairy cow, claw, amputation, freestall barn

Introduction

This report shows that it is possible to successfully perform claw amputations in practice even on farms that do not have ideal conditions for sick cows. The biggest management challenge on the farm was the lack of a sick cow pen. The cows had to go back immediately to the freestall with straw-bedded cubicles.

Objectives

The procedure of claw amputation and the aftercare can pose several challenges when performedin practice. This report of four cases managed on a farm addresses these challenges and shows how they can be successfully overcome.

Materials and Methods

On a farm milking approximately 545 dairy cows located in central Slowakia, claw amputations were performed in two multi-parous pregnant Holstein Friesian (HF) cows, one Simmental x HF and one Sim-mental cow in their first lactation (Table 1).

In all four cases the clinical findings were complicated claw horn lesions affecting deeper structures and all cows showed a lameness score of 5 out of 5 (Sprecher et al. 1997). The claw amputations in the medial phalanx were performed after regional intravenous anesthesia. Perioperatively, the cows received Oxytetracycline and Ketoprofen for 3 days.

After three days the cows were reexamined, the bandage was changed and it was decided wetherthe medical treatment was continued for another 2 days. After that bandages were changed every five to seven days.

Table 1. Overview of cows with digital amputation

Nr	Breed	Lactation	DIM	DCC	Lesion location
1	HF	2	226	152	Left hind lateral claw
2	HF	5	321	142	Left hind medial claw
3	HFxS	1	106	-	Right hind lateral claw
4	S	1	21	-	Right hind lateral claw

HF: Holstein Friesian; S: Simmental; DIM: days in milk; DCC: days carrying calf

Results

The biggest management challenge on the farm was the lack of a sick cow pen. The cows had to go back immediately to the freestall with straw-bedded cubicles.

At the time of submission of this abstract of the four cows treated three were stillalive 146, 228 and 163days after the surgery, respectively. Both pregnant cows had healthy calves and the Simmental x HF cow is currently pregnant. Despite intensive treatment the Simmental cow did not recover, most likely due to her progressed stage of disease which had manifested systemically.

Conclusions

This report shows that it is possible to successfully perform claw amputations in practice even on farms that do not have ideal conditions for sick cows.It also emphasized the importance of lameness detection: these cows should have been detected before they were extremely lame.

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Prevalence of thin soles in dairy cows housed on mastic asphalt floors

Masztix aszfalton tartott tejhasznú tehenek vékony talpúságának előfordulása

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Abstract

Mastic asphalt (MA) is a mixture of crushed stone gravel with bitumen and is known for its high abrasiveness (Steiner et al. 2008). In this field study two groups were defined, including cows kept on fully- or semi-full floored mastic asphalt area. A sole horn thickness (SHT), measured by ultrasound, under or equal 4 mm was defined as "thin sole" (TS). A higher prevalence for thin soles (52 %) was found in cows kept on fully floored MA areas. Furthermore there was an impact on lameness (higher locomotion-score) and higher prevalence of claw diseases (i.e. toe ulcer, white line disease). Claw measurements (dorsal wall length, heel height) were positively correlated with SHT. As a consequence, the recommendation can be given that for new barn installations only semi-full floored mastic asphalt areas should be planned and hoof trimming technique have to be adapted according to the different shape of the claws (Shearer & Van Amstel 2009; Kofler 2015)

Key words: mastic asphalt, sole horn thickness, ultrasound measurement, thin sole

Introduction

The sole horn thickness in bovine claws is a function of the growth versus the wear rate (Van Amstel et al., 2004; Sanders et al., 2009). Thin soles (TS) describe an inadequate (≤ 5 mm) thickness of the sole horn, whereas 7 mm are regarded as adequate sole horn thickness in adult Holstein-Friesian and Simmental cows (Van Amstel et al., 2004; Nuss & Paulus, 2006; Kofler, 2015). The following predisposing factors have been reported as being causative for the development of TS: overtrimming of sole horn, overstocking and long walking distances leading to increased sole horn abrasion, and in particular excessive sole horn wear due to abrasive floors. The latter condition is common in new floor installations in free stall barns where freshly hardened concrete creates a particularly abrasive surface ("new concrete disease") and in particular on mastic asphalt floors, which get increasingly rougher with time (Van Amstel et al., 2004; Sanders et al., 2009; Shearer & Van Amstel, 2009; Kofler, 2015, 2017). The objective of the present study was to evaluate the prevalence of thin soles (≤ 4 mm) in cows housed on mastic asphalt floors in Austrian dairy farms.

Experimental methods

Ninety-seven cows from six different dairy farms were examined in this study, starting in spring and ending in autumn 2016. The farms were split up in two groups. Cows from the experimental-group (EG; n = 57) were housed on fully-floored mastic asphalt at four farms, and cows of the control-group (CG, n = 40) were housed on semi-full floored mastic asphalt in two other farms. Several data from the farms were documented i.e. age, breed and lactation data for each cow. All the cows were scored using the locomotion scoring system (Sprecher et al., 1997), and then the cows were restrained in lateral recumbency on a tilt table for further examination. The claws were cleaned with water and the following measurements were taken in all the claws of the rear limbs only: the dorsal wall length, the heel height, the sole horn thickness at the tip of the claw (SHT1) and 3 cm caudal (SHT2) using a 7.5 MHz linear ultrasonographic probe and a compression test was performed by finger palpation and the hoof tester. Markings on the dorsal wall were made for repeated measurement two months later for the calculation of the net growth rate of the hoof horn. Afterwards the cows were trimmed by a professional hoof-trimmer and all the claw lesions observed were documented using the computerised hoof trimming data base program "Klauenmanager" (Kofler et al., 2011; Kofler, 2013). Finally, certain defined body-size measurements (i. e. chest-depth, chest girth, belly girth) were taken to calculate the body-weight by a formula (Ledinek 2014). Statistical analysis of the data was carried out using the software IBM SPSS Statistics 24®. A mixed ANCOVA-model was created for SHT by using the following covariates:

numbers of lactation ("NoL"), days in milk ("DIM") and logarithmized body weight ("log(BW)"). Main factors were, "group", "farm" and "breed". The effect of farm was nested in the effect of group (using term "group*farm"). Differences in prevalence of TS and claw lesions were tested using a chi^2-test. Differences of locomotion score were tested by Mann Whitney U-test. Correlation between claw measurements and SHT were calculated by Spearman correlation test. All results in this study were considered significant at $p < 0.05$. All claws were classified as thin soles at a sole horn thickness less or equal 4 mm at site 1 (SHT1).

Results

In 32 from 97 cows a total of 65 claws with TS were assessed. The control-group had significantly less cows with TS (n = 2, i.e. 5%) than the experimental-group (n = 30, i.e. 52.6%). Locomotion scores were observed to be significantly higher (p = 0.01) in cows housed on fully-floored mastic asphalt (mean score: 1.97 +/-0.11) compared to the cows of the control-group (mean score: 1.09 +/-0.04; p = 0.01). There was a significantly higher prevalence of white line lesions (25 % vs. 59.6 %, p = 0.01) and toe ulcers (0 % vs. 12.3 %, p = 0.02) in the experimental group. Net growth rate per month of the hoof horn was significantly lower in the EG (1.94 ±0.64 mm) compared to the CG (2.65 ±0.64 mm; p = 0.01). Dorsal wall length (r = 0.54; p = 0.01) and heel height (r = 0.43; p = 0.01) showed a positive correlation with SHT1.

Discussion

In cows of the experimental group, housed on fully-floored mastic asphalt, a signi-ficantly higher prevalence of white line disease and toe ulcers was determined compared to cows in the control-group, which were housed on semi-full floored mastic. Mastic asphalt is recognised as a highly abrasive surface, causing a lower net growth rate of claw horn and a higher incidence of lameness (Van Amstel et al., 2004; Telezhenko et al., 2008, 2009; Sanders et al., 2009; Shakespeare, 2009; Shearer & Van Amstel, 2009). From the results of this study it has to point out that it is very important to adapt the hoof trimming technique according to the shape of the claws, which is a function of the present flooring system in the farm (Kofler, 2015, 2017). The dorsal wall length acts as a proper indicator for the sole horn thickness, because the dorsal wall-length and the heel height were positively correlated with the sole horn thickness, which was observed even by other authors (Telezhenko et al., 2009). From the results of this and previous studies (Shearer & Van Amstel, 2009, Kofler, 2015) the recommendation can be given that for new barn installations only semi-full floored mastic asphalt areas should be planned. High traffic areas, such as the aisle in front of the feeding fence and the waiting area in front of the milking parlour, should be floored for example with rubber mats.

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Concentrations of cortisol during claw treatment under pain control in meat Merino ewes

Kortizol koncentráció alakulása fájdalomcsillapítással végzett csülökezelés során hústípúsú Merinó juhokban

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Abstract

The occurrence of claw disorders, for example footrot, in sheep is associated with pain and consequently with stress. The intravenous regional anaesthesia is a frequently performed procedure in cattle to desensitize the distal limb. In sheep this kind of anaesthesia is not commonly used under field conditions. Our objective was to examine the effect of claw trimming for footrot treatment under intravenous regional anaesthesia (5 ml procainhydrochloride) and xylazinhydrochlorid injection (0.1 mg/kg) on the stress response.

In total 36 meat merino ewes with contagious footrot and 13 healthy sheep as control animals (control animals: placebo xylazinhydrochlorid, placebo intravenous regional anaesthesia) were included in the study. Claws were trimmed and footrot was treated under the following conditions A: xylazinhydrochlorid, intravenous regional anaesthesia, B: placebo xylazinhydrochlorid, intravenous regional anaesthesia, C: placebo xylazinhydrochlorid, placebo intravenous

regional anaesthesia. Before claw treatment the concentrations of cortisol in blood and lacrima were measured at three times (0, 10, 20 min) until claw treatment, during (30 min) and two times (40, 60 min) after treatment. The treatment group A showed decreased levels of cortisol over all sampling time points compared to group C. Moreover plasma cortisol levels in group A were significantly lower at time point 10, 20, 30, 60 minutes, than in the control group ($P < 0.05$). In the lacrima similar differences between the treatment groups A and C were detected.

In conclusion, the treatment of footrot lesions is accompanied with stress and leads to a quantifiable hormonal stress response. Xylazinhydrochlorid enhances the effect of the intravenous regional anaesthesia.

Development of a system analysis for problem-centered data acquisation of cow- and housing-related risk factors for infectious claw diseases in dairy farms

Rendszerelemzés kidolgozása csülökbetegséggel terhelt tehenészeti gazdaságokban a tehén és a tartásához kapcsolódó kockázati tényezők értékelése céljából

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Abstract

Reasons for bovine hoof disorders are multifactorial and can be divided in cow-related and housing-related risk factors. The aim of the study was to identify causes for bovine hoof disorders, especially for the digital and interdigital dermatitis-complex.

From December 2016 to February 2018 we performed a system analysis on eleven dairy farms (46 to 1646 dairy cows per farm, 7768 kg to 11216 kg 305-day milk yield), which stated to have a problem with infectious hoof disorders. Herd data and external test results were analysed in preparation of the farm visit. During the visits information about housing and management, staff, animals and workflow were gathered by interviewing the responsible persons, inspecting the farm and scoring animals and their housing environment. Based on the gathered data a strategy plan was developed.

Between 6% to 57% of the cows were lame. Farms that worked with multiple high-qualified hoof trimmers had less lame cows. There was no sufficient documentation about claw diseases. New cases of lame cows were treated between once per week and once per day. At the beginning of the study, only five of eleven farms involved veterinary competence in the claw health management. At the end of the study, eight of eleven farms involved a veterinarian in the claw health management.

To assess possible risk factors for bovine infectious hoof disorders, an objective system analysis is needed. The effectiveness of an analysis and subsequent decisions depends on the quality of documentation as well as the qualification of the personal and should be steadily optimized. Besides factors of housing, feeding and breeding, health care management has a big influence on bovine hoof disorders. Especially the veterinary expertise, intensity of hoof trimming and treatment as well as a high degree of qualification of the staff are required.

Effects of different dosages of propylene glycol in cows according to calving

Az elléskor adagolt különböző propilénglikol dózisok hatása tehenekben

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Abstract

The application of propylene glycol (PG) supporting the cows energy metabolism during periods of negative energy balance has already been used since the 1950s. Although PG has been used over decades the literature provides controversial information about dosages, application methods and differences in efficacy of PG at different periods of lactation. The aim of the study was to investigate the metabolic effects in dairy cows at different times around the transition period giving different doses of PG (Mauer et al., 2017).

We hypothesized that the temporary insulin resistance during the transition period may cause significant differences in the efficacy of PG at the four sampling periods (dry cows, close up cows, fresh cows and lactating cows) and that in some cases this effect will be dose dependent (100 ml, 300 ml and 500 ml, per os, one application per day).

A convenience sample (animals which were in a suitable stage of pregnancy at that period before starting their 2nd to 5th lactation) of 7 pregnant cows was selected for the study. The cows entered the study according to their pregnancy status. Eighty four sampling sets were generated by studying the 7 multiparous Holstein cows repeatedly at 4 sampling periods of 3 days length (dry cows: days 40, 39 and 38 *antepartum*; close up cows: days 10, 9 and 8 *antepartum*; fresh cows: days 3, 4 and 5 *post-partum*; lactating cows: days 38, 39 and 40 *post-partum*). On each of these days three

hours after morning feeding propylene glycol was drenched in different dosages of 100, 300 or 500 ml once per day (cross over study). The different doses were applied in an alternating order (Latin square). Blood samples were taken before, every 30 min up to four hours, after six hours and twelve hours after PG application. Following parameters have been measured: insulin, non-esterified fatty acids (NEFA), betahydroxybutyrate (BHB), bilirubin, cholesterol, potassium, aspartate aminotransferase (AST) and glutamate dehydrogenase (GLDH). Revised Quantitative Insulin Sensitivity Check Index (RQUICKI) was calculated.

It was found that glucose, insulin, NEFA, BHB, bilirubin and potassium concentrations were influenced differently by the three defined dosages of propylene glycol at the four different sampling periods. Whereas RQUICKI, cholesterol, AST and GLDH did not differ between the sampling periods and treatments. The major results of the study are that the effect of PG is dose-dependent and that the effect of PG is depending on the time of application according to calving. It can be concluded that in fresh cows higher dosages are necessary to provoke similar effects in comparison to dry, close up and lactating cows. Although the present study did not compare to PG used as topdressing or mixed in the TMR there is strong evidence that the bolus application of a certain volume PG (depending on lactation status) is necessary to provoke an insulin peak which results in the major effect on the metabolism.

Summarizing the study showed that for the major metabolic parameters the impact of PG application is dose dependent and that the effect of PG is also dependent on time to or from calving. Fresh cows require higher dosages of PG to provoke similar effects in comparison to dry, close up or lactating cows. These findings might explain some of the conflicting reports about efficacy of PG application volume of 500 ml PG for post calving cows seems the most appropriate whereas during dry period or later in lactation dosage of 100 or 300 ml are sufficient. The required dosage of PG has to be given as bolus to generate the desired effects on metabolism.

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A bendőfolyadék pH és a drencselés kapcsolatának vizsgálata az ellés körüli időszakban

The effect of drenching on the changes of rumen fluid pH in dairy cows around parturition

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Összefoglalás

A kísérlet egy nagyüzemi tejelő tehenészetben zajlott 12, többször ellett Holstein-fríz tehén bevonásával. A tehenek bendőjébe körülbelül 14 nappal az ellés előtt reticuloruminalis pH- és hőmérséklet-mérő bólust helyeztünk. Drencs-kezelést 8, véletlenszerűen kiválasztott tehénen végeztünk (D csoport), a kontroll csoport (K) 4 állatból állt. Az adatok elemzésekor a teheneket a szubakut bendőacidózisra (SARA) való érzékenységük alapján két csoportra (érzékeny – É és toleráns – T) osztottuk, majd a SARA és a kezelési csoportokat kombináltuk (ÉD, ÉK, TD, TK). A pH minden állatban csökkent az ellés után, a pH=5,8 alatt töltött időszak pedig nőtt. AZ ÉD csoport pH átlagai szignifikánsan alacsonyabbak voltak a többi csoportétól (P<0,01), a pH=5,8 alatt töltött időszak pedig itt nőtt a legjobban (P<0,01). A TD és a TK csoport között nem volt jelentős különbség, viszont a TD csoport pH=5,8 alatt töltött ideje két nappal a TK után lépte át a határértéket, ami egybeesett a drencskezelés idejével, habár az összefüggés nem volt szignifikáns.

Abstract

The study was conducted on a commercial dairy farm with 12 multiparous Holstein-Frisian cows. Approximately 14 days before calving reticuloruminal boluses were placed in the animals via balling gun. These boluses recorded the reticuloruminal pH and

temperature. Drenching was performed on eight, randomly selected cows (D), while 4 cows served as controls (K). During the data analysis, the cows were assigned to sub-acute ruminal acidosis (SARA) susceptible (É) and tolerant (T) groups, and these were combined with the treatment groups (ÉD, ÉK, TD, TK). Reticuloruminal pH decreased in all animals after calving, and time spent below pH 5.8 increased. Group ÉD had a significantly lower pH after calving than any other group (P<0.01), and the time spent below pH 5.8 was the highest in this group (P<0.01). TD and TK had no major differences in their values, but the average time spent below pH 5,8 in Group TD passed the threshold two days later than TK, on which 2 days the treatments were conducted, but the difference was not significant.

Bevezetés

Az ellés körüli időszakban jelentkező számos változás közül jelentős, hogy a tejtermelés miatt az állatok energiaigénye megnő. A takarmányadagban az abrak részarányának növelése ezt az igényt igyekszik kielégíteni, de mindeközben fontos, hogy a bendő megfelelő működését megőrizzük, ezáltal elkerüljük az olyan problémákat, mint a szubakut bendőacidózis (SARA). A SARA számos negatív következménnyel bír: csökkent takarmányfelvétel és tejzsírtermelés, hasmenés, laminitis (Plaizier et al., 2008; Zebeli & Meltzer-Zebeli, 2012). A probléma diagnosztizálására az utóbbi évekig nem volt megbízható módszer (Zebeli et al., 2008). A reticulorumen pH-ját mérő bólusok megjelenésével azonban lehetővé vált az állatok bendőműködésének valós idejű monitorozása.

A drencs-kezelések évtizedek óta használatosak az állatorvosi gyakorlatban. Számos hatóanyag és receptúra kapható kereskedelmi forgalomban világszerte. Az aktív száraz élesztő bizonyos tanulmányok szerint jótékony, stabilizáló hatással van a bendő pH-jára (Miller-Webster et al., 2002; Jouany, 2006). A kalcium-propionát, bár a bendő pH-jára nincs direkt hatással, hozzájárul a tehén energiaigényének kielégítéséhez (Liu et al., 2010), ezáltal az állat metabolikus egyensúlyának megőrzéséhez.

Anyag és módszer

A kísérlet egy nagyüzemi tejelő tehenészetben zajlott 12, többször ellett Holstein-fríz tehén bevonásával. A tehenek bendőjébe körülbelül 14 nappal az ellés előtt reticuloruminalis pH- és hőmérséklet-mérő bólust (SmaXtec Animal Care Sales GmbH, Graz, Ausztria) helyeztünk. A bólus 10 percenként rögzítette az állatok reticuloruminalis pH-ját és hőmérsékletét, amit az ellés után kb. 30 nappal, egy kézi adatleolvasó műszerrel olvastunk le.

A drencs-kezelést 8, véletlenszerűen kiválasztott tehénen végeztük el (D csoport), a

kontroll csoport (K) 4 állatból állt. A drencseléshez használt takarmány-kiegészítő kalcium-propionátból, magnézium-szulfátból, kálium-kloridból, nátrium-kloridból, és élesztőből állt, amit 25 liter langyos vízben oldottunk fel. A kezelést háromszor végeztük el, körülbelül 24 óránként. Az első drencs az ellés utáni 24 órán belül történt. A drencs pontos idejét feljegyeztük. A K csoporttal való bánásmód a drencsen kívül mindenben megegyezett a D csoportéval.

Az adatok elemzésekor a teheneket a SARA-ra való érzékenységük alapján két csoportra (érzékeny – É és toleráns – T) osztottuk. A SARA-ra való érzékenységet korábbi irodalmi adatok alapján határoztuk meg: ha a reticuloruminalis pH az ellés előtti egy hétben legalább egy napon 5,8 alá csökkent több, mint 330 percig (Zebeli et al., 2008), az állat az érzékeny csoportba került. A határértéket el nem érő állatok alkották a toleráns csoportot. Létrehoztunk egy második csoportosítást is, ahol a pH határértéket felemeltük 6-ra. A SARA és a kezelési csoportokat kombináltuk (ÉD, ÉK, TD, TK). Csoportonként kiszámítottuk a pH, és a határérték alatt töltött percek átlagát napi, illetve heti bontásban, ezután kétmintás t-próbával hasonlítottuk össze a csoportok értékeit.

Eredmények

A 12 állat a következőképpen oszlott meg a pH<5,8-cal számított SARA-érzékenység és a kezelés alapján alkotott csoportok között: ÉD: n=5, ÉK: n=0, TD: n=3, TK: n=4. A pH 6-ra emelésével ez a következőképpen változott: ÉD: n=6, ÉK: n=0, TD: n=2, TK: n=4.

Az pH<5,8 alapján kialakított csoportok reticuloruminalis pH-ja az ellés előtti, illetve utáni héten a következően alakult: ÉD: -7.0. nap: 6,15; 1-7. nap: 5,61. TD: -7-0.nap: 6,39; 1-7. nap: 6,03. TK: -7-0. nap: 6,34; 1-7. nap: 5,99. A pH<6 alapján osztott csoportokban az előzőhöz képest nem történt jelentős változás: ÉD: -7.0. nap: 6,15; 1-7. nap: 5,68. TD: -7-0.nap: 6,38; 1-7. nap: 5,93. TK: -7-0. nap: 6,27; 1-7. nap: 5,99. A pH=5,8 alatt töltött percek számának az átlaga a következő: ÉD: -7.0. nap: 298 perc; 1-7. nap: 991 perc. TD: -7-0.nap: 26 perc; 1-7. nap: 234 perc. TK: -7-0. nap: 96 perc; 1-7. nap: 397 perc.

A pH<5,8 alapján kialakított csoportok pH-értékeinek összehasonlításakor szignifikáns különbség volt kimutatható az ÉD-TD (P<0,01), illetve az ÉD-TK csoportok között (P<0,01). A két toleráns csoport pH-ja között nem volt szignifikáns különbség. Ez akkor sem változott, amikor a pH<6 alapján osztott csoportok pH-ját hasonlítottuk össze. A pH=5,8 alatt töltött idő tekintetében az ÉD csoport értékei jelentősen különböztek a többitől (P<0,01), a TD-TK közti eltérés viszont nem volt szignifikáns (P=0,051). Viszont a pH=5,8 alatt töltött időt napi bontásban vizsgálva a TD és TK csoport értékei között egy nem szignifikáns, de jelentős különbség látszik az

ellés utáni 1-3 napon: TD: 1. nap: 30 perc, 2. nap: 13 perc, 3. nap: 543 perc. TK: 1. nap: 455 perc, 2. nap: 425 perc, 3. nap: 513 perc. A TK csoport átlagai két nappal korábban lépték át a 330 perces határértéket, mint a TD csoport tehenei.

Következtetések

Az adatokból jól látszik, hogy a csoportok beosztásától függetlenül a pH csökkenése és a pH= 5,8 alatt töltött időszak minden állatnál megnő az ellés utáni napokban. A leg nagyobb mértékű pH-csökkenés az ÉD csoportban alakult ki. Ez szignifikáns eltérést okozott az ÉD és a többi csoport pH-ja, illetve pH=5,8 alatt töltött ideje között is.

Ennek a drencs-kezeléssel való összefüggését, ÉK csoport híján, nem tudtuk vizsgálni. A TD és a TK között megjelent különbség viszont egybeesik a drencs-kezelés idejével. Lehetséges, hogy a drencs-készítmény élesztőtartalma a kezelés után képes volt rövid időre stabilizálni a reticuloruminalis pH-t, elodázva a SARA megjelenését a kezelt csoportban.

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The influence of pre-partum rumination on post-partum metabolic parameters in dairy cows

Az ellés előtti kérődzés hatása a metabolikus paraméterekre az ellés körüli időszakban tejelő tehenekben

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Abstract

The study was carried out on a commercial dairy farm and involved 159 Hungarian Holstein Frisian cows. The rumination time was recorded using an automatic system from Day 14 before to Day 4 after calving, in 2-hour intervals. Herd average was calculated and cows were assigned to high (H) or low (L) rumination group. Blood samples were taken on Days 1, 2, 3, 7 and 12 after calving and BHB, NEFA, total calcium and magnesium levels were measured. BHB concentrations were significantly lower in Group H (P=0.018), and cows of Group H had significantly higher odds of remaining free from SCK (P<0.01). Both calcium and magnesium concentrations were significantly higher in Group H (P<0.01). Day of sampling also influenced concentrations of all parameters significantly. According to the data, Group H could maintain better metabolic health after calving than Group L.

Összefoglalás

A kísérlet egy nagyüzemi tejelő tehenészetben zajlott 159 Holstein-fríz tehén bevonásával. A kérődzési időt egy automata rendszer mérte kétórás intervallumokban az ellés előtti 14.-től az ellés utáni 4. napig. A telepi átlag kiszámítása után a teheneket magas (H) vagy alacsony (L) kérődzési csoportba osztottuk. Az ellés utáni 1., 2., 3., 7. és 12. napon vérvétel történt BHB, NEFA, összes

kalcium és magnézium koncentráció meghatározására. A vér BHB-koncentrációja a H csoportban szignifikánsan alacsonyabb volt, mint az L csoportban (P=0,018), és a H csoport állatai szignifikánsan kisebb eséllyel szenvedtek szubklinikai ketózistól (P<0,01). A kalcium és magnézium koncentrációk is magasabbak voltak a H csoportban (P=0,01). A mintavétel napja is szignifikánsan befolyásolta az eredményeket. Az adatok szerint a H csoport az ellés után jobban volt képes megtartani a metabolikus egyensúlyát, mint az L csoport.

Introduction

There is a growing body of literature concerning cows' health in relation to their rumination times. There is a close relationship between rumination times in the peripartal period and the occurrence of clinical diseases. In one study it was found that cows spending less time with rumination in the peripartal period than the herd average are more affected by periparturient diseases than the ones whose rumination exceeds the herd average (Calamari et al., 2014).

It is also known that animals suffering from subclinical diseases have shorter rumination times than their healthy counterparts (Soriani et al., 2012).

In a recent study (Stangaferro et al., 2016) an automatic health monitoring system was used to identify cows suffering from metabolic problems. The algorithm included rumination times, activity and health index score (HIS), and had a sensitivity of 93% to signal metabolic and digestive disorders (left displaced abomasum, ketosis, and indigestion). Animals that were flagged by the system had lower rumination times, activity and HIS than the healthy ones.

In terms of blood metabolic parameters the same results were published. In a study cows with subclinical ketosis (SCK, blood BHB ≥1.2 mmol/l) ruminated 25 ± 12.8 min/d less than healthy ones, and animals suffering from SCK plus another disease had a rumination time 44 ± 15.6 min/d less than the healthy cows. This difference occurred from -1 to 1 week around calving (Kaufman et al., 2016). In another study, precalving rumination times of cows suffering from SCK were lower than those of the healthy group (Schirmann et al., 2016).

These data indicate that measuring the rumination times in the peripartal period can be useful for the identification of cows at risk for clinical and subclinical problems.

Materials and methods

The study was carried out on a commercial dairy farm and involved 159 Hungarian Holstein-Frisian cows. The rumination time was recorded using an automatic system (Hr-Tag, SCR Engineers Ltd., Netanya, Israel), which was placed on the cows approximately 2 weeks before calving and was removed 4 days after calving. The data were recorded in 2-hour intervals. The

rumination times of the last 4 days pre-partum were summarized and the herd mean was calculated. Cows below the herd average were assigned to the low rumination (L) group, and those above the herd average were assigned to the high rumination (H) group.

After calving, blood samples were taken from the tail vein on Days 1, 2, 3, 7 and 12, respectively. The samples were taken to our laboratory and the concentrations of beta-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), total calcium (Ca), magnesium (Mg) and several other parameters were measured.

Post-partum drenching (14% calcium, 42% propionate, 6% magnesium, 6% potassium, 3% sodium, 9% chloride and 20% yeast were dissolved in 25 liters of lukewarm water) was performed on randomly selected cows (n=87, D), and the rest of the cows served as controls (n=72, C). Drenching was performed three times approximately 24-hour apart. The exact time of the drenching was recorded. The first drench had to be done in the first 24 hours after calving. Cows in the C group were handled exactly the same way except for drenching.

For the statistical analysis linear models were used. The response variable was one of the biochemical parameters, and the explanatory variables in all cases were the rumination group (H/L), treatment group (D/C), day of the sampling (D01-D12), and lactation number (factor). The BHB and NEFA results were categorized by being below (B) or above (A) the given threshold (1.2 mmol/l and 0.4 mmol/l for the BHB and NEFA, respectively), and logistic regression models were also used with the same formula.

Results

The mean rumination time of the herd before calving was 1967 min/96 h. Group L consisted of 70 cows and Group H had 89 cows. Their mean rumination times were 1699 min/96 h and 2176 min/96 h, respectively. Lactation number and treatment had no influence on any of the parameters examined.

The mean BHB concentrations of Groups H and L were 0.94 and 1.07 mmol/l, respectively. There was a significant difference between the two groups ($P=0.018$), and the result was also influenced by the different days of sampling ($P<0.01$, for Days 3, 7 and 12). The odds of cows in Group H not suffering from SCK on the first 12 days of lactation was 1.94 compared to Group L ($P<0.01$).

The mean concentrations of NEFA in Groups H and L were 0.79 and 0.74, respectively. There was no significant difference between the two groups ($P=0.09$), but the values were influenced by the day of sampling ($P<0.01$ for Days 2, 7 and 12).

The concentrations of total Ca were higher in Group H compared to Group L (2.19 and

2.15 mmol/l, respectively, P<0.01), and these were also heavily influenced by the day of sampling (P<0.01 for Days 3, 7 and 12). Mg concentrations of Groups H and L were 0.91 and 0.98, respectively and there was a significant difference between the groups (P<0.01) and also between the days of sampling (P<0.01 for Days 2, 3, 7 and 12).

Conclusion

All parameters measured had significantly different values for the two groups. Group H had lower HBH and NEFA, but higher Ca and Mg levels compared to Group L, indicating that cows that were healthier before calving can maintain a better metabolic status in the first 12 days of lactation. We can also conclude that monitoring the rumination times before calving can be useful to find cows at risk for subclinical metabolic disorders.

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The capability of blood gas analysis *ante partum* for the prediction of hypocalcaemia (milk fever) in cows

Ellés előtti vérgáz-analízis lehetősége hipokalcémiás tehenek (ellési bénulás) előrejelzésére

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Introduction

The aim of this study was to evaluate a possible association between the pH value of the blood gas analysis in cows *ante partum* and the blood calcium level *post partum*. Thereby, it should be determined, if blood gas analysis in dry cows can serve as a possible indicator for the risk of the occurrence of clinical or subclinical hypocalcaemia (milk fever) in cows after parturition. Furthermore, the association between the results of the blood gas analysis and the blood calcium level *post partum,* as well as the concentration of the alkaline phospahatase (AP) *ante partum* and the blood calcium level after parturition should be determined.

Materials and Methods

Dairy farms holding Austrian Simmental cows in the region of the Innviertel (Upper Austria) were enrolled in the study. The farms were selected because of a known high incidence of hypocalcaemia of 23–30%. In total, 101 cows in different lactations and without receiving any prophylactic measures against hypocalcaemia were included in the study. Animals were sampled twice before parturition (6 and 2 weeks), as well as within one day after giving birth. The statistical analysis was performed using SPSS Statistics for Windows as well as Microsoft Excel 2010.

Results and Discussion

The results of this study indicate, that the pH-value and the bicarbonate concentration in blood samples collected *ante partum*, as well as the urinary acid-base status, are not suitable for the early prediction of hypocalcaemia in cows, due to their limited sensitivity and specificity.

The results of the present study further show, that the blood calcium and AP level *ante partum* may serve as early diagnostic predictive values for possible hypocalcaemia in post partal cows, with a high sensitivity and specificity.

As only 34.6% of cows included in the present study showed hypocalcaemia, the significance of the results is limited. To verify these results, additional studies, including more cows with and without hypocalcaemia are needed. With an increased number of cows enrolled, the deviations of the results would also be compensated to a higher extend. Nevertheless, it remains doubtful if the blood gas analysis has the potential to serve as a reliable early diagnostic predictive value for hypocalcaemia in cows, as blood gases, especially the pH value, are regulated in very tight limits by the organism.

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Hipokalcémia monitoring. Helyszíni gyorsvizsgálatok tejhasznú tehénállományokban

Hypocalcemia monitoring. On-farm quick testing in dairy herds

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Összefoglalás

A hipokalcémia az ellés után gyakran előforduló anyagcsere-zavar, ami súlyosabb esetben ellési bénuláshoz és elhulláshoz vezethet. Szubklinikai formában hajlamosít számos más anyagforgalmi megbetegedés vagy fertőző betegség kialakulására és negatívan hat a tejtermelésre és a szaporodóképességre (Seifi et al., 2011; Rodríguez et al., 2017; Goff, 2008; Martinez et al., 2012). A hipokalcémia diagnosztizálásának alapját a vér kalcium koncentrációjának mérése adja. A vérből meghatározható az összes kalcium koncentráció, illetve a metabolikus szempontból valójában aktív, ionizált kalcium (iCa) szintje. Az iCa hipokalcémia diagnózis felállítására használt határértéke 1,05 mmol/l (Oetzel, 2004).

A vizsgálatok célja egy olyan vizsgálati protokoll fejlesztése, amely alkalmas a tejelő tehenek hipokalcémiájának és az arra hajlamosító metabolikus veszélyhelyzetek ill. kockázatok helyszíni állományvizsgálatára. A szerzők bemutatják e fejlesztés előzetes eredményeit.

Vizsgálat állományonként öt előkészítős (prepartum 1-2 hét) és öt frissen ellett (postpartum 0-6 nap) klinikailag egészséges tehénre terjed ki. Az ellés előtti teheneknek a mintavételt megelőzően minimum 72 órája az előkészítős takarmányadagot kell kapnia.

A tehenektől vér és vizeletmintákat gyűjtünk. A helyszíni vérvizsgálatokat szobahőmérsékletű, alvadásban gátolt vénás vérből végezzük iSTAT Handheld (*ABBOTT Point of Care Inc. 400 College Road East,*

Princeton, NJ 08540) típusú véranalizátorral és a hozzá tartozó egyszerhasználatos iSTAT Chem-8 tesztpatronnal. A meghatározott paraméterek: ionizált Ca [mmol/l], Na [mmol/l], K [mmol/l], Cl [mmol/l], glükóz [mmol/l], karbamid [mg/dl], összes szén-dioxid (TCO_2) [mmol/l], kreatinin [mg/dl], hematocrit [%PCV], hemoglobin [g/dl], AnGap = (Na+K) – (Cl-(TCO2-1)) értékek.

A vizelet kémhatását kalibrált, hőmérséklet kompenzációra képes, nagy pontosságú műszerrel határozzuk meg a helyszínen. Az ÁTE laboratóriumában meghatározott paraméterek a vérplazma összes Ca és Mg [mmol/l] és a vizelet a nettó sav bázis ürítést (NSBÜ) [mmol/l] koncentrációi. A vizsgálatokkal egyidőben helyszíni telep- és állományszemle keretében vizsgáljuk a legfontosabb tartástechnológiai elemeket, különös tekintettel azokra, amelyek közvetett vagy közvetlen módon befolyásolhatják az állatok egészségi állapotát, termelékenységét.

Az előzetes eredmények alapján a hipokalcémia előfordulása a frissen ellett tehenekben akár 50%-ot is meghaladhatja egyes állományokban. A hipokalcémia kialakulására hajlamosító metabolikus állapotok közül több esetben lehetett az ellés előtt a savbázis anyagcsere alkalikus irányú eltolódását (magas vizelet pH és NSBÜ) továbbá az ellés előtt és a frissen ellett tehenekben hipomagnezémiát megállapítani.

Kulcsszavak: hipokalcémia, tejelő tehén, helyszíni állomány monitoring

Abstract

Hypocalcemia is a common metabolic disorder that may lead to clinical milk fever or death. The subclinical form of the hypocalcemia may predispose to other fresh cow diseases and has negative effect on milk production and reproduction (Seifi et al., 2011; Rodríguez et al., 2017; Goff, 2008; Martinez et al., 2012).

The diagnosis of the hypocalcaemia based on the determination of the total Ca and/or ionised Ca (iCa) concentration of the blood. The hypocalcemia diagnosed when the iCa concentration – the active form of Ca for metabolic processes- is <1.05 mmol/l (Oetzel, 2004).

The aim of the work is to develope a practical protocol for the on-farm monitoring of hypocalcemia and those metabolic risk factors may contribute to the development of the disease. Authors present the preliminary results of the research.

Clinically healthy cows selected randomly from close up period (n=5; 1-2 weeks prepartum) and from fresh cows group (n=5; 0-6 days postpartum) at each examined farms. Dry cows should get close up diet minimum 72 hours before sampling, fresh cows sould be min. 12 hours after parturition.

Blood and urine samples were taken for metabolic analysis. The heparinised, room temperature whole blood samples were analised on-farm by the iSTAT Handheld

(*ABBOTT Point of Care Inc. 400 College Road East, Princeton, NJ 08540*) analyser equipment withthe usage of iSTAT Chem-8 one way test cartridge. Parameters were analysed: iCa [mmol/l], Na [mmol/l], K [mmol/l], Cl [mmol/l], Glucose [mmol/l], BUN [mg/dl], TCO_2 [mmol/l], Creatinine [mg/dl], %PCV, [mmol/l], Hgb [g/dl]. AnGap = (Na+K) – (Cl-(TCO2-1)) values.

The urine pH were measured on-farm by a calibrated pH analiser. The blood plasma total Ca, Mg [mmol/l] and urine Net Acid Base Excretion (NABE) [mmol/l] were determined in the laboratory of UVM. Ont the day of sampling a farm- and herdvisit was performed to check the management and nutrition related isk factors may contribute to the developement of the hypocalcemia.

According to our preliminary results the prevalence of hypocalcemia may exceed 50% in Hungarian dairy herds. Metabolic risk factors (prepartum alcalosis, Mg shortage before and after calving) may contribute to the developenemt of the hypocalcemia also were detected.

Key words: Hypocalcemia, dairy cow, on-farm monitoring

Köszönetnyilvánítás

A bemutatott eredmények részét képezik annak a projektnek amely az Európai Unió támogatásával, az Európai Szociális Alap (ESZA) társfinanszírozásával valósul meg. (A támogatási szerződés száma: EFOP-3.6.2-16-2017-00012. A projekt címe: „Funkcionális, egészséges és biztonságos élelmiszer termékpálya modell kidolgozása a szántóföldtől az asztalig elv alapján, tematikus hálózatban”.

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Effect of a prepartal treatment with Vitamin D_3 on early postpartum uterine contractility and blood ionized calcium concentrations in dairy cows

Ellés előtt adott D_3-vitamin kezelés hatása az ellést követő méhkontraktilitás és a vér kalciumion-koncentrációjának alakulására tejhasznú tehenekben

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Abstract

Proper involution is necessary for the success of a subsequent insemination in due time after preceeding calvings in dairy cows. During the puerperal changes the role of uterine muscle activity is primary. It is mainly responsible for the expulsion of uterine contents, including foetal membranes and lochia. However, in case of disturbed myometrial activity the process of involution prolongs. During this time, metabolic disorders are often found in the background, and the consequences are poor reproductive performance or even further health problems of the affected cows. Hypocalcaemia represents one of such metabolic disorders, which may appear in its severe form as parturient paresis (milk fever) affecting mainly older, high producing dairy cows during their first few days postpartum (Oetzel, 1988). Numerous possibilities are used in the field to overcome or to diminish the negative effects of severe hypocalcaemia, among them prepartal treatment with Vitamine D_3, as one option (Thilsing-Hansen et al., 2002; Horst et al., 2003; Goff, 2008), has been tested under farm conditions in this study.

Myometrial contractions of normocalcaemic (Group NC, n=8; blood Ca^{2+}>1.06 mmol/l)

and hypocalcaemic Holstein-Friesian cows (Group HC, n=12; blood Ca^{2+}<1.06 mmol/l) at a large-scale Hungarian dairy farm were examined during the years 2014 and 2015, using an open tip catheter system, which was suitable for digitally measuring intrauterine pressure (IUP) changes (Bajcsy et al., 2005a,b). Further cows were intramuscularly treated with a single dose of 10 ml Vitamine D_3 inj. (1 million IU colecalciferol/ml, CP-Pharma, Burgdorf, Germany; Group D, n=9) 7 days prior to expected calving, which was calculated using the farm average (280 days). Only cows that had shed their foetal membranes within 12 hours after calving were involved in the current evaluations to exclude the obvious influence of placental retention on contractility patterns.

IUP recordings with 4 hour initial durations were started 14-17 hours after calving and further three recordings, each with a duration of 1 hour, were performed in 12-hour intervals from the beginning of the previous sessions. Pressure data were collected from the previously pregnant uterine horn, and were analysed using a semiautomatic, operator-made software (LabVIEW® 5.0, National Instruments, Austin, TX). Mean contraction frequency (FREQ), amplitude (AMP), duration (DUR), mean and total area under the pressure curves (AUC and TAUC) of the pressure cycles were calculated.

Coccygeal blood samples were withdrawn at the beginning and at the end of the first, and at the end of all other recordings, and their Ca^{2+}-concentrations were analysed within 30 min. of sampling, using a portable blood gas and electrolyte analyser on site (ABL™ 77, Radiometer, Copenhagen, Denmark).

Repeated measures analyses of variance were used to calculate group- and time-related differences. Tukey post-hoc tests were performed to explore where significance between groups, recording times or their interactions appeared (Statsoft, Inc. 2004. STATISTICA v. 6.1. www.statsoft.com).

After treatment with Vitamin D_3 at day 273 of pregnancy, actual calvings occurred between 1 and 9 days after treatment, which gave an average of 4.00 (±2.87) days (mean±SD).

From the IUP parameters, significant group differences only occurred in TAUC with significantly higher values in Group D throughout the entire period ($p=0.0131$). Although this group had the highest means of FREQ, AMP and AUC, group differences were statistically not significant. The values of DUR were not different among the three groups. In all IUP parameters a consequent and significant decline over time was evident ($p<0.001$).

Mean blood Ca^{2+}-concentrations remained within a narrow range in all groups, and did not decline below 1.04 mmol/l at any stages in Group D. In this parameter, significant

group differences could be detected between Groups D and HC (p=0.0034), and between Groups NC and HC (p=0.0029), with Group HC in both comparisons being lower, while no difference was found between Groups D and NC. Blood Ca^{2+}-concentrations gradually declined until 25 hours with a consecutive increase until 49 hours postpartum (p=0.0039).

In conclusion, prepartum treatment with Vitamin D_3, 7 days prior to expected calving (in average 4 days before actual calving) turned out to effectively prevent severe postparturient decline in blood Ca^{2+}-concentrations during the first two days postpartum in dairy cows. It was also beneficial in maintaining a higher myometrial contractility (with significant changes only in TAUC values) in treated cows than in untreated ones, irrespectively of the initial blood Ca^{2+}-concentrations of those, during the observed early puerperal phase. Futher evaluations are though necessary to clarify these effects.

Összefoglalás

A szabályos involúcióhoz a méhizomzat megfelelő működése elsődleges fontosságú tejhasznú tehenekben, hiszen elsősorban ennek köszönhető a magzatburkok eltávozása és a lochia kiürülése is. A méhizmok működési zavara esetén az involúció gyakran elhúzódik romló szaporodásbiológiai teljesítményt eredményezve, mely folyamat hátterében gyakran metabolikus zavarok állnak. Egyik ilyen jelentős metabolikus zavar a hipokalcémia, mely súlyos esetben ellési bénuláshoz vezet, és főleg a nagy termelésű, idősebb tehenekben fordul elő, általában röviddel az ellést követően. Megelőzésére számos módszer ismert, egyik közülük az ellés előtt végzett D_3-vitamin kezelés.

Egy magyarországi nagyüzemi szarvasmarhatartó telepen 2014-2015-ben preventív D_3-vitamin kezelés hatását vizsgáltuk az ellést követő első 2 napban Holstein-fríz teheneken, melyek a magzatburkaikat 12 órán belül elvetették. A véletlenszerűen kiválogatott tehenek 10 millió NE D_3-vitamint kaptak intramuszkuláris kezelés formájában várható ellésük előtt 7 nappal (n=9), ami a bekövetkezett ellések ismeretében 4 napos átlagnak felelt meg (±2,87 nap). További két csoportba normokalcémiás (n=8) és hipokalcémiás (n=12) teheneket válogattunk azok vérmintáinak Ca^{2+}-koncentrációja alapján, mely mintákat ellést követően a 14-17. óra között a farokból vettük. E csoportosítás határértékeként 1,06 mmol/l Ca^{2+}-koncentrációt választottunk. A méhkontraktilitás alakulását az első vérminta levétele után először egy 4 órányi, majd azt 3, egymást 12 óránként követő, 1-1 órányi belső méhnyomás-mérés segítségével követtük nyomon.

Megállapítottuk, hogy a D_3-vitaminnal ellést megelőzően kezelt tehenekben a vér Ca^{2+}-koncentrációjának csökkenése mérsékeltebb maradt a vizsgált időszakban. Egyidejűleg azt is megfigyelhettük, hogy a kezelés mind a normo-, mind a hipokalcémiás tehenekhez

képest valamelyest aktívabb méhkontraktilitást eredményezett a puerperális időszak első két napjában, legalábbis a méh-kontraktilitás jellemzésére használt egyik fontos paraméter, a TAUC tekintetében. A kezelések pontos hatásának megismeréséhez azonban további elemzések szükségesek.

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A hőstressz hatása tejelő tehenek egyes anyagforgalmi mutatóira és az anyagcserezavarok előfordulási gyakoriságára – retrospektív vizsgálat

The effect of heat stress on some metabolic parameters and prevalence of metabolic disorders in lactating cows – a retrospective study

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Összefoglalás

A tejelőállományokban a nyári melegben tapasztalható étvágycsökkenés miatt csökkent energiabevitel, és a tejtermelés, illetve az élettani testhőmérséklet fenntartására irányuló fokozott hőleadás energiaigénye hajlamosító tényező az anyagcserezavarok, elsősorban a bendőacidózis, illetve az energiahiány következtében fellépő (szubklinikai) ketózis kialakulásában. A közelmúltban végzett kutatások tanúsága szerint hosszan tartó hőstressz hatására az inzulin vérben mérhető koncentrációja emelkedik, ami korlátozza a zsírbontást és fokozza a glükóz felhasználását. A glükoneogenezis prekurzorai az izomfehérjék fokozott bontásából is származnak. Az elmúlt 8 évben végzett állomány-egészségügyi vizsgálatok adatai alapján vizsgáltuk azt a hipotézist, hogy a hőstressz okozta tendenciózus változások telepi körülmények között is megfigyelhetők.

A szabad-zsírsavak, a glükóz, a béta-hidroxi-butirát (BHB) és a karbamid vérplazmában mért koncentrációit, a vizeletben mérhető nettó sav-bázis ürítés (NSBÜ) mértékét, valamint a szubklinikai ketózis (BHB>0,8 mmol/l), illetve a savterhelés (NSBÜ<100 mmol/l) előfordulási gyakoriságát hasonlítottuk össze. Hősemleges környezetnek a március-áprilisi (46 telep, 1371 állat), a feltehetően hőstresszt okozó időszaknak pedig a nyári hónapokat (66 telep, 2146 állat) tekintettük. Az adatokat az R statisztikai szoftver segítségével, lineáris kevert modell alkalmazásával hasonlítottuk össze az egyes termelési csoportok (előkészítő, frissen ellett, fogadó, nagytejű) függvényében.

A plazma nyári időszakban mért szabadzsírsav tartalma a számszerű különbségek ellenére nem különbözött a tavaszi időszaktól az előkészítő csoportban, a frissen ellett, illetve a csúcslaktációjú állatok között, azonban 20%-kal alacsonyabb volt a fogadó csoportban (p<0,001).

A vérplazma glükózkoncentrációja a nyári időszakban a frissen ellett állatok kivételével minden csoportban alacsonyabb volt (p<0,0001) a tavaszi időszakhoz viszonyítva. A BHB nyáron mért koncentrációja némileg (10%) alacsonyabb volt a fogadó csoportban (p=0,014), azonban nem különbözött a többi csoportban. A szubklinikai ketózis gyakoriságában nem volt évszakos különbség egyik csoportban sem. A nyári időszakban számszakilag magasabb karbamid-koncentrációkat mértünk, a különbség azonban csak a frissen ellett állatok között volt statisztikailag igazolható (p<0,05). A NSBÜ mértéke átlagosan 20 mmol/l-rel (p<0.05) volt alacsonyabb a frissen ellett csoportban nyári időszakban, azonban a többi csoportban nem volt különbség. Ezzel összhangban a savterheléses esetek is a frissen ellett állatok között voltak gyakoribbak (odds ratio: 1,81, p<0,05).

A csökkent zsírmobilizáció és fokozott glükózfelhasználás tendenciája megmutatkozik a telepek átlagában. A feltehetően hőstresszt okozó időszakban végzett állomány-egészségügyi vizsgálatok során figyelembe kell venni, hogy az adott telepen alkalmazott takarmányozási stratégia, valamint az istállóhűtési technológia módosíthatja az elemzés során becsült, szakmailag talán kevéssé releváns különbségeket. A hőstressz enyhítése különösen fontos a frissen ellett állatok esetén.

Köszönetnyilvánítás

A projekt az Európai Unió támogatásával, az Európai Szociális Alap (ESZA) társfinanszírozásával valósul meg (a támogatási szerződés száma: EFOP-3.6.2-16-2017-00012). Jurkovich Viktor az MTA Bolyai János kutatási ösztöndíja (BO/29/16/4) támogatta.

Abstract

Negative energy balance resulting from decreased dry matter intake coupled with the energy demands of maintaining physiological body temperature and milk production makes heat stressed cows more prone to metabolic disorders. Quite paradoxically, it was shown that in prolonged heat stress circulating insulin concentrations increase that limit the rate of fatty acid utilization and increase glucose disposal. The increased demand for gluconeogenic precursors usually induce increased protein catabolism. Data from regular herd health monitoring visits in large scale dairy herds in the past 8 years were analyzed to study whether heat induced changes are reflected in the metabolic profiles of cows in field conditions.

Data on metabolic parameters were collected from thermoneutral (March-April, 1371

cows, 46 farms) and potentially heat stress conditions (June-July, 2146 cows, 66 farms). Animals in similar stage of lactation (dry cows, DIM 1-5, DIM 6-40 and DIM >40, respectively) were compared. Herd bias was controlled for in the statistical analysis.

Summer average of plasma free fatty acid concentrations were not different in dry and fresh cows and in the DIM>40 group, however, it was lower by 20% in the DIM 6-40 group ($p<0.001$). Glucose concentrations measured in summer were slightly lower in all groups ($p<0.001$) except fresh cows. A moderate decrease (10%) in beta-hydroxy-butyrate concentrations was observed in hot weather in the DIM 6-40 group ($p=0.014$), but not in other groups. Urea concentrations were numerically higher in summer conditions in all groups, the difference was significant in fresh cows ($p<0.05$).

Prevalence of ketosis did not differ significantly by season in any of the groups. Mean net acid base excretion (NABE) in urine was on average 20 units lower ($p<0.05$) in fresh cows, but did not differ in other groups. In paralell to this, the occurence of acid load cases (0<NABE<100) were more frequent in fresh cows (odds ratio: 1.81, $p<0.05$).

In general, the tendency of reduced lipolysis and increased glucose utilization could be confirmed, however, the clinical relevance of observed differences are questionable. Between-farm variances might reflect the differences in actual temperature at the time of sampling and the efficiency of cooling technologies as well as potential nutritional interventions. The severity of heat stress should be considered when evaluating energy and nitrogen balance in dairy herds in the summer. Heat stress abatement is particularly important in the periparturient period.

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Influence of dietary clinoptilolite supplementation on milk fat to protein ratio and β-hydroxybutirate serum levels during transition period in dairy cows

A klinoptilolit (CPL) takarmány-kiegészítő hatása tejelő tehenek a tej zsír:fehérje arányára és a szérum β-hidroxibutirát-szintjére az ellés körüli időszakban

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Abstract

The aim of the study was to establish the influence of dietary clinoptilolite (CPL) supplementation on milk fat-to-protein ratio (FPR) and serum β-hydroxybutirate (BHB) levels during transition period in dairy cows. Thirty pregnant cows for 240 days of the Holstein-Frisian breed, aged from 3 to 5 years and kept in a farm near Đurđevac, Croatia, were used in the trial. The cows were randomly assigned into two groups (either in-feed CPL treated; CPL group or control non-treated; CON group) comprising 15 cows each. The CPL group received 100 g of natural modified clinoptilolite preparation (Vibrosorb, Viridisfarm, Podpićan, Croatia) in the ratio for dairy cows on a daily basis. Blood samples were collected from the cows in the period from day 240 to 260 of pregnancy, after parturition (day 0), and on days 12, 26, 40 and 60 of lactation. During lactation, milk samples were taken (same days when a blood samples were taken) for milk fat and milk protein analyses. The average value of BHB was higher in the CON than in the CPL group (0.99±0.10 *vs.* 0.81±0.99 mmol/L). The highest concentration of BHB was obtained on days 0, 12 and 26 of lactation in the CON group (1.83±0.35, 1.55±0.35, and 1.62±0.35 mmol/L, respectively) and in the CPL group

after parturition, on day 0 (1.79±0.41 mmol/L). The FPR was significantly higher in the CON group on day 0, ($P<0.05$) as well as on days 12 and 26 slightly higher than in the CPL group. However, on day 10 after parturition FPR was slightly higher in the CPL group then in the CON group. Such similar trends are associated with decreasing level of BHB following parturition. Hence, by determining of the BHB levels (in blood, urine or milk), the FPR could be used for an early diagnosis of subclinical ketosis. Also, the results of this study indicated that CPL applied as dietary supplement could be effective in reducing clinical signs of ketosis.

Key words: dietary clinoptilolite, β-hydroxybutirate, milk fat-to-protein ratio, transition period, dairy cows

Összefoglalás

A kutatás célja a klinoptilolit (CPL) takarmány-kiegészítő hatásának vizsgálata a tej zsír:fehérje arányára (FPR) és a szérum β-hidroxibutirát-szintjére (BHB) az ellés-körüli időszakban tejelő tehenekben.

A vizsgálatban 30, vemhes (240. nap), 3-5 év közötti Holstein-fríz tejelő tehén vett részt a horvátországi Đurđevac-ban található tehenészetben. Az állatokat random két csoportba osztották; klinoptilolittal etetett (CPL) és kontroll, vagyis klinoptilolittal nem etetett (CON) csoportba, egyenként 15-15 egyeddel. A kezelt csoport naponta kapott 100 g klinoptilolitot a takarmányadagjába (Vibrosorb, Viridisfarm, Podpićan, Croatia). Az állatoktól a következő időközönként vettek vért: a vemhesség 240-260. napja között, az ellés napja (0. nap), a laktáció 12., 24., 40. és 60. napján. Ezen kívül a laktáció vérvételi napjain tejmintákat is gyűjtöttek. A BHB átlagos értéke magasabb volt a CON csoportban, mint a kezelt (CPL) csoportban (0.99±0.10 *vs*. 0.81±0.99 mmol/L). A legmagasabb BHB koncentrációk a 0., 12. és 26. napokon voltak mérhetők a CON csoportban (1.83±0.35, 1.55±0.35, and 1.62±0.35 mmol/L) és a CPL csoportban az elléskori, 0. napi mintákban (1.79±0.41 mmol/L). A tejzsír:tejfehérje aránya szignifikánsabban magasabb volt a 0., míg enyhén magasabb volt a 12., és a 26. napon a CPL csoporthoz képest. Mindazonáltal a FPR magasabb volt a CPL csoportban az ellés utáni 10. napon, mint a CON csoportban. Ehhez hasonló trendek a BHB-szint csökkenésénél figyelhetők meg az ellés után. Ennek megfelelően a BHB-szintek meghatározásával (vérből, vizeletből vagy tejből) idejekorán jelezhető lenne a szubklinikai ketózis. Eredményeink alapján arra a következtetésre jutottunk, hogy a klinoptilolit takarmány-kiegészítőként alkalmazva hatékony lehet a ketózis klinikai tüneteinek csökkentésében.

Kulcsszavak: klinoptilolit takarmány-kiegészítő, β-hidroxibutirát, tejzsír:tejfehérje arány, ellés körüli időszak, tejelő tehén

Introduction

Transition period is defined as the period from 2 to 4 weeks prior to calving and 2 to 4

weeks after calving (LeBlanc, 2010). Energy requirements increase during the last month of pregnancy and during early lactation causing mobilization of body fat, which increases non-esterified fatty acid (NEFA) and concentrations of β-hydroxybutyrate (BHB) in plasma (Duffield et. al., 2009). Consequently the milk protein content slight decreases, while fat levels tend to increase, hence increasing the fat : protein ratio of milk (FPR) (Toni et al., 2011). Čejna & Chládek (2005) established optimal FPR values of 1.05 to 1.18 while the FPR values higher than 1.3 or 1.5 suggest a severe negative energy balance (NEB) and the subclinical ketosis (SCK) if ketone bodies are increased (Duffield et al., 1997; Krogh et al., 2011). Prevalence of the SCK in Croatia is more than 14.75% (Đuričić et al., 2015). Toni et al. (2011) found the incidences of retained placenta membranes, left displaced abomasum, clinical metritis and endometritis increased with FPR ≥2.00 in cows. Therefore, the FPR could be used by bovine practitioners for evaluation of the energy status of cows (Schcolnik, 2016) and to predict the fertility problems in dairy herds (Podpečan et al., 2013).

In case of the increased sanitary problems in intensive farming of food animals have been overcome thus far by adding sub-therapeutic doses of antibiotic growth promoters (AGP) infeed to enhance production efficiency by increasing growth rate, improving feed utilization and reducing mortality from clinical disease is well documented in dairy cows (Kotsoulos et al., 2006). However, the consumers especially in developed countries are becoming increasingly concerned about drug residues in milk, meat and other animal products (Zarcula et al., 2010). As a result, the USA banned dietary AGP effective January 1, 2017. However, much earlier total ban of AGP in the EU countries has been affective since January 1, 2006. Consequently, the need to develop alternative dietary and non-dietary strategies to evaluate the potential antimicrobial agents for replacement of AGP (Karatzia, 2010). Among many substances of natural origins, promising results have been obtained with natural, synthetic or modified clay minerals, zeolites (Laurino & Palmieri, 2015) among which clinoptilolite (CPL) is the best known as zootechnical and biomedical feed ingredient widely reported in the scientific literature and used in farm animal nutrition as candidate to replace AGP in farm animals (Papatsiros et al., 2013). The CPL is thought to modulate metabolic, endocrine and antioxidative status in dairy cows, and thus improving their health, fertility and milk yield (Valpotić et al., 2017). Zeolites are microporous, crystalline, hydrated aluminosilicate minerals commonly used as commercial adsorbents and catalysts (Kralj & Pavelić, 2003). Among the 140 types of natural zeolites, clinoptilolite is the most widespread scientifically studied substance used in farm animal nutrition (Papatsiros et al., 2013) because it positively influences growth and reproductive performance.

The aim of the study was to establish the influence of dietary zeolite clinoptilolite on the milk fat-to-protein ratio and serum BHB levels during transition period in dairy cows.

Materials and methods

Thirty cows of the Holstein-Frisian breed were used in the study. The cows aged 3 to 5 years were pregnant for 8 months and kept at a farm near Đurđevac, Croatia. The cows were assigned into two groups, the control (CON; n=15) and the CPL-fed group (CPL; n=15). The CPL group received 100 g of vibroactivated and micronized clinoptilolite preparation (Vibrosorb, Viridisfarm, Podpićan, Croatia) in the ratio for dairy cows on a daily basis. Blood samples were collected from the cows in the period from day 240 to 260 of pregnancy, after parturition (day 0), and on days 12, 26, 40 and 60 of lactation. The serum concentration of BHB was determined by spectrophotometry using an Olympus AU 600 analyzer (Olympus Diagnostica GMBH, Hamburg, Germany). During lactation, milk samples were taken (same days when a blood samples were taken) for milk fat and milk protein analyses. The obtained data were statistically analysed using the ANOVA method with repeated measurements and differences were considered significant at $P<0.05$.

Results

The average value of serum BHB was higher in the CON than in the CPL group (0.99±0.10 *vs.* 0.81±0.99 mmol/L), as well as average value of FPR (1.46 *vs.* 1.29). The highest serum BHB levels were obtained on days 0, 12 and 26 of lactation in the CON group and in the CPL group after parturition (day 0). All data are presented in Table 1. Three cows from the CON group and only 1 cow from the CPL group suffered of SCK.

Table 1. Serum concentrations of BHB and FPR in the CON and in the CPL group in dairy cows on days 240 and 260 day of pregnancy, after parturition (day 0), and on days 12, 26, 40 and 60 of lactation

Group	CON	CPL	CON	CPL
days	Parameters			
	BHB (mmol/L)		FPR	
-30	0.45±0.34[a]	0.53±0.37[a]	1.27±0.16[a]	1.16±0.09[a]
-10	0.49±0.35[a]	1.30±0.35[b]	1.26±0.14[a]	1.40±0.19[a]
0	1.83±0.35[a]	1.79±0.41[a]	1.85±0.24[a]	1.43±0.24[b]
12	1.55±0.35[a]	0.85±0.35[b]	1.50±0.19[a]	1.28±0.10[a]

Group	CON	CPL	CON	CPL
days	Parameters			
	BHB (mmol/L)		FPR	
26	1.62±0.35[a]	0.89±0.36[b]	1.39±0.22[a]	1.17±0.13[a]
40	0.64±0.33[a]	0.61±0.37[a]	1.16±0.14[a]	1.19±0.14[a]
60	0.55±0.38[a]	0.62±0.38[a]	1.21±0.15[a]	1.20±0.15[a]

Values with different superscripts in the same column differ significantly (P<0.05)

Discussion

Optimal FPR value is below 1.2 while the FPR value higher than 1.33 may suggests SCK (Duffield et al., 1997; Čejna & Chládek, 2005; Krogh et al., 2011). Unfortunately, the determination of the cows suffered from the SCK only on the basis of elevated FPR is not a relevant diagnosis (Garcia et al., 2015). The interpretation of the results is not so evident. Just on the basis of the FPR >1.42 it could not be certainty claiming that çows suffered of the SCK, because there are false positive results in 35% of cases, and the rest of cows (65%) suffered from the other metabolic or reproductive disorders (Jenkins et al., 2016). In our study three cows from the CON group (20%) and one cow from CPL group (6.67%) suffered of SCK and had serum BHB level higher than 1.4 mmol/L. The increase in ketone bodies concentration in the serum of cows immediately after parturition has a negative impact on health of the cows, and is associated with reduced milk production (Kuterovac et al., 2005; Duffield et al., 2009; Toni et al., 2011; Folnožić et al., 2015). The peak prevalence of the SCK in cows occurred between 4 and 19 days after parturition (Garro et al., 2014), or in average on 5 days in milk (DIM) (McArt et al., 2012), when 28.9% of the cows had a SCK-positive test (McArt et al., 2012). Early detection of ketosis, including the SCK, may enable earlier intervention and minimize the loss in milk production (Duffield et al., 2009). Thus, it is very likely that by determining of the BHB levels (in blood, urine or milk), the FPR could be used for an early diagnosis of the SCK. Also, the results of this study indicated that CPL applied as dietary supplement could be effective in reducing clinical signs of ketosis.

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Verification of an on-farm ketone measurement device for the detection of betahydroxybutirat concentration in blood in dairy cattle

Egy hordozható keton meghatározó készülék ellenőrzése a vér béta-hidroxi-butirát koncentrációjának megállapítására tejelő szarvasmarhákban

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Abstract

The subclinical ketosis is an important metabolic disease in dairy cattle. The objective of this study was to test a new on-farm ketone measurement device for the detection of subclinical ketosis (WellionVet BELUA®).

Practicability and applicability under field conditions were evaluated using different ways of blood sampling. In the study 80 German Holstein cows (transit phase), were included. The blood samples were analysed for betahydroxybutyrat (BHB) concentrations in mmol/l either in the laboratory using a Cobas Analyzer (Roche Diagnostics Deutschland GmbH) or using the on farm device. Blood from the v. jugularis and v./a. coccygea was analysed in the laboratory, and blood from the v./a. coccygea med. and capillary blood was tested with the on-farm device.

We considered as gold standard the BHB values in blood received from the v. jugularis measured in the laboratory. BHB values in the v./a. coccygea measured in the laboratory were 0.07 higher ($P = 0.03$) than in the v. jugularis. A close correlation $r = 0.93$ ($P < 0.001$) between measurements was present. Blood which was analysed with the on farm device from the v. coccygea had 0.13 lower BHB values ($p<0.001$) than BHB values measured in the laboratory in the v. jugularis. Again a close correlation $r = 0.94$ ($p<0.001$) was present. In the capillary blood BHB values assessed using the on farm device were 0.12 higher than BHB values measured in the laboratory in the v. jugularis, similarly with a close correlation $r = 0.94$ ($p<0.001$).

In conclusion we have seen small differences in BHB values between measurement methods and ways of blood sampling with a close correlation between methods. Therefore with the correct use of the device, the system is a good diagnostic tool for subclinical ketosis in dairy cows on a farm.

Egy lehetséges módszer az oxidatív stressz tejelő állományokban történő in-vivo meghatározására: Előzetes eredmények

A possible method for in vivo determination of the oxidative stress in dairy herds: Preliminary results

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Összefoglalás

A szerzők előzetes adatokat közölnek a FRAS4, (H&D srl., Parma, Italy) az oxidatív stressz és az antioxidáns kapacitás in-vivo mérésére szolgáló, validált berendezés tejelő szarvasmarha állományokban való alkalmazhatósának vizsgálatából. A dROM (Reaktív Oxigén Metabolitok), a PAT (Plazma Antioxidáns Kapacitás) értékek és a kalkulált OSI (Oxidatív Stressz Index) utalnak a szervezet redox állapotára. Még nem közöltek szarvasmarhákra vonatkozó referencia-értékeket. A vizsgálatokat hazai nagyüzemi szarvasmarha-állományokban végezték itatásos borjak (n=7), <14 nappal ellés előtti (n=22), és <7napja ellett (n=8), 8-30 napja ellett (n=30) és nagytejű tehenek (n=16) körében. Klinikailag egészséges állatok tőgyvénájából levett, heparinnal alvadásban gátolt vérmintákat vizsgáltak. Az elletőben volt a legmagasabb a dROM értékek átlaga (uCarr 131, sd 44). A PAT a fogadóban volt a legalacsonyabb (uCor 2828, sd 199). A csúcslaktáció időszakában találták a legalacsonyabb dROM (uCarr 101, sd 21) és a legmagasabb PAT (uCor 2891, sd185) értékeket. A csoportok között nem volt szignifikáns különbség, amit magyarázhat az alacsony mintaszám.

Kulcsszavak: oxidatív stressz, in-vivo mérés, tejelő szarvasmarha

Abstract

Preliminary data of the investigation of applicability of FRAS4 (H & D srl., Parma, Italy) equipment for in vitro measurement of oxidative stress and antioxidant capacity in dairy herds. dROM (Reactive Oxygen Metabolites), PAT (Plasma Antioxidant

Capacity) and calculated OSI (Oxidative Stress Index) refer to the redox status. Bovine reference values have not yet been reported. The study were performed in large-scale dairy herds among <60 days old calves (n = 7); cows <14 days before calving (n = 22); DIM <7 days (n = 8), DIM 8 to 30 days (n = 30) and in peak lactation (n = 16). Blood samples were obtained into tubes with heparine from the udder vein of clinically healthy animals. The dROM was the highest in the DIM <7 days goup (uCarr 131, sd 44) and the PAT was the lowest in the DIM 8 to 30 days group (uCor 2828, sd 199). The lowest dROM (uCarr 101, sd 21) and the highest PAT (uCor 2891, sd185) were found during peak lactation. There was no significant difference between the groups that could be explained by the low number of samples.

Key words: oxidative stress, in-vivo measurement, dairy cow

Irodalmi áttekintés

Oxidatív stressz (OS) éri az állati szervezetet minden olyan esetben, amikor a reaktív oxigén metabolitok koncentrációja a testfolyadékokban eléri, illetve meghaladja a szervezet antioxidáns védelmi rendszereinek kapacitását. Az OS tejelő tehenekben gyakran kialakulhat az ellés körüli, illetve a hőstresszes időszakokban, de a mikotoxinokkal szennyezett takarmányok etetése is hajlamosító tényező. Előidézhet tőgygyulladást és szaporasági zavarokat is, amelyek negatívan hatnak az egészségi állapotra és mind közvetlenül, mind közvetett módon rontják a termelékenységet (Turk et al., 2011, 2012, 2017).

Az elléskörüli időszak egészségi problémáinak (metritis, mastitis, oltógyomor helyzetváltozás, ketózis) kifejlődésében az elsődleges tényező az állatot érő metabolikus stressz. A megnövekedett NEFA koncentráció OS-t indukálhat (Bernabucci et al., 2005; Shi et al., 2014; Xu et al., 2014). Az állatokat érő metabolikus stressz valójában a megváltozott tápanyag-metabolizmus (NEB), az immun-diszfunkció és az OS együttes hatása (Sordillo & Mavangira, 2014; Talukder et al., 2015). A metabolikus stresszt, illetve annak élettani következményeit jelző biomarkerek egy részét a gyakorlatban használják az OS kimutatására is.

A hőstressz egyre nagyobb kihívást jelentő környezeti tényező a hazai tejtermelő tehenészetek számára is. Térségünkben az állatok nyári időszakban átlagosan napi 6-10 órát töltenek hőstressznek kitéve. A Zimbelman & Collier (2011) által közölt metodikán alapuló kalkulált tejveszteség jelentős mértékű, mintegy 3kg tej/tehén/nap. (Zimbelman & Collier, 2011). Hőstressz alatt a tehenek esetében a vörösvértestekben magasabb antioxidáns enzimaktivitási értékeket mértek, ami arra utal, hogy nyáron, az elléskörüli időszakban lévő tehenek jelentős OS-t szenvednek el. (Bernabucci et al., 2002). A percenkénti légzésszám növekszik, a hőmérséklet-páratartalom index (THI) növekedésével összefüggésben (Zimbelman & Collier,

2011). Feltételezik, hogy az erythrociták oxidációjának kockázata nagyobb a hőstressz hatására megnövekedett légzésintenzitás következtében, mivel ilyenkor magasabbá válik az oxigén-nyomás, ami intenzívebb $O_2\bullet^-$ formációt okozhat (Lacetera et al., 2003). Hőstressz miatt csökken a szárazanyagfelvétel is (Zimbelman & Collier, 2011), ami a korábban már említett metabolikus változásokon keresztül idézhet elő OS-t. A hőstressz hatását érdemes figyelembe venni az OS diagnosztizálására választott biomarkerek normálérték-tartományának megállapításakor.

Az elmúlt évek adatai alapján úgy tűnik, hogy a mikotoxin szennyezettség egyre növekvő veszélyt jelent az állatok egészségi állapotára és ennek a megtermelt élelmiszereken keresztül közegészségügyi vonatkozásai is vannak. Mára tudott, hogy egyes mikotoxinok szervezetbe való bejutása a citokróm P-450 izoenzim aktivitásának fokozása révén OS-t indukál (Miller et al., 1993). A korábban említett normálérték-tartományok meghatározásakor erre a tényezőre is érdemes figyelemmel lenni.

A fenti néhány példa rámutat arra, hogy a tejtermelő teheneket érő élettani- és/vagy környezeti stresszhatások közvetve vagy közvetlenül OS kialakulását idézhetik elő, ami veszélyeztetheti az állatok egészségét, ronthatja a termelékenységet. A fokozott ROS termelődést, illetve a szervezet csökkent antioxidáns kapacitását időben észlelve hatékonyan be lehet avatkozni a folyamatba és ezzel visszaállítható az egyensúly. Ehhez szükséges ismerni a fajra, korcsoportra, élettani állapotra illetve termelési csoportokra jellemző normálérték tartományokat.

Több módszer is alkalmas az oxidatív stressz fennállásának egyedi kimutatására, de ezek általában nem teszik lehetővé az állomány szintű monitoring vizsgálatok elvégzését. A preventív szemléletű állatorvosi gyakorlatba illeszkedő, gyakorlati körülmények között jól használható, olcsó és megbízható állománydiagnosztikai módszerek kifejlesztésére jelentős igény mutatkozik, így ez a fejlesztések egyik lehetséges iránya (Leblanc, 2006; Sordillo & Mavangira, 2014).

Célkitűzések

A FRAS4 módszer tejelő szarvasmarha állományokban való alkalmazhatóságának vizsgálatát több lépésben tervezzük elvégezni: 1) az analitikai- és a biológiai variancia meghatározása 2) a dROM, PAT és OSI referenciaérték tartományok meghatározása. 3) A módszer szenzitivitásának és specificitásának kiszámítása 4) állomány szintű mérések a redox-státusz megállapítására és egyes antioxidáns takarmánykiegészítők hatásának vizsgálata üzemi körülmények között.

Anyag és Módszer

A vizsgálatokat hazai nagyüzemi szarvasmarha telepek állományainak bevonásával végezzük. A telepek előzetes megkeresést

követően, önként vállalják a vizsgálatokban való részvételt, ez biztosítja a véletlenszerűséget, azaz az esetleg érvényesülő, egy-egy gazdasághoz köthető torzító hatást.

Klinikailag egészséges állatok tőgyvénájából levett, heparinnal alvadásban gátolt vérmintákból történik a vizsgált biokémai markerek megállapítása a FRAS 4 (H&D srl, Parma, Italy) berendezéssel.

1. Szakasz: az analitikai- és biológiai variancia, illetve a referenciaérték-tartományok megállapításának lépései:

Elővizsgálatok az analitikai-, illetve a populációra vonatkozó biológiai variancia meghatározására. Referencia-értéktartomány meghatározása a megfelelő számítási módszerrel.

Előzetes eredmények és megbeszélésük

1. táblázat. 2017-ben gyűjtött 83 vérmintából mért eredmények

Termelési csoport/OS paraméter	Borjak (n=7)	Előkészítő <14 nappal ellés előtti (n=22)	Ellető DIM[4]<7 (n=8)	Fogadó DIM[4] 8-10 (n=30)	Nagytejű (n=16)
dROM [uCARR][1]	144 (sd 33)	113 (sd19)	131 (sd 44)	113 (sd 36)	101 (sd 21)
PAT[uCORR][2]	2932 (sd 167)	2838 (sd 210)	2885 (sd 229)	2828 (sd 199)	2891 (sd 185)
OSI[3]	49,2 (sd 12,0)	40,0 (sd 7,3)	46,0 (sd 17,7)	39,6 (sd 11,9)	34,8 (sd 6,8)

[1]1 U.Car=0,08mg/100ml H_2O_2, [2]1 U.Cor=1.4 µmol/l Vit C, [3]OSI= dROM/BAPx1000
[4]DIM=laktációs napok száma

A tehéncsoportok dROM és PAT és OSI értékek átlagait általános lineáris kevert modell illesztésével összehasonlítva nem kaptunk szignifikáns különbséget (P>0,05).

A dROM (Reaktív Oxigén Metabolitok), a PAT (Plazma Antioxidáns Kapacitás) értékek és a kalkulált OSI (Oxidatív Stressz Index) utalnak a szervezet redox-egyensúlyi állapotára (Abuelo et al., 2013). A módszert elektronspin rezonancia módszerrel validálták (Alberti et al., 2000). A vizsgálat eredményei az irodalomban közölt adatokhoz hasonlóak (Celi et al., 2011). A teheneknél az elletőben volt a legmagasabb a dROM, amit az ellés OS

indukáló hatása magyarázhat (Gaál et al., 2006). A PAT a fogadóban volt a legalacsonyabb. Feltételezhető, hogy ekkorra kimerül a szervezet antioxidáns kapacitása. A csúcslaktációra helyreáll a szervezet energiamérlege. Ekkor mértük a legalacsonyabb dROM és a legmagasabb PAT értékeket. Az eredmények alapján a csoportok között tendenciózus különbséget állapítottunk meg, de az nem volt szignifikáns, aminek egyik magyarázata lehet az eddig elvégzett vizsgálatok alacsony száma.

Az előzetes eredmények alapján indokoltnak tartjuk a vizsgálatok folytatását. A továbbiakban tervezzük a referenciaérték-tartományok meghatározását, csoportonként összesen mintegy 80-100 egészséges állat bevonásával, illetve kiszámoljuk a módszer szenzitivitást és specificitást a plazma izoprosztán (8-izo-PGF2α) koncentrációjának párhuzamos mérésével kapott eredmények felhasználásával. Az így kapott eredményekkel megállapítható lesz, hogy az állatok redoxstátusza megfelelő-e vagy fenn áll-e az OS.

A vizsgálat tervezett utolsó szakaszában állományszintű méréseket végzünk a berendezéssel az oxidatív státusz megállapítására, illetve üzemi körülmények között vizsgáljuk majd egyes antioxidáns takarmánykiegészítők hatását a hatékonyabb preventív védekezés érdekében, amivel javítható az állatok egészségi állapota és a termelt élelmiszerek biztonsága és minősége.

Köszönetnyilvánítás

A projekt az Európai Unió támogatásával, az Európai Szociális Alap (ESZA) társfinanszírozásával valósul meg (a támogatási szerződés száma: EFOP-3.6.1 -16-2016-00024, címe: Intelligens szakosodást szolgáló fejlesztések az Állatorvostudományi Egyetem és a Széchenyi István Egyetem Mezőgazdaság- és Élelmiszertudományi Karának együttműködésében).

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Haptoglobin concentration in various inflammatory diseases in German Holstein cows under field conditions

Haptoglobin koncentráció különböző gyulladásos betegségekben gyakorlati körülmények között németországi Holstein tehenekben

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Abstract

Haptoglobin is an acute phase protein responsive to infectious and inflammatory diseases in cattle. This field study was conducted to evaluate the relationship of haptoglobin as inflammatory indicator and clinical parameter. In 12 German Holstein cows, involved in a study assessing the stress response during lameness treatment, the haptoglobin concentrations were measured in blood and milk over 10 days. Daily sample collection and clinical examinations were performed and haptoglobin was analysed by ELISA. During the observation period we measured a quantifiable increase in the haptoglobin concentration associated with clinical mastitis, thrombophlebitis and vaccination. The haptoglobin response was related to the severity of inflammation and showed a quantifiable decrease under antibiotical treatment in cases of mastitis and thrombophlebitis. In conclusion, results of our study show that inflammatory and infectious processes in dairy cows are associated with an increase in haptoglobin. Severity and antibiotic treatment affect the haptoglobin concentration in the observed cases. Acute phase proteins as haptoglobin are useful biomarkers of inflammation, infection or trauma in dairy cows and can be measured in blood and milk.

A borjak ad libitum tejitatásának hatása – szakirodalmi összefoglaló

The effects of ad libitum milk feeding on calves – review of literature

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Összefoglalás

A jelenlegi gyakorlat szerint a borjak a születésük után egyedi ketrecben vannak és tejet vagy tejpótlót kapnak naponta két alkalommal (visszafogott takarmányozás). Emellett étvágy szerint, a telepek adott technológiája alapján starter tápot, szénát, esetleg szilázst is fogyaszthatnak. Újabban terjedőben van az ad libitum tej- vagy tejpótló itatás, mint technológia.

A szakirodalom szerint a születés utáni időszakban a takarmányfelvétel nagyobb intenzitása az érzékeny időszakban rövid távú, esetenként hosszabb távú hatással is lehet az állatra. A jelenséget „takarmányozási programozásnak”, „fejlődési programozásnak” vagy „metabolikus bevésődésnek” ('nutritional programming', 'develpomental programming', 'metabolic imprinting') nevezik (Guilloteau et al., 2009; Kaske et al, 2010).

Ezek alapján több kísérletet végeztek, melyekben a borjak ad libitum (igény szerinti) tejitatását vizsgálták a visszafogott itatáshoz képest.

A legtöbb szerző egyetért abban, hogy rövid távon szignifikáns hatása van az ad libitum tejitatásnak. Az elfogyasztott tej mennyisége jelentősen nagyobb az igény szerinti itatás során, ennek megfelelően a testtömeg gyarapodás is jobb a választásig (Appleby et al., 2001; Jasper & Weary, 2002; Kiezebrink et al., 2015; Maccari et al., 2015). Az igény szerinti itatás során a borjak kevesebb starter tápot fogyasztanak, de ez a különbség a választás után eltűnik (Appleby et al., 2001, Kiezebrink et al., 2015).

A szerzők vagy nem találtak különbséget a borjak egészségi állapotában, vagyis a hasmenés előfordulási gyakorisága hasonló volt a két csoportban (Appleby et al., 2001;

Jasper & Weary, 2002), vagy pedig az igény szerinti itatáskor alacsonyabb volt a hasmenés előfordulási gyakorisága (Maccari et al., 2015). Maccari et al. (2015) vizsgálatában az igény szerinti itatás hatására a borjakban magasabb volt a vérplazma glükóz, total protein és albumin koncentrációja, valamint magasabb volt az inzulin, IGF-1 és növekedési hormon (GH) koncentráció is. Appleby et al. (2001) a borjak viselkedését is vizsgálták. Az igény szerinti itatás során a borjak több időt töltöttek tejivással (egy szopási periódus hosszabb volt, összehasonlítva a vödörből itatással), és naponta többször látogatták meg (3-6 alkalommal) a tejforrást. A viselkedés jobban hasonlított a természetes szopási viselkedésre, mint az vödörből itatás során tapasztalható.

Többen vizsgálták az igény szerinti itatás hatást a laktációs termelésre. Az eredmények gyakran ellentmondásosak. Több szerző úgy találta, hogy az igény szerinti itatás nincs hatással az első laktációra (Morrison et al., 2009; Raeth-Knight et al., 2009; Terre et al., 2009; Kiezebrink et al., 2015, Korst et al., 2017). Mások (Bar-Peled et al., 1997; Shamay et al., 2005; Moallem et al., 2010) viszont jobb első laktációs teljesítményről számoltak be.

Látható, hogy a szakirodalom nem egységes az eredmények tekintetében, további kutatások szükségesek az ad libitum tejitatás hatásainak megismeréséhez.

Köszönetnyilvánítás

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Abstract

It is a general practice in Hungarian farms that calves are reared in individual cages after birth and fed milk or milk replacer two times a day (restricted feeding). Additionally starter feed, hay or silage may also be offered ad libitum. The technology of ad libitum feeding of milk (replacer) is applied on a smaller but increasing number of farms.

Increased dry matter intake in the first days of life can have a short or probably long-term effect on the organism, often called as „nutritional programming", „developmental programming" or „metabolic imprinting" (Guilloteau et al., 2009; Kaske et al., 2010).

Most of the studies that compared ad libitum milk feeding to restricted feeding agree on the short term effect of ad libitum feeding. The amount of milk consumed is considerably higher when offered ad libitum, so as average daily weight gain (Appleby et

al., 2001; Jasper & Weary, 2002; Kiezebrink et al., 2015; Maccari et al., 2015). The amount of starter feed consumed is lower in calves fed ad libitum milk (replacer), however, the difference diminishes after weaning (Appleby et al., 2001, Kiezebrink et al., 2015).

In terms of health status, the prevalence of diarrhoea was similar or lower in ad libitum fed calves as compared to restricted fed animals (Appleby et al., 2001; Jasper & Weary, 2002; Maccari et al., 2015). In the study of Maccari et al. (2015) plasma glucose, total protein and albumin concentrations, as well as insulin, IGF-1 and growth hormone concentrations were in correlation with the amount of milk intake. Appleby et al. (2001) have also observed feeding behaviour and found that ad libitum fed calves made a higher number of visits to the feeder (3-6 daily) and spent a longer time feeding that more resembles natural suckling behaviour than bucket feeding.

The effect of ad libitum feeding on lactational performance has also been studied. Several authors have found that ad libitum feeding had no effect on milk yield (Morrison et al., 2009; Raeth-Knight et al., 2009; Terre et al., 2009; Kiezebrink et al., 2015, Korst et al., 2017), however, others reported on improved first lactational performance (Bar-Peled et al., 1997; Shamay et al., 2005; Moallem et al., 2010). Contradicting results necessitate further studies on the effects of ad libitum feeding of calves.

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Miért pont az ELISA? Rövid betekintés a szerodiagnosztikai vizsgálatok kulisszái mögé

Why ELISA? In-depth look into serological diagnostics

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Összefoglalás

A fertőző állatbetegségekkel kapcsolatos laboratóriumi szerodiagnosztikai vizsgálatokat legjellemzőbben a mentesítési programok elengedhetetlen részeként, vagy vakcinázási programok során, a vakcinázás hatásának ellenőrzése céljából végezzük. A klasszikus szerológiai próbák (agglutináció, precipitáció, komplementkötési próba, vírusneutralizáció) mellett már régóta egyre nagyobb teret hódít a jelzéses módszerek közé tartozó ELISA. A klasszikus módszerek általában idő-, anyag- és munkaigényesek, nem egy esetben élő kórokozóval dolgoznak, eredményük elbírálása pedig nagymértékben szubjektív. Az ELISA módszerrel kivitelezett vizsgálatokra jellemző, hogy járványügyileg és a vizsgálószemélyzetre tekintve egyaránt biztonságosak, jól standardizálhatóak és reprodukálhatóak, tömeges mintaszám esetén is jól kivitelezhetők, eredményük pedig objektív műszeres mérésen alapul. A klasszikus módszerek kivitelezésében magasabb a hibakockázat, mint az ELISA módszerrel történő vizsgálatok esetében. Az igen nagy számban alkalmazott ELISA módszer térnyerése ellenére azonban a klasszikus módszerek sem szorulhatnak ki a szerodiagnosztikából, mert előfordul, hogy az immunválasz különböző stádiumaiban más-más módszer alkalmazható az áthangolódás kimutatására, emellett az egyes eljárások szenzitivitásukban és specificitásukban is különböznek egymástól. Bizonyos esetekben csak több vizsgálómódszer együttes alkalmazásával állítható fel pontos szerodiagnózis.

Abstract

Serological diagnostics of infectious diseases of animals are essential to monitor disease eradication or to determine efficacy of a vaccination program. In addition to the classical serodiagnostic methods (agglutination, precipitation, complement fixation, and virus neutralization), ELISA has become more widespread in recent years. Classical serodiagnostic methods are time-

consuming and require various reagents and man-hours. They often require working with live pathogens and the read-outs often have an element of subjectivity. ELISA, on the other hand, poses no threat of infection, can be easily standardized and reproduced, has a high through-put, and the results are measured by a machine. In addition, compared to classical serodiagnostic methods, ELISA is less error-prone. Despite the popularity of ELISA, the classical serodiagnostic methods have certain advantages over ELISA, since they can be more advantageous in probing certain phases of the immune response. In addition, classical serodiagnostic methods vary in sensitivity and specificity; therefore certain instances require a combination of different serodiagnostic methods for accurate diagnosis.

Bevezetés

A dolgozat célja a fertőző állatbetegségek állatorvosi in vitro szerológiai rutindiagnosztikája során leggyakrabban alkalmazott laboratóriumi vizsgálómódszerek irodalmi összefoglaláson alapuló, tematikus rendszerezése és áttekintése különös tekintettel azok mechanizmusának megértetésére és lényegi megvilágítására. A gyakorló állatorvosok leggyakrabban a különböző mentesítési programokkal kapcsolatosan, kevésbé gyakran egyéb, diagnosztikai indíttatású vizsgálatok kapcsán találkoznak a szerológiai laboratóriumi eredményekkel. A szakmai látókört és az általános szakmai műveltséget bővítheti, a gyakorló praxis és a laboratórium közötti hidat erősítheti, ha a laboratóriumi eredmény kézbevételekor a gyakorló állatorvos kolléga alapvető szinten tájékozott a laboratóriumi eredmény mögött meghúzódó folyamatokkal kapcsolatban. Dolgozatomban nem térek ki az egyes vizsgálatok kivitelezésének részletes labortechnikai ismertetésére. A dolgozat nem tárgyalja a rutindiagnosztikai vizsgálatok körébe nem tartozó (például kutatási célú) szerológiai vizsgálómódszereket és a celluláris próbákat.

Megbeszélés

Az immundiagnosztikai vizsgálatok közé a következő eljárások tartoznak: 1. klasszikus szerológiai próbák, 2. szerológiai céllal alkalmazott jelzéses módszerek, 3. immunhisztokémia, 4. celluláris próbák (az eddig felsoroltak mind in vitro), 5. allergiás dg. próbák (in vivo).

Az immundiagnosztikai vizsgálatok közül rutin szerológiai céllal tehát a klasszikus szerológiai próbákat és a jelzéses módszereket alkalmazzuk. A szerológiai vizsgálat klasszikus értelemben eredetileg vérszérumból történő ellenanyag (Ea) kimutatást jelentett. Értelmezése mára kissé kibővült, mert nem csak szérumból, hanem egyéb testfolyadékokból (pl. tejből, liquorból, has- vagy mellüregi izzadmányból, húsléből) is történhet a vizsgálat, illetve antigén (Ag) kimutatást is magába foglalhat (pl. BVD Ag vérből). Mindemellett szerológiai vizsgálatnak minősül az is, ha ismert típussavóval biológiai mintákból kórokozó Ag-jét mutatjuk ki illetve azonosítjuk (pl. salmonel-

la kimutatás, tipizálás tárgylemez-agglutinációval), hiszen a szerológiai vizsgálat fogalmának lényege, hogy az Ag-Ea kölcsönhatáson alapul. A szerológiai próbák során az I. fázisban megtörténik a molekuláris szintű Ag-Ea kötődés, ami még nem jár látható eredménnyel. A II. fázisban vagy az immunkomplexben (IC) spontán bekövetkező kolloidkémiai változások hatására (ülepedés, csapadékképződés), vagy az általunk a rendszerhez adott jelzőrendszer hatására látható (hemolízis vagy színreakció), esetleg mérhető változások következnek be. Szerológiai vizsgálatokat ma leggyakrabban és legnagyobb mennyiségben egy bizonyos kórokozóval, vagy annak antigénjével szemben termelődött Ea-ok vérszérumból történő kimutatására használunk, amiből következtetni lehet az egyed (vagy az állomány) esetleges fertőzöttségére, vagy fertőzéstől való mentességére (bizonyos esetekben a vakcinázás hatékonyságára). A szerológiai vizsgálatok a legtöbbször informatív választ adnak az általunk jól feltett kérdésre! Fontos, hogy jól (és jó sorrendben) kérdezzünk a rendszertől, ami azt jelenti, hogy minden szükséges feltételt mi biztosítunk, a kérdéses egyet (a vizsgálandót) kivéve, ami az állatból származik. Ha Ea jelenlétére vagyunk kíváncsiak, akkor mi adjuk az Ag-t, ha Ag jelenléte a kérdés, akkor mi adjuk az Ea-ot. Ha ismerjük a szerodiagnosztikai vizsgálat mechanizmusát, akkor az ismert paraméterek birtokában a reakció negatív és pozitív kontrollokhoz viszonyított kimeneteléből következtetni tudunk az egy ismeretlenre. Egy szerodiagnosztikai eredményre mindig pillanatfelvételként kell tekintenünk: a mintavétel pillanatának kimerevítése, ami a folyamatról, a változás irányáról nem mindig ad információt. Utóbbira indokolt esetben egy savópár vizsgálat már többet enged következtetni. Szem előtt tartandó emellett az is, hogy az immunválasz stádiumától is függően (IgM, IgG, inkomplett Ea-ok termelődésének esetleges dominanciája miatt) a különféle szerológiai próbák eredményei nem mindig fedik egymást, a próbák a fertőzés más-más szakaszában adhatnak pozitív eredményt.

A szerológiai próbák közé tartoznak 1. a klasszikus szerológiai próbák (agglutináció, precipitáció, komplementkötési próba, vírusneutralizáció) és 2. a szerológiai céllal alkalmazott jelzéses módszerek (elsősorban az ELISA).

Az agglutináció és a precipitáció mechanizmusának kulcsmozzanata egyaránt az, hogy az Ag-ek Ea-ok közvetítésével nagyobb rács- vagy hálózatszerű, terjedelmes méretükből adódóan kicsapódásra hajlamos egységekké kapcsolódnak össze. Ha az Ag-ek viszonylag nagy (sejtes) méretűek, akkor agglutinációról, ha sejteknél sokkal kisebbek (makromolekuláris méretűek), akkor precipitációról beszélünk.

Az agglutináció baktériumok, vagy hemagglutináció (HA) esetében vörösvértestek (vvt), mint Ag-ek (agglutinogének) Ea-ok (agglutininek) közvetítésével történő összecsapódását jelenti (I. fázis). Az agglutináció során összecsapódott Ag-Ea-komplexhálózat a II. fázisban válik láthatóvá, amikor

a komplexből képződött aggregátum baktérium Ag-ek esetén fehér (vagy megfestett) pelyhes vagy rögös üledék formájában a szuszpenzióból kiválik, miközben körülötte az eredeti Ag-szuszpenziótól opálos közeg feltisztul. Az agglutináció reakcióhelyszíne szerint vannak cső-agglutinációs és tárgylemez-agglutinációs (TA) próbák.

Cső-agglutinációt kémcsőben vagy 96 lyukú mikrotitráló lemez (plate) mélyedéseiben kivitelezünk. A B. abortus szérum agglutinációs teszt során a vérszérumban lévő Ea-ok agglutinálják a B. abortus Ag-eket. Az Abortus Bang Ring (ABR; Fleischhauer-féle gyűrűs próba) próbában pedig a friss tejhez adott, vörösre festett, elölt brucella baktérium szuszpenzió pozitív esetben a tejben lévő Ea-okkal agglutinálódva a tej felfölöződésével a zsírcseppekkel együtt a csőben lévő tej felszínére emelkedik és a feltisztuló tejoszlop tetején vörös gyűrűt alkot, míg negatív esetben a tej egyneműen rózsaszín marad a festett Ag-szuszpenziótól, a felszínen pedig fehér tejszíngyűrű keletkezik. A cső-agglutináció sajátos változata a leptospira mikroagglutinációs teszt: a plate lyukaiban élő leptospira levestenyészethez, mint ismert Ag-forráshoz vizsgálandó vérsavót adva pozitív esetben sötétlátóteres mikroszkóp alatt látható mikroszkopikus méretű, gömbszerű, fehér agglutinátum-felhők keletkeznek.

A TA a legegyszerűbben kivitelezhető próbák egyike. Tárgylemezen néhány csepp vérsavót és Ag-szuszpenziót elegyítünk. Pozitív esetben a festetlen vagy valamilyen festékkel megfestett élő, vagy elölt baktérium szuszpenzió fehér, illetve a festésnek megfelelő színű pelyhek, rögök formájában válik ki az addig egynemű elegyből, negatív esetben pedig egynemű szuszpenzió marad (pl.: mycoplasma TA baromfi vérsavó mycoplasma-Ea-ainak kimutatására; Rose Bengal Teszt B. abortus Ea-ok helyszíni kimutatására egyes országokban). A TA fordítottan feltett kérdés megválaszolására is alkalmazható, amikor általunk biztosan ismert típussavóval (Ea-okkal) hozzuk össze a kérdéses biológiai mintát (pl. baktérium tenyészetet); pozitív reakció esetén tudjuk, hogy a típussavónak megfelelő (pl. salmonella) baktériumokkal van dolgunk.

HA próbát legjellemzőbben csőben (ill. plate-en) kivitelezünk. Pozitív esetben a vvt-ektől rózsaszín-opálos folyadékközeg feltisztul és kivilágosodik, miközben a hálózattá összekapcsolódott vvt-agglutinátum a reakciócső alján összeálló üledéket képez, ami enyhe megdöntés hatására a rácsszerkezetnek köszönhetően nem folyik meg, alakját megtartja. A vvt-k a természetes süllyedés miatt idővel negatív esetben is a cső aljára ülepednek, ám ilyen esetben a közöttük lévő összeköttetés hiánya miatt az üledékgomb megdöntés hatására könnycseppszerűen megfolyik lefelé.

Tárgylemezen kivitelezett HA esetén a vvt-aggregátumok a lemezen lévő cseppen belül vörös pelyhek, rögök formájában válnak ki és mutatkoznak meg. Ilyen próba az, amikor kutyák vértranszfúziója előtt ún. három csepp próbát (összeférhetetlenségi próba) végzünk: egy csepp donor vér, egy csepp

recipiens vér és egy csepp fiziológiás sóoldat összekeverése után pár percen belül a próba pozitív, ha szabad szemmel összecsapódás látszik, negatív, ha az elegy egynemű marad. A humán ABO vércsoport-meghatározás szintén HA-n alapul.

Vannak direkt hemagglutináló képességgel rendelkező kórokozók (pl. baromfipestis vírus, sertés parvovírus, mycoplasmák), amelyek agglutinálják meghatározott állatfajok vvt-eit. A jelenséget kórokozó kimutatására nem jellemzően, de az ilyen kórokozók elleni Ea-ok kimutatására HA-gátlási (HAG) próbában baromfipestis szerodiagnosztikájában pl. ma is alkalmazzuk. Ha a vizsgálandó vérsavó mintát első inkubációban ismert vírusmennyiséget tartalmazó szuszpenzióval hozzuk össze, akkor a vizsgálandó mintában lévő specifikus Ea-ok lekötik a vírus vvt-receptorokhoz kapcsolódó epitopjait, ezért a második lépcsőben a rendszerhez adott vvt-ek agglutinálódása pozitív esetben elmarad. Ea-ok hiányában a vírus a vvt-ek hozzáadásakor zavartalanul agglutinálja azokat.

Agglutinációban a korai típusú IgM és a késői típusú IgG Ea-ok egyaránt részt vesznek, de pentamer formációjuk miatt az IgM Ea-ok sokkal hatékonyabb agglutinogének (az ötös, monomerenként is bivalens Ea-alakzatok sokkal több Ag-t tudnak magukhoz kötni), mint a monomer szerkezetű IgG Ea-ok. Az agglutináció diagnosztikai jelentősége emiatt elsősorban az, hogy fertőződést követően korán pozitívvá válik.

A Coombs-teszt (antiglobulin próba) inkomplett Ea-ok kimutatására szolgál. Az inkomplett Ea-ok kettő helyett csak egy Ag-kötő hellyel rendelkeznek (univalensek). Ezért, bár specifikusan kötődnek a sejtes Ag-ek felületéhez, több Ag-t nem képesek összekapcsolni egymással rácsszerű alakzattá, így láthatóvá váló reakciót sem okoznak, sőt kötődésük által megakadályozzák bivalens, komplett Ea-ok további megkötődését (blokkoló Ea-ok). A blokkoló hatású Ea-ok kötődését úgy tudjuk láthatóvá tenni, hogy a vizsgált állatfaj Ea-ai, mint Ag ellen termelt komplett, bivalens Ea-okat (antiglobulin savót) adunk a rendszerhez, ami az Ag-ekhez kötődött inkomplett Ea-okhoz kötődik és összekapcsolja, ezáltal agglutinálja azokat. Az eljárás a nagyon régi keletű fertőzések esetében használható, amikor már csak inkomplett Ea-ok termelődnek (pl. brucellózis).

Precipitáció során az oldott makromolekuláris méretű Ag-ek az első fázisban Ea-okkal oldott IC-t (precipitátumot) képeznek, ami a II. fázisban fizikai-kémiai folyamatok hatására oldhatatlan csapadékként kicsapódik és láthatóvá válik. Elsősorban IgG, részben IgM típusú Ea-ok vesznek részt a folyamatban. Közegét tekintve megkülönböztetünk folyadék fázisban kivitelezett cső-precipitációt és gél közegben kivitelezett agargél-precipitációs próbát. Utóbbira legismertebb példa a lovak fertőző kevésvérűségének szerológiai vizsgálatára alkalmazott radiális kettős immundiffúzió (Coggins-teszt). A módszer során agargélbe

fúrt lyukak közül az egyikbe vírusAg-t, a másikba vizsgálandó vérsavót mérünk. Pozitív esetben a lyukakból sugárirányban szétdiffundáló Ag-ek illetve Ea-ok a két lyuk között összetalálkozva (az ekvivalencia zónában) szabad szemmel látható precipitációs vonalat képeznek.

Cső-precipitációra példa a B. anthracis hőstabil Ag-jének kimutatására alkalmas Ascoli-féle termoprecipitációs próba. A kérdéses mintából főzéssel készített kivonatot ismerten pozitív B. anthracis sejtfalhaptén elleni hiperimmun savóra rétegezzük. Pozitív esetben a két oldat elegyedési határán az ekvivalencia zónában néhány perc alatt opaleszkáló precipitációs gyűrű jelenik meg.

Agglutináció és precipitáció esetén egyaránt szem előtt tartandó diagnosztikai csapda a zóna jelenség problémája. Nagymértékű Ag vagy Ea felesleg esetén IC-ek bár keletkeznek, de a rácsszerkezetű, nagyméretű komplexek kialakulása elmarad, a képződött kisebb méretű komplexek pedig nem csapódnak ki. A nagyon erősen pozitív savó tehát paradox módon fals negatív reakciót ad. A jelenség a Marrack-féle rácselmélettel magyarázható, miszerint a komplett Ea-nak két, az Ag-nek legalább négy valenciája, azaz kötődésre alkalmas aktív csoportja van. Egy Ea molekula két Ag-el, az Ag pedig még további két Ea-al tud kapcsolódni, ekvivalencia esetén így jön létre a rácsszerkezet. Az egyik partner nagymértékű feleslege esetén az optimális kötőhely-arány felborul, kiterjedt rácsszerkezet nem alakul ki. A jelenségre a minták hígítása (titrálás) a megoldás. (A jelenség in vivo is lezajlik, ezért van az, hogy a szérumbetegség jelentkezése leginkább a 7-14. napok körüli időre tehető, az Ea-ok elleni Ea (anti-immunglobulin) termelés ugyanis ebben a fázisban ad kiterjedt IC-képződéshez kedvező optimális Ag-Ea arányt. Előtte kevés, utána pedig túl sok a recipiensben a savó elleni Ea).

A komplementkötési próba (KK) leginkább oldott Ag és Ea reakcióján alapul, komplett IgG Ea-ok kimutatására alkalmas direkt változatának mechanizmusa a következő. A reakció I. fázisában plate lyukaiban együtt inkubáljuk az ismert Ag-tartalmú oldatot ismert mennyiségű (általunk hozzáadott) komplementtel és a kérdéses (vizsgálandó) vérsavó mintával. (A komplementrendszer a veleszületett, nem specifikus immunitás részét képezi, a vérsavóban fiziológiás körülmények között jelen van. Ezért a vizsgálandó szérum saját komplementjét előzetesen a minta 56 Celsius fokon történő 30 perc hőkezelésével inaktiváljuk). Ha a minta tartalmaz az Ag-re specifikus Ea-okat, akkor azok IC-t képeznek és aktiválják, azaz kapcsolódási felületükön megkötik a komplementet. A II. fázisban a rendszerhez adjuk a hemolitikus jelzőrendszert (juh vvt + hemolizin, azaz juh vvt elleni Ea komplexe). Pozitív reakció esetében szabadon maradt komplement hiányában (hiszen a minta Ea tartalmából képződött IC-k felhasználták) nem történik hemolízis: a vvt-ek az oldat aljára ülepednek. Negatív esetben a hemolitikus jelzőrendszer kapcsolódik a szabadon maradt komplementtel, komplement mediált

hemolízis történik. Az eljárást pl. brucellózis, takonykór, fertőző tenyészbénaság szerodiagnosztikájában alkalmazzuk.

Az indirekt KK próba inkomplett Ea-ok kimutatására alkalmas. Az inkomplett Ea-oknak az Ag-el képzett IC-e nem köti meg a komplementet, így direkt KK próbában fals negatív eredményt ad. A próba I. fázisában az Ag kötődik az inkomplett (blokkló) Ea-okkal. A II. fázisban az elegyhez az Ag ellen komplett Ea-okat, emellett komplementet tartalmazó oldatot adunk: az előzetesen a blokkoló Ea-ok által lefedett kötőhelyekhez a komplett Ea-ok már nem tudnak kötődni, ezért nem kötődik meg a komplement sem. A III. fázisban a rendszerhez adott hemolitikus jelzőrendszer szabad komplementtel kerül kapcsolatba, megköti azt és hemolizál (pozitív). Ha a vizsgált savóban nem voltak Ea-ok, akkor a II. fázisban hozzáadott komplett Ea-Ag kötődés megköti a komplementet is, a II. fázisban ilyenkor nem fog bekövetkezni a hemolízis (negatív). A reakció végkimenetele negatív-pozitív esetben tehát fordított, mint a direkt KK esetében.

A vírusneutralizációs (VN) teszteket vírusok ellen termelődött Ea-ok kimutatására használjuk. Lényege, hogy a vizsgálandó vérszérumot fertőzőképes, komplett vírusokkal inkubáljuk, majd olyan élő sejtekkel hozzuk össze, amelyekben a vírus replikálódni képes. Ez lehet szövettenyészet, fiasított tyúktojás és kísérleti állat. Ezekben a vírus hatásait figyeljük (cytopathogen hatás, megbetegítő hatás) és annak bekövetkeztéből vagy elmaradásából következtetünk a vírus hatását semlegesítő (vírusneutralizáló) Ea-ok jelenlétére. Pozitív esetben a vírus hatása elmarad. Az eljárás munka és időigényes, emellett a virulens kórokozó multiplikálódásával is jár.

A jelzéses módszereket a szerológiai vizsgálatokon kívül más céllal (például élelmiszer allergének, toxinok kimutatása) is alkalmazzuk. Szerodiagnosztikai célra ma a legnagyobb számban alkalmazott vizsgálómódszer az ELISA (Enzyme Linked Immunosorbent Assay). A módszert leginkább Ea-ok kimutatására alkalmazzuk, de alkalmas és használatos Ag-ek kimutatására is (utóbbiak közt nem csak fertőző ágensek, hanem pl. a reakcióban Ag-ként viselkedő hormonok, tumormarkerek, akut fázis fehérjék, vemhességi fehérje). Az ELISA elengedhetetlen feltétele a már említett plate, amit jelen esetben ELISA lemeznek nevezünk. A gyári reagens-készletekben jól standardizált, minőségellenőrzött és minőségtanúsított formában beszerezhető tesztekben megfelelő Ag-el érzékenyített plate-ek furataiban zajlanak a reakciók.

Ha a vizsgálandó savó specifikus Ea-ot tartalmaz, akkor az első lépésben kötődik a plate-hez rögzített Ag-el. Második lépésben indirekt ELISA módszer esetén az illető állatfaj Ea-ai ellen termelt monoklonális, enzimmel jelzett antiglobulin Ea-ok (konjugátum) kötődnek a vizsgált mintából kötődött Ea-ok nehéz láncához, gátló (vagy kompetitívnek is nevezett) ELISA módszer esetén pedig a minta Ea-ai által szabadon hagyott rögzített Ag-ekhez. Az egyes lépé-

sek között mosással távolítjuk el a megadott inkubációs idő alatt nem kötődött reakció-partnereket. A jelölő enzim (leginkább tormagyökér-peroxidáz vagy alkalikus-foszfatáz) megfelelő szubsztrátját harmadik lépcsőben a rendszerhez adva (előhívás) a kikötődött konjugátum jelölőenzim-aktivitásával arányos mennyiségű színes, oldható termék keletkezik. Az oldat szín-intenzitása arányos a megkötődött konjugált Ea mennyiségével, ami indirekt módszernél egyenesen, blokkoló módszernél fordítottan arányos a minta specifikus Ea-tartalmával. Az oldat színének megfelelő hullámhosszon (az abszorpciós maximumon) speciális fotométer (leolvasó berendezés) méri meg a létrejött színintenzitást (abszorbancia, vagy optikai denzitás, OD), ami alapján (a negatív és pozitív kontrollok színintenzitásához viszonyítva) értékelő szoftverben rögzített algoritmus szerint elvégzi az értékelés eredményét adó számítást.

Ag kimutatása esetén a plate az Ag-re specifikus ún. elkapó (capture) Ea-okkal előkészített, amelyek kihalásszák a specifikus Ag-jeiket a mintából. A próba az Ag megfelelő epitopjai ellen termelt konjugátum hozzáadásával az előbbiekben az indirekt eljárásnak megfelelő mechanizmus szerint folytatódik. Mivel az Ag-t végül két Ea molekula keretezi, a módszert szendvics ELISA-nak nevezik.

A radioizotópos (RIA: Radioimmune Assay; IRMA: Immunoradiometric Assay) és a fluoreszcens jelzéses (ELFA: Enzyme Linked Immunofluorescent Assay) módszerek ultraszenzitívek, különleges műszert illetve sugárvédelmet igényelnek, elsősorban a rutindiagnosztikán túlmutató célokra alkalmazottak.

Említést érdemel egy az eddigi kategóriákba nem sorolható, lateral flow működési elven alapuló egylépcsős immunkromatográfiás teszt. A pár perc alatt, speciális szaktudás nélkül kivitelezhető, ún. asztal melletti gyorstesztek alkalmasak pl. parvovírus, FeLV, FIV, E. coli pilus Ag-ek és egy sor más Ag kimutatására. (A hCG kimutatáson alapuló humán terhességi tesztek is ide tartoznak). A biológiai mintába mártott tesztcsíkon a folyadék a kapillaritás jelen-ségének köszönhetően fut végig. Az illető Ag-re specifikus Ea-okkal bevont színes mikroszemcsék úsznak a folyadékkal, miközben az Ag-ek az Ea-okon keresztül kötődnek azokhoz. A teszt (T) zónában rögzített, az Ag-re specifikus Ea-ok pozitív esetben az Ag-hez kötődve kihalásszák a folyadékból az Ag-t megkötött színes mikroszemcséket (Ag csapda), ezért ott színes csík jelenik meg. A teszt belő pozitív kontrollcsíkjában (C) a mikroszemcsék felületén rögzített Ea elleni antitestek vannak, amik a még szabadon maradt mikroszemcséket fogják ki. Pozitív esetben két, negatív esetben egy (csak a kontroll csík) jelenik meg a tesztablakban.

A klasszikus szerológiai próbák többségükben (a TA kivételével) munka- és időigényesek, több esetben is soklépcsős előkészítést igényelnek (komplement titrálás, a hemolitikus jezőrendszer ellenőrzése, Ag vagy Ea

titrálás, élő leptospira levestenyészet fenntartása [zoonosis!]), elbírálásuk pedig a vizsgáló némileg szubjektív megítélésén múlik. Az ELISA módszer biztonságos, jól reprodukálható, nagy tömegben is fennakadásmentesen, kedvező idő/mintamennyiség arányban kivitelezhető, értékelése mentes minden szubjektív tényezőtől, ezért kedvelt és igen elterjedt vizsgálómódszer.

A módszerek két nagyon fontos tulajdonsága a szenzitivitás és a specificitás. A szenzitivitás (érzékenység) annak a mérőszáma, hogy az illető eljárás mennyire érzékeny, a betegek hány százalékát mutatja ki. A szenzitív mérés eredményében optimális esetben benne van minden pozitív, de vannak benne fals pozitívok is. Magas szenzitivitású mérés esetén tulajdonképpen a fals pozitívok árán vesszük meg azt a biztonságot, hogy ne eresszünk el tévesen megnyugtató (fals negatív) eredménnyel kórokozót hordozó egyedet. Az nagy baj, ha negatívnak minősítünk valójában pozitív egyedet! Sokkal kisebb baj (pl. brucellózis esetében), ha fals pozitívok vannak (amelyeket további vizsgálatokkal lehet tisztázni), mint ha fals negatívat hagyunk az állományban, ami valójában kórokozót hordoz és üríthet is! A specifikus teszt kevés egyedre pozitív, nincs benne egészséges (fals pozitív), alkalmas a magas szenzitivitású tesztek által kifogott pozitív egyedek további tisztázására.

A ma világszerte használt, milliószám gyártott és nélkülözhetetlen, többször is említett laboratóriumi kellék, a mikrotitráló lemez eredetileg dr. Takátsy Gyula humán orvos, virológus 1950-es évekbeli találmányának, a Takátsy-féle mikrotitrátornak volt része, az abban használt laboratóriumi mikrolap (microplate) utódja (Braun & Lomniczi, 2014). A mikrotitrátornak a kapillaritás és a felületi feszültség elvén működő, kalibrálható hígító kacsait felváltották a modern pipetták, de a plate lényegében változatlan és ma is töretlen világsikerű. A szerencsétlen szabadalmaztatási körülmények miatt azonban sajnos ő maga nem élvezhette találmánya hozadékát.

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Danish guidelines for the use of antibiotics for the treatment of mastitis in dairy cattle

Dán iránymutatás a tőgygyulladások kezelésére használt antibiotikumokra vonatkozóan tejhasznú tehenészetekben

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Abstract

Antibiotic treatment of mastitis represents one of the major parts of antibiotic use in dairy cattle. Most of udder infections, where treatment with antibiotics promotes healing, are caused by bacteria which are sensitive to simple Penicillin. Therefore, the first choice for the treatment of mastitis, as a rule, is simple penicillin. In exceptional circumstances, herd veterinarian using his background knowledge of herd conditions can choose a broad-spectrum antibiotics for an initial treatment of the acute mastitis.

In acute cases, where general condition of the cow is affected (fever, swelling, tenderness, etc.) start treatment immediately with simple Penicillin as drug of your first choice. However, treatment with broad-spectrum antibiotics is acceptable when there is a strong suspicion of coli- form mastitis. As soon as laboratory results (including susceptibility testing) are performed, the choice of antibiotics need to be reconsidered. If there is a discrepancy between laboratory results and the selected antibiotic, drug must be selected in accordance with laboratory results or in some cases treatment can be discontinued. When there are serious professional reasons for an improved treatment outcome, simple penicillin can be replaced with broad-spectrum antibiotics. Furthermore, in case of mild and subclinical mastitis after careful evaluation of the prospect of bacteriological cure, assessment should be performed to proceed with a treatment or without treatment. The assessment is based on laboratory results on milk sample, a former SSC and previous treatments. It should be mentioned, that in many cases of mild mastitis, milk changes disappear without treatment during a few days period. Treatment should be carried out in strict accordance with the microbiological diagnostics.

Finally, it is important, that antibiotic treatment of acute mastitis with clear inflammatory changes will be supplemented with appropriate supportive therapy (NSAD, fluid & etc.) as this may have important implications for treatment success and welfare of the animal. Continuous use of bacteriological testing (cultivation or PCR) of milk samples from cows with mastitis is a valuable tool for creating an overview of heard patterns for udder infections and is thus a crucial for implementing the best possible prevention in the individual dairy herd. Examination of milk samples can thereby help reduce the consumption of antibiotics.

The guidelines discussed below were drawn up in collaboration between: *The Danish Veterinary Association, Knowledge Center for Agriculture, Cattle, KU Sund* (as a basis for cattle veterinarians education) and *Aarhus university*

Basic conditions that should always be considered in the treatment of mastitis with antibiotics.

Mastitis treatment with antibiotic represents by far the largest proportion of antibiotic consumption in dairy cattle. Totally 12920 kg active antimicrobial agents (kg active compound) were sold for parenteral and intramammary treatment of cattle in Denmark in 2016. (DANMAP 2016). The majority of antimicrobial agents 10127 kg, were used for the treatment of dairy cows and mainly prescribed for mastitis (9629 kg were used for parenterally and 498 kg for intramammary treatment).

Most cases of udder infections where treatment with antibiotics can promote healing, are caused bacteria which are sensitive to penicillin. Therefore a basic rule for treatment of udder infection, drug of the first choice, should be penicillin. Under certain circumstances, based on knowledge of conditions in the herd, the herd veterinarian may choose a broad spectrum of antibiotics for the initial treatment of mammary gland infection. All treatments of mastitis with antibiotics should be done according to the following general guidelines:

1. In acute cases where the cow is sick (fever, swelling, pain, etc.), treatment is started immediately. As a rule, simple Penicillin is drug of first choice.

2. In case of severe suspicion of acute coli-mastitis, treatment with broad spectrum antibiotics can be considered.

3. As soon as laboratory results (including resistance testing) are available, the choice of antibiotics is reconsidered. If there is no correspondence between the laboratory results and the selected antibiotic, the antibiotic selection is carried out according to the laboratory response or the treatment is stopped. When there is a sure veterinary-professional justification for achieving improved treatment results, Penicillin may be replaced with broad spectrum antibiotics.

4. In the case of mild and subclinical cases of mastitis, treatment as a general rule should only be initiated after careful assessment of the prospect of bacteriological healing with or without treatment. The assessment may include knowledge about laboratory results of milk sample, previous cell count or any previous treatments. In many mild mastitis cases, milk changes disappear within a few days without treatment. Otherwise, treatment must be carried out in strict accordance with microbial diagnostics.

5. It is important that an antibiotic treatment of mastitis with clear inflammatory changes, as appropriate, be supplemented with appropriate supportive treatment as this may be of major importance to the treatment outcome and *cow's welfare*. Continuous use of bacteriological testing (cultivation or PCR) of milk samples from cows with mastitis is a valuable tool for creating an overview of heard patterns for udder infections and is thus a crucial for implementing the best possible prevention in the individual dairy herd. Testing for bacteria by cultures or PCR from cows with mastitis also effects the selection of cases suitable for treatment. Examination of milk samples can thereby help reduce the consumption of antibiotics. Laboratory for mastitis diagnosis must be part of a recognized ongoing quality system (e.g., the Ring test offered by the DTU Veterinary Institute).

Definition

Subclinical mastitis:
Changes in milk can only be found by measuring inflammation markers like cell count, CMT reaction and conductivity, as well as in the cultivation of bacteria

Mild clinical mastitis:
Changed milk with color change or clots without swelling of mammary gland

Moderate clinical mastitis:
Changed milk and concomitant swelling of udder, possibly fever, but the cow has not affected general condition (normal appetite and milk volume).

Serious clinical mastitis:
Modified milk, swelling of the udder and affected general condition.

Subclinical mastitis

Treatment of subclinical mastitis should be avoided as far as possible during lactation, as treatment success and the results in many cases questionable. Treatment should most often be based on a bacteriological study (culture or PCR) of a milk sample and from an assessment of the individual cow. In cases where laboratory examination shows infection with ß-hemolytic streptococci, *Strep.uberis or Strep.dysgalactia*, treatment shold be considered in lactation. Treatment of *Staph.aureus* is only recommended in young cows without previous history of increased cell count. In connection with remediation programs, it is also possible to treat moderate

infections with ß-hemolytic streptococci. In addition, treatment of subclinical infections with all other bacteria should be avoided. Treatment can be done as dry cow therapy.

Mild clinical mastitis

Treatment of mild clinical mastitis should most often be avoided, as the cow is usually able to recover from the infection. Treatment should be based on a bacteriological diagnosis by cultivation or PCR examination of a milk sample and from an evaluation of the individual record history according to the above mentioned instructions (under subclinical mastitis).

Severe clinical mastitis

In case of severe clinical mastitis, early supportive treatment, in the form of fluid and painkillers treatment, is often essential for the course of the disease. It should be described in herd diagnoses how pain or supportive treatment must be manage on farm. For example, the veterinarian may give fluid and calcium administrated intravenously. In addition, fluid may be given orally, like 20-40 liters of lukewarm water (pumped with vom probe). The effect of broad spectrum antibiotics against penicillin-resistant bacteria is often very poor, and rarely determines recovery of the cow. Therefore simple Penicillin is always first and wise choice also in severe clinical mastitis therapy. However, with severe suspicion of severe coli mastitis, treatment as a initial point is treatment with broad spectrum antibiotics in one day. However, a milk sample should be taken for bacteriological examination (PCR or cultivation). In cases where laboratory result on the day after start of treatment shows no growth or few *coliform* bacteria, treatment with antibiotics should be stopped. In cases where cultivation response the day after initiation of treatment shows treatment-requiring infection with bacteria that are not sensitive to penicillin, in some cases it may make a difference if the treatment is changed to broad spectrum antibiotics.

Antibiotics Dry Cow Therapy (ADCT)

In the single dairy herd, the vet. and the farmer collectively select the cows that can be expected to be successful in ADCT. Typically, cows with elevated cell counts (e.g., over 100,000 cells per.ml or CMT value above 1) on one or more teats in the period before the drying off. ADCT requires bacteria to be found in a milk sample before treatment, according the current Danish regulations. Examination can be done either by PCR study or by cultivation.

If the examination of the sample shows a treatment-requiring occurrence of bacteria, a tube of dry cow antibiotics will be given as imm. application in all teats immediately after the last milking. The treatment can be supplemented with internal teat sealants (ITS). Several studies indicate that ITS have the effect on ADCT and treatment can be improved.

Occurrence of oxacillin/methicillin resistant Staphylococci (ORS) in Austrian ruminants and New World camelids

Oxacillin/meticillin rezisztens Staphylococcus-fajok (ORS) előfordulása osztrák kérődzőkben és újvilági tevefélékben

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Abstract

The aim of this study was to determine the prevalence and the antimicrobial resistance pattern of methicillin resistant *Staphylococci* from Austrian ruminants and New World camelids that were treated at the University of Veterinary Medicine, Vienna. Between April 2014 and January 2017, 708 nasal swabs originating from ruminants and New World camelids were examined. Antimicrobial susceptibility testing was conducted by agar disk diffusion. ORS was detected 202 animals and the majority were categorized as multi-drug resistant pathogens. Still the information of methicillin resistant non-*S. aureus Staphylococci* (MRnSA) from Austrian ruminants and New World camelids are scarce therefore further studies are necessary.

Key words: ruminants, *Staphylococci*, multi-drug resistance

Összefoglalás

Vizsgálatuk célja az volt, hogy a Bécsi Állatorvosi Egyetemen kezelt kérődzőkből és újvilági tevefélékből (New World camelids, NWC) izolált meticillin rezisztens *Staphylococcus*-fajok prevalenciáját és antimikrobiális szerekkel szemben mutatott rezisztenciaprofilját meghatározzuk. 2014. áprilisa és 2017. januárja között összesen 708, kérődzőkből és újvilági tevefélékből származó orrtampon-mintát vizsgáltunk.

Az antibiotikum érzékenységi vizsgálatot korong-diffúziós módszerrel végeztük. Oxacillin rezisztens *Staphylococcus*-t (ORS) 202 állat esetében találtunk, ezek többsége pedig multidrog-rezisztens kórokozóként volt azonosítható. Ezzel együtt az osztrák kérődzőkből és újvilági tevefélékből származó meticillin rezisztens nem-*S. aureus Staphylococcus*-okkal kapcsolatos adataink hiányosak, így e témakörben további vizsgálatok szükségesek.

Kulcsszavak: kérődzők, *Staphylococcus*-ok, multidrog-rezisztens

Introduction

Staphylococci are Gram-positive, facultative anaerobe and catalase-positive bacteria. Up till now, more than 50 validly described species belong to the genus *Staphylococcus* (http://www.bacterio.net/staphylococcus.html). *Staphylococci* are commensals of the skin and mucosa and are considered to be opportunistic pathogen in mammals. Well known is methicillin resistant *Staphylococcus* (*S.*) *aureus* (MRSA) causing severe infections in humans and animals, but also non-*S. aureus* S*taphylococci* (nSA) can cause problems especially when they are multi-drug resistant. There are only a few documented studies on the occurrence of multi-drug resistant bacteria in ruminants and New World camelids in Austria. Loncaric et al. (2013) reported the occurrence of MRSA in a goat, Schauer et al. (2018) described the isolation of MRSA from ruminants and New World camelids. Interestingly, in the latest study both mec allotypes, *mecA* and *mecC* which represent first description of *mecC* positive MRSA in a domestic animals in Austria.

The information regarding isolation of methicillin resistant non-*S. aureus Staphylococci* (MRnSA) from Austrian ruminants and New World camelids are practical non-existent. Therefore, the objective of the present study was to assess the prevalence of MRS in the population of Austrian ruminants and New World camelids presented to the University clinic for ruminants.

Materials and Methods

The study was discussed and approved by the institutional ethics and animal welfare committee in accordance with GSP guidelines and national legislation.

From spring 2014 until January 2017 a total of 708 nasal swabs were collected from ruminants including cattle (n=220), calves (n=143), goats (n=86) and sheep (n=131), as well as from New World camelids such as alpacas (n=98) and llamas (n=30), all presented as patients to the clinic for ruminants at the University of Veterinary medicine in Vienna.

All nasal swabs were incubated overnight and subsequently streaked onto selective agar for the detection of Oxacillin resistant *Staphylococci* on the following day.

Antimicrobial susceptibility testing was performed for all oxacillin resistant *Staphylococci* by agar disk diffusion according to guidelines of the CLSI (CLSI 2017). Oxacillin resistant S*taphylococci* were genotyped by PCR's and DNA-Microarray analyses (Monecke et. al. 2008).

Results and Discussion

Oxacillin resistant S*taphylococci* were detected in 202 out of 708 nasal swabs indicating a nasal carriage rate of 28.53%. Oxacillin resistant *Staphylococci* was isolated from all tested animals e.g. cows, calves, sheep, goats, llama and alpacas. The majority 90.59% of detected ORS were categorized as multidrug-resistant pathogens which mean they were resistant to at least 3 antimicrobial classes (Schwarz et al. 2010). Overall the results showed that more than 25% of all sampled ruminants and New World camelids are carrier of multi-drug resistant bacteria in the upper respiratory tract. More systematic studies are needed to unravel the role of ruminants and New World camelids as reservoir for multi drug resistant *Staphylococci* and their potential risk for zoo-anthropogenic transmission especially with the upcoming of more "pet" farm animals in Austria.

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Meticillin rezisztens *Staphylococcus aureus* törzsek hazai tejelő szarvasmarha állományokban az elmúlt 15 évből – kitekintés és újabb eredmények

Methicillin-resistant *Staphylococcus aureus* strains isolated from Hungarian dairy herds in the past 15 years – an outlook and further results

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Összefoglalás

A *Staphylococcus aureus* (SA) az emberben és a gazdasági haszonállatokban is sokféle fertőzés kialakítására képes. A legtöbb SA genotípus fejlődéstörténete egy vagy csupán néhány gazdafajjal együtt élve zajlott, evolúciós értelemben véve hosszú időn keresztül. Ez a kialakult genotípusok esetében gazdafaj-specifikusságot eredményez, jóllehet kivételek akadnak. Egyes genetikai variánsok képesek gazdafajt váltani, megtelepedve, illetve akár fertőzést is okozva az új gazdafajban, ahogy azt különféle meticillin rezisztens *Staphylococcus aureus* (MRSA) genotípus esetében korábban már bizonyították.

A *Staphylococcus aureus* okozta tőgygyulladás világszerte komoly problémát jelent a tejelő szarvasmarha ágazatban. Az első, 1972-ben publikált eset óta Európa több országából jelentették állati eredetű (livestock-associated, LA-) MRSA törzsek felbukkanását klinikai, illetve szubklinikai tőgygyulladásból izolálva. Jóllehet az

MRSA jelentősége a tőgygyulladások kóroktanában még nem tisztázott, de az eddigi eredmények alapján nem tér el a meticillinre érzékeny *Staphylococcus aureus* (methicillin-susceptible *S. aureus*, MSSA) törzsekétől. Ismert, hogy az MRSA képes betörni az élelmiszerláncba, és izolálható akár a kiskereskedelmi tejtermékekből is, következésképp – a genotípustól függően – komoly kockázatot jelenthet a humán egészségügyre nézve. A vonatkozó hazai adatok hiánya miatt a különböző állati eredetű MRSA genotípusok jelenlétére vonatkozóan csak találgathatunk.

Egy nemrégiben kezdődött monitoring program keretében részletesen megvizsgáltuk a Nemzeti Élelmiszerlánc-biztonsági Hivatal Állategészségügyi Diagnosztikai Igazgatóságának törzsgyűjteményéből származó meticillin rezisztens *S. aureus* izolátumokat, hogy képet kaphassunk a különféle genotípusok jelenlétéről a hazai tejelő gazdaságokban.

A 2003-2018 közötti időszakban 10 állomány összesen 27 egyedi, tehéntejből izolált MRSA törzse került a NÉBIH ÁDI törzsgyűjteményébe. Ezen törzsek reprezentatív tagjait vizsgáltuk fenotípusos (szelektív tenyésztés, antibiotikum érzékenység meghatározás), illetve számos genetikai módszerrel (*mecA*, illetve *mecC* PCR, *spa*-tipizálás, MLST, SCC_{mec}-tipizálás). Az eredményeket a törzsek adatait figyelembe véve értékeltük.

Ugyan hazai viszonylatban a szarvasmarha eredetű MRSA az elmúlt 15 évben is sporadikusnak bizonyult, de néhány egyedi eset ráirányítja a figyelmet az MRSA eredetű tőgygyulladás halmozódásának, illetve a tehén-ember keresztfertőződésének lehetőségére.

Abstract

Staphylococcus aureus (SA) causes an array of infections in humans and in economically important livestock animals. Most SA genotypes have co-evolved with either a single or only with few host species for a long time in evolutionary terms, and thus leading to host specialisation and restriction, though some exceptions may emerge. These genetic variants have the potential to switch host species and colonise or even infect the new host, respectively, as it was demonstrated in case of some distinct methicillin-resistant *Staphylococcus aureus* (MRSA) genotypes.

Staphylococcus aureus mastitis is a major economic burden on the global dairy industry. Since first documented in 1972, livestock-associated (LA) MRSA of clinical and subclinical mastitis origin has been reported from numerous country of Europe. Although the pathogenic potential of MRSA in cattle is still not well declared, it seems that it does not differ from that of methicillin-susceptible *Staphylococcus aureus* (MSSA). MRSA may also enter the food-chain and sporadically could be isolated from retail dairy products, consequently - depending on the

genotype - posing risk to human health as well. Due to lack of data the occurrence of LA-MRSA genotypes in Hungary could only be assumed.

As a part of a recently started monitoring programme, we have investigated the conserved bovine MRSA isolates of the Veterinary Diagnostic Directorate (VDD), National Food Chain Safety Office, Budapest to get an insight in the occurrence of MRSA genotypes circulating in Hungarian dairy farms in the past 15 years.

Between 2003 and 2018 overall 27 MRSA strains originating from 10 dairy farms were deposited in the culture collection of the VDD. Representative strains were selected and investigated with selective culturing, polymerase chain reaction for the presence of *mecA* and *mecC* (formerly $mecA_{LGA251}$), genotyping (*spa*-typing, MLST, SCC_{mec}-typing) and antibiotic susceptibility testing, respectively. Results were evaluated regarding supplementary data of isolates.

Although in the last 15 years MRSA bovine origin has seemed to be sporadic in Hungary, some cases raise the possibility of mastitis outbreaks caused by MRSA and even of cow to human transmission.

Financial evaluation of vaccination against Staphylococcus and E. coli mastitis on a commercial Hungarian dairy farm

Staphylococcusok és E. coli által okozott tőgygyulladás elleni vakcinás védekezés gazdasági elemzése egy hazai nagy létszámú Holstein-fríz tehenészetben

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Abstract

The aim of our study was to quantify the production effects and the economic benefit of mastitis vaccination that was started in 2010 against *Staphylococcus aureus*, coagulase-negative *Staphylococci* (CNS) and *Escherichia coli* in a 700-cow Holstein-Friesian dairy herd. The major milk production indices and udder health parameters were surveyed. In the economic analysis the reduction of losses due to clinical mastitis after the initiation of the vaccination programme was considered as benefit and the expenditures of vaccination were taken into account as cost. The average daily number of cows with clinical mastitis decreased significantly after 2010 from over 30 to 5-10 cows a day. The monthly number of mastitis treatments decreased from more than 800 in 2006 to less than 300 in 2014. The mastitis vaccination yielded € 50.7 average annual profit per cow between 2011 and 2014. The vaccination as an investment had 3.37 benefit-cost ratio and 236.8% ROI.

Key words: dairy cattle, mastitis, vaccination, economics, cost-benefit analysis

Összefoglalás

Kutatásunk célja az volt, hogy számszerűsítsük a *Staphylococcus aureus*, a koaguláznegatív *Staphylococcus*-ok és az *Escherichia coli* ellen 2010-ben indított vakcinázási program termelési és gazdasági hatásait egy 700 tehenes hazai holstein-fríz állományban.

A fő termelési és tőgyegészségügyi mutatók számszerűsítésén túl elvégeztük a vakcinázási program gazdasági elemzését is, amelyben a klinikai tőgygyulladásokból származó veszteség csökkenését tekintettük a program hasznának, a vakcinázás miatt kiadásokat pedig költségnek. A klinikai tőgygyulladásos esetek napi átlagos száma 2010 után 30-ról napi 5-10 tehénre csökkent. A tőgygyulladások miatt 2006-ban több, mint 800 kezelés történt, ami 2014-re 300 alá csökkent. A tőgygyulladás elleni vakcinázás 15,2 ezer Ft hasznot eredményezett tehenenként évente 2011 és 2014 között. A vakcinázás költség-haszon aránya 3,37, a befektetés megtérülése (ROI) 236,8% volt.

Introduction

Mastitis has always been responsible for one of the most significant production losses in the dairy industry (Ózsvári et al., 2003a; Ózsvári et al., 2003b; Pfützner & Ózsvári, 2017). According to the recent surveys, the coagulase-negative *Staphylococci* (CNS) and *Staphylococcus aureus* are responsible for most mastitis cases in Hungary. The aim of our study was to present the production effects and the economic evaluation of mastitis vaccination against *S. aureus*, CNS spp. and *Escherichia coli* in a large-scale Holstein-Friesian dairy herd.

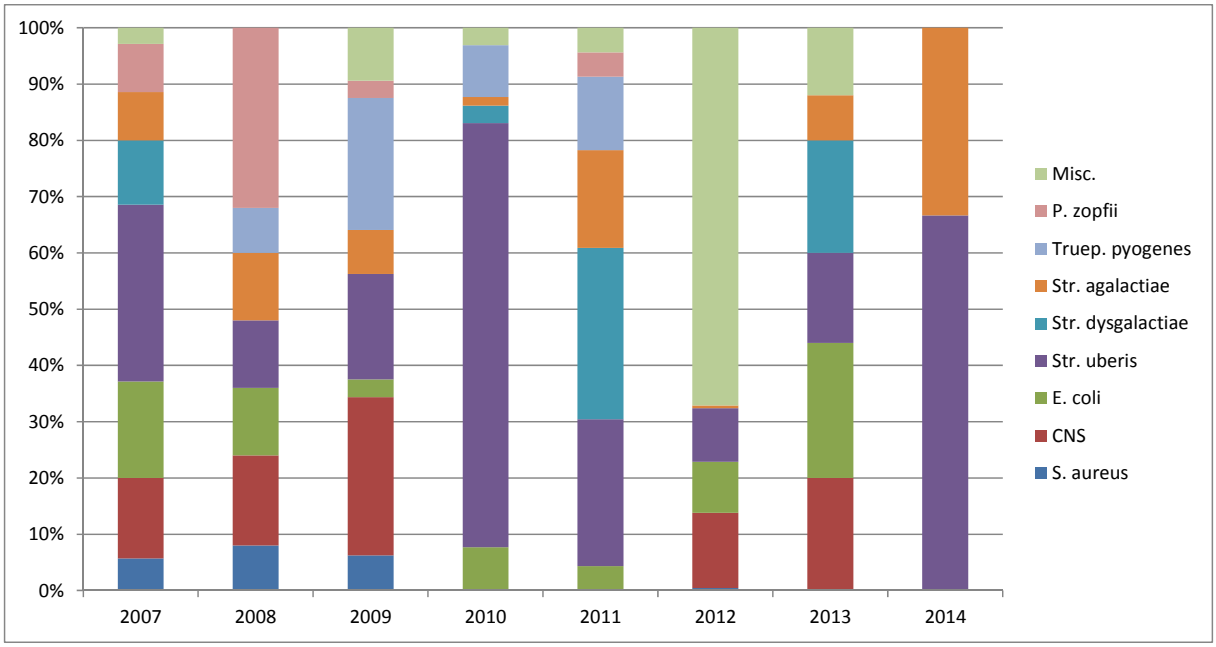
Materials and Methods

The study was carried out in a 700-cow Hungarian dairy herd, in which a large number of mastitis cases, mainly caused by *S. aureus* and CNS spp., resulted in high milk somatic cell counts (SCC), high culling rate in cows and significant treatment costs. The mastitis vaccination was started in 2010 on the farm. We surveyed the major milk production indices (average lactation and daily milk production, monthly SCC) and udder health parameters (occurrence and distribution of mastitis pathogens, number of clinical mastitis cases and udder treatments, mortality and culling rate in cows due to mastitis) and their economic impact on the farm between 2006 and 2014. Thus, we were able to compare the losses caused by clinical mastitis in the 4-year-long period before the vaccination (2006-2009) with those in the 4-year-long period after the entire cow population was vaccinated (2011-2014) in order to conduct the cost-benefit analysis of the mastitis vaccination. In the economic analysis the reduction of losses due to clinical mastitis was considered as benefit and the expenditures of vaccination were taken into account as cost. All the benefits and costs occurred over the 9 years of the survey period were adjusted for the general price level in 2014.

Results and Discussion

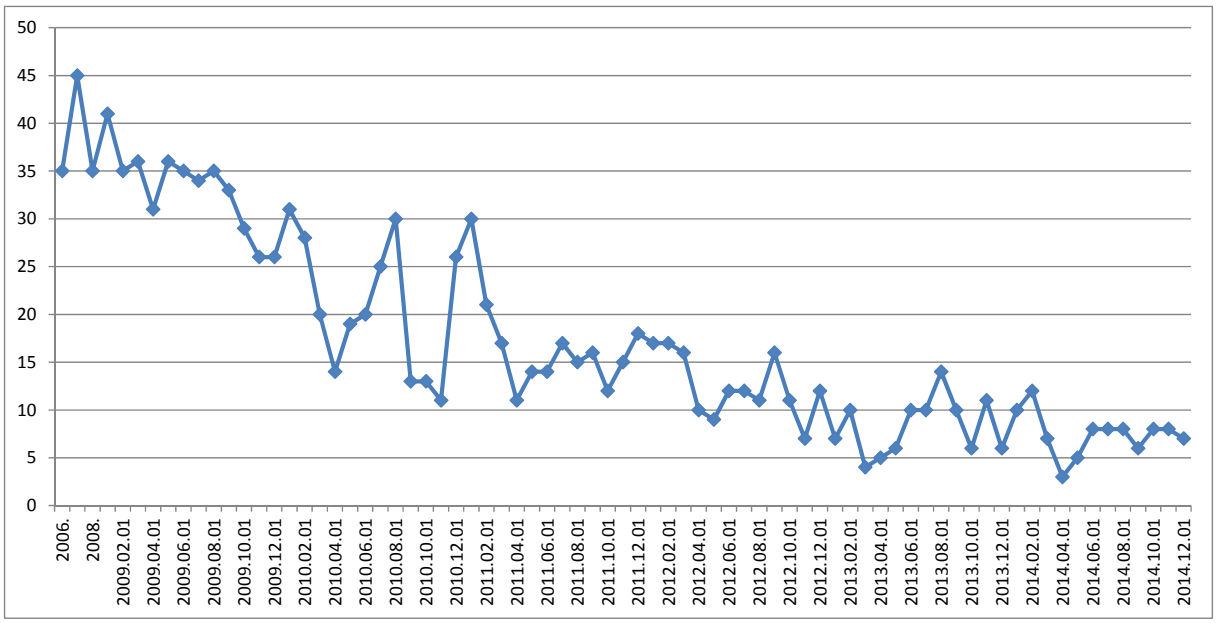
After vaccination the incidence and proportion of the CNS spp. in the mastitis cases has dropped moderately, nevertheless, the clinical mastitis cases caused by *E. coli* have disappeared and the occurrence of *S. aureus* remained low throughout the entire vaccination period (Figure 1).

Figure 1. The distribution of identified udder pathogens between 2007 and 2014 (n=450)



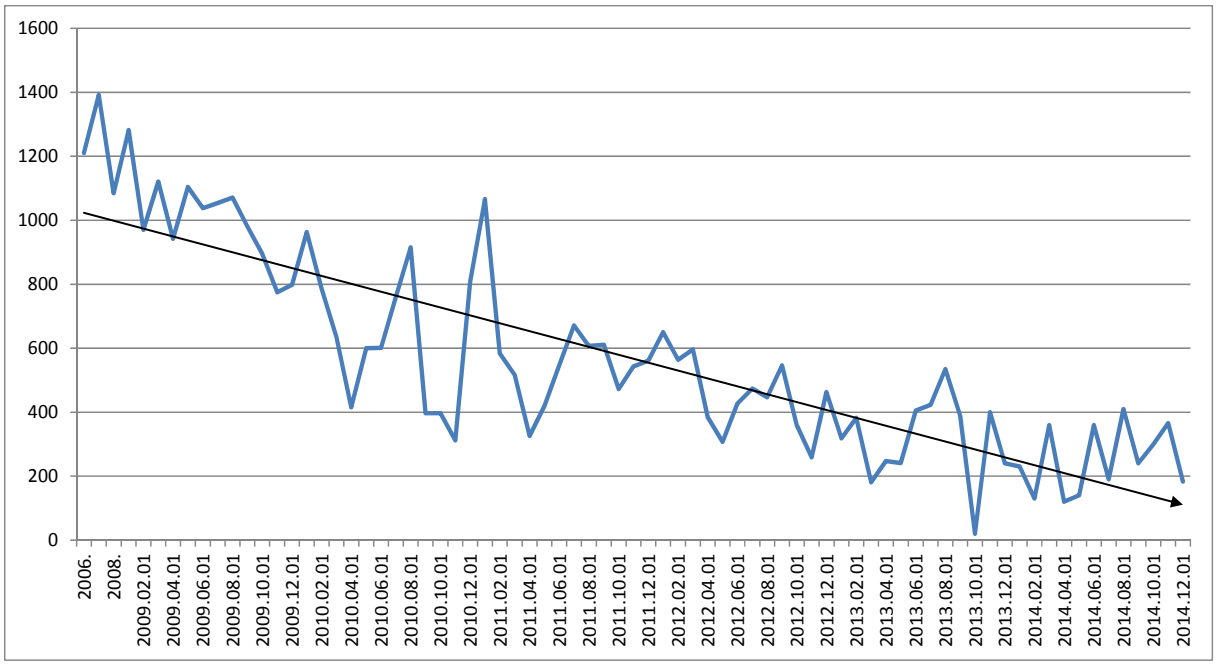
The average daily number of cows with clinical mastitis decreased significantly after 2010 from over 30 to between 5 and 10 cows a day (Figure 2).

Figure 2. The average daily number of separated cows with clinical mastitis between 2006 and 2014 (cows/day)



The monthly number of mastitis treatments decreased, as well: from more than 800 in 2006 to less than 300 in 2014 (Figure 3).

Figure 3. The monthly number of udder treatments between 2006 and 2014 (treatments/month)



Thus, the quantity of discarded milk also decreased greatly (from 6.1% to 1.2% of the total milk production), and so did the curative mastitis treatment cost (from € 18.3 to € 10 per cow). At 2014 price level the cost-benefit analysis of mastitis vaccination showed that the yearly loss caused by clinical mastitis in the 4-year-long period before the vaccination (2006-2009) was € 206.3 per cow which decreased to an annual amount of € 138.0 between 2011 and 2014 when all the cows were vaccinated. It included the significant decrease in the cost of discarded milk and mastitis treatments (Table 1).

Table 1. The losses owing to mastitis and the costs of vaccination at price level of 2014

Parameter	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**
Value of discarded milk (thousand EUR/farm)	90.8	103.0	106.0	90.0	56.0	59.9	43.8	32.5	31.3
Drug cost of mastitis treatments (thousand EUR/farm)	18.4	12.4	20.2	15.6	10.3	9.1	8.4	7.5	7.6

Parameter	2006	2007	2008	2009	2010	2011	2012	2013	2014
Cost of culling (thousand EUR)	52.1	14.7	19.9	31.9	29.9	45.9	26.5	38.7	66.5
Total losses due to mastitis (thousand EUR/farm)	**161.3**	**130.1**	**146.0**	**137.4**	**96.1**	**114.8**	**78.6**	**78.8**	**105.5**
Total losses due to mastitis (EUR/cow)	225.0	184.3	209.7	206.3	158.7	181.3	123.0	113.7	136.7
Cost of Startvac® vaccination (thousand EUR)					3.4	13.3	14.7	17.5	13.1
Cost of Startvac® vaccination (EUR/cow)					5.7	21.0	23.0	25.3	17.0

The mastitis vaccination yielded € 50.7 average annual profit per cow between 2011 and 2014. The vaccination as an investment had 3.37 benefit-cost ratio and 236.8% ROI (Table 2).

Table 2. The economic analysis of the Startvac® vaccination in the surveyed herd

Parameter	Before initiating vaccination (2006-2009)	After initiating vaccination (2011-2014)	Difference
Total losses due to clinical mastitis (thousand EUR/farm/year)	143.7	94.4	-49.3
Loss per average cow (EUR/year)	206.3	138.0	-68.3
Farm-level cost of Startvac® vaccination (thousand EUR/year)	0.0	14.6	14.6
Farm-level profit of Startvac® vaccination (thousand EUR/year)		34.7	34.7
Profit of Startvac® vaccination (EUR/cow/year)		50.7	50.7
Benefit-cost ratio			3.37
Return on investment (%)			236.8

The findings of the case study show that the mastitis vaccination significantly reduced the occurrence of clinical mastitis cases caused by *S. aureus*, CNS spp. and *E. coli* in the herd. According to the result of the economic analysis the mastitis vaccination was profitable on the surveyed farm, as the decrease in the loss due to mastitis coming from the improved udder health parameters exceeded the mastitis vaccination costs.

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Emergence of enterococcal mastitis and its clinical implications

Enterokokkális masztitisz megjelenése és annak klinikai következményei

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Abstracts

Totally 280 milk samples collected from mastitic cows in three provinces of western China were examined for the prevalence of enterococci. Sixty enterococci strains were verified with traditional biochemical tests and 16S rRNA sequences. Resistance to seven antibiotic agents were analyzed with microdilution method, and the frequency of seven virulence genes were screened with previously published primers (Table 1). Their biological risks were evaluated in Kunming mice (Table 2).

Among the isolates, 41 (68.3%) were *Enterococcus hirae*, 15 (25%) were *Enterococcus faecium*, *Enterococcus durans* and *Enterococcus mundtii* were 2, (3.3%) and 2, (3.3%), respectively. A total of 83.3% of the isolates were resistant to penicillin, whereas 15.0% were resistant to ampicillin, 15.0% to vancomycin, 6.7% to tetracycline and 25.0% to ciprofloxacin 0 (Table 3). The gene asa1 was detected in all enterococcal isolates, whereas 66.7% of strains harbored three or more virulence factors and 56.7% were asa1-ccf-gelE-positive (Table 4). In pathogenicity tests, isolates harboring more virulence factors genes did not show greater invasiveness than isolates harboring fewer virulence traits against Kunming mice. Compared with control group (PBS), the health status of all inoculated mice declined rapidly in the 24 h, and most mice gradually recovered in 72 h.

In conclusion, as an opportunistic pathogen, enterococci appeared as one of the main pathogens causing dairy cow mastitis. The number of virulence factors does not appear to predict the risk of enterococcal infection.

Isolates were commonly resistant to penicillin and sporadically to ampicillin and vancomycin (which never been used in dairy farm in western China), and majority of the isolates are sensitive to tetracycline. These results suggest that the use of gentamycin, streptomycin and ciprofloxacin against enterococci should be avoided in the sampled farms. Additionally, farm using of antibiotic drugs should be under proper guidance, and the veterinary organizations should invest more develop non-antibiotic methods to reduce antibiotic drug usage in clinical practice.

Key words: antibiotic resistance, pathogenic enterococci, prevalence, virulence

Table 1. Primers of the virulence gene screening and 16S rDNA sequencing

Virulence factors	Primer (5'-3')	Product (bp) length
CylA	ATGGATGGGACAGATGGAAA AGCTGCGCTTACTTCTGGAG	519
GelE	ACCCCGTATCATTGGTTT ACGCATTGCTTTTCCATC	419
Ccf	GGGAATTGAGTAGTGAAGAAG AGCCGCTAAAATCGGTAAAAT	543
Agg	AAGAAAAAGAAGTAGACCAAC AAACGGCAAGACAAGTAAATA	1553
Asa1	GCACGCTATTACGAACTATGA TAAGAAAGAACATCACCACGA	378
Esp	AGATTTCATCTTTGATTCTTGG AATTGATTCTTTAGCATCTGG	510
Ace	6GGAATGACCGAGAACGATGGC GCTTGATGTTGGCCTGCTTCCG	616
16S rDNA	AGAGTTTGATCCTGGCTCAG TACGGCTACCTTGTTACGACTT	around 1492

Table 2. Virulence profile of the isolates inoculated to Kunming mice

	Esp	*GelE*	*Ccf*	*CylA*	*Asa1*	*Ace*	*Agg*	Virulence count	Identification	
MS7	-	+	-	-	+	-	-	2	*E. hirae*	NGVI
MS10	-	-	-	-	+	-	-	1	*E. hirae*	NGVI
MS14	-	+	-	-	+	-	-	2	*E. hirae*	NGVI
MS17	-	-	-	-	+	-	-	1	*E. hirae*	NGVI
MS50	-	-	+	-	+	-	-	2	*E. faecium*	NGVI
MS38	-	+	+	-	+	+	-	4	*E. hirae*	GVI
MS39	-	+	+	-	+	+	-	4	*E. hirae*	GVI
MS42	-	+	+	+	+	-	-	4	*E. hirae*	GVI
MS46	+	+	-	-	+	+	-	4	*E. durans*	GVI
MS62	+	+	+	-	+	-	-	4	*E. faecium*	GVI

"+++", stands for the isolates that were positive to asa1, ccf and gelE positve.
GVI, genetically virolent isolates. NGVI, non-genetically virulent isolates.

Table 3. Resistance of the four enterococcal species (n = 60) to seven antibiotic agents

	E. hirae		*E. faecium*		*E. mundtii*		*E. durans*		total R
	n = 45		n = 15		n = 2		n = 2		
	I	R	I	R	I	R	I	R	
Pen	2	38	0	11	0	1	0	1	51 (85%)
Amp	6	6	1	2	0	0	0	1	9 (15%)
Van	2	7	0	2	0	0	0	0	9 (15%)
Tet	2	3	0	1	0	0	0	0	4 (6.7%)
Cip	10	11	2	3	0	0	0	1	15 (25%)
Gen[a]	0	25	0	4	0	0	0	1	30 (50%)
Str[b]	0	12	0	1	0	0	0	0	13 (21.7%)

Pen: penicillin, Amp: ampicillin, Van: vancomycin Tet: tetracycline, Cip: ciprofloxacin,
Gen: gentamycin, Str: streptomycin

Table 4. Frequencies of virulence factor of the 60 enterococcal isolates

Species	*Esp*	*GelE*	*Ccf*	*CylA*	*Asa1*	*Ace*	*agg*
E. hirae	5	31	28	5	41	9	0
E. faecium	2	11	9	0	15	1	0
E. mundtii	0	2	2	0	2	0	0
E. durans	1	1	0	0	2	1	0
Total	8	45	39	5	60	11	0
Positive rate	13.3%	75.0%	65.0%	8.3%	100.0%	18.3%	0

A mintavétel szerepe egyes tőgypathogén kórokozók kimutathatóságában

The effect of sampling procedures on the identifications of some mastitis pathogens

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Összefoglalás

Kísérleteinkben két jelentős tőgypathogén kórokozó, a *Staphylococcus aureus* és a *Prototheca zopfii* alga kimutathatóságát vizsgálatuk különböző mintavételi módszerek alkalmazásával. Ennek során azt találtuk, hogy gyakorlati körülmények között kielégítő az elegytej minták vizsgálata mindkét kórokozó esetén.

Abstract

In our experiments the effectiveness of before and after milking composite and individual quarter samples were examined. In every case the examination of individual quarter samples was more effective, but in the daily routine the composite samples are the advisable ones. In case of *S. aureus*, the individual samples weren't much more effective while the cost of laboratory examinations are four times more than the composite samples. In case of *Prototheca* algae the composite samples showed the presence of the algae in 80% of the cases, while the quarter samples were positive in approx. 90% of the cases.

Key words: individual quarter sample, composite sample, *Staphylococcus aureus*, *Prototheca zopfii*

Bevezetés

A tőgygyulladás a tejelő szarvasmarha állományok egyik legjelentősebb megbetegedése. A konkrét kártétel mértékében jelentős szereppel bír a betegség hátterében álló kórokozó. Különösen nagy kártétellel bírnak az állatról-állatról terjedő kórokozók, melyek a higiéniai hiányosságok esetén sok állatot betegíthetnek meg. Tovább fokozza a gazdasági veszteséget, ha a gyógykezelés korlátozott hatásfoka, és az állomány védelme érdekében a megfertőzött állatok jelentős részét selejtezni kell. Ezért fontos tudni, hogy a rutin tenyésztéses eljárások használatával milyen esélyeink vannak a kórokozó kimutatására a fertőzött tehenekből vett tejmintákból.

Anyag és módszer

Staphylococcus aureus

18 ismerten fertőzött és elkülönített tehenet vontunk be a vizsgálatba, mely 5 héten át tartott. Ennek keretében minden héten mintát vettünk a kísérleti állatokból. Minden állatból elegytej mintákat vettünk a fejés előtt, a tőgyelőkészítés és az első tejsugarak kifejése után. A mintavételt megismételtük a fejés végeztével is. Az elegytej minták vétele során egy állat minden fejt tőgynegyedéből tejet vettünk egy mintavételi csőbe. Ezen túl a fejés végén egyenként, külön mintavételi csőbe mintát vettünk minden egyes tőgynegyedből is. A tejminták vizsgálata eszkulinos Columbia agaron történt. Nyílt láng felett sterilizált fém oltókacsok segítségével 10 µl tejet szélesztettünk szét az agar felületén a tejminta homogenizálása után. Ezen túl vizsgáltuk, hogy ugyanazon minta háromszori vizsgálata, az elegytej minták laboratóriumi előállítása (minden tőgynegyedből garantáltan egyenlő mennyiségű tejek összekeverése), illetve az fejés előtti és fejés utáni elegytej minták 1:1 arányú keverése milyen hatással van a vizsgálatok eredményére. A feldolgozott táptalajokat 37°C-on 48h át tartó inkubációt követően értékeltük.

Prototheca zopfii

24 ismerten fertőzött állatot vontunk be a kísérletbe. 4 héten át hetente 1x majd pedig az 5. héten 5 egymást követő napon vettünk tejmintákat. Mind a fejés előtt, mind pedig a fejés után elegytej és külön mintavételi csövekben, egyenként tőgynegyedtej mintákat is vettünk, a *S. aureus* mintavételénél említett módon. A tejminták vizsgálata Sabouraud agaron történt. Nyílt láng felett sterilizált fém oltókacsok segítségével 10 µl tejet szélesztettünk szét az agar felületén a tejminta homogenizálása után. A feldolgozott táptalajokat 37°C-on 48h át tartó inkubációt követően értékeltük.

Eredmények

Staphylococcus aureus

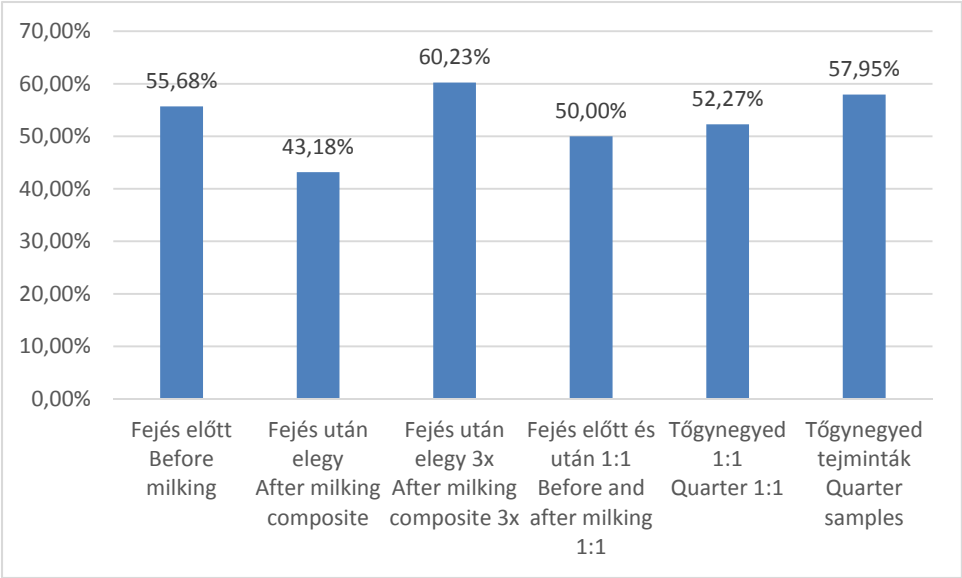
A *Staphylococcus aureus* kimutathatóságára vonatkozó vizsgálatok eredménye az 1. ábrán látható. Vizsgálataink azt mutatták, hogy a közhiedelemmel ellentétben a fejés után vett tejminták nem voltak hatékonyabbak a *S. aureus* fertőzött állatok azonosításában. Emellett, különösen a kórokozót csak kis számban ürítő állatok esetében a minták többszöri vizsgálatával is javítani lehetett a hatékonyságon, mert többször vizsgálva egyazon mintát nagyobb eséllyel került kórokozó a táptalajon szétszélesztett 10 µl tejbe. Egyes állatok csak a fejés előtti, más állatok csak a fejés utáni elegytej minták vizsgálata során adtak pozitív mintát. Ezen tejminták elegyítése során nem összeadódtak a pozitív eredmények, hanem a két módszer hatékonysága közötti eredményt kaptunk. Ugyanígy nem volt jelentősen hatékonyabb a tőgynegyed tejminták elegyítése sem. Leghatékonyabb módszer, nem meglepő módon, a tőgynegyedek egyenkénti vizsgálata volt. Viszont a hatékonyság béli különbség nem volt olyan jelentős, hogy nagy számú állat vizsgálata esetén indokolhatóvá tette volna a jelentős, négyszeres laboratóriumi költségeket. Ezért nagy számú állatok vizsgálata során, állományszűrések alkalmával, a gazdasági tényezőket is figyelembe véve elegendőnek tartjuk a fejés előtti elegytej minták vizsgálatát. Egyes nagy értékű állatok, vagy a legnagyobb hatékonyságra törekvés esetén azonban mindenképpen az egyes tőgynegyedek egyenkénti vizsgálatával érhető el a legjobb eredmény.

Prototheca zopfii

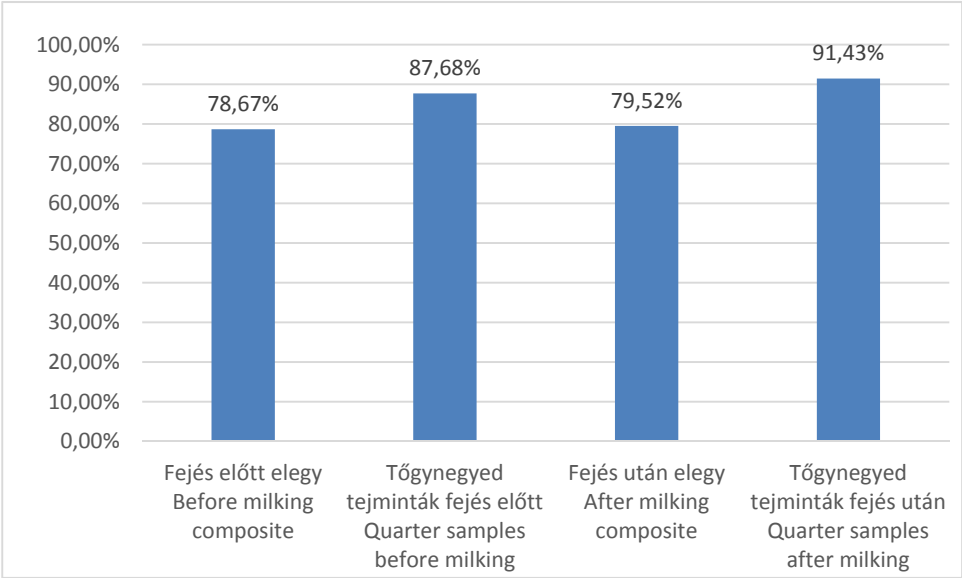
A *Prototheca zopfii* kimutathatóságára vonatkozó vizsgálatok eredménye az 2. ábrán látható. A vizsgálatok szerint nem volt jelentős eltérés a fejés előtt és a fejés után vett tejminták eredményei között. Mindkét esetben, nem meglepő módon, a tőgynegyed tejminták egyenkénti vizsgálata bizonyult hatékonyabbnak, azonban átlagban az elegytej minták is kb. 80%-ban alkalmasak voltak a fertőzött állatok azonosítására. Kicsit árnyalja a képet, ha figyelembe vesszük, hogy hány fertőzött tőgynegyeddel rendelkeztek a vizsgált állatok, de még ekkor is hatékonynak tekinthetjük az elegytej mintákat. A fejés előtt is és fejés utáni mintákat nézve 75,44%/73,77%-ban pozitívnak bizonyultak az elegytej minták 1 fertőzött

tőgynegyed, és 84,44%/81,25%-ban 2 fertőzött tőgynegyed esetén. 3 és 4 fertőzött negyed esetében 99%/100% és 100%/100%-ban jelezték az elegytej minták az állatok fertőzöttségét.

1. ábra. S. aureus kimutathatóságára vonatkozó vizsgálatok eredménye
The results of the examination of S. aureus positive animals



2. ábra. P. zopfii kimutathatóságára vonatkozó vizsgálatok eredménye
The results of the examination of P. zopfii positive animals



A tőgybimbót érő káros fizikai hatások vizsgálata nagyüzemi tehenészetekben (Irodalmi áttekintés)

The examination of teat-end condition on large scale dairy farms (Literature review)

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Összefoglalás

A tőgygyulladás az egyik legjelentősebb gazdasági kátételt okozó betegség a tejtermelő szarvasmarhatenyésztésben, melynek kialakulásáért többnyire bakteriális fertőzések felelnek. A veszteség forrása, a gyulladás következtében fellépő termelés csökkenés, a gyógykezelés költsége, valamint a kieső és a fogyasztásra nem értékesíthető tejmennyiség árbevétel hiánya. A tőgybimbó sérülése, károsodása következtében a bimbócsatorna (a fertőzés elleni harc első vonala) védelme gyengülhet. Ezért a tőgygyulladással szembeni ellenállóképesség egyik jelentős eleme az egészséges, ép tőgybimbó. A tőgybimbó állapotának felmérése segít a felmérni azokat a romboló hatásokat, melyeket a nem megfelelő környezeti tényezők, a hibás fejéstechnológia, vagy fejőberendezés válthat ki. Egy egyszerű, könnyen elsajátítható osztályozási metódus áll a tenyésztők rendelkezésére, azonban az a tapasztalat, hogy a telepek nem fordítanak kellő figyelmet a bimbók egészségi állapotára. A tanulmány célja bemutatni az említett problémákat és bemutatni az osztályozás menetét.

Kulcsszavak: tőgygyulladás, hiperkeratózis, tőgybimbó pontozás, fejéstechnológia

Abstract

Mastitis is one of the most significant economic damages in cattle breeding due to reduced milk production, treatment costs, and discarded milk. The major causes of mastitis are the infections with pathogenic bacteria, which entered the udder trough the teat end canal. The teat end damage might result in the colonisation of pathogenic organisms thus the defence system of the teat canal will weaken.

Therefore, one important resistance factor against mastitis is the good condition of the teat end. The degree of teat-end hyperkeratosis (roughness, cornification or callosity) can be a major factor in udder health status. Classification of teat condition is a good method to assess the effects of environment, milking management, or milking equipment on teat tissue and the risk of mastitis. A simplified scoring method is commonly used for the routine evaluation of teat conditions such as teat hyperkeratosis. However, the use of the method is not widespread on the dairy cattle farms in the practice. The aim of the study is to determine the causes of these problems and to provide advice to farmers on how to use this method.

Key words: mastitis, hyperkeratosis, teat conditions, milking performance, milking management

Bevezetés

A tőgygyulladás az egyik legjelentősebb gazdasági kátételt okozó betegség a tejtermelő szarvasmarhatenyésztésben, melynek kialakulásáért többnyire bakteriális fertőzések felelnek. A veszteség egyik forrása a gyulladás következtében fellépő termelés csökkenés, a gyógykezelés költsége, valamint a kieső és a fogyasztásra nem értékesíthető tejmennyiség árbevétel hiánya. A járványügyi és más állategészségügyi fontosság mellett ezek azok a gazdasági megfontolások, melyek kihangsúlyozzák a megelőzés szerepét a tőgyegészségügyi helyzet szinten tartásában.

A tőgy egészségét befolyásolja a fejési technológia (Neijenhuis et al., 2004), a fejések gyakorisága mellett a fejőberendezés típusa, szakszerű üzemelése, üzemeltetése. A fejőgép közvetlenül és közvetve is komoly szerepet játszik a szarvasmarha-állományok tőgyegészségügyi helyzetében. Hatással van a fejés minőségére a fejési vákuum (Besier & Bruckmaier, 2016), a pulzáció (Rasmussen & Madsen, 2000), a kollektor súlya (Hillerton et al., 2000) és a kehelygumik minősége, mérete (Gleeson et al., 2004) egyaránt.

A gyulladás megelőzése érdekében nagy szerepe van az egészséges, sérülésmentes, jól ápolt tőgybimbónak. Mivel a tőgygyulladás elleni védelem első vonala a tőgybimbó záróizma, annak károsodásával megnő a betegség kialakulásának kockázata. Jelen tanulmány célja bemutatni a tőgybimbót a fejés alatt érő hatásokat, valamint a bimbók fizikai elváltozásának értékelési rendszereit. A bemutatott értékelési rendszert a Wisconsin egyetem (https://milkquality.wisc.edu/teat-end-health/) által összeállított ajánlások alapján szemléltetjük.

A fejést követően látható, nem maradandó elváltozások

A fejőberendezés hibája, vagy a helytelen fejési technológia okozhatja a tőgybimbó színének, bőrének, alakjának elváltozásait. A tőgybimbók rendszeres vizsgálatával kiküszöbölhető, hogy az elváltozások ne legyenek maradandók. Számos osztályozási rendszer létezik a tőgybimbó bőrének és a

bimbóvégek állapotának értékelésére. A leggyakoribb módszerek pontozásos értékelésén alapszanak. Az értékelés alapfeltétele a rendszeres ellenőrzés és az adatok folyamatos rögzítése, eredményességüket meghatározza a gyakorlati tapasztalat, az ismételhetőség és a precizitás. A vizsgálatokat úgy kell beiktatni a telepi munkába, hogy azzal a napi fejési rutint ne zavarjuk.

A tőgybimbó színének változása

Esetenként a fejőkészülék levétele után közvetlenül látható pirosság a bimbó végén, vagy az egész bimbón. Máskor a készülék levételét követő 30-60 másodpercben következik be a pirosság. Szélsőséges esetben a bimbók színe szinte kékessé válik. A színelváltozás oka lehet a túlfejés, a nem megfelelő méretű és formájú kehelygumik használata, a túl nehéz kollektor, a magas fejési vákuum, vagy a rosszul beállított pulzáció.

A színváltozás osztályozásakor a pigmentált, vagy fekete színű bimbók nem értékelhetők. A szín szerint három fő csoportot lehet kialakítani:

- Normál, rózsaszín
- Kivörösödött (részben, vagy egészben
- Kékes, vagy lilás elváltozás (részben, vagy egészben).

A gyakorlatban elterjedt egy egyszerűsített megoldás, mely két osztályra sorolja a tőgybimbót a színe alapján, ezek a normál és eltérő színű (vörös, vagy kék, esetleg kékes-lila).

Duzzanat, gyűrű kialakulása a tőgybimbó alapjánál

A tőgybimbó alapjánál látható gyűrű oka a hosszantartó magas vakum a kehelygumi szájnyílásánál. Amennyiben a szájnyílás alatti vákuum túl hosszú ideig magas, abban az esetben a kehelygumi felkúszik a tőgybimbó alap felé, összeszorítja azt, akadályozva a tejleadást, ezáltal megnő a visszatartott tej mennyisége is. Ennek megakadályozására a forgalmazók a bimbóhoz jobban idomuló kehelygumik fejlesztésével próbálkoznak.

Az állományok értékelésekor a duzzanat, illetve a gyűrű megjelenését nézzük a kehely eltávolítása után 1 percen belül.

Általában három kategória kialakítása célszerű.

- Normál (nem látható duzzanat és gyűrű a bimbó alapjánál.
- Vékony csík formájában látható a kehelygumi nyoma
- Duzzadt, tapintható vastagabb gyűrű marad a bimbó alapon

Különösen fontos az ellés utáni időszakban figyelemmel kísérni a jelenség kialakulását, amikor a tőgy ödémásodásra hajlamos.

Nyitott bimbócsatorna

Közvetlenül a fejés után a tőgybimbócsatorna nyílása bezár, de megfigyelhető a nyitott bimbócsatorna is, mely szélsőséges esetben

akár gyufafej nagyságú is lehet. Nyitott bimbócsatorna esetén megnő a fertőzésveszély kockázata, különösen fontos a vizsgálata a szárazra állítási terápia megkezdése előtt.

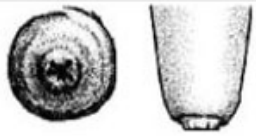
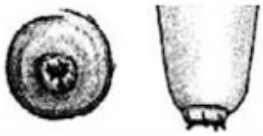
Hajlamosító tényező lehet a magas fejési vákuum, a túlfejés, a nem megfelelő méretű és alakú fejőgumi használata, a túl nehéz kollektor.

Az osztályozásnál két csoportot képezünk:

- zárt bimbócsatorna
- nyitott bimbócsatorna (több mint 2 mm széles, vagy mély nyílás)

A vizsgálat előtt egy tiszta tőgytörlő papírral távolítsuk el a tejmaradványt a bimbóról. A szemrevételezéskor összehasonlíthatjuk a bimbócsatorna nyitottságát egy gyufaszál méretével, melynek a szára körülbelül 2 mm, a feje 3 mm vastag.

1. ábra. A tőgybimbó hiperkeratózisának pontozásos értékelése (Mein et al., 2001)

Pontszám	Leírás	Ábra
1 (N)	A bimbó vége sima és kicsi, a bimbócsatorna nyílása szabályos.	
2 (S)	A bimbócsatornát enyhén érdes tapintású, kiemelkedő gyűrű veszi körül. Elhalt szövetrészek alig érzékelhetők.	
3 (R)	Megemelkedett érdes tapintású, jól elkülönült gyűrű, mely 1-3 mm-re kitüremkedik a bimbó nyílásától.	
4 (VR)	Nagyon érdes gyűrű. Az akár 4 mm-re is kidomborodó gyűrű pereme érdes, repedezett, alakja „virágos” kinézetű.	

Idült, hosszabb ideig fennálló elváltozások

A legfontosabb krónikus tőgybimbó elváltozás a bimbóvég hiperkeratózisa (1. ábra). A hiperkeratózis jelentkezhet gyűrű formájában vagy rojtszerű nyúlványokként a bimbócsatorna nyílása körül Mértéke (érdesség, vagy kérgesedés) kedvezőtlen környezeti feltételek (bélsár, napsugárzás, hideg, rossz alom) mellett egyedileg és állomány szinten is gyorsan, akár napokon belül is változhat. Általánosságban 2-8 hét között változik a kialakulás tartama.

A környezeti tényezők mellett a hiperkeratózis kialakulásáért felelős genetikai és egyedi tényezők a bimbó mérete és alakja, a

termelési szint és a laktáció stádiuma. Meghatározza a fejési technológia és a fejőberendezés hatása, különösen a lassú fejés és a túlfejés. Súlyosbíthatja a helyzetet a bimbó szövetét erősen irritáló fertőtlenítő szerek magas koncentrációban történő alkalmazása.

A bimbó végének kérgesedését befolyásolja a fejés előtti tőgyelőkészítés, a kehely felhelyezésének időzítése és az automatikus kehelylevevő készülék beállítása.

- Ajánlások a tőgybimbók állományszintű vizsgálatára
- A vizsgálatokat közvetlenül a kehely levétele után végezzük, azonban, ha a bimbóbőrének állapotát kívánjuk felmérni, akkor a fejés előtt kell megvizsgálni azt.
- Először érintés nélkül nézzük meg, véleményezzük a bimbó állapotát, azután töröljük szárazra, hogy a bimbó nyílása is látható legyen.
- Minden oldalról nézzük meg a bimbót.
- Ha nincs elég fény, akkor használjunk fejlámpát, a kézben tartott lámpa balesetveszélyes lehet.
- Ha 80 tehénlétszám alatti az állomány, akkor az összes tehenet vizsgáljuk meg, 80-400 tehén esetében minimum nyolcvanas mintát nézzünk véletlenszerűen, ennél nagyobb létszám esetében az állomány legalább 20 %-át vizsgáljuk.
- Az eredményeket rendszeresen jegyezzük fel, ha egy személy vizsgál, akkor célszerű a diktafon használata.

További vizsgálatokra, esetleg beavatkozásra van szükség, ha:

- A vizsgált egyedek több mint 20%-ánál figyelhető meg vöröses, vagy kékes elszíneződés a bimbón.
- Az esetek 20%-ánál nagyobb az előfordulása a bimbó alapnál kialakuló gyűrűnek.
- A tehenek több mint 20%-ának van egy, vagy több nyitott bimbónyílása.
- A vizsgálat során 10 %-nál több bimbón látható vérömleny.
- A tehenek több mint 5% -ánál egy, vagy több bimbón látható nyílt seb.
- A hiperkeratózis tüneteiből a tehenek több, mint 20% -ánál van egy vagy több bimbó, amelyek pontszám értéke 3 (R), vagy 4 (VR) pontszámú, esetleg több mint 10%, amely 4-es (VR) értéket kapott.

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Assessment of udder health in dairy cows with claw diseases by using of mastitis scoring system

Tőgyegészségügy értékelése masztitisz pontozási rendszer segítségével csülökbetegségekben beteg tejelő tehenekben

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Abstract

The aim of this study was to assess the udder health in dairy cows suffered from claw diseases by using of Mastitis scoring system. 366 dairy cows were used in the study. For an analysis of mastitis rate in cows with claw diseases a six-point Mastitis Scoring system (MS) was used. This scoring system is using data of clinical examination of mammary gland and milk. Results were analysed statistically. The claw diseases were found in 122 cows (33.3%). The most frequent claw diseases were digital dermatitis (27.1 %) and interdigital phlegmon (21.3 %), followed by white line abscess (18.9%), sole ulcers (14.8 %), toe necrosis (13.9 %), and laminitis (4.1%). The prevalence of mastitis in the cows with claw diseases was 50.8 % (n 62). The clinical

mastitis was detected in 13 (21 %) and subclinical mastitis in 49 (79 %) dairy cows. The Mastitis score was significant higher in cows affected with claw diseases (P<0.05) than in the healthy ones. There was no significant difference in the mastitis score among the groups of cows with different claw diseases. In conclusion, our results indicate a potential risk of claw diseases for development of an intramammary infection in dairy cows.

Key words: mastitis, claw disease, dairy cattle

Introduction

Worldwide, bovine mastitis have been presented as an important welfare problem of dairy cows and one of the biggest economic problems in dairy industry (Hogeveen et al., 2011). Mastitis may be classified according to two different criteria: either according to the clinical symptoms (clinical and subclinical) or depending on the mode of transmission (contagious and environmental; Kováč et al., 2001). Subclinical mastitis are more prevalent than clinical form (to every clinical mastitis there are 15 to 40 cases of subclinical mastitis; Jones, 2009). In the case of subclinical mastitis the milk is without sensory changes. A typical sign of subclinical mastitis is an increased somatic cell count (Auldist & Hubble, 1998). Affected cows with subclinical type serve as a reservoir of pathogens that can infect other animals in the herd (Barrett, 2005) and causes considerable indirect and direct losses of milk yield (Detilleux, 2018). The globe prevalence of mastitis in dairy herds is different and ranges from a few percent to more than half of herds (Hisira et al., 2013). It depends mainly on dairy farm management and is related to many risk factors, which can be divided into two groups: individual risk factors and environmental risk factors (Nayan et al., 2014). Lameness could be regarded as one of the risk factors responsible for distribution of mastitis in herds, because cow with severe lameness spend on lying longer time (Chapinal et al., 2010). Therefore, there is higher risk of bacterial contamination of teats and formation of mastitis.

The goal of this work was to investigate the effect of claw diseases on prevalence of mastitis in cows of a dairy herd in Eastern Slovakia.

Experimental methods

366 dairy cows were used in the study. Clinical examination of the mammary glands was carried out during orthopaedic examination of cows in the claw crush. Milk samples were evaluated according to physical properties (as a colour, viscosity and smell) and pathological content (flakes, floccules, pus or blood). No changed milk samples were tested by CMT.

For analyses of mastitis rate and grade a six-point Mastitis Scoring system (MS) was used. This scoring system is using data of clinical examination of mammary gland and milk (Table 1).

Table 1. Mastitis Score (MS)

Score	Description	CMT
1	Healthy milk	Negative
2	Doubtful mastitis of one quarter	Trace: 200,000 – 400,000
3	Weak Subclinical mastitis	1: 400,000 – 1,200,000
4	Distinct Subclinical mastitis	2: 1,200,000 – 5,000,000
5	Strong Subclinical mastitis	3: over 5,000,000
6	Clinical mastitis	

MS results were analysed statistically be using student T test and one-way ANOVA test.

Results

Of 366 cows claw diseases were found in 122 cows (33.3 %; Table 2). The most frequent claw diseases were digital dermatitis (27.1 %) and interdigital phlegmon (21.3 %), followed by white line abscess (18.9 %), sole ulcers (14.8 %), toe necrosis (13.9 %), and laminitis (4.1 %; Tab. 3). The prevalence of mastitis in the cows with claw diseases was 50.8 % (n=62, Table 3). The clinical mastitis was detected in 13 (21 %) and subclinical mastitis in 49 (79 %) dairy cows. The Mastitis score was significant higher in cows affected with claw diseases ($P<0.05$) than in the healthy ones (Table 2).

There was no significant difference in the mastitis score among the groups of cows with different claw diseases. In conclusion, our results indicate a potential risk of claw diseases for development of an intramammary infection in dairy cows (Tables 3 and 4).

Table 2. Mastitis score in dairy cows

	Number of dairy cows	Mastitis Score (x±sd)
Claw diseases	122	8.14 ± 4.357*
No claw diseases	244	7.23 ± 3.459*

Table 3. Udder health in dairy cows suffered from claw diseases

	Number of dairy cows	Mastitis	Clinical mastitis	Subclinical mastitis	Mastitis score
Digital dermatitis (DD)	33 (27.1%)	14 (43.8 %)	4	10	7,3 ± 3.94
Interdigital phlegmon + hyperplasia	26 (21.3%)	14 (53.9%)	4	10	8.3 ± 4.59
White line abscess (WLA)	23 (18.9%)	12 (52.2 %)	4	8	8.8 ± 5.54
Sole ulcer (SU)	18 (14.8%)	10 (55%)	4	6	9.1 ± 4.85
Toe necrosis (TN)	17(13.9%)	10 (58.8 %)	0	10	8.2 ± 3.01
Laminitis	5 (4,1%)	2 (40 %)	0	2	7.0 ± 0.98
Total	**122**	**62 (50.8%)**	**13**	**49**	**8.14 ± 4.357**

Table 4. Mastitis score in lame dairy cows

	Number of dairy cows	Mastitis Score (x±sd)
Corium inflammation	67	8.43 ± 4.46
Digital dermatitis	59	7.80 ± 4.25

Discussion

Prevalence of mastitis is affected by many risk factors. Lameness could be viewed as one of them. Claw diseases are regarded as the predominant causes of lameness in dairy cows. In U.S. study claw problems (sole ulcers and white line disease) accounted for 63 percent of the reported cases in lame cows. Digital dermatitis and foot rot presented for 20 percent, and 17 percent of the cases, respectively (Stokka et al., 1996). Animals suffered for lameness spend more time lying down that increases risk of mastitis compared to healthy cows (Chapinal et al., 2010). Hygiene of environment and surface area of animals play important role in exposition of udder by intra-mammary pathogens and it is predictor for the occurrence environmental mastitis (Bartlett et al., 1992). The close relationship between poor hygiene of dairy cow and increasing of

SCC was revealed in a Canadian study (DeVries et al., 2012). According to previous data claw diseases predicted as one of main causes of lameness increase risk of bacterial contamination of teats and mammary gland and probably there is higher risk of intra-mammary infection. Therefore, claw disease may be regarded as one of the most common risk factors responsible for distribution of mastitis in dairy herds with increased prevalence of lameness. The similar finding as our observation was detected in previous works (Peeler et al., 1994; Sato et al., 2008). There was noted association between lameness and mastitis, whereby prevalence of mastitis was very high in cows suffered from claw diseases (74.3 %). In dairy herds in the southwest of England a negative effect of lameness on mastitis was demonstrated. Significance among claw diseases and mastitis incidence rate was also found out in Danish study (Sato et al., 2008). In Swedish observation a close connection has also been documented between poor foot health and a high incidence of clinical mastitis (Arvidson, 2000). Moreover a significant difference in mastitis rate between healthy and lame cows was reported (Mudron, 2016).

However, in some previous studies no association between lameness and subclinical mastitis was presented (Mitev et al., 1998; Olechnowicz & Jankowski, 2012; Hultgren, 2004). In Bulgarian research prevalence of mastitis in lame cows was 5.5% (Mitev et al., 1998). In Polish Holstein Friesian dairy herds no confirmation of statistical significance in SCC between clinically lame cows and healthy cows were determined (Olechnowicz & Jankowski, 2012). According to study in 102 Swedish dairy herds no associations have been proved between sole ulcer and clinical mastitis or high milk cell counts. This might be explained as a result of relatively mild lameness of affected cows. Possibly, only severe lameness impairs the cow's locomotion and increases the risk of teat injuries and subsequent mastitis. The analyses indicated that teat injury was not an intervening variable between sole ulcer and mastitis or high cell counts, no a confounder (Hultgren, 2004).

In conclusion, our observation noted close relationship between claw diseases and mastitis by using of mastitis scoring system. Claw diseases as predominant causes of lameness may be characterised as one of the risk factors contributing to the increased prevalence of mastitis in dairy herds. However, no significant differences were observed in severity of mastitis in dairy cows with different type of claw diseases.

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Cardiac responses to milking in dairy cows milked in conventional milking systems with stationary and rotary milking stalls

Tejelő tehenek szívműködési értékeinek változása hagyományos halszálkás, parallel és karusszel fejési rendszerekben

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Abstract

Step behavior, heart rate (HR), power spectral components of heart rate variability (HRV) as acute and fecal cortisol concentrations as chronic measures of stress as well as rumination behavior during milking were investigated in dairy cows milked in lower- and higher-capacity herringbone (HMS1, n = 20 and HMS2, n = 51) and parallel milking systems (PMS1, n = 27 and PMS2, n = 33) with stationary milking stalls and a higher-capacity rotary milking system (RMS, n = 49) operated in five different working farms. Heart rate, the high frequency (HF) component of HRV and the ratio of the low frequency (LF) and HF components (LF/HF ratio) were analyzed for the stages of the evening milking process. Feces samples for analysis of cortisol

metabolites were collected between 0630 and 0800 h and following the evening milking (between 1900 and 2030 h). During the stage of driving HR increased from baseline in all milking systems reaching higher values compared to all other stages. After driving a gradual decrease in HR was observed for all milking systems. The HR increase was higher in cows milked in PMS1 and RMS and remained higher compared to baseline in these animals during the further stages of the milking process. Only cows milked in the RMS showed a decrease in vagal tone (indicated by HF) compared to baseline with the onset during the stage of driving, with a gradual increase during milking and returning to baseline during the stage of waiting. In HMS1 HF was lower compared to baseline during driving, holding area and waiting suggesting stress during these stages. Compared to baseline HF was lower at waiting in PMS2 as well. In HMS2 HF fell below baseline during milking but not during any of the other stages of the milking process. HF did not change throughout the milking process in PMS1. LF/HF ratio indicated increased sympathetic activity of cows milked in the RMS starting during driving with a gradual decrease from milking and returning to baseline during the stage of waiting. The LF/HF ratio did not differ from baseline in any of the milking stages in cows milked in both PMS. In HMS1 the LF/HF ratio was higher compared to baseline in the holding area and during waiting indicating higher levels of stress during these stages. In HMS2, LF/HF ratio was higher compared to baseline during milking. Cows milked in PMS1 exhibited steps more often during milking compared to cows milked in the RMS or HMS1. Stepping frequency did not differ across the milking systems during the stage of waiting. The probability of performing rumination during milking was 7.8, 5.2 and 2.5 times higher in the RMS compared to PMS2, HMS2, and PMS1. Fecal cortisol concentration values were lower in RMS compared to PMS2 and HMS2. Milking in a RMS seems the less challenging for cows than in milking parlors with stationary milking stalls irrespective of stall arrangement or capacity as indicated by adequate autonomic stress response during milking, frequency of steps during milking and fecal cortisol levels. Cows milked in larger-capacity milking parlors with stationary milking stalls showed an inadequate autonomic response to milking and a possible sign of chronic stress. Stress during waiting can occur either in herringbone and parallel parlors irrespective of parlor capacity.

Key words: herringbone milking parlor, parallel milking parlor, rotary milking system, heart rate variability, step behavior, dairy cows.

A szelekciós és támogatott reprodukciós technológiák alkalmazása a tőgygyulladás kezelésére egy tejhasznú tehenészetben

Using selection and assisted reproductive technologies to deal with mastitis on a dairy farm

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Összefoglalás

Hazánkban a tejkvóta megszüntetése miatt kialakult kedvezőtlen tejár ellenére is növekszik a szarvasmarha állomány és a tejtermelés. A megtermelt tej egyre nagyobb részét exportra értékesítjük. Ezért is, illetve a hazai igények minőségi kielégítése miatt kiemelten fontos az Európai Unió irányelveinek megfelelő termelési feltételeket, ezáltal biztonságos élelmiszert előállítanunk. Az utóbbi években kiemelt fontosságot kapott az antibiotikum-felhasználás, így az antibiotikum-rezisztencia kockázatának csökkentése. A szarvasmarha-ágazat két legköltségesebb területe éppen a jelentős antibiotikum- és hormonfelhasználás miatt a tőgyegészségügy és a szaporodásbiológia. Mindkettő alapjaiban határozza meg egy telep jövedelmezőségét, hiszen kellő számú és magas genetikai értékű nőivarú állat fog egyre több tejet termelni és még jobb adottságokkal bíró utódokat a világra hozni. Kutatásunk e két terület együttes fejlesztését célozza: asszisztált reprodukciós eljárások felhasználásával kívánjuk egy adott állomány tőgygyulladással szembeni ellenállását növelni. Céljaink közt szerepel egy célszerűen kiválasztott szarvasmarha-állomány egyedeinek tőgyegészségügyi szempontból történő osztályozása, a mastitis szempontjából legjobb rezisztenciával bíró egyedeinek kiválogatása, azok genetikai tulajdonságainak feltérképezése és tőlük nagy számú olyan utód létrehozása, melyeknek várhatóan jobb lesz a tőgypatogénekkel szembeni ellenállása, ami által csökkenthető a tőgykezelésekre fordított antibiotikum mennyisége.

A kísérletek során a kiválasztott donorállatokat szuperovuláltatjuk és olyan bika spermájával termékenyítjük, amely szintén jó tőgyegészségügyi tulajdonságokat örökít, majd az embriókat kinyerjük, in vitro tovább

tenyésztjük, vizsgáljuk osztódási mintázatukat, ami által értékelni tudjuk életképességüket, prekondícionáljuk és mélyhűtjük őket, valamint génállományukban mastitis rezisztencia markerek jelenlétét kutatjuk. Célunk a projekt szempontjából releváns alkalmazott biotechnológiai eljárások alkalmazása és finomhangolása, gyakorlatban használható protokollok fejlesztése.

Végeredményként létre hozunk egy több száz beültethető, nőivarú, mastitisre vélhetően rezisztens embrióból álló mélyhűtött állományt, amellyel egy generáció alatt nagymértékű genetikai előrehaladást érhetünk el a tőgyegészségügyben és csökkenthetjük az adott telep antibiotikum-felhasználását, javíthatjuk a selejtezési arányt, növelhetjük az értékesített tej mennyiségét, valamint jó minőségű, biztonságos alapanyagot állíthatunk elő az élelmiszeripar számára. A kutatások záró szakaszában az embriók beültetésre kerülnek, és az utódok nyomon követésével kívánjuk validálni a feltételezéseink gyakorlati megvalósulását.

Kulcsszavak: tőgygyulladás, embriótranszfer, mastitis rezisztencia

Abstract

Regardless of the repealed dairy product quota and the consecutive low milk prices cattle herds and milk production are slowly growing in Hungary. Export trade is also increasing together with the growing production. The safety of production is highly regulated, as it is fundamental to produce safe food based on European Union's directives. One of the focuses is to decrease the use of antibiotics. In the dairy sector mastitis and reproduction have the highest costs by the common application of antibiotics and hormones. These two factors basically determine a farm's profitability as cows in adequate number and genetic value can produce more milk and bred better offspring. Our proposed research involves both fields: our target is to improve mastitis resistance in a specific herd by targeted selection and assisted reproduction techniques. Our procedures include classification of the individual cows in the particular dairy herd by udder health, selection of the best 2% of the animals for mastitis resistance, chart their genetic properties, create embryos using selected donors, vitrify then warm and transfer then breed offspring from them. Thus, we hypothesize to create a population with higher resistance against udder pathogens, consequently the application of antibiotics would be considerably reduced.

In our experiment we induce superovulation on the selected cows (donors) and inseminate them with sexed sperm of selected bulls (inheriting good udder health). We flush the embryos, culture them in vitro, examine their morphokinetics by time-lapse equipment whereby we can evaluate their viability. We then precondition them to improve viability and then vitrify the created blastocysts. Our target here is to define a practical protocol.

Finally we establish a transferable, presumably mastitis resistant frozen stock with hundreds of female embryos, which can presumably improve a particular herd's udder health in a short time. In the long run we can reduce application of antibiotics, advance culling rate, increase the amount of sold milk and produce better-class, safe milk for the dairy industry. At the end of our study the embryos will be transferred and we follow up the offspring for the occurrence of clinical mastitis to validate our hypothesis.

Tejelő szarvasmarha állományok esetében kijelenthető, hogy a teljes antibiotikum-felhasználás több mint fele a tőgy, ezen belül is mastitis kezelésére fordítódik. Egy 2012-ben lezárt, hét évig tartó, 92 tehenészetet magában foglaló vizsgálat adatai szerint a teljes antibiotikum felhasználás 68%-a fordítódott tőgyegészségügyre, 24%-a klinikai mastitis kezelésére (Kuipers et al., 2016). A klinikai tőgygyulladások értékelése során kiderült, hogy az esetek közel 80%-ában a kezeléseket a klinikai ajánlásoktól függetlenül, rutinszerűen meghosszabbítják az állatorvosok, illetve a farmerek (Swinkels et al., 2015). Mindezen tendenciák annak ellenére megfigyelhetők, hogy az Európai Unió egyértelműen állást foglalt az antibiotikumok használatának csökkentése érdekében (EMA, 2014). Mi több, az élelmiszeripar és a vásárlói szokások is egyre inkább megkövetelik az antibiotikumok élelmiszer előállító állományokban történő limitált felhasználását.

Az elmúlt évtizedben a tőgygyulladással szembeni rezisztencia fontos szelekciós szemponttá vált. Hagyományosan, a szomatikus sejtszámmal, illetve a tőgy morfológiájával közelítették a tőgy egészségére irányuló szelekciót. Ezeket a tulajdonságokat egészíthetjük ki a klinikai mastitis korábbi előfordulásának illetve a test kondíció indexnek vizsgálatával. Ezen tulajdonságok összességével mintegy 70%-ban magyarázható a mastitis rezisztencia genetikai varianciája (CDN, 2013, 2014). További szelekciós lehetőséget kínálnak a genetikai markerekhez kötött módszerek. A betegség összetett oktanában a genetikai háttér vizsgálata se mutat egyértelmű irányt a megfelelő szelekciós potokoll kialakításához, ugyanis a mastitis poligenikus hátterű (Hayes et al., 2010). Mindezeket nem figyelmen kívül hagyva, számos dolgozat jelent meg potenciális, mérhető genetikai markerekkel kapcsolatban: TLR 4 (toll-like receptor 4) (Deb et al., 2013; Ingham & Menzies, 2006); NLR (nod-like receptor) (Pant et al., 2007); Lactoferrin (Pawlik et al., 2009); lizozim (Chen et al., 2013); kemokinok pl: IL-17 és IL-8 (Lewandowska-Sabat et al., 2012); BoLA-DRB3 (Ramirez et al., 2014) egyéb jelöltek mellett.

A kutatások nehézségét az adja, hogy az egymást befolyásoló vagy együtt öröklődő tulajdonságok között tudunk-e egyensúlyt teremteni vagy genetikai kiskaput találni, ugyanis az utóbbi években a tenyésztők főként a magas tejtermelésre szelektálták az

állataikat, ami viszont alacsonyabb tőgy-egészségügyi státuszt eredményezett, hiszen ezen két tulajdonság negatív korrelációban van egymással (Oltenacu & Broom, 2010).

A szaporodásbiológiát támogató asszisztált reprodukciós eljárások észszerű eszköztárat biztosítanak egy kedvező genetikai változás elindítására és meghonosításásra. A már hagyományosnak tekinthető in vitro fertilizáció, mesterséges embriótenyésztés majd a preimplantációs stádiumú embriók mélyhűtése mellett az utóbbi években további lehetőségekkel bővült az asszisztált reproduckció. A szuperovuláltatás során a természeteshez képes sokkal több petesejt kerül a petevezetőbe, és mindezeken túl e petesejtek zöme termékenyíthető állapotban van. Az érett petesejtek mesterséges termékenyítését követően egy ovulációból tehát nem egy, hanem potenciálisan akár húsz embrió is születhet. Noha a genetikai háttere (szülei) e ~20 embriónak megegyezik, mégis mindegyik embrió más továbbfejlődési és genetikai potenciállal bír. A beültetés akkor végezhető el a legjobb hatékonysággal, ha az embriók közül kiválasztható a legjobb fejlődési potenciállal bíró darab. Ennek eszköze az embriók in vitro fejlődését folyamatosan, az inkubátorban nyomon követő automata digiális time-lapse mikroszkóp. A rendszer az embriók fejlődéséről 10 percenként felvételt készít, mely képsorozat a mikroszkóphoz kapcsolt személyi számítógép segítségével elemezhető. Az elemzés különös figyelmet szentel az embriók osztódásainak szabályosságának (pl. tripoláris osztódások felismerése), a sejtciklus egyes részeinek (S fázis, citokinezis) időtartamainak, valamint egyéb rendellenességeknek (fragmentáció, vakuolizáció) a meglétére és dinamikájára. Az ily módon összegyűjtött adatok lényegi információt szolgáltatnak az embriók bírálatához, a beágyazódási esélyük megbecsüléséhez, valamint a beültetésre megfelelők kiválasztásához (Pribenszky et al., 2010, 2017).

Embrió mélyhűtés egy évtizedek óta rutinszerűen használt eljárás, mely során preimplantációs stádiumú embriókat megfelelő krioprotektánsokkal kiegészített oldatokban eljárástól függő sebességgel, illetve hordozón a folyékony nitrogén hőmérsékletére (-196 C) hűtjük. A mélyhűtés során az embriók életképessége számtalan ponton (membránok, belső struktúrák épsége; fehérjék, enzimek szerkezete; metabolizmus épsége), számtalan ok mentén (hidegstressz, mechanikus stressz, ozmotikus stressz, toxikus anyagok direkt hatásai) sérül. A rutin mélyhűtési eljárások lépéseinek technikai finomhangolása mellett felmerül az embrió felkészítése, prekondícionálása az életképességet direk és indirekt módon romboló, a mélyhűtéssel szükségszerűen együtt járó hatásokkal szembeni rezisztenciájának növelésére (Pribenszky et al., 2014; Jiang et al., 2016).

Csoportunk azt a célt tűzte ki maga elé, hogy egy adott tejelő szarvasmarha telepen a saját állomány szelekciójával definiál egy mastitis rezisztencia szempontjából optimális donor

populációt. E donorok, szuperováltatást követően, mastitis rezisztencia szempontjából kedvező tulajdonságokkal bíró bikák (kedvező szomatikus sejt- illetve tőgy indexek) örökítő anyagával termékenyítésre kerülnek. A létrejött embriók, kimosást követően, folyamatos time-lapse megfigyelés alatt fejlődnek inkubátorban egészen a blasztociszta stádiumig. A time-lapse felvételek elemzését követően az embriók értékelésre kerülnek beágyazódási potenciáljuk szempontjából. Az ültethető embriók biopsziát követően genetikai tipizálásra majd lefagyasztásra kerülnek. A mélyhűtést megelőzően az embriókat prekondícionáljuk, hogy a mélyhűtéssel járó szükségszerű káros hatásokkal szemben az ellenállóképességük a lehető legjobb legyen.

Anyag és módszer

Projektünk során egy szarvasmarha állomány egyedeit osztályozzuk tőgyegészségügyi szempontból a Canadian Dairy Network ajánlása szerint figyelembe véve a szomatikus sejtszámot, a tőgykezelések számát, illetve a tejtermelést.

Az állatok adatait a Riska telepirányítási rendszerből nyerjük ki és egy több szempontos rangsorolásnak vetjük alá (Tofallis, 2013). Pozitív elbírálás alá esik a minél magasabb tejtermelés, minél kevesebb tőgykezelés és minél alacsonyabb szomatikus sejtszám, mindezek minél kisebb szórás értékekkel. Az állomány legjobb 2%-át szuperovuláltatjuk a gyakorlatban használt hormonkezelési protokoll szerint, majd olyan bika ondójával termékenyítjük a kiválasztott teheneket, mely szintén jó tőgyegészségügyi tulajdonságokat örökít (somatic cell score, illetve udder indexes).

Az így létrejövő korai stádiumú embriókat kimossuk a méhből, in vitro tovább tenyésztjük WOW edényekben (Well-Of-The-Well dish, Vitrolife AB, Svédország) és fejlődésüket time-lapse technológia segítségével (Primo Vision Embryo Monitoring System, Vitrolife AB, Svédország) monitorozzuk, hogy információt nyerjünk a szarvasmarha embrió korai osztódási szokásairól és invazív beavatkozás nélkül tudjuk értékelni életképességüket (Pribenszky et al., 2010, 2017). Meghatározzuk az embriók ivarát és DNS-üket szekvencia analízisnek vetjük alá mastitis rezisztencia markerek felkutatása érdekében. A mélyhűtésre történő előkészítés során szubletális dózisban nagy hidrosztatikai nyomással kezeljük az embriókat (PTAT – Pressure Triggered Acquisition of Tolerance, Pribenszky et al., 2010, Jiang et al., 2016) a jobb túlélés érdekében, majd mélyhűtjük őket vitrifikációs eljárással (Rapid i, Vitrolife AB, Svédország).

Projektünk végén a rendelkezésünkre álló életképes, feltételezhetően mastitisre rezisztens, nőivarú embriókat beültetjük recipiensekbe, így évekkel később nyomon tudjuk követni az utódok életteljesítményét és tőgyegészségügyi állapotát.

Rövid és hosszú távú célok

Kutatásunk rövid távú célja, hogy a minta-állományban, az állomány saját genetikai erőforrásait felhasználva jelentősen növeljük a tőgygyulladással szembeni rezisztenciát, következésképpen jelentősen csökkentsük a tőgygyulladás kezeléséhez felhasznált antibiotikumok mennyiségét. A projekt hosszú távú célja az, hogy egy gyakorlatban alkalmazható protokollt alakítsunk ki, melynek alkalmazásával bármely Holstein-fríz állományban nagy mértékű javulást tudunk elérni a tőgyegészségügyben asszisztált reprodukciós eljárások segítségével.

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Teat structure in Brazilian dairy buffalos assessed using ultrasonography

Brazíliai tejhasznú bivaly tőgybimbó szerkezetének megállapítása ultrahangvizsgálattal

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Abstract

Differences exist in udder and teat characteristics between buffalos (*Bubalus bubalis*) and cattle (*Bos taurus*) and they are important to consider when milking them with the machine. Dairy buffalos are known to be slow and hard to milk, especially with mechanical devices, in comparison with dairy cows. We aimed to describe udder and teat characteristics of dairy buffalos in Brazil, a country with a very recent history of buffalo husbandry. Udder typing and ultrasonography of the teat was performed in 63 dairy buffalos of the breed pure Murrah and Mediterranian × Murrah on three farms with different management systems. Udder types were different on different farms. Rear-heavy udder type was the most prevalent udder type on two farms, and ball-shaped udders were observed often on the third farm. Ultrasonographic measurements of the teat were performed on one farm, and correlations between width of the teat tip,

teat canal length and teat wall thickness and teat length were found. During ultrasonographic examination of the teat, hyperechogenic membrane-like structures narrowing the transition between teat und and glandular cistern in the region of the annular folds were noted in at least one teat in 98 % of the examined cows. The functionality of the folds and the relevance our findings for the mechanical milking process are not clear to date. Further studies evaluating their influence on milk flow characteristic during mechanical milking in buffalos are needed.

Replacement heifer rearing

Tenyészüszők felnevelése

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The crop of replacement heifers produced by a dairy is a crucial resource that needs to be managed with great care. The replacement heifers will be responsible for the dairy's future performance. These animals should represent the best genetics in the herd. Managing properly to bring them into milk production quickly, and in top health, is a key performance driver for dairy farms. In this paper, I will describe, in detail, the steps we take on our two dairies to bring these young animals into the herd.

The first step in our process has to do with the dam carrying the unborn calf. Our prefresh vaccination program includes Guardian (Merck) vaccine. The purpose of this is to increase the dam's colostral levels of antibodies for K99 *E. coli* as well as Rota and Corona virus. By doing so, the calf will be protected against these scours-producing organisms by consuming its colostrum. Our calves receive four (4) liters of colostrum within twenty (20) minutes of birth. The colostrum is milked from the dam immediately after freshening while still in the maternity pen. The colostrum is tested for antibody levels with a Brix refractometer. If the colostrum is of

sufficient quality, four (4) liters are transferred immediately to an esophageal feeder and the calf is fed while the colostrum is still very close to body temperature. If excess colostrum of sufficient quality is produced, the excess is saved in a single use plastic bag where it is refrigerated for up to one day. If a calf's dam does not produce colostrum of sufficient quality or quantity, the saved colostrum will be warmed up and fed to the calf. If there is no colostrum of sufficient quality available, the calf will be fed a commercial colostrum replacer (Secure). The esophageal feed bottle and tube are fully sanitized between each use, as is the milking equipment. All calves are tested for serum protein between 3-7 days of age, to assess the colostrum program's results (Figure 1).

At birth, before colostrum feeding, the newborn calf is stimulated to establish regular respiration. The calf is then dried off, weighed on a drive-over scale, and placed in a neonatal stall. The calf's naval will be thoroughly dipped with iodine before feeding. The calf will be processed with plastic and RFID ear tags at the time and will have all pertinent information entered into her Dairy Comp 305 records (Figure 2). About twelve (12) hours after birth, the calf will be transferred to the nursery barn. In cold weather conditions, we will put a jacket on the newborn to help maintain body temperature.

Dehorning

At the time the neonatal calf is moved, the hair over its horn buds will be clipped. At one day old, we will apply dehorning paste to the horn buds. The calf will be examined for failure of the paste dehorning at six (6) weeks of age. If paste dehorning was not successful, the calf will receive a lidocaine block and have the remaining horn tissue cauterized.

Figure 1. Serum protein report

Nov	CPROT	BDAT	CMTR	CMAN	DID
23593	6.7	11/1/2017	29	A	20786
23594	6.4	11/1/2017	29	A	16140
23595	6.3	11/1/2017	27.5	A	18468
23596	4.5	11/2/2017	1	L	17778
23597	6.5	11/2/2017	31.5	L	17778
23598	5.3	11/2/2017	26	A	20570
23599	6.9	11/2/2017	26	A	19375
23600	4.3	11/2/2017	1	V	15060
23601	4.9	11/2/2017	1	V	15060
23602	5.6	11/2/2017	27	L	19182
23603	6.8	11/2/2017	26.5	L	17471
23604	6.8	11/3/2017	26.5	A	20879
23605	6.6	11/3/2017	28	V	20871
23606	5.9	11/3/2017	30.5	V	16905
23607	5	11/4/2017	1	L	16990
23608	4.8	11/4/2017	1	L	17549
23609	6.4	11/4/2017	24.5	L	20753
23610	5.9	11/4/2017	28	A	15771
23611	6.6	11/5/2017	25	L	19307
23612	6.5	11/5/2017	26.5	L	20722
23613	6.4	11/5/2017	25	L	20329
23614	7	11/5/2017	24	L	17775
23615	6.1	11/6/2017	24	A	16476
23616	6.7	11/6/2017	24.5	A	17759
23617	4.7	11/6/2017	1	V	20863
23618	4.4	11/6/2017	26.5	L	17866
23619	6.3	11/6/2017	26.5	L	17866
23622	5.7	11/7/2017	23.5	L	19377
23623	7.8	11/7/2017	28	A	20619
23624	7.7	11/7/2017	24.5	V	17371

CPROT= Serum protein value
BDAT= Birth date
CMTR= Refractometer reading of colostrum fed
CMAN= Manager that fed colostrum to calf
DID= Ear tag number of the calf's dam

Figure 2. Fresh cow sheet

Fresh cow sheet – Dairy Dreams

Date/Fecha	Cow ID/Identida Arete	Pen/corall	M/F - sexo	Calf ID	Cassa de vecerras	Difficulty - Dificultad	Time Born - horan	Time Fed - comir	Colostrum – total leche	Colostrum Meter Reading – mide leche	Feder – quien - comir	Pullet – quien saca	Teats - teta	Calf Weight	Calcium bolus
6-16-12	11255	11	M		15	1	2:00	2:20	2	60	O	O	4	96	B.C
	4471	11	M		14	1	3:35	4:10	2	60	O	O		90	B.C
	7263	11	M		13	1	3:40	4:15	2	60	O	O		82	B.C.
	11051	11	F	15213	11	1	6:15	6:40	2	60	JS	Ju		88	B.C
	12587	9	F	15214	12	1	6:30	7:00	2	70	Ju	Ju		79	
	10519	11	M		16	1	6:40	7:20	2	60	Ju	Ju	3	83	B.C.
	9772	11	F	15215	10	1	9:00	9:20	2	60	Ju	Ju	4	90	B.C.
	7571	11	F	15216	8	1	10:10	10:20	2	60	Ju	Ju	4	104	B.C
6-17-12	12521	9	M		7	1	1:55	2:20	2	70	Ju	Ju	4	91	
	9627	11	M		3	1	4:50		2	60	Ju	Ju	3	69	
	10636	11	M		2	2	9:50	10:10	2	80	O	Ps	4	101	

Feeder= Individual who fed colostrum to the calf
Puller= Individual who was responsible for monitoring birth
Teats= Number of functional teats on the dam

Feeding

Calves will be started on a diet of pasteurized hospital milk and feed twice (2X) daily. Hospital milk is supplemented with a high protein powder and water. This increases the protein level and decreases the fat level of the milk. After consuming their milk, the calves are offered warm water twice daily. Calves are also offered a start grain and consumption is visually monitored. Milk is offered at these rates:

Day 1-4	2.0 liter	2X
Day 5-10	2.5 liter	2X
Day 11-21	3.0 liter	2X
Day 22-35	4.0 liter	2X
Day 36-43	3.0 liter	2X
Day 44-49	3.0 liter	1X
Day 50-63	no milk	

Calves are moved from the nursery barn to group housing at 57-63 days. The calves are weighed before moving and the weight is recorded on their cow card. An entire nursery barn is weaned and moved at the same time. This happens weekly. The animals are moved from their individual pens to group housing, in groups of eight (8). The groups are selected by weight, so the largest eight (8) calves will be penned together. Each subsequent group of calves is also sorted by weight. The group pens are bedded with a base of straw covered with manure biosolids from digested manure.

Group feeding

The calves in the group pens are fed their same starter grain for the first week. After one week, they have soft, grassy hay offered with the starter grain for the second week. For the third week, they are fed starter grain along with a TMR and by the fourth week, they are fed only TMR. They will remain in the group housing until five (5) months of age, at which time they will be moved into free stall housing. They are weighed for a third time as they move at five (5) months.

From five (5) months until 365 days of age, the heifers are moved through a progression of larger free stall groups. At 365 days, the heifers are moved into the breeding pen and weighed one last time. A heifer must be 750 pounds (340 kg) before she is first bred. Animals that are not of sufficient weight of 750 pounds (340 kg) will be withheld from breeding until they reach the desired weight.

We find the four (4) individual weights to be very valuable data. By having weights at birth, weaning and five (5) months, we can determine whether growth rates are satisfactory, or whether we need to make some adjustments in the milk feeding program. We are consistently striving to feed sufficient milk for early development, but not so much as to hamper the intake of starter grain. We find that consumption of adequate starter grain is crucial to post-weaning growth rates. The growth rates also provide excellent data for determining which calves should be culled. The animals with the lowest ADGs, generally below 1.5 lb (0.68 kg)/day, will be sold (Figure 3).

By the time heifers reach breeding weight, they are separated into two different populations, based on genetic merit. Genetic merit is determined by their parent average genetic merit. We do this so we can make an accurate assessment of genetic merit without the expense of actual genetic testing. Use of the parent average genetic data requires absolutely correct semen identification and absolutely correct identification of the correct dam for each newborn calf. This data is used to determine which heifers provide offspring to the herd. The heifers in the lower fifty percent (50%) of the genetic merit pool will serve as surrogate uteruses for a genetics company. The offspring are returned to the company for a fee. The heifers in the top of the genetic merit pool are bred with sexed semen. These animals provide all the offspring that comes from the heifer population. Significant effort goes in to

maximizing the economic benefit of the surrogate embryo program, while at the same time ensuring a full complement of replacement animals for the dairy (Figures 4,5,6 and 7).

In conclusion, our replacement animals are a highly important group of animals. Much management effort goes into providing the maximum number of healthy heifers to continue the genetic progress of the herd of the future. Accurately tracking body weights and average daily gains (ADG) is a critical tool for us to measure our results, track the effect of management changes and identify underperforming animals at a relatively early age (Figures 8,9,10,11,12 and 13).

Figure 3. Calf growth rates

ID	SID	DDINX	AGEDY	NEWWT	WEIGT	HEIGT	ADG	ADGI	XPNEU	LMSDT
23627	11H11745	620	140	78	263	41	1.33	1.01	0	3/27/2018
23645	511H11727	717	137	86	314	43	1.68	1.22	0	3/27/2018
23631	11H11765	624	140	94	338	43	1.76	1.89	0	3/27/2018
23618	511H11602	650	142	48	297	42	1.77	1.94	2	3/27/2018
23633	511H11727	620	139	84	329	43	1.78	1.49	0	3/27/2018
23616	511H11727	650	142	82	334	43	1.79	1.76	0	3/27/2018
23619	511H11602	650	142	44	297	42	1.79	1.92	0	3/27/2018
23623	11H11691	614	141	54	307	42	1.81	1.71	0	3/27/2018
23611	511H11727	712	143	86	353	44	1.88	2.17	0	3/27/2018
23632	511H11499	690	139	96	363	44	1.93	2.07	0	3/27/2018
23614	11H11676	531	143	80	356	43	1.94	2.03	0	3/27/2018
23628	11H11676	602	140	94	367	43	1.96	1.86	0	3/27/2018
23643	511H11602	645	137	84	350	43	1.96	1.58	0	3/27/2018
23634	11H11745	567	139	86	358	44	1.97	2.06	0	3/27/2018
23622	511H11727	667	141	80	357	43	1.98	2.06	0	3/27/2018
23635	511H11499	701	139	86	366	44	2.03	2.29	0	3/27/2018
23637	511H11727	663	139	108	390	44	2.04	1.67	0	3/27/2018
23615	11H11750	498	142	98	393	44	2.09	2.39	0	3/27/2018
23626	511H11499	599	140	78	370	44	2.1	2.15	0	3/27/2018
23638	511H11727	641	138	80	368	43	2.1	2.29	1	3/27/2018
23644	511H11727	660	137	92	378	44	2.1	2.39	0	3/27/2018
23613	511H11499	679	143	66	367	43	2.12	2.19	1	3/27/2018
23641	511H11499	628	138	84	374	44	2.12	2.38	0	3/27/2018
23612	511H11499	679	143	78	381	44	2.13	2.29	0	3/27/2018
23624	11H11781	652	141	80	378	44	2.13	2.18	0	3/27/2018
23625	11H11765	617	141	90	388	44	2.13	2.26	0	3/27/2018
23642	11H11781	664	137	82	376	44	2.16	2.22	0	3/27/2018
23629	511H11727	663	140	80	382	44	2.17	2.33	0	3/27/2018
23630	511H11602	687	140	92	393	44	2.17	2.14	0	3/27/2018
23640	511H11499	634	138	76	377	44	2.2	2.4	0	3/27/2018
23636	11H11765	664	139	92	410	45	2.3	2.46	0	3/27/2018
23617	511H11499	657	142	76	415	45	2.4	2.58	0	3/27/2018
32 total Avg		642	140	81.7	359	43	1.99	2.04	1.3	100

ID= Calf ID
SID= Sire ID
DDINX= Estimated genetic score
AGEDY= Calf age in days
NEWWT= Birth weight of calf (pounds)
WEIGHT= Current weight of calf (pounds)
HEIGHT= Height of calf (inches)
ADG= Average daily gain (pounds/day) from birth to current
ADGI= Average daily gain (pounds/day) from previous weight to current weight
XPNEU= Number of treatments for respiratory disease
LMSDT= Most recent date measured prior to current date

Figure 4. Projected heifer calvings Dairy Dreams

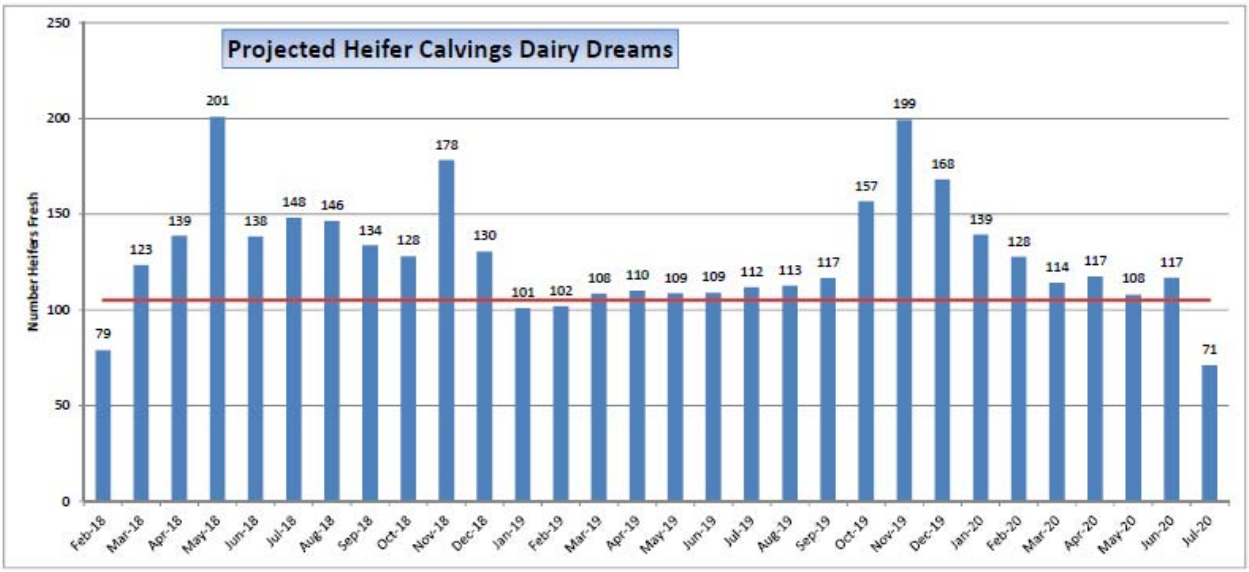


Figure 5. Dairy Dreams hutch calves – 56d in hutches

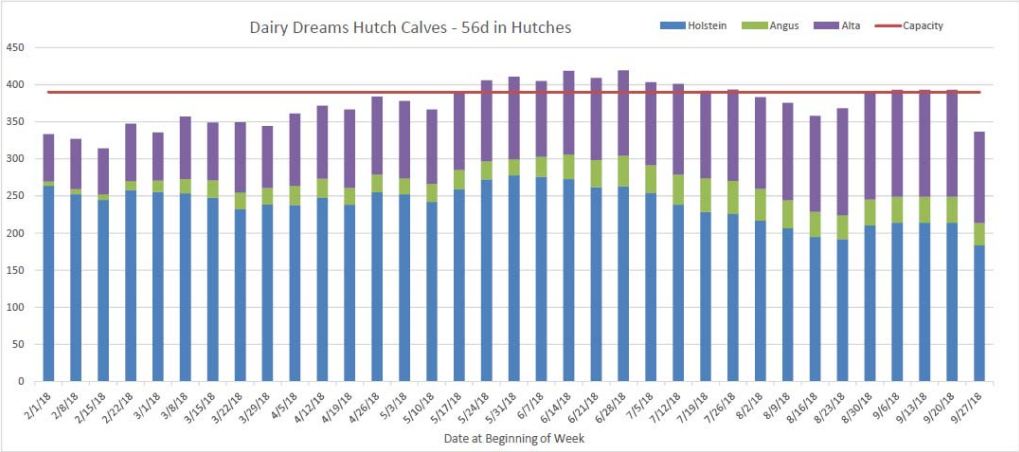


Figure 6. Dairy Dreams hutch calves – 63d in hutches

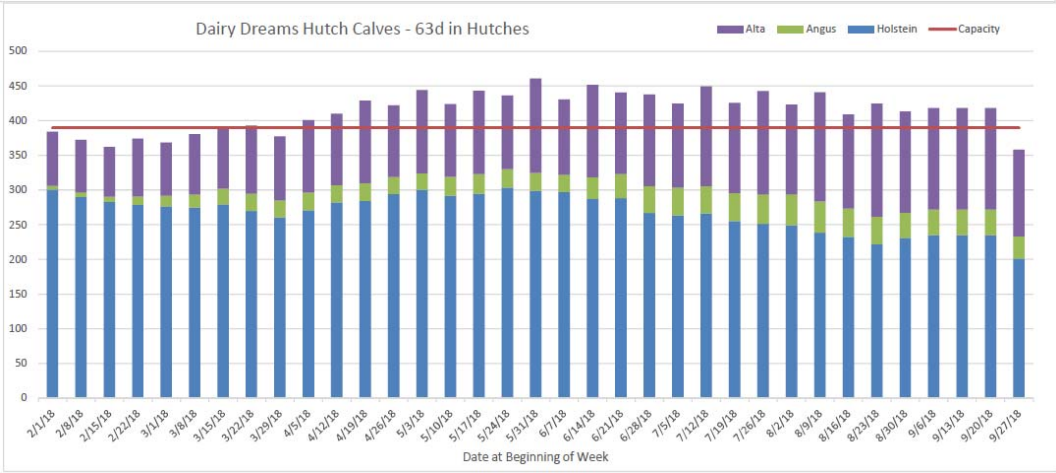


Figure 7. Dairy Dreams hutch calves – 70d in hutches

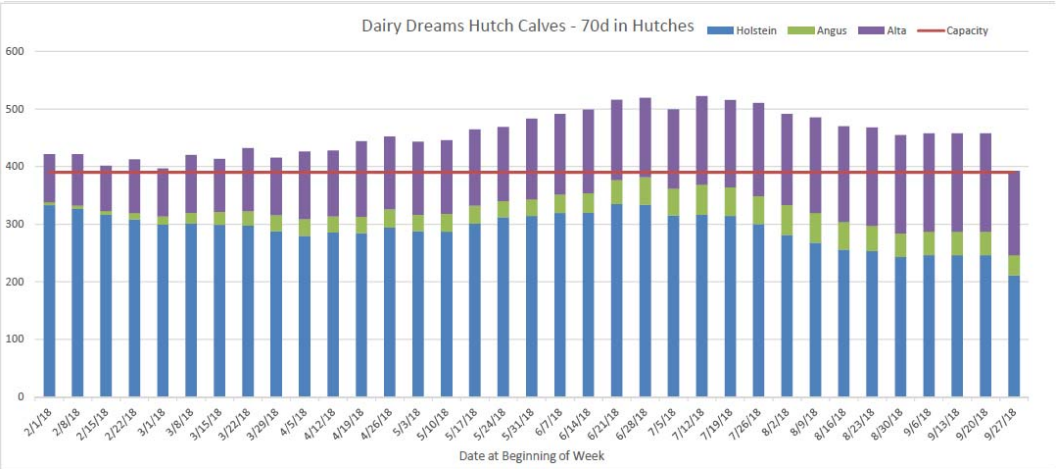


Figure 8. Six month survival rates of calves

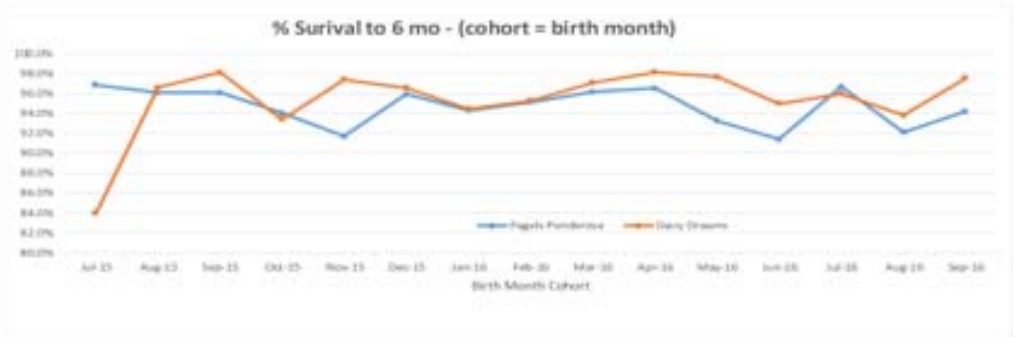


Figure 9. Cull (sold and dead) rates for first 30 days

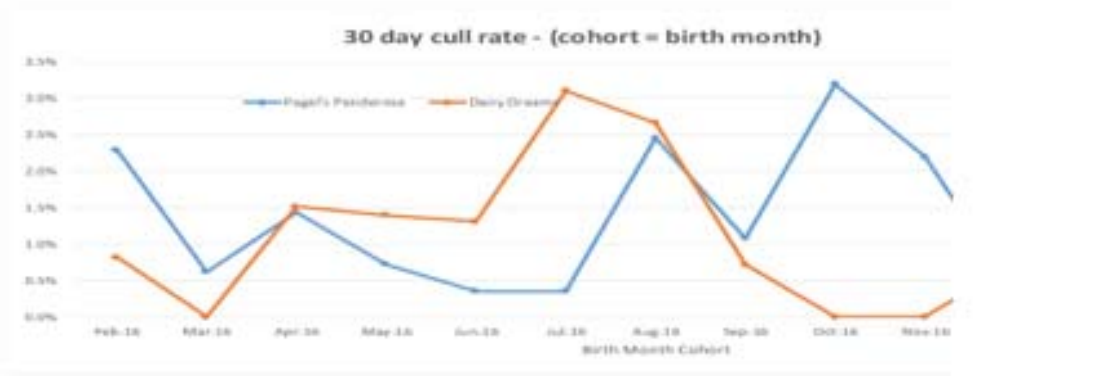


Figure 10. Respiratory disease percentage in first 60 days

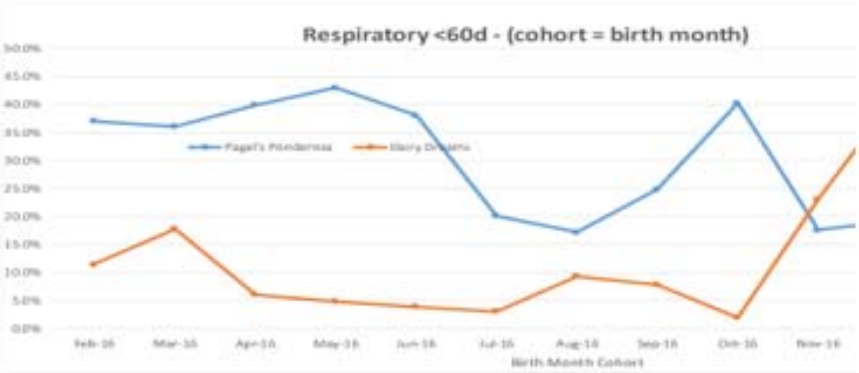


Figure 11. Hutch average daily gain

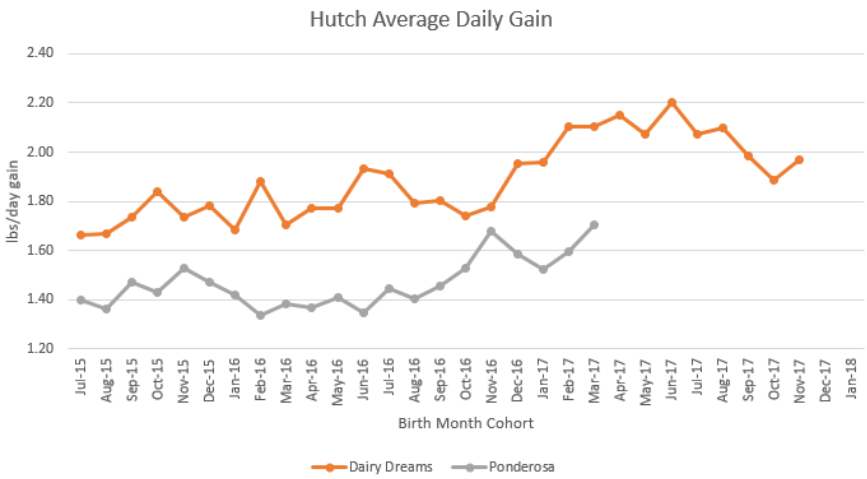


Figure 12. Lifetime gain at 2nd body weight measurement (~150 days of age)

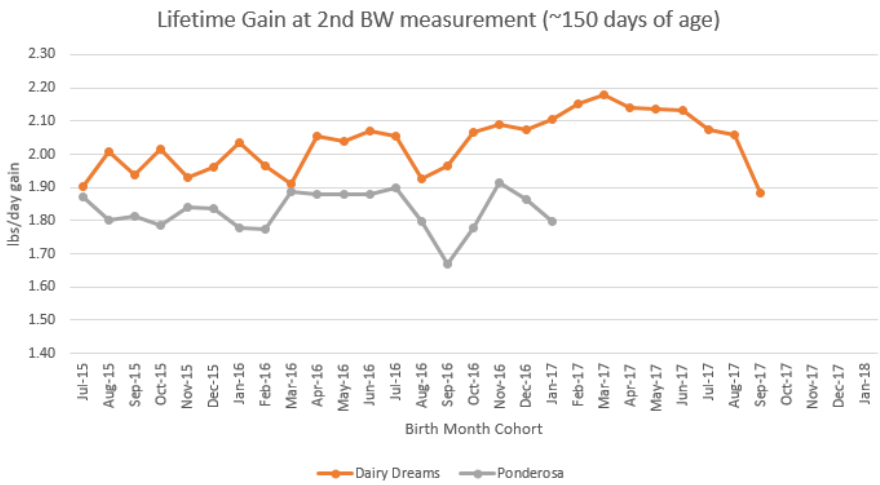
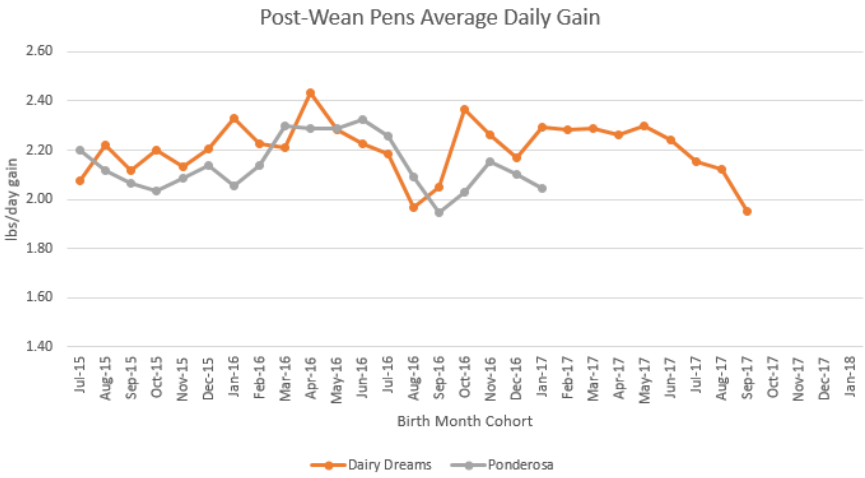


Figure 13. Post-wean pens average daily gain



A magzati jóllét vizsgálatának lehetőségei ultrahangkészülékkel szarvasmarhában

Ultrasonographic assessment methods of foetal well-being in cattle

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Összefoglalás

Szarvasmarhák magzatainak morfometriai vizsgálatával az 1980-as évek óta foglalkoznak, azonban ennek ellenére e fajban a magas vemhesség során végzett magzat-jólléti vizsgálat nem terjedt el széles körben a klinikákon kívül. A borjúmagzat szívverését a vemhesség 24. napjától kezdve lehet mérni, azonban telepi gyakorlatban a 30. nap kö-

rüli vemhesség vizsgálat terjedt el. A vemhesség későbbi stádiumában azonban újra meg lehet erősíteni a vemhesség tényét, amire a magzati mortalitás jelensége miatt lehet szükség. Szarvasmarhákon végzett vizsgálataink azt bizonyítják, hogy a vemhesség utolsó szakaszában végzett rektális vizsgálat során, a borjúmagzat metacarpalis/metatarsalis csontátmérőjéből, és az anyai testtömegből számított index (MCTI) diagnosztikai jelentőséggel bír a nehézellés előrejelzése szempontjából. A terület jelentősége miatt szükségesek lennének további, nagy létszámon végzett, ultrahang-diagnosztikai vizsgálatok a magzati perinatális morbiditás és –mortalitás csökkentése céljából.

Abstract

Studies investigating bovine morphometric parameters have been published since 1980's, but ultrasonographic methods assessing foetal well-being were not widely used except of the clinical approach. Foetal heart rate can be detected as early as 24th day of gestation, but the pregnancy examination is usually performed approx. Day 30 under practical circumtances. Additional sonographic examinations are advisable later in gestation because of the possible pregnancy loss. Our study showed that near-term rectally performed foetal examination of the metacarpal/metatarsal bone diameter combined with maternal weight (MCTI) can be a possible predictor of dystocia in dairy cattle. The significance of this area indicates further large-scale studies evaluating ultrasonographic methods in order to decrease perinatal morbidity and mortality in the bovine.

Bevezetés

Jelen dolgozatunk célja a szarvasmarhában, a magzati jóllét és egészségi állapot vizsgálatára alkalmas ultrahang-készülékkel végezhető telepi vizsgálatok összefoglalása, ill. kutatócsoportunk e területen végzett vizsgálatainak (2013-2017) rövid ismertetése.

Szaporodásbiológiai célú ultrahangvizsgálatokat szarvasmarhában az ultrahang-készülékek széles körű elterjedése óta nagy számban végeznek az egész világon. Ezek a vizsgálatok a telepeken alapvetően rektális vizsgálatok, a rutin szaporodásbiológiai gondozás elemei (ciklusdiagnosztika, vemhesség-vizsgálat, anösztrusz kóroktani vizsgálata). Tudományos igényű vizsgálatokat Európában 1980-as évek óta közölnek (Kähn, 1988) a témában, az USA-ban az első összefoglaló jellegű ultrahangdiagnosztikával kapcsolatos, a mai napig hasznos információkat tartalmazó művet 1998-ban adta ki Ginther (1998). Bár a felvételek minősége az akkori készülékek felbontóképességének felel meg, az összegyűjtött információk a mai napig helytállóak. Az első időben az embrió, majd később a magzatok szívverésének detektálása jelentette a biztos vemhességi módszert a vemhesség 24. napjától. A magzatok *in utero* ivarmeghatározásának módszerét szintén a 1990-es években dolgozták ki, abban az időben azonban a korlátozott felbontó-

képességű eszközök miatt egy viszonylag szűk időkeret (kb. 50-65 vemhességi nap) állt rendelkezésre (Curran et al., 1986). Bár lovakban az elmúlt években a magzat ivarmeghatározásának módszerét tovább fejlesztették, és ennek eredményeként gyakorlatilag az 50. vemhességi naptól egészen a vemhesség utolsó harmadáig meghatározható az ivar, szarvasmarhában ilyen irányú vizsgálatok ezt még nem tették lehetővé. Az ehhez használt módszer a vemhes állatok nem rektális, hanem transzabdominális módon történő vizsgálata, amely, figyelembe véve a jellegzetes anatómiai struktúrákat (pl. a bendő miatt a magzat a jobb oldalon helyeződik), alkalmas a magzat jóllétének, vagyis egészségi állapotának vizsgálatára. A magzati morfológiai mérések mellett a magzati szívfrekvencia (FHR, foetal heart rate), a magzatok száma, mozgása, a magzati környezet (magzati folyadékok mennyisége, minősége, méhpogácsák mérete, struktúrája, stb.) is vizsgálható.

A vemhesség vége közeledtével a figyelem elsősorban a magzat méretének meghatározására irányul, amelyre az utóbbi években próbáltak megbízható módszereket kidolgozni (Takahashi et al., 2001, 2005; Buczinski et al., 2007, 2009, 2011). Az egyik lehetséges módszer a magzati, kiágazódásnál mért aorta átmérőjének mérése, amely azonban úgy tűnik, nem kiforrott módszer (1. vizsgálatunk), a másik lehetőség az ellés előtt álló anyaállatok magzatainak rektális módon történő *metacarpalis* csontok átmérőjének mérése az anya testtömegéhez viszonyítva (2. vizsgálatunk).

Anyag és módszer

Az 1. vizsgálatunkban magas vemhes tehenekben és üszőkben mértünk magzati aortaátmérőket a vemhesség napjaival és a később megszületett és lemért borjak testtömegével összefüggésben (Buczinski, 2009; Baska-Vincze et al., 2014; Vincze et al., 2017) transzabdominális módon.

Második vizsgálatunkban a Takahashi et al. (2001, 2005) által használt rektális metacarpus/metatarsus csontátmérőket mérő módszert fejlesztettük tovább (Vincze et al., 2017). A módszer segítségével 97 üszőt és tehenet vizsgáltunk vemhességük utolsó időszakában; az anyaállatok testtömegét és a borjak ivarát, az ellés körülményeit (könnyű/nehézellés), az elérhető magzati lábvég metacarpus vagy metatarsus csontátmérőjét, és az anyaállatok interischiadicus és intercoxális távolságát, valamint a kondíciópontokat jegyeztük fel és elemeztük statisztikai módszerekkel (logisztikus regresszió).

Eredmények és megbeszélés

A magzati ultrahangvizsgálat témakörben néhány dolgozat foglalkozik csak a magzati jóllét vizsgálatával, ezek közül a legfrissebbet Kutatócsoportunk közölte (Vincze et al., 2017). Összefoglaló dolgozatként két közlemény jelent meg, Buczinkski munkája 2009-ben, és egy lóval és szarvasmarhával foglalkozó összefoglaló mű (Baska-Vincze et al., 2014). Szarvasmarhák ultrahangvizsgálatának legújabb módszereivel egy

2016-os cikkgyűjtemény foglalkozik, de sajnos nem szerepel benne magzati ultrahang-vizsgálatra vonatkozó rész (Buczinski, 2016). Szükség van tehát olyan vizsgálati eredményekre, amelyek nagy létszámú állatokon végzett, gyakorlatias ismereteket közvetítenek a tudományos világ és egyúttal a praktizáló állatorvosok felé.

Ez a megfigyelés indította el vizsgálatainkat 2013-ban, amikor egy korábban publikált, de csak kevés állatra vonatkozó tudományos eredményt szerettünk volna felhasználni további módszerbeli fejlesztésekhez. A borjú-magzatok aortaátmérőjét mértük meg 44 vemhes állaton, és ezt hasonlítottuk a születéskori testtömeghez. Azonban a mi eredményeink nem támasztják alá a korábbiakat, vagyis az aorta-átmérők nem voltak összefüggésben a magzatok testtömegével (Vincze et al., 2017). Így ez a módszer nem alkalmas a magzatok méretének, testtömegének becslésére, így arról sem ad információt, hogy várható-e esetleg nehézellés az adott állatban.

Tovább dolgozva kerestünk egy olyan mérhető morfometriai paramétert, amely gyakorlati szempontból is beilleszthető a telepi gyakorlatba, és nem igényel többéves ultrahangos tapasztalatot a használójától. Takahashiet al. (2001) vizsgálati módszerét továbbfejlesztve végeztünk 2016-ban egy ultrahang-diagnosztikai kísérletet, ahol az ellés előtti időszakban vizsgálva a vemhes anyaállatokat azt találtuk, hogy az üszők nagy részében a magzatok végtagjait rektálisan lemérve a csontátmérők lemérhetők voltak, egy rövid (kb. 5 perc/állat) vizsgálat alkalmával. Mivel a magzat metacarpalis/ metatarsalis csontátmérője önmagában nem ad információt az anya-magzat egymáshoz viszonyított méretéről, így a kalkulációba bele kellett vennünk egy anyai paramétert, a legutolsó testtömeg értékét. Ebből a két paraméterből számított MCTI (metacarpus/ tarsus-testtömeg index) viszont szignifikáns összefüggést mutatott az ellésnél a segélynyújtás szükségességével. Kisebb MCTI esetén valószínűsíthető volt a nehézellés a vizsgált Holstein-fríz tejelő állományban.

A szerzők következtetése az, hogy a jövőben szükséges kiterjeszteni a magzatok egészségét vizsgáló módszerek továbbfejlesztését és validálását, ahogy az más állatfajokban (pl. ló) megtörténik, mert a fejlett diagnosztikai eljárások ellenére a tejelő szarvasmarha állományokban a néhol jelentős magzati morbiditás és -mortalitás jelentős gazdasági károkat okozhat a telepek tulajdonosainak.

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Heart rate variability, respiratory rate and rectal temperature in heat stressed dairy calves with or without supplemental shade

Árnyékolás és elmaradásának hatása hőstressznek kitett tejhasznú borjak szívritmus-változékonyságára, légzési frekvenciájára és rektális hőmérsékletére

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Abstract

Changes in non-invasive stress biomarkers were studied in shaded (n = 8) and non-shaded (n = 8) Holstein bull calves exposed to extreme heat load conditions in a continental region. Ambient temperature and humidity data were recorded for the S and NS hutch and exercise pen environments. Temperature-humidity-index (THI) was also calculated. Respiratory rate, rectal temperature, heart rate, and the root mean square of successive differences between R–R intervals (RMSSD) were recorded as animal-based indicators during three periods: (1) day 1, *control day*, during which all calves were shaded for 24 h (shade removal from non-shaded calves at 2400 h); (2) day 2, *heat stress day*, with shade over shaded calves; (3) days 3–5, *post-stress period*, with shade over shaded calves. On the heat stress day, maximum temperatures were 44.3 and 46.7 °C for the non-shaded hutch and pen environments, respectively. The temperatures were with 6.2 and 6.9 °C (P = 0.015 and P = 0.008) and the THIs were with 5.9 and 4.2 units higher (P = 0.020 and P = 0.032) in the non-shaded than in the shaded environment for the hutch and exercise pen, respectively. Shaded calves had with 42.3 ± 3.2 breaths/min higher respiratory rate than non-shaded ones on the heat stress day at 1200 h (P = 0.001), which was moderated to 20.1 ± 2.4 breaths/min at

1600 h (P = 0.023). Significant differences in respiratory rate occurred earlier than in any other animal-based parameter between shaded and non-shaded calves on day 2. The only significant group difference in rectal temperature was found at 1200 h on day 2 when THI exceeded 91 units, with 0.59 °C higher values for non-shaded calves (P = 0.045). The heart rate on days 2 and 3 was higher for non-shaded calves than for shaded ones. Group differences were also significant at 0800 h (18.2 ± 1.2 beats/min, P = 0.008), 1200 h (22.3 ± 1.4 beats/min, P = 0.003), 1600 h (15.3 ± 0.8 beats/min, P = 0.012), and 2000 h (19.0 ± 1.1 beats/min, P = 0.010) on day 2. Following a rapid daytime reduction, RMSSD showed a nighttime overcompensation in non-shaded calves on day 3 (between 0000 and 0600 h) and day 4 (between 0000 and 0800 h), exceeding the levels recorded on day 1 (control) and the levels for shaded calves, thus suggesting a recovery of the autonomic nervous system from heat stress. Based on our results, shading effectively reduced heat stress as evidenced by heart rate and RMSSD in addition to traditional measures of heat stress. Respiratory rate and heart rates exhibited by non-shaded calves support that the well-being of pre-weaned calves can be impaired in continental regions during following a heat stress day without providing shade.

Key words: dairy calves, temperature-humidity index, heart rate, respiratory rate, heat stress, supplemental shade

Microbial contamination of colostrum from Czech dairy herds

Cseh tejhasznú tehenészetekből származó kolosztrum mikrobiális szennyezettsége

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Abstract

The assessment of immunoglobulin content in colostrum is a routine procedure within many Czech farms but little attention is paid to its microbial quality despite the fact microbial contamination can reduce immunoglobulin absorption. The aim of our study was quantitative and qualitative evaluation of bacteria present in freshly milked colostrum in Czechia.

In 38 dairy herds (21 Czech Fleckvieh, 14 Holstein, 3 both breeds), 823 colostrum samples (20 per farm) were taken from 2015 to 2017. Farmers collected the samples from the first milking after the calving into sterile 30 mL vessels. Subsequently, the samples were frozen and transported into the laboratory. Plate count agar was used for total plate count (TPC) and MacConkey agar was used for coliform count (CC) and Gram negative non-coliform count (NCC) determination in 1 mL of colostrum. In 108 samples out of a total of 823 (from 18 farms, 6 samples each), the species of microorganism were determined using MALDI-TOF and findings were classified into 4 groups: faecal contaminants, environmental contaminants, skin and mucosal microorganisms, and Gram positive (G+) mastitis agents. The data was evaluated in Statistica CZ 10.0.

Only 27.7% samples met the requirement of TPC below 100,000 cfu/mL (median 420,000 cfu/mL) but 72.5 and 83.7% samples were below the limits of 10,000 and 5,000 cfu/mL for CC and NCC, respectively (median 190 and 120 cfu/mL, respectively).

TPC was significantly lower in the Holstein herds (median 299,000 compared to 550,000 cfu/mL).

The proportions of samples containing faecal contaminants, environmental contaminants, and skin and mucosal microorganisms were comparatively high with 79.2%, 72.9%, and 80.4%, respectively. The most frequently isolated representatives of the above groups were *Enterococcus spp*. (in 71.0% of samples), *Moraxella osloensis* (42.1% samples) and *Staphylococcus spp.* (41.1% samples). G+ mastitis agents were detected in 20.6% of samples, *Streptococcus parauberis* in 10.3%, *Streptococcus uberis* in 6.5% and *Staphylococcus aureus* in 5.6% of samples.

Basing our data on species composition, the large amounts of bacterial contamination within most of the samples of colostrum were most likely caused by poor hygiene within the housing and an insufficient preparation of the udder during the first milking after parturition in which protocol normally states is done outside the main parlour. Poor sanitation of the separated milking devices used can also be key factor.

Acknowledgments

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The experimental phage therapies of diarrhoea in newborn calves

Kísérleti fágterápia hasmenéses újszülött borjakban

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Abstract

Escherichia coli is responsible for a wide variety of enteric and extraintestinal infections in humans and animals. The calf diarrhoea, which is a commonly reported disease in young animals, and still a major cause of productivity and economic loss to cattle producers worldwide. Antibiotic therapy used in the treatment of calf diarrhoea is no longer effective due to the increasing antibiotic resistance of bacteria and therefore new treatments are sought. Phage therapy and applications of probiotics are alternative tools to control bacterial infections. The aim of the study was to evaluate the therapeutic and preventing effect of different bacteriophages treatments in calves with diarrhoea.

The study were carried out with twenty two female HF calves, aged from 1 to 14 days, with clinical signs of diarrhoea. The animals received per rectum for 5 days suppositories with Lactobacillus spp. and bacteriophages cocktails specific for pathogenic *E. coli* strains. The observation of health status was carried out during the eleven days after first day of treatment and after the next two weeks after experiment. The obtained results showed a significant therapeutic and preventing effect on calves which was

shown in decreasing of rectal temperature, reduction or elimination of diarrhoea within 24-48 hours. It was also confirmed, that used treatment had a significant effect on the stimulation of humoral specific and non-specific mechanisms of the immune response of the calves.

The experimental calf treatment is beneficial in control of bacterial diarrhoea infection in young calves.

Key words: bacteriophages, calves, diarrhoea, *E. coli*, infection, therapy

Introduction

Diseases of the digestive system in calves manifested by symptoms of diarrhoea constitute a significant health problem in dairy and beef cattle herds, thus contributing to the reduction of animal welfare, especially during the first weeks after birth. The consequence of the occurrence of diarrhoea in calves in the first days of life is the deficit of passive immunity transfer resulting in reduced immunity and immunosuppression, increased morbidity and mortality, reduced feed absorption and weight gains and prolonged of growth and maturation (Urban-Chmiel et al., 2016). Over half of the diagnosed cases show the polyethiological character of the disease and the presence of more than one pathogen. Among the factors predisposing to the occurrence during of diarrhoea in calves should also include a group of non-infectious agents (Muktar et al., 2015), including: poor resistance of newborns - errors in the control of the transfer of colostrum, poor quality of colostrum, failure of passive transfer; poor sanitary and hygienic conditions of the housing systems, social stress - excessive crowding of the calves in stalls, mixing of age-different calves with different immune status, inadequate microclimate of housing systems (temperature, humidity, air movement, dust content, microflora) and inadequate motivation and commitment of service staff in caring for calves (Larson &Tyler, 2005).

Diarrhoea caused by *E. coli* is one of the most common diseases of calves. As confirmed by numerous reports in newborn calves occur in the age of 9-10 days and usually characterized by fast course and high mortality. Usually it's begins within 36-72 hours after birth, and falls occur within the next 2-3 days. It should be noted that the first falls, being a result of toxic shock, are already observed a few hours after the appearance of disease symptoms (Muktar et al., 2015)

E. coli bacteria, based on their virulence factors, were classified into the following pathogenicity groups (Cho et al., 2014)

• Enterotoxigenic E. coli (ETEC);

• Shiga toxin-producing E. coli shiga toxin;

• Enteropathogenic E. coli (EPEC);

• Enteroinvasive E. coli (EIEA);

• Enteroaggresive E. coli;

• Enterohaemorrhagic E. coli (EHEC);

The enterotoxic, enteropathogenic and enterohaemorrhaege strains are classified as diarrheal types whose presence has been demonstrated in livestock. The most common cause of newborn diarrhoea are ETEC strains that produce K99 antigen and thermostable enterotoxin (Mukatar et al., 2015; Kaper et al., 2004)

The widespread increase in antibiotic resistance among pathogenic bacterial strains which causing infection in humans and animals significantly limits the effectiveness of therapy. Threats resulting from a significant reduction or inability to eliminate infections resulted in increased interest in the use of diverse, alternative biological methods that could be used in combating infectious diseases. Within the alternative scope or supporting activities, among others, probiotic cultures, natural immunostimulators, herbal extracts, immunoglobulins, during the last decade have again been turned towards bacteriophage therapy (Meril et al., 2003; Denou et al., 2009)

Material and Methods

In the study 20 female HF calves, aged from 1 to 14 days old, weight from 35 to 50 kg with clinical signs of bacterial diarrhoea were used. The animals received per rectum for 5 days suppositories with bacteriophages cocktail specific for pathogenic E.coli strains mixed with Lactobacillus spp. strains isolated from cattle. The medicine was prepared with a formula mixer which was carried out at Department of Pharmacology University of Life Science in Lublin with the Sub Department of Veterinary Prevention and Avian Diseases (appl. Patent No P.424314).

The observation of health status was carried out during the eleven days after first day of experiment. Additionally, after the end of experiment all calves used in the study were checked for the presence or absence of diarrhoea symptoms for the next 3 weeks after treatment.

The obtained results were then compared to the results obtained in other study with treatment of calves with bacteriophages presented by Smith & Huggins (1983) and Smith et al. (1987). In the study of cited authors the newborn calves received by oral administration a different phage cocktails specific for pathogenic E.coli strains. The administration procedure was connected with using a different formula, time of application and the number of applications (Table 1).

Table 1. The procedures of phages application in different experimental phages therapy in newborn calves

Experimental Group	Kind of challenge	Schemat aplikacji	The therapeutic effect	References
Calves at 24h life	ETEC O9:K30.99; $3 \cdot 10^{9}$ CFU, p.os.	1. Phage cocktail B44/1, B44/2, 10^{11} PFU, one-time oral application after challenge, before diarrhoera occurence. 2. Phage cocktail B44/1, B44/2,10^{11} PFU, one-time oral application after diarrhoea occurence 3. Phage cocktail B44/1, B44/3, 10^{11} PFU, one-time oral application after diarrhoea occurence	1. Total elimination of diarrhoea among two days after treatment. Reduction of mortality about 30%. śmiertelności. 2. The diarrhoea in 67% of experimental calves. The presence of resistance to B44/phage E.coli strains. 3. The clinical signs of diarrhoea in all experimental calves. Reduction of mortality in 15% of calves.	Smith & Huggins, 1983
Lambs at 24h.	ETEC O8:K85.99; S13,$3 \cdot 10^{8}$ CFU p.os.	Phage S13, 10^{9}, 10^{10} PFU oral once time after challenge.	Reduction of diarrhoea about 50%, reduction of mortality more than 25%. Reduction of *E.coli* strains isolated from feaces.	Smith & Huggins, 1983
Calves at 24h	Multiple oral challenge ETEC strains	1.Phage cocktail 10^{9} PFU once time during in time of diarrhoea 2.The differen doses of bacteriophages applicated directly after challenge. 3.Phages applicated with milk 10^{5} PFU during different time after challenge. 4.Phages obtained from housing systems seawage applicated on litter at 10^{5}-10^{9} CFU/m^{2}.	1. A single or phage cocktails reduced diarrhoea and mortality in calves. 2.The reduction of clinical signs (fever, diarrhoea) after application of phages 10^{2} i 10^{5} PFU (6h to 10min) before challenge and (10-12h) after challnege. 3. High efficacy in reduction of clinical signs after administration with milk 4h before and 10-12h after challenge. 4. High efficancy in protection after treatmen within 3 h after challenge. 5. Protection of calves after application on liter 6-0 h. before challenge.	Smith et al., 1987a
Calves during two first weeks of life	ETEC *strains*	Oral application of phages: 1.The influence of pH in gastric environment. 2.The influence of colostrum immunoglobulins on phages viability and activity. 3. The range of optimal temperature for virulence of phages	1. Inactivation of phages in pH ≤3. 2. Antibodies active against phages in the gut after feeding with milk or colostrum containing calcium carbonate 3. An increased of body temperature in newborns have a significant effect on the virulence of phages.	Smith et al., 1987b

Results and Discussion

The obtained in the present study results showed the significant reduction of *E. coli* in experimental calves. These results confirm the results obtained in the other studies that the application of bacteriophage in young animals in drinking water throughout the production cycle can be an effective way to reduce the occurrence of *E. coli* O157: H7 and K99 and other E.coli pathogenic strains which was shown in Table 1.

In the present own study was also observed a significant therapeutic effect on calves which was shown in reduction of clinical signs such as fever, diarrhoea within 24-48 hours in treated calves and significant influence on changes in body weight (Table 2). It should be noted that used in experiment procedure of five-day per rectum application of bacteriophages cocktails mixed with probiotic strains and stabilised by glycerine was beneficial in elimination of clinical signs and mortality in experimental calves. The application of the medicine was carried out after clinical signs of diarrhoea occurrence. The similar results was observed in the study Smith & Huggins (1983) after oral application of phage cocktail B44/1, B44/2, 10^{11}PFU in one day old calves. However the treated calves didn't' show a clinical signs of diarrhoea. In the same study after application of different phage cocktails in calves after challenge with clinical signs of diarrhoea the therapeutic effect was very negligible. The similar results were obtained in the other study after multiple oral application of phage cocktails in newborn calves with diarrhoea. In the treated calves the reduction of diarrhoea about 50%, reduction of mortality more than 25% was observed, however only in case of application phages at the same time after challenge (Smith et al., 1987a)

Table 2. The chosen clinical parameters observed in experimental calves

Experimental group	AW (kg) 1 d	AW (kg) 11 d	Average Rectal Temp. °C							The % calves with diarrhoea
			1	2	3	4	5	7	11	
Gr.1 Control	43.8±5	51.4±6[a]	38.5±0.2	38.5±0.3	38.4±0.3	38.4±0.3	38.7±0.1	38.8±0.1	38.9±0.1	0
Gr. 2 Control Positive	43±10	47.2±12	38.8±0.2	38.5±0.2	38.7±0.3	38.4±0.2	39.02±0.3[ab]	39.2±0.1[ab]	39.1±0.2[ab]	100
Group 3 Healthy	41.4±8	48.25±7,5	38.5±0.3	38.5±0.2	38.3±0.1	38.6±0.1	39.2±0.2[ab]	38.8±0.1[a]	38.5±0.1[a]	0
Group 4 with diarrhoea	42.4±10	49.1±12	38.6±0.1	38.8±0.2	38.6±0.1	38.6±0.2	38.7±0.1	38.5±0.2	38.9±0.1[a]	20

Significant differences p≤0.05 in comparison to the first day of experiment.
Significant differences p≤0.05 in comparison to the first group of calves.

The present own study also showed a protective effect as increasing the resistance of experimental calves to infections caused by pathogenic strains of E. coli- no clinical signs of diarrhoea during the next two weeks of housing. The experimental treatment confirmed also the preventive effect which was shown as a significant stimulation of humoral specific and non-specific mechanisms of the immune response of the calves. Analysis of humoral immunological response stimulation in calves after treatment confirmed the significant $p \leq 0.05$ increase in the concentration of IgA and IgG immunoglobulins among groups of medicine treated calves in comparison to not treated control positive and control healthy calves. A significant differences in the obtained values were depending on the health status of animal and used treatment. The used experimental treatment had also a significant influence on non- specific immunological response which was demonstrated in significant $p \leq 0.05$ increase of lysozyme level in medicine treated calves in comparison to not treated animals and significant $p \leq 0.05$ increase of serum interferon gamma (IFNγ) concentration in experimental treated calves which confirmed the stimulation of antiviral immunological response in experimental calves (data obtained in our study, not yet published).

It should be also noted that it was the first time procedure with using of suppositories and no side effects, both general and local, in calves was observed.

Conclusions

To sum up it should be noted that using of the treatment with this experimental phage therapy procedure is beneficial in control and prevention of bacterial diarrhoea infections in young calves. However this study should also be continued in another cattle housing systems.

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Limb fractures in 32 small ruminants – Treatment and outcome

Végtagtörés 32 kiskérődzőben – Gyógykezelés és annak kimenetele

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Introduction

The objective of this study was to evaluate the records of 32 small ruminant patients treated due to limb fractures during a 14-years period.

Material and Methods

The records of 32 patients of our Clinic suffering from limb fractures were analyzed retrospectively regarding the species, breed, and age of the animals, age, localization and

type of fractures, the applied treatment method, and the final outcome.

Results

The patients were 16 goats and 16 sheep (18 male, 14 female) with a mean age of 1.5 years and a mean body weight of 37.8 kg showing in total 35 fractures. The age of the fracture ranged between one and six days in 23 animals, and from 12 to 60 days in the other. Fractures involved most frequently the femur (28.6%), the radius and ulna (22.8%), the metatarsus (20.0%) and the metacarpus (14.3%). Five animals were not treated due to very poor prognosis.

The success rate by conservative treatment (17 animals) was 94.1%, and 81.9% by surgical treatment (11 animals). In six animals (22.2%) complications were observed during and after fracture treatment, however four out of them had a good final outcome. The age and the body weight of the animals and the age of the fracture were not statistically correlated with the treatment success. However, a general tendency was observed that the success rate decreased with a higher age of fracture.

Discussion

The overall success rate of 92.6% for treatment of limb fractures in small ruminants from this study should encourage to apply even in practice the conservative treatment of metacarpal, metatarsal, phalangeal fractures in sheep and goats. In contrast, for proximal limb fractures, the internal fixation (Kaneps et al., 1989; Anderson & St-Jean, 1993, Nuss et al., 2011; Piórek et al., 2012) is the applied method of choice. Small ruminants are ideal fracture patients due to their low body weight (Kaneps et al., 1989).

The complete results of this study had been published in: KOFLER et al. Frakturen von Gliedmaßenknochen bei 32 kleinen Wiederkäuern – Methoden und Ergebnisse der Behandlung. Tierärztliche Praxis Grosstieren 2017.45.201-212.

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Effectiveness of mineral supplementation in sheep

Ásványi anyag kiegészítés hatékonysága juhokban

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Objectives

The aim of this study was to determine the effectiveness of ruminal bolus administration in adult sheep.

Methods

Group of 3 healthy domestic-breed sheep were administered mineral, ruminal bolus of 180 days dissolving time. The blood samples were taken 6 times in 30-day intervals to estimate the Co, Se and Zn levels in serum. First sampling occurred before bolus administration. Blood was collected from the jugular vein using 1,2mm needle into test-tubes containing clot activator. The blood was centrifuged at 3000 rpm for 15 minutes. The serum was put in clean tubes and then tested using atomic emission spectrometry method for cobalt, selenium and zinc levels.

Results

Obtained results were analyzed with Statistica software using t-test in order to compare results between samplings. Ruminal bolus administration appears to influence Co, Se and Zn concentrations in blood serum of sheep even though the obtained results oscillate around reference values. Such form of long-acting mineral supplementation seem favorable because of its time of effectiveness and easiness in administration.

Sheep 1						
Sampling*	1	2	3	4	5	6
Co (ug/l)	0,4	0,4	0,3	0,3	0,3	0,4
Se (ug/l)	36	39	32	42^{x}	49^{x}	84^{x}
Zn (ug/l)	443	408	505^{x}	510^{x}	601^{x}	499

Sheep 2						
Sampling	1	2	3	4	5	6
Co (ug/l)	0,7	0,4^{x}	0,6	0,4^{x}	0,4^{x}	0,5
Se (ug/l)	41	57^{x}	88^{x}	77^{x}	104^{x}	114^{x}
Zn (ug/l)	594	700^{x}	691^{x}	901^{x}	826^{x}	737^{x}
Sheep 3						
Sampling	1	2	3	4	5	6
Co (ug/l)	0,5	0,3^{x}	0,7^{x}	0,5	0,4	0,3^{x}
Se (ug/l)	46	56^{x}	69^{x}	66^{x}	68^{x}	90^{x}
Zn (ug/l)	670	604^{x}	613^{x}	631	746^{x}	662^{x}

*Blood samples were taken in 30-day intervals
^{x}statistically significant difference at $p \leq 0.05$

Effect of β-hydroxy β-methylbutyrate acid (HMB) for growth and health traits in young goats

β -hidroxi-β-metil-vajsav (HMB) hatása a fiatal kecskék növekedésére és egészségügyi állapotára

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The aim of the study was to investigate the influence of HMB, for growth and health traits in organism of young goats. The study include 26 goats, separated from mothers in age about 30 days, divided into two analogous groups: I - control and II - experimental. For 60 days, group II were fed with the addition of HMB (50 mg / kg body weight). The following traits were analyzed: body weight, daily increases of weight, as well as haematological and biochemical blood indicators. The results of the study were statistically analyzed by ANOVA method using Statistica 10.0 Soft Incorp program.

A significant predominance in terms of body weight in the group II was found, both after 30 and 60 days of experiment, respectively: 0.34 kg and 0.57 kg ($p \leq 0.01$). The daily gain of the experimental group of young goats, was significantly ($p \leq 0.01$) higher, than in the control group, throughout 60 days of experiment. There were also significant differences in hematological and biochemical blood tests. The young goats from the supplemented group showed higher level of red blood cell counts and lymphocyte levels in comparison with the control group. These differences were statistically significant ($p \leq 0.01$). There were also significant differences in the level of triglycerides, inorganic phosphorus and protein level. The acid-base balance test, as well as the control group's ionogram, showed a higher pH level ($p \leq 0.05$), act.$HCO3^-$, std.$HCO3^-$, BE, TCO2, SAT, K +, Cl^- ($p \leq 0.01$), while the anion gap, Na + indices were significantly lower ($p \leq 0.01$).

Calves- and lamb-level association between serum 25-hydroxyvitamin D concentrations and cryptosporidiosis

Szérum 25-hidroxi-D-vitamin-koncentráció és a cryptosporidiosis közötti kapcsolat borjakban és bárányokban

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Abstract

Cryptosporidiosis, a frequently detected enteric diseases, has been commonly observed among ruminants in Eagean Region of Turkey. This study was dedicated to analyze relationships among selected calves (n=50) and lambs (n=20) populations

diagnosed within cryptosporidiosis in Aydin, Turkey to those of serum 25-hydroxyvitamin D concentrations

In the present study diagnosis was based on microscopical examination of diarrheic calves (n=40) and lambs (n=10). Diseased calves belong to 6 different farms, whereas 2 sheep flock were involved. Aged matched healthy controls (n=10 calves and n=10 lambs) were also involved for comparative analysis. Diagnosis of cryptosporidiosis was made based on rapid diagnostic test kits and modified Kinyoun acid-fast staining.

Serum 25-hydroxyvitamin D concentrations (mean±SD) among diseased and healthy calves were 22.39±10.15 and 64.33± 26.52 mg/dl, respectively. On the other hand regarding diseased and healthy lambs serum 25-hydroxyvitamin D concentrations ranged 19.08±5.55 and 56.91±23.41, respectively.

Especially in field conditions, Veterinary Surgeons who could not possibly evaluate Vitamin d profiles, should aware of deficiency, consequently controlled treatment with correct dosages should be considered in cryptosporidiosis.

Key words: neonatal calves, lamb, vitamin D.

Introduction

Cryptosporidium parvum a highly zoonotic, worldwide observed pathogen (Chalmers & Giles, 2010) is responsible for enteric diseases of neonatal calves and lambs (De Graaf et al., 1993; Ayana & Alemu, 2015; Bjorkman et al., 2003, 2015) worldwide and Eagean Region of Turkey (Sarı et al., 2008; Özdal et al., 2009; Erman et al., 2000). Cryptosporidiosis characterized by greenish-yellow watery diarrhea followed by loss weight, dehydration, depression and similar symptoms. (Bjorkman et al., 2015). In sheep cryptosporidiosis and its impact on health when compared to those of cattle has usually been asymptomatic (Imre et al., 2013).

In ruminants given vitamin D is synthesized within two routes; 7-dehydrocholesterol converted vitamin D3 with ultraviolet light in the skin and vitamin D2 derived from forages (Vasconcelos & Galyean, 2007; McDowell, 1989). On the other hand suckling neonatal calf and lambs are required vitamin D for growth and development (Rupel et al., 1932; Bechdel et al., 1937; Bonniwell et al., 1988; McErlean & McAllister, 1982). In diarrheic neonates vitamin levels might alter, in which this study aimed at detecting serum 25-hydroxyvitamin D concentrations among calves and lambs populations diagnosed within cryptosporidiosis in Aydin, Turkey.

Materials and Methods

In the present study 40 calves and 10 lambs feces were collected directly from rectum into clean caps with gloves from 6 different farms and 2 flocks located in Aydin, Turkey. On the other hand for the analysis of vitamin

D blood samples were withdrawn from *Vena jugularis* to 4 ml serum tubes. All blood and feces samples were carried with cold chain to Adnan Menderes University, Laboratory of Veterinary Faculty. Diagnosis of *C. parvum* was based on microscopical examination of diarrheic neonatal calves (n=40) and lambs (n=10) feces stained with Kinyoun acid-fast and rapid diagnostic test kits (Bovid-5 AG test kits, Anigen). Aged matched healthy controls (n=10 calves and n=10 lambs) were also involved for comparative analysis.

Vitamin D [25(OH)D3] analysis practiced with separated serum samples on device [Savant POCT analyzer (Beijing Savant Biotechnology Co., Ltd., China, Turkish side distributor RDA Group, Istanbul, Turkey)] based on fluorescence immuno-chromatographic method.

Results

Serum 25-hydroxyvitamin D concentrations (mean±SD) among diseased and healthy calves were 22.39±10.15 and 64.33± 26.52 mg/dl, respectively. On the other hand regarding diseased and healthy lambs serum 25-hydroxyvitamin D concentrations ranged 19.08±5.55 and 56.91±23.41, respectively.

Conclusion

In general function of Vitamin D is enhanced plasma calcium and phosphorus levels by different stimulator activity and finally normal mineralization of bone (Horst et al., 2003). Strikingly it was denoted that vitamin D participates on growth, health and innate immune system in newborns (Clancy et al., 2013) and similar effect was presented in adult cattle (Nelson et al., 2012). Immune system of neonatal calves is immature even they born and this situation predispose them to neonatal disease (Cortese, 2009). Furthermore as was observed within the cell culture, antibacterial peptite cathelicidin might have effects on cryptosporidium sporozoite regarding its replication (Giacometti et al., 2003). Vitamin D might affect stimulation of cathelicidin expression consequently initiates variable defense cells alteration (Valencia et al., 2011; Wang et al., 2004) as well as indirectly vitamin D effect of different immune function (chemotaxis, cytokine release, inflammation) through cathelicidin (Niyonsaba et al., 2006) and intestinal collectivity (Otte et al., 2009).

In our study vitamin D levels were considerably lower in infected with *C. parvum* calves and lambs in contrast to healthy animals. Given aforementioned studies, it might be suggested that vitamin D might effect in the development of the disease among infected neonates. Furthermore taking into account vitamin D deficiency as well as depend on the lack of positive effects of vitamin D on immune system, it may be the reason why the neonatal animals predispose to the disease. Through the lack of absorption of vitamin D, taken with orally or by the milk, from the

intestine may be seen as a co-factor for development vitamin D deficiency in the diarrheic neonates. Finally especially in field conditions, Veterinary surgeons who could not possibly evaluate Vitamin D profiles, should be aware of deficiency, consequently controlled treatment with correct dosages of cryptosporidiosis.

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A borjak újszülöttkori pancytopeniája néven ismert kórkép egy szokatlan formájának hazai előfordulása

Occurrence of an unusual form of bovine neonatal pancytopenia in a Hungarian herd

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Összefoglalás

Korábban, 2007. és 2012. között Európa-szerte, több országban észleltek fiatal borjakban egy addig ismeretlen kórképet. A kórkép az esetek túlnyomó többségében egészségesen, megfelelő fejlettségi állapotban, a fajra jellemző vemhességi idő után született, jó kondícióban levő borjakon jelentkezett, és általában az igen jó menedzsmentű, átlagon felüli állat-egészségügyi ellátásban részesülő állományokban, jó tartási feltételeket biztosító gazdaságokban okozta a legnagyobb károkat.

A borjak újszülöttkori pancytopeniája (bovine neonatal pancytopenia, BNP) a legelfogadottabb magyarázat szerint immunpatológiai kórkép, amelyet az anyai szervezetbe (vélhetőleg vakcinázással) bejuttatott alloantigének váltanak ki, és amely a föcstej kiszopása után jelentkezik olyan borjakon, amelyek az apai vonal révén a tehénbe (az anyába) bejuttatott alloantigénnel megegyező típusú MHC-antigéneket hordoznak sejtjeik felületén.

Esetleírásunkban egy olyan halmozottan jelentkező kórképet ismertetünk, mely egy nem vakcinázott állományban lépett fel. Ez egyben azt is bizonyítja, hogy a kórkép „spontán” is felléphet, a korábban a kialakulásáért felelősnek tartott vakcina alkalmazásától függetlenül. A kórkép „spontán” előfordulásának ez az első hazai bizonyítéka.

Kulcsszavak: bovine neonatal pancytopenia, BNP, vaccination, immunopathology

Summary

Between 2007 and 201 throughout Europe a previously unknown disease was reported among young calves, which occurred mostly in well-developed calves which were born in time healthily and were in good condition. The problem caused high economic losses mostly in well managed herds, where veterinary care and keeping conditions were above average level.

The disease was called bovine neonatal pancytopenia (BNP) and is considered to be an immunopathological disease caused by antibodies transmitted with the colostrum which were generated by alloantigens administered with vaccines to the cows. The problem appeared clinically only in calves inheriting alloantigens from the paternal side corresponding with the alloantigen residues present in the vaccines produced in tissue cultures.

We report a case which appeared in a non-vaccinated herd, and proves that the disease may appear spontaneously, without application of the vaccine which was previously blamed to induce it. This is the first report on the spontaneous occurrence of BNP in Hungary.

Bevezetés

A szarvasmarha újszülöttkori pancytopeniája kórképet néhány éven keresztül (2007-2011 között) Európa szerte, több országban észlelték fiatal borjakban (Friedrich et al., 2009). Az első németországi előfordulásokról megjelent beszámolót követően egyre több közlemény jelent meg, melyek szerint előfordult Belgiumban (Pardon et al., 2010), Franciaországban és az Egyesült Királyságban is (Penny et al., 2009). A kórképet több néven említették, ezek közül a szélesebb körben ismertek: a borjak vérzéses betegsége (bleeding calf syndrome), ismeretlen oktanú vérzéses diatézis (idiopathic haemorrhagic diathesis, „blood sweating disease”), neonatal haemorrhagic diathesis (NHD) (Pardon et al., 2010), a legáltalánosabban elfogadottá vált elnevezés, a szarvasmarha újszülöttkori pancytopeniája, vagy BNP (Bell, 2011). A kórkép az esetek túlnyomó többségében egészségesen, megfelelő fejlettségi állapotban, a fajra jellemző vemhességi idő után született, jó kondícióban levő borjakon jelentkezett. A betegség általában az igen jó menedzsmenttel rendelkező, átlagon felüli állat-egészségügyi ellátásban részesülő állományokban, jó tartási feltételeket biztosító, és BVDV ellen vakcinás védelmet folytató gazdaságokban okozta a legnagyobb károkat. Az utóbbi miatt a kezdetektől felvetődött és folyamatosan vizsgálat tárgyát képezte a kórkép esetleges kötődése a borjak vírusos hasmenését okozó vírus okozta fertőzéshez. Ennek az a magyarázata, hogy a vérzéses diathesis halmozott előfordulása igen gyakran társult az erősen virulens BVDV-1 vagy a BVDV-2 által okozott fertőzésekhez (Corapi et al., 1989). Ugyanakkor a BVDV kimutatására irányuló vizsgálatok sorra sikerteleneknek bizonyultak a BNP esetekből. Mint később

kiderült, a kórkép nagy valószínűséggel immunpatológiai alapon fejlődhetett ki egy nagy hatékonyságú adjuvánst és inaktivált BVDV-t tartalmazó vakcina hatására (Sauter-Louis et al., 2012), amelynek piacról való visszavonása óta nem figyeltek meg újabb esetet.

2016. nyarán egy Bács-Kiskun megyei állományban egymás után számos borjú hullott el rövid időn belül egy olyan kórok miatt, melynek hátterében nem a fiatal (néhány napos korú) borjak körében szokványos és emiatt általában viszonylag könnyen kivédhető ok állt. A kezdeti vizsgálatok után, a jellegzetes klinikai tünetek és kórbonctani elváltozások miatt felmerült ugyan a BNP újabb előfordulásának gyanúja, de a Pregsure vakcina kereskedelemből történő kivonása óta már több év telt el úgy, hogy az e vakcina alkalmazásához kötött kórkép előfordulásáról nem számoltak be. A korábban hibáztatott vakcina tehát biztosan nem álhatott az adott állományban halmozottan előforduló elhullások hátterében.

Anyag és módszer

Állomány

A vizsgálatunk alapját képező telepen 109 tehenet számláló állományt tartottak, melyben három bika, három, nagyjából azonos létszámú tehéncsoporthoz beosztva (37, 37 és 35 tehén), csordaszerű („háremszerű") tartásban látta el reprodukciós feladatát, így csoportonként nyomon tudták követni, hogy melyik csoportban melyik bikától származtak a borjak. Az egyik ilyen csoportban a bikától a csoportot alkotó 37 tehén vemhesült. Az általunk végzett vizsgálatok lezárásáig 30 tehén jutott el az ellésig, a született 30 borjú közül 14 hullott el 2016. június 01. és 2016. július 20. között. További hét tehén a vizsgálatok lezárása után ellett meg. A 14 elhullott borjú közül az utolsó ötöt küldték vizsgálatra az Állatorvostudományi Egyetem Járványtani és Mikrobiológiai Tanszékére; a többi (korábban elhullott) állatot vagy nem küldték vizsgálatra, vagy más diagnosztikai intézetekbe küldték. Ez utóbbi esetekben az elvégzett vizsgálatok alapján változatos diagnózisok (tüdőgyulladás, septicaemia, coli-hasmenés stb.) érkeztek vissza a telepet ellátó állatorvoshoz.

A telepen kizárólag a Merial által gyártott Trivacton-6 oltóanyagot alkalmazták a borjúkori hasmenés kivédésére. Az oltóanyag a borjú szövettenyészeten elszaporított, inaktivált rotavírust és coronavírust, továbbá *Escherichia coli* antigéneket tartalmaz. A vakcinával a teheneket kétszer oltották: egyszer 3 hónappal a hárembe helyezés után, másodszor pedig közvetlenül az ellés után. (Vagyis a vakcina használati utasításától igen eltérő, de igen kevés munkatöbblettel járó, „kényelmes" vakcinázási protokollt alkalmaztak.)

Vizsgálati anyag

Vizsgálatainkat az Állatorvos-tudományi Egyetem Járványtani és Mikrobiológiai Tanszékére küldött öt borjútetemen, továbbá három alvadásgátolt vérmintán végeztük.

A vérminták két, jellegzetes klinikai tünetet mutató borjúból, valamint az egyik borjút ellő tehénből származtak

Boncolás, kórszövettani vizsgálat

A beküldött, elhullott borjak külső vizsgálata során látható elváltozások makroszkópos megfigyelése és képi rögzítése után, az állatokat felboncoltuk, a megfigyelt elváltozásokat ugyancsak dokumentáltuk, majd a különböző szervekből mintákat vettünk kórszövettani és kiegészítő patológiai vizsgálatok céljára. A rutinvizsgálat során megszokott eljárás szerint a vizsgálatokra vett mintákat 4%-os pufferolt formalaldehid-oldatban fixáltuk, majd ezekből a mintákból 5 µm vastagságú metszeteket készítettünk, és ezekből hematoxilin-eozin festést végeztünk a rutindiagnosztikában megszokott eljárás szerint.

Ezt követően rutin bakteriológiai vizsgálatokat végeztünk aerob körülmények között, véres agar lemezeken. A makroszkópos vizsgálatok során vett, de formalinnal nem fixált minták homogenizátumaiból molekuláris virológiai (PCR) vizsgálatokat végeztünk a BVDV kimutatása vagy jelenlétének kizárása érdekében egy már korábban leírt vizsgálati módszer felhasználásával (Kovago et al., 2013).

Eredmények

Klinikai tünetek

A borjak egészségesen, életerősen születtek, de a kolosztrum kiszopását követően, pár napos korban legyengültek, kedvetlenné és étvágytalanná váltak, megbetegedtek és végül elpusztultak.

A tünetek jellemzően 1–4 hetes borjakon jelentkeztek, és a kórképet a kifejezett vérzékenység és anaemia uralta. A beküldött három vérminta (két beteg borjú és egy tehén) vizsgálatával a borjakban rendkívül alacsony vörösvérsejt-számot (alacsony hematokrit értéket) tapasztaltunk.

A véralvadási zavart jelezte a minden bőrsérülést (injekció beadását, vérszívó rovarok szúrását) követő tartós vérzés mellett a spontán vérzések megjelenése (vérvizelés, orrvérzés, ínyvérzés stb.). A klinikai tünetek jelentkezése után a borjak általában rövid időn belül (2–7 nap alatt) elhullottak. Az elhullások aránya igen nagy volt, a megbetegedő borjak többsége a gondos állapotjavító (palliatív) kezelés dacára elpusztult.

Kórbonctan, kórszövettan

A külső vizsgálattal a beküldött öt állatból egyben hasmenés jeleit (bélsárral szennyezett farok és végbéltájék), kiszáradást (beesett szemgolyók, száraz nyálkahártyák) és a kornak megfelelő állapotú, de enyhén gyulladt köldöktájékot láttunk.

A boncolásnál valamennyi tetemben rosszul alvadt vért, kitágult, folyékony vért tartalmazó köldökvénát, a bendő falában, a szívburok és a mellhártya alatt pontszerű és diffúz vérzéseket találtunk. A boncolások során kiterjedt vérzéseket lehetett látni testszerte: a bőrben, a bőr alatti kötőszövetben, a nyálkahártyák alatt, az emésztőszervek falában, a

zsigerekben. A savós testüregekben vörhenyes exsudátum halmozódott fel.

A máj világos színű, enyhén megnagyobbodott, szakadékony állományú volt. Az előgyomrok, az oltógyomor és a zsigerek (lép, máj, vese stb.) falában a savóshártya felől is jól látható nagy kiterjedésű vagy petecsszerű vérzések voltak megfigyelhetők. A vékonybélben a nagy mennyiségű gázt tartalmazó, világos, híg tartalmat és enyhén kipirult nyálkahártyát láttunk (hurutos bélgyulladás) a külső vizsgálattal hasmenés-gyanúsnak ítélt állatban.

Az öt borjúból egy másikban a hasüreg felnyitásakor a hasüregben nagy mennyiségű (kb. 1,5 liter) zavaros exudatumot, a hashártya fali és zsigeri lemezeire tapadva vaskos álhártyákat láttunk (súlyos, fibrines hashártyagyulladás). A savóshártyák alatt és a szövetek között kiterjed vérzéseket lehetett látni.

A szívizomzatban egy esetben világosabb csíkok és foltok voltak megfigyelhetők A légcsőben nagy mennyiségű habos tartalmat találtunk, a tüdőben sötétvörös, tömött tapintatú területek, légtelen lebenyek voltak láthatók, a metszéslapról közepes mennyiségű, habos tartalom volt levonható (hurutos-kruppos tüdőgyulladás).

Kórszövettani vizsgálattal a tüdőparenchyma vérereiben súlyos fokú vérérfal-károsodást és multiplex trombusképződés (disseminált intravasalis coagulopathia, DIC) jeleit figyeltünk meg.

A kórszövettani vizsgálat során a már felsorolt elváltozásokon kívül a nyirokszervekben (thymus, lép, nyirokcsomók) súlyos fokú lymphoid depletiot, a csontvelőben a myeloid állomány kiterjedt pusztulását, a vesében a tubulusok üregében vörösvértest szétesésre utaló hemoglobin cilindereket és következményes tubulushám-elfajulást figyeltünk meg.

Bakteriológiai vizsgálatok

A bakteriológiai vizsgálat során tüdőmintákból *Mycoplasma bovis* és *Escherichia coli* baktériumok voltak kitenyészthetők, amelyek tipizálását az Állatorvos-tudományi Egyetem Járványtani és Mikrobiológiai Tanszéke, illetve az MTA Agrártudományi Kutatóintézete végezte el. Az *in vitro* végzett gyógyszer-érzékenységi vizsgálat során az *E. coli* baktériumtörzs a legtöbb kipróbált antibiotikummal (amoxicillinnel, neomicinnel, oxytetraciklinnel, streptomicinnel, penicilinnel, marbofloxacinnal, flumequinnel, flórfenikollal, gentamicinnel, enrofloxacinnal, cefquinonnal) szemben rezisztensnek, doxiciklinnel és cefalexinnel szemben érzékenynek bizonyult.

Virológiai vizsgálatok

A borjak vírusos hasmenését okozó vírus (bovine viral diarrhoea virus, BVDV) kimutatására irányuló polimeráz láncreakcióra (PCR) alapozott vizsgálat valamennyi állat esetében negatív eredménnyel zárult).

Megbeszélés

A kórelőzmény és a fenti vizsgálatok eredménye alapján egyértelműen megállapítható, hogy az állományban a borjak újszülöttkori pancytopeniája (bovine neonatal pancytopenia, BNP) néven ismert kórkép jelentkezett. (Mellékleletként szelénhiányos szívizomelhalást, másodlagos fertőzésként *E. coli* okozta bélgyulladást, illetve szövődményként fibrines hashártyagyulladást, köldökgyulladást és kruppos tüdőgyulladást is megállapítottunk.) A diagnózist a korábban részletezett vizsgálati eredményeken kívül a tulajdonostól és a kezelő állatorvostól kapott szóbeli (telefonos) adatok is alátámasztották.

A kórelőzmény szerint a borjak egészségesen, életerősen születnek, de a kolosztrum kiszopását követően, pár napos korban legyengültek, kedvetlenné és étvágytalanná váltak, megbetegedtek és végül elpusztultak. A kórkép kizárólag az egyik bika mellé beosztott tehenek borjain jelentkezett, de ebben a tehéncsoportban sem minden tehén borja betegedett meg.

A kórkép nem jelentkezett az adott bika mellé beosztott tehéncsoport minden borjában, mert (1.) az elsőborjas tehenek még esetleg nem hangolódtak át az adott bika MHC antigénjei ellen, vagy (2.) MHC típusuk azonos volt a bikáéval, esetleg (3.) a vakcina nem megfelelő alkalmazása miatt egyes tehenek nem hangolódtak át a reziduális sejtmaradványok ellen.

A megelőzés megoldható az adott bika kizárásával a tenyésztésből. Az esetleírásban szereplő állományban például a három tenyészbika közül csak egynek az utódaiban jelentkezett a kórkép. A tulajdonos tanácsunknak megfelelően selejtezte az adott bikát, és azóta újabb BNP eset nem fordult elő az állományban. Ugyancsak tanácsunk alapján a diagnózis ismeretében az adott bika mellé beosztott 37 tehén közül még nem ellett 7 tehén borjait (lásd Anyag és módszer, Állomány fejezet) dajkásította, vagyis olyan, ugyanabba a csoportba tartozó tehenekkel neveltette fel, melyek borjain nem jelentkezett a BNP, így tejük vélhetőleg nem tartalmazott a bika MHC antigénje ellen képződött, és ezért a borjakra veszélyes ellenanyagokat. A hét borjút veszteség nélkül felnevelte, bár tartásuk fokozott gondosságot igényelt, hiszen nem kolosztrumot kaptak, hanem olyan tehén tejét fogyasztották, amelyik 2-6 héttel korábban ellette a saját borját. A BNP jelentkezése mind a hét borjú esetében elkerülhető volt. Ezek a gyakorlati tapasztalatok is alátámasztják a kórkép immunpatológiai eredetét, kolosztrum (illetve anyai ellenanyagszint) dependenciáját, illetve a diagnózisunk helyességét.

A kórelőzményben szerepelt az is, hogy a teheneket a Merial által gyártott Trivacton-6 oltóanyaggal immunizálták a borjúkori hasmenés elkerülése érdekében. Bár a tulajdonosnak ezt nem tanácsoltuk, a vakcinázást azonnal megszűntette, mihelyt a diagnózisunkat megkapta.

A Trivacton-6 nem tartalmaz BVD elleni komponenst, illetve adjuválószerként is más adalékanyagot tartalmaz, mint a Pregsure, így semmiképpen nem tartottuk indokoltnak a vakcinázás megszűntetését. Jelen helyzetben így egyértelműen nem dönthető el, hogy a BNP kórkép megszűnése kizárólag a bika tenyésztésből való kizárása miatt szűnt meg, vagy a Trivacton-6 vakcina alkalmazásának felfüggesztése is szerepet játszott a veszteségek megszűntetésében.

Az eset kapcsán mindenképpen megállapítható az, hogy a BNP kialakulásában korábban egyértelműen felelőssé tett Pregsure vakcina alkalmazása nélkül is kialakulhat a kórkép. Hogy ez önállóan, csupán az apai és anyai vonal eltérő MHC haplotípusának következtében is kialakulhat, vagy kell hozzá valamilyen immunstimuláló és/vagy adjuválószert tartalmazó vakcina alkalmazása is, az a jelen eset kapcsán a fentiek miatt nem eldönthető, további vizsgálatokat igényel. Az mindenesetre leszögezhető, hogy a Pregsure vakcina forgalomból való kivonása nem jelenti a kórkép eltűnését, és a BNP állományok vonatkozásában sporadikus, de adott állományban halmozottan jelentkező kártételével a továbbiakban is számolni lehet.

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Comparative study on correlations between coproscopic examination and organ diagnosis for trematodes and lungworms in slaughtered lambs

Vágóhidi bárányok koproszkópos és szervdiagnosztikai vizsgálati eredményeinek összehasonlítása trematodák és tüdőférgek tekintetében

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Introduction

One of the main health challenges facing sheep producers worldwide is how to control endoparasites, which may cause massive economic losses. The gold standard in endoparasitic diagnosis is coproscopic examination. In this study we set out to estimate if the results of coproscopic examination correlated with liver and bile organ diagnosis in cases of fasciolosis and in lungs in cases of lungworm disease. We also sought to determine if prevalence estimates from faecal tests correlate with clinical signs in lambs during live animal studies at the slaughterhouse.

Materials and Methods

In total 221 lambs were investigated from two slaughterhouses in Styria (Austria) between June and November 2016. Faecal samples, condemned lungs and livers and the bile of all randomly selected animals were collected during the slaughter process. *Fasciola hepatica* and *Dicrocoelium*

dentriticum were the target species. Lungworm larvae were differentiated into large (*Dictyocaulus* spp.) and small (family Protostrongylidae) species.

Results

F. hepatica and *Dictiocaulus* spp. were not detected in any of the samples taken. The total prevalence of *D. dentriticum* was 14.5 %. *Protostrongylus rufescens* was detected in five out of 221 animals, corresponding to a prevalence of 2.3 %.

All samples where negative for *F. hepatica* in faeces, biliary fluid and liver. Overall, we observed that prevalence from coproscopic examinations was higher than prevalence estimated from *post mortem* examination. No significant association could be established between infestations of livers and the detection of adult leeches in the biliary fluid. Infestations of *D. dendriticum* could not be detected purely by macroscopic observation of organs.

We detected a significant correlation between eggs in faeces and bile and adult infestations in the gall bladder. Sedimentation gave more sensitive results. However, we did not find a correlation between clinical parameters, such as body condition score or conjunctival colour, and infestations with small liver flukes or lung worms. While we found a correlation between abnormal pulmonary sounds and lungworm infestation, we could not conclude from the clinical examination alone the degree of infestation with *D. dendriticum*. We could detect *D. dendriticum* in intensive as well as extensive herds. Female animals were more frequently infested with flukes than males, in contrast to the results for lungworm disease. Only young animals between 143 and 195 days were infested with the small lungworm. Further, this study revealed a seasonal correlation with egg numbers in the biliary fluid and faeces. Highest numbers for *D. dendriticum* occurred in September.

Conclusion

The fact that cattle appear more susceptible than sheep to *F. hepatica* most likely reflects different immunities, and accounts for enormous contaminations of sheep pastures. Consequently, we can assume that the sheep used for this study were not co-pastured with cattle. Alternatively, sheep appear to be more susceptible to dicrocoeliosis compared to goats and bovines. In this respect the different feeding behaviours likely play a role. Thus, a consequence of anthelmintic treatment is that while it may lead to a complete reduction in adult worm numbers, faecal larvae excretion could be detectable for up to three weeks post-treatment.

A possible explanation for seasonal differences in prevalence could follow from migration of adult parasites from the gall bladder into the hepatic bile ducts. Results suggest that climate change and projected temperature increases of between 1 °C and 4

°C will lead to a change in the epidemiology and intensity of lungworm infestations.

Clinical anatomy: a glance in the abdomen of the cattle

Klinikai anatómia: pillantás a szarvasmarha hasüregébe

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Abstract

Clinical anatomy is the study of anatomy as it relates to clinical practice. It is not designed to give general anatomical knowledge, but it focuses on specific structures and issues which clinicians may encounter in a clinical setting.

Although clinical anatomy is usually considered a subject to be taught during the academic studies, it should be considered of paramount importance in the context of the continuing education of veterinary practitioners. First of all because it is very exciting exploring anatomy with a clinical experience in mind. Secondly because this practice is very useful to clarify clinical uncertainties by studying in depth specific anatomical position of the single organs. With this kind of analysis it is possible to better understand body systems interconnections and to try to go back to clinical doubts that clinicians may have experienced in clinical practice. Therefore, clinicians are prompted to practice it whenever possible, for example each time it occurs that an examined patients dies, or had to be put down due to adverse prognosis. In these cases, the post mortem investigation should be addressed not only to the investigation of the specific pathology affecting the animals, but also to a broader exercise of the topographical anatomy of other organs. That means, practitioners should take advantage of the availability of cadavers, wherever they origin, to practice clinical anatomy addressed to uncertainties or doubts experienced in the past.

The presentation will give an example of the potentiality offered by this kind of practice: in this respect, the presentation will introduce the participants to the abdomen of the cattle. Different clinical findings and diseases of the abdomen will be considered by the way of an anatomical intra-abdominal exploration.

Does current veterinary medical education meet the future requirements of cattle practice?

A jelenlegi állatorvosi képzés megfelel-e a szarvasmarha-gyakorlat jövőbeni követelményeinek?

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Abstract

In view of the enormous increase in knowledge and the increased demands, the question arises whether the current ideal of omnicompetence, aimed at the German and most European veterinary schools is still realistic. The goal of these studies was therefore to get the opinion of representatives of cattle clinics at German universities, bovine practitioners and farmers on this topic. For this purpose, eight university professors of all German veterinary colleges were personally interviewed, and questionnaires were used to conduct a survey among practitioners (n = 174) and farmers (n = 232).

Almost all university professors and practicing veterinarians consider that the current broad based curriculum does not provide the essential knowledge and skills required of a newly qualified veterinarian. Most respondents would prefer clinical tracking while maintaining general approbation (licensure).

According to this survey, traditional veterinary activities continue to play a major role in bovine practice. However, the importance of production medicine and counseling will increase, with veterinarians and farmers taking the view that veterinary graduates are less competent in such areas as feeding and milking techniques than non-veterinary consultants.

Key words: veterinary curriculum, tracking, cattle practice, skills, competences

Introduction

In the Federal Republic of Germany, five universities offer veterinary medicine as a course of study. The first semester of study is always the winter semester, whereby each

year 1,064 new admissions are available (Berlin 167, Giessen 210, Hannover 256, Leipzig 141, Munich 290). Objectives and organization of veterinary training are standardized by the Approbation Ordinance for Veterinarians (TAppV). According this ordonance, the objective of the studies is an academically and practically trained veterinary surgeon who is capable of practising the veterinary profession responsibly and independently and of undergoing further training and ongoing advanced training. The duration of the studies including the time for the final examination is 5½ years. All subjects listed in Directive 2005/36/EC and in the document "Evaluation of Veterinary Training in Europe, Principles and Process of Evaluation 2007" are basically covered. The course of study is generalized throughout, there is no priority training or species or specialty specialization (tracking). After successfully passing the exams the graduate receives the general approbation, allowing him or her to practice veterinary medicine with no restrictions.

For years there have been discussions as to whether this aim of training omnicompetent veterinarians is realistic, in light of the enormous growth of knowledge in all areas, as well as modern intellectual requirements, or if a tracking system that allows students to follow a particular "track" of specialization would not better suit the requirements of the practice. This is especially true in regard to present and future farm animal practices. The ongoing change in agriculture structure is affecting cattle farming in Germany with increases in size of herds and increasing application of technology. At the same time demands are increasing for higher quality of the foods and food products produced. Aspects such as biosecurity, reduction of antibiotic use and animal welfare are gaining in importance. Simultaneously, livestock owners must compete internationally, in regard to production prices. Parallel to this is the diversification of production into conventional and organic farming. Although there has always been criticism that upon completion of training veterinary graduates do not possess the required capabilities, and in particular not the skills necessary to perform their professional tasks, in traditional veterinary medicine these deficits could usually be compensated for by in-depth job training and practical experience. In the meantime, however, livestock owners are no longer lay persons, but are increasingly educated agriculturists, often with an academic background. What is more, competition is arising in these areas from other occupational groups, beginning with agricultural scientists and artificial insemination organizations to consultants from the feed industry. Suggestions on improvement of methods for imparting the knowledge and skills required in farm animal practices range from compulsory and continuing postgraduate advanced training to a fundamental restructuring of veterinary studies.

The aim of this present report is to compile the collective opinion of faculty representatives from veterinary colleges, practitioners and cattle owners regarding their assessment of future developments in this field and the strategies and solutions they would suggest.

Methods

To obtain the opinions of university faculty representatives eight buiatric professors were personally interviewed using a standardized survey. Bovine practitioners were surveyed using a structured questionnaire made available at two large national buiatric congresses. In addition it was possible to fill out the questionnaire online, available on the internet portal of a trade journal "Der Praktische Tierarzt." A total of 174 bovine practitioners took part in this survey. The points of view of cattle owners were assessed by means of an online survey that was made available on the website of an agricultural trade journal. A total of 232 farmers in all regions of Germany took part in the survey. The focus of this survey was to estimate the competence of veterinarians in the various areas of cattle production and the expectations that cattle owners have of veterinarians.

Results

Results of the interviews with representatives of veterinary faculties

Almost all German buiatric professors (7/8) consider the present state of veterinary training outdated. All of those questioned wish to see a reorganization of the course of study including, to a greater or lesser degree, a curricular concentration. Five out of eight desire the possibility of an optional immersion module in the last year of study, similar to that as now practiced in Vienna, for example. The others support the idea of a more comprehensive solution by breaking down the course of study into basic training and specialization. Nonetheless, almost all (7/8) of those questioned considered it important that the general approbation be maintained. Two German professors supported the "Dutch Model," i.e. a specialization during the course of study, with general approbation, however with the limitation that the graduates only practice in his or her area of specialization. In regard to buiatric training all of the professors interviewed expressed their conviction of the continuing importance of training on individual animals (individual-animal examination and treatment), on the one hand because this is the basis of herd health management, but also on the grounds of animal protection. At the same time more emphasis must be made on herd health management and production medicine, either for all students or just for those who are interested. The question as to whether graduating students are adequately qualified to meet the future demands of a farm-animal practice was universally answered in the negative. All of those interviewed expressed the opinion that the training program must urgently be adapted to new needs, either on

the level of pre- or postgraduate studies. In addition, an expansion of subject matter is necessary. Prophylactic measures, herd health management and evaluation of housing systems need to be more intensely considered in the course of study. Courses on feeding, including feed evaluation need to more specifically deal with the requirements of cattle farming. All-in-all, an improvement in knowledge relating to agricultural economics is necessary.

In addition, the majority of those interviewed favor improved advanced training in bovine medicine. As long as the course of study is generalized and multi-species oriented post-graduate specialization will be indispensable.

In regard to the future importance of the bovine practice all of those interviewed assume that the significance will generally remain unchanged, whereby this will depend upon the training and consequently on the competence of veterinarians in this field and the resultant market position of the veterinary profession.

Results of the Practicing Veterinarians Surveyed

The majority of the 174 bovine veterinarians (69.6%) surveyed had graduated between the years 2000 and 2017. Of these, 58.6% were employed in a practice, the rest were owners or co-owners of a practice. Most of the practices (40.1%) employed between 3½ and 6 veterinarians. The range of employed veterinarians was from 1 to 20, with an average female proportion of 60.1%. Only 22.5% were pure bovine practices. The rest were mixed veterinary practices with a very high bovine proportion. Most considered their competence upon graduation retrospectively as adequate at best, and that primarily in theoretical ability. In regard to their abilities upon beginning work in their profession they noted massive deficits, in particular regarding practical procedures in surgical interventions (43% of those surveyed), in the area of herd care (45%), in evaluation of feed and feed practice (61%) as well as in the areas of animal husbandry (56%), milking technique (44%) and business management (53%). A number of reasons were given for the poor degree of competence in these areas that are important in a bovine practice: Inadequate teaching of theoretical knowledge (60%), inadequate practical intramural (63%) and extramural (40%) training, but also a lack of practical experience (72%). Overall, 62.6% of the practicing veterinarians would consider it useful to offer elective courses of specialization. Nonetheless, the large majority considers it essential to retain the general approbation.

Of all subjects that should receive greater emphasis in the future, business management was in first place (according to 55% of those surveyed) followed by communication with owners and farmers (37.5%). Far behind that were herd care (14.8%) and surgery (13.9%). The activities most commonly carried out in the bovine practice remain treatment of

individual or groups of animals (96.6%), followed by consultation in regard to prophylactic measures (85.5%).

In the meantime, however, herd health management is of considerable importance (78.2%). Consultations regarding housing, feeding and milking technique, however, are only offered by 43.1% of those surveyed. In the opinion of the large majority of those surveyed (83.3%) curative activities were of importance, as was herd health management, the latter considered very important by almost all those surveyed (97.1%). In regard to the academic standard of graduates only 1.7% of the practitioners assume they are both very well equipped theoretically and practically. Of those surveyed 60.9% feel, at least, that graduates have the necessary basic foundation, however more than a quarter (28.7%) are not convinced of this.

More than half of those surveyed worry that in certain areas there will be increasing competition from service providers other than veterinarians. This is especially the case in areas such as feeding (77.6%), claw treatment (65.5%) and reproduction (57.5%). This type of development, however, is strictly rejected by only 39%, whereas 32% of the practicing veterinarians would welcome a closer cooperation with other service personnel. The chance of obtaining new assistants for a bovine practice is considered very difficult by 52.9% to nearly impossible by 15.5%. The main reasons for this would appear to be poor working conditions (43.1%), work in rural regions, considered unattractive by some young veterinarians (42%) as well as insufficient training in farm animal medicine in college (27.6%). Only 19.5% consider the large proportion of female veterinary students as a cause of the lack of young veterinarians in bovine practices.

Results of the online survey of farmers

The majority of the 232 participants in this survey were dairy farmers mostly with herds of 50 to 150 cows. Only 7% had more than 400 animals. Just fewer than 40% of the participants indicated that they wished to increase their herd size in the next five years. In response to the question of allocation of tasks on the farm veterinarians are still responsible for the tasks they have always taken care of such as diagnosis (67.1%), treatment of serious diseases (e.g. uterine prolapse, as many as 82.7% by the veterinarian) and surgical interventions. Rather surprisingly only 91% of caesarian sections or abomasal displacement surgery were performed by veterinarians and as few as 8.3% of claw treatments. However, on 60% of the farms veterinarians are responsible for pregnancy examinations and treatment of fertility disorders, but artificial insemination is carried out by veterinarians in only 17%. The farmers considered veterinarians very competent in all of the areas mentioned above, and these classical veterinary activities are judged to continue to be very important in the future by 95% of those surveyed. In contrast, farmers see deficits in the veterinarians in the areas of feeding (50.0%), business

management (59.6%) and milking technique (44%). In particular, however, they complained about lack of understanding of production conditions and economic relationships. The reasons given for enlistment of non-veterinarian consultants were: Greater competence (43.7%), less expensive (38.8%) and enhanced accessibility (13.4%). At least, however, 40% of those surveyed would be willing to call on a veterinarian for consultations in the future if the veterinarian was equally competent or if they even offered such consultation at all (16.8%). However, 19.4% noted that this would be question of cost. In the end, however, 38.4% said they could not even imagine that a veterinarian would take on these activities.

The importance of integrated veterinary herd health management or production medicine was estimated by 47% of the participants as important to very important and according to 69% this importance will increase in the future. In particular, farmers with large herds affirmed this question almost unanimously (connection of herd size with response to this question was significant; p=0.001). Nonetheless, single animal treatment was judged to remain important to very important in the future as well by almost all of the farmers questioned (96%).

Discussion

The majority of buiatric professors at German universities consider the course of veterinary studies to be in urgent need of reform, whereby most believe there is no way around specialization during the time of study. The default goal of training omnicompetent veterinarians at the university, as it stands in the German approbation ordinance is considered to be absolutely unrealistic. The majority of practicing bovine veterinarians is of the same opinion. The Dutch or Viennese models are suggested as possible alternatives (Eysker, 2002; van Beukelen, 2004; Murauer, 2013). Nonetheless, in spite of expressing support for such focus or specialization during training almost all implore that the general approbation be retained.

Complaints of ostensibly inadequate first-day competence are not limited to bovine practices in Germany, but are also expressed by small-animal and equine practitioners (Hällfritzsch et al., 2005; Veith, 2006). Introduction of a "tracking system" in which undergraduates can chose a particular species track, as discussed both positively and negatively in the international literature, is offered as a solution to this problem (Vecerek, 2006; Klostermann et al., 2009; Walsh et al., 2009; Crowther et al., 2014 a,b). At the same time it is generally accepted that in light of the enormous growth of material it is impossible to teach students all possible methods and techniques in all areas of a wide-ranging veterinary education in the 5 to 6 year course of studies. According to the report of Bravo Moral (2016) 25 of 74 European veterinary

schools already practice such a system of specialization tracking. The main areas of specialization offered are companion animal medicine, farm animal medicine, food safety and/or veterinary public health and research. The often mentioned disadvantage of this kind of specialization is the fact that young students must decide too soon on a particular field of activity. Another alternative would be to accept the fact that due to the large diversity of areas in veterinary medicine the course of study cannot provide job proficiency, but rather job qualification so that, just as in human medicine, advanced and ongoing training is obligatory (Hoffmann, 2011).

In spite of the long-standing discussion about veterinary herd health management the present survey clearly shows that the in general the focus for German bovine practitioners is still traditional veterinary activity, and in the opinion of veterinarians and cattle owners that is not likely to change in the near future. In contrast to other food animals such as pigs and poultry, for cattle individual animal examination and treatment is very important. That said, herd health management is also gaining in importance in the cattle industry. To date, however, most farmers are of the opinion that veterinarians are less competent than other consultants in important related areas such as housing conditions, feeding, milking technique, breeding, etc. The surveyed practicing veterinarians are of the same general opinion and advocate an increased consideration of these areas in undergraduate training. It is, however, not a secret that similar demands are being expressed in other areas such as small-animal medicine, equine medicine, food science, etc. so that one comes to the conclusion that in Germany, and in other countries as well, a reform of under- and postgraduate veterinary studies should be considered.

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A survey on teaching of veterinary students on ruminant medicine in veterinary schools in Europe

Felmérés az állatorvostan-hallgatók kérődző gyógyászati oktatásáról az európai állatorvosi egyetemeken

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Abstract

The changing background and the high percentage of female veterinary students has raised concerns about the decreasing number of graduates who are entering food animal practice. On the other hand it has been shown that an attractive and high-quality teaching of food animal medicine may result in increased interest even in students without farm background.

The aim of the present survey was to obtain an overview by questionnaire about ruminant medicine teaching at European and North American Veterinary Schools and to analyse this comparing the two and attempt to define best practice.

The overall response was 46.9% (38 of 81 surveys, Europe 29 of 42, 69%; North America 9 of 39 surveys, 23%). Minor differences have been found in theoretical teaching; however organisation of practical teaching (e.g. group size, inclusion of the students, special ruminant track) differed significantly. Many vet schools reported increasing difficulties to ensure a sufficient case load for teaching and increasing financial pressure. Veterinary Schools running a ruminant hospital (>500 cases/year) seem able to offer better condition for practical teaching. Additionally it seems that the ability to offer a transportation service to transport cattle to the veterinary hospital is important in obtaining sufficient cases.

We conclude that to ensure that a sufficient number of graduates enter a career in food animal medicine a high quality teaching is necessary and this requires financial and personal commitment by the university and its staff since given the current prices for milk and meat, ruminant veterinary hospitals have to be subsidised to deliver their teaching.

Veterinary medical education for cattle at the University of Veterinary Medicine Hannover

A szarvasmarha-egészségügy oktatása a Hannoveri Állatorvosi Főiskolán

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The Clinic for Cattle of the University of Veterinary Medicine Hannover is a traditional veterinary teaching hospital with unit for herd health management and production medicine applying newest standards. The core curriculum for bovine species currently includes:

- Internal medicine and surgery (propaedeutics, organ-, infectious and metabolic diseases, surgical procedures, including pain management, diagnostic imaging) in theory and clinical training.
- Theriogenology (propaedeutics, diseases of the mammary gland, gynaecology, andrology, obstetrics, neonatology, biotechnology) in theory and clinical training.
- Herd and production medicine including practical training in herd health management.

Forms of teaching are lectures, seminars, self-directed learning, hands-on clinical training and e-learning. The Clinical Skills Lab, a central unit of the University enables the graduate students to gain practical skills enhancing basic diagnostics procedures in order to increase the efficiency when working with living animals. Preparation of written case reports are mandatory for 3rd and 4th year students. Elective courses are offered in small groups for 3rd or 4th year students (maximum 8 students/group) in form of practicals, farm visits, seminars and "problem-based learning" courses. Four courses of 10 weeks of specialized clinical training (maximum 20 students/course) are offered for 5th year students with an intraclinical rotation including orthopaedics and radiology / aseptic surgery and anaesthesia / internal medicine, diagnostic imaging, laboratory medicine / gynaecology, obstetrics and udder

diseases / reproduction biotechnology / herd health management. Students are expected to take (supervised) case responsibility from admission to the clinic until release or referral to the Department of Pathology (including participation in necropsies). Surgical trainings (rumenotomy, caesarean section, procedures on lower legs, genital tracts, udders, heads, tails) and interdisciplinary seminars in preventive herd health management are organized. Clinic owned cows and bulls are kept to improve students' skills.

The Clinic for Cattle is open all year round and provides a 24h/7d emergency service. It offers a service to transport animals into and out of the clinic. The current case load covers a wide variety of different cattle diseases and is used for teaching undergraduate and graduate students. Herd health aspects are integrated into the curriculum and are taught directly on farms. An own diagnostic laboratory is open for samples aquired from patients of the Clinic, research projects, and field practitioners, their results serve educational purposes as well. The Clinic collaborates intensively with other veterinary faculties (national or international) and enables training for their students.

There are numerous differences between a hospital-based and an ambulatory-based training. A hospital-based training form permits more students to see learning cases under clinical conditions than ambulatory services, ensuring for the students sufficient time for case management in the hospital. Teaching hospitals provide students to understand all disease characteristics when having the possibilities of controlling the cases daily, which is usually not given in ambulatory services. However, disadvantageous is that hospitalized animals are seen as individuals without their herd mates and are not seen in their original environment, including housing, feeding, milking, calving and hygienic conditions. Aspects of biosecurity provide major challenges in particular in teaching hospitals but also in ambulatory trainings.

Agricultural production is in a transition from family operated farms to agribusiness enterprises. The experience of the Clinic shows that for economic and welfare reasons also at large-scale dairy farms a profound veterinary service for individual cases is demanded, in particular for the top 25% of breeding cows. Large farms often provide a well equipped section for the veterinarian to allow high quality internal, surgical and obstetrical interventions. Today herd owners and farm managers often have a high level academic background and long-standing experience. They are seeking intensive advice in many aspects to improve herd health, welfare and productivity (e.g. nutrition and feed management, stall design and housing, milking, disease control and prevention, welfare, biosecurity, waste management, product quality and safety). In the consulting service veterinarians are in direct competition with consultants having

other academic backgrounds (e.g. agro-engineers). To keep future veterinarians, competitive aspects of farm management need to be strengthened in veterinary undergraduate and postgraduate education. The optimal way of veterinary teaching appears to be a combination of a hospital-based profound basic veterinary training and an ambulatory on-farm training covering also aspects of herd and farm management.

The Clinic for Cattle in Hannover expects the following perspectives regarding veterinary medical education for cattle:

- keeping theoretical and practical education based on individual clinical service of intra- and extramural patients,
- core facility of the education of diseases for undergraduate students remains the veterinary teaching hospital, but possibilities of the ambulatory service will be enhanced,
- considering aspects of infectious disease control, treatment, specific and non-specific issues of prevention together with aspects of non-infectious diseases including nutrition, production, reproduction and environmental circumstances involving biotechnological methodologies in close cooperation with other units of the production animal education at the University,
- covering topics of public health, zoonotic diseases, waste management, animal welfare, food security, biosecurity,
- managing herd health, population health and epidemiology,
- enhancing national and European accredited clinical and laboratory capacities,
- enabling and supporting internships for local and international undergraduate students and residency programs for postgraduates,
- offering courses of specific subjects for practitioners in form of continuous education (e.g. meetings, seminars, workshops),
- maintenance and extension of research conditions related to the above mentioned fields, enabling thereby a direct implementation of their results into the education.

Currently the duration of the veterinary curriculum in Hannover is 5.5 years, which includes teaching in basic veterinary science, farm and companion animals as well as public health. Knowledge is increasing very rapidly in all veterinary disciplines. Doubts exist whether the increasing depth in the entire veterinary education can be maintained in all aspects of the profession within the existing frame of the curriculum. Thus, a discussion appears necessary on either extending the duration of the training period or splitting the curriculum in a common „basic education” and a further more specialized training, e.g. „farm animals and public health” and „veterinary medicine of companion animals”.

The Clinic of Ruminants in Kosice (Slovakia) – teaching of the ruminant medicine

Kérődzők Klinkája Kassán (Szlovákia) – a kérődző-egészségügy oktatása

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Education in the ruminant medicine at the ***Clinic of Ruminants*** in Košice is based on the lectures and practical training of the obligatory subject *Diseases of ruminants*, which includes all main specialties, i.e. internal medicine, surgery, orthopaedics, reproduction, andrology and artificial insemination, and the obligatory subjects *Andrology* and *Artificial insemination*, and *Herd health management*. The obligatory elective subjects provide the students with opportunity to gain hands-on experience in the subjects *Diagnostics of the metabolic disorders in animals* and *Assisted reproduction* (Table 1.). Practical training in the diseases of ruminants in the 5th year of study (semester 9 and 10) are done in-house in the form of demonstrations of patients in a clinical auditorium and also externally on the nearby livestock and small ruminant farms. The exercises can take various forms (examination of sick animals, sampling of blood or other biological material, detection of pregnancy, drug administration, orthopaedic surgery, treatment of puerperal diseases, etc.) In the subsequent semesters (11 and 12) in the 6th year of study, the teaching of *Diseases of ruminants* has the form of six-week blocks with a quarter of the students of the 6th year (20 – 25 students/block). The block combines two subjects: Diseases of Ruminates and Herd health management (3 days on the university farm), and the Clinical practice (one week at the Clinic). The university farm is provided by the orthopaedic unit (two claw crushes), surgery room (caesarean sections, rumenotomy, and omentopexy), and laboratory. The subject *Diseases of ruminants* is completed after the six-week block by taking a state examination (commission exam). Practical exercises under the subject *Andrology and artificial insemination* are scheduled during the first ten weeks and take place in-house, in the purpose-designed practice rooms. The in-house exercises are attended by two groups in parallel and last two hours. The practical

exercises in the last three weeks are conducted outside the university on the university farm. Biological material for training purposes is mainly obtained from bulls, which are kept at the Clinic of Ruminants. Other animals include male goat, ram and dog, which also belong to the clinic. The practical training in *Herd health management* is divided into the activities on the clinic's premises and analyses of farms with dairy herds. At the clinic, the students practise using clinical indices in the evaluation of condition, nutrition and feeding of the animals, their hygiene and welfare as well as the risks of mammary gland diseases. They also become acquainted with the basic documentation and its processing. This experience is later used by the students on the farms to analyse the animal holdings with the aim of providing advice. A protocol is drawn up at the end of the practical training, with records of the findings from the farm and recommendations to the farm's management. The practical training in *Diagnostics of the metabolic disorders in animals* takes place in the winter semester of the 5th year of study and includes collection and analysis of biological material from the clinic's patients and the animals from the university farm with a subsequent interpretation of the results. At the end of a semester, the students work in groups of 2 – 3 to analyse the results obtained from the patients and assess the severity of the metabolic disorders. The subject *Assisted reproduction* is included in the studies in the 10th semester. It informs and acquaints the students with the basic and currently most widely used methodological procedures in assisted reproduction both in animal and human medicine. Practical exercises are conducted in clinical and laboratory rooms of the Clinic of Ruminants and on the university farm, where the students train the basic assisted reproduction methods.

In addition, a total of 90 hours of the obligatory **Clinical practice** is prescribed at the ***Clinic of Ruminants*** in the 5th and the 6th year of study. The clinical training starts in the 5th year (semester 10) and continues in the 6th year (semester 11 and 12). The clinical practice lasts one week within a predefined period and is done in small groups (5 – 10 students). The clinical training takes place on the clinic's premises which are used for clinical activities and animal housing. The students engage in diagnostics and therapy of the hospitalized animals under supervision of a veterinary surgeon on duty. They perform all necessary tasks and activities relating to diagnostics and therapy. In addition to the clinical training at the clinic, the students also do field practice on the university farm or other farms around Košice as required by the breeders. They are supervised by a teacher of the clinic. Emphasis is placed on the acquisition of additional skills and experience in the area of production diseases of dairy cows, the ability to solve reproduction problems, orthopaedic diseases, diseases of young animals, and

taking preventive measures to decrease morbidity and losses in production.

Table 1. Subjects of the Clinic of Ruminants with number of hours per semester (13 weeks/semester)

Subject	**Semester**									
	VIII		IX		X		XI – XII		Total	
	L	P	L	P	L	P	L	P	L	P
DR			**3**	**3**	**3**	**3**	**1**	**1**	**85**	**85**
A&AI	2	2							26	26
HHM							0	2	0	26
DMD			1	2					13	26
AR					1	2			13	26
CP						30		60		90
								Total	137	253

L – lecture; P- practical; DR – Diseases of ruminants; A&AI – Andrology and artificial insemination; HHM – Herd health management; DMD – Diagnostics of metabolic disorders; AR – Assisted reproduction; CP – Clinical practice

Effect of post-parturient metabolic disorders on reproductive performance in dairy cows

Az ellés utáni anyagforgalmi zavarok hatása a tejelő tehenek szaporodásbiológiai teljesítményére

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Abstract

The successful genetic selection for higher milk production caused a dramatic decline in the reproductive performance of dairy cows all over the world during the last decades. Achievement of optimum herd reproductive performance (calving interval of 12 or 13 months) requires concentrated management activities especially during the first 100 days following calving. The following management activities are needed to pursue during the early postpartum period to reach or approach the optimal reproductive performance such as careful surveillance and assistance at calving, prevention of periparturient metabolic diseases, early diagnosis and treatment of post parturient uterine diseases, accurate detection of oestrus, correct timing of insemination, reducing the effect of heat stress and early pregnancy diagnosis. Among these main activities only early diagnosis, and treatment of periparturient metabolic diseases and their effects on reproductive performance and milk production are discussed.

Fertility in dairy cows reflects the cumulative influence of metabolic, endocrine, and postpartum health components. Energy imbalance seems to be one of the most important factors, but the complex interactions of the aforementioned factors can be considered in order to be able to improve fertility, at the same time body condition score (BCS), glucose, non-esterified fatty acid (NEFA), insulin-like growth factor-1 (IGF-1), leptin concentration from calving to AI cannot explain the low fertility rate.

Cows should be challenge-fed during the dry-off period and early lactation to prevent

the incidence of metabolic disorders of the puerperal period such as milk fever, acidosis, ketosis and fat cow syndrome. These diseases can increase the incidence of reproductive diseases and reduce reproductive performance. Prevention is more preferable than treatment and requires close attention to nutrition and management. The maintenance of good body condition at calving and the provision of a high-density energy diet that does not produce a fatty liver in early lactation are also very important to minimize the detrimental effect of NEB on the return of oestrous cycle after calving.

Key words: dairy cow, negative energy balance, ketosis, fatty liver, fertility, milk production

Összefoglalás

A nagyobb tejtermelésre irányuló sikeres genetikai szelekció miatt az elmúlt évtizedekben a tejelő tehenek reproduktív teljesítménye az egész világon drámaian csökkent. Az optimális állományszintű szaporodásbiológiai teljesítmény (12-13 hónapos két ellés közti időszak) elérése szempontjából fontos az ellést következő 100 nap teendőinek összehangolt szervezése.

Az ellés utáni időszakban a következőkre kell kiemelt figyelmet fordítanunk, hogy az optimális szaporodásbiológiai teljesítményt elérjük: gondos és szakszerű ellési felügyelet, valamint szülészeti segélynyújtás, az ellés utáni anyagforgalmi megbetegedések megelőzése, a méh eredetű megbetegedések korai diagnózisa és kezelése, az ivarzók felismerése, a mesterséges termékenyítés helyes időzítése, a hőstressz hatásainak mérséklése, és a korai vemhességvizsgálat. Ezek közül most csak az ellés körüli időszak anyagforgalmi megbetegedéseit és azoknak a fogamzásra gyakorolt hatásait tárgyaljuk.

A tejelő tehenek fertilitása tükrözi a szevezet anyagcseréjének, endokrin helyzetének, valamint az ellés utáni általános egészségügyi állapot együttes hatását. Úgy tűnik, hogy az energiai egyensúlyi zavar az egyik legfontosabb tényező, de a fertilitás javítása érdekében a fentiek összetett kölcsönhatásait is figyelembe kell vennünk. Ugyanakkor az elléstől a MT-ig terjedő időszakban a kondíció (BCS), a glükóz, a nem-észterezett zsírsav (NEFA), az inzulin-szerű növekedési faktor-1 (IGF-1), és a leptin koncentráció változásaival nem lehet megmagyarázni az alacsony termékenyülési eredményeket.

A szárazon álláskor és a laktáció korai szakaszában a tehenek szükségletének megfelelő takarmányozással az ellés körüli időszak metabolikus megbetegedéseinek (ellési bénulás, acidózis, ketózis, zsírmáj szindróma) előfordulása csökkenthető. Ezek a betegségek növelhetik a szaporodás-biológiai problémák megjelenését és csökkenthetik a szaporodás-biológiai teljesítményt. A megelőzés előnyösebb a kezelésnél, ezért az első 100 nap takarmányozására és a menedzsmentre kiemelt figyelmet kell fordítanunk. Az elléskori jó kondíció megtartása és magas energiatartalmú takarmány adása,

amely a laktáció korai szakaszában nem okoz májelzsírosodást, nagyon fontos, hogy minimalizáljuk a negatív energia egyensúly-nak az ellés utáni ciklusba lendülésre kifejtett káros hatását.

Kulcsszavak: tejelő tehén, negatív energiai egyensúly, ketózis, zsírmáj, termékenység, tejtermelés

The successful genetic selection for higher milk production in Holstein cows has nearly doubled the average milk production in the United States since 1960, to over 11,000 kg/year. Over the same time period, there has been a dramatic decline in the reproductive performance of dairy cows (Butler, 2000). The conception rate has decreased from 66% since 1948 (Trimberger, 1948), to about 50% until 1975, and further more until recently to about 33.1% in Spain (López-Gatius, 2003), 33.4% in Israel (Galon, 2008), 37% in Canada (Bouchard and Du Tremblay, 2008) or 41% in Japan (Nakao, 2008). The average number of days open (interval from calving to conception) and the number of services per conception have increased substantially (Snijders et al., 2001). In order to decrease the longer lactations and the number of cows culled for reproductive reasons it is very important to improve our reproductive management practices (Silva, 2003). Achievement of optimum herd reproductive performance (calving interval of 12 or 14 months with the first calf born at 24 months of age) requires concentrated management activities especially during the first 100 days following calving. Early postpartum breeding of dairy cows results in more calves, and higher milk production per lactation (Britt, 1975). Poor reproductive performance can reduce the number of calves born and milk production and may increase the cost of therapy and semen.

The following management activities like careful surveillance and assistance at calving, prevention of post-parturient metabolic diseases, early diagnosis and treatment of post-parturient uterine abnormalities, accurate detection of oestrus, correct timing of insemination, reducing the effect of summer heat stress and early pregnancy diagnosis are needed to pursue during the early postpartum period to reach or approach the optimal calving interval (Szenci, 2008). Among these main activities, only the effect of post-parturient metabolic diseases on fertility will be discussed.

Dairy cattle are usually in negative energy balance (NEB) in the first weeks of lactation because of energy intake during this period is less than half of the energy requirements for milk production. Therefore the gap between energy input and output during early lactation must be met through increased non-esterified fatty acid (NEFA) production. On the other hand the energy requirements of dairy cows is met in 60-70% by volatile fatty acids (acetate, propionate and butyrate) fermented in the rumen therefore ruminal fluid is one of the most important source of energy metabolism in the dairy cow. In the periparturient period feed intake is

physiologically suppressed which will cause a lack of dietary energy intake which results in a lack of gluconeogenesis and in turn, a lack of glucose to allow for complete oxidation of NEFA. The incomplete oxidation of fatty acid will contribute to the increased production of ketone bodies (β-hydroxybutyrate /BHB/, acetone, acetoacetate), which may cause ketosis and fatty liver. According to Oetzel (2004) two types of ketosis may develop: "Type I ketosis" with low blood glucose due to lack of precursors for gluconeogenesis without fatty liver occurring at 3 to 6 weeks postpartum; "Type II ketosis" associated with fatty liver just before or at calving manifesting 5 to 15 days after calving. Increasing amount of ketone bodies may also contribute to suppress feed intake. Salivation is also decreased during calving due to break of chewing or reduced period and intensity of chewing. This may contribute to the development of clinical or subclinical rumen acidosis especially when the ration of concentrate is not limited for a few days around calving. Ruminal acidosis may also negatively affect rumen motility and appetite. Similarly according to Geishauser et al. (2000) severe hypocalcaemia may be associated with decreased abomasal motility but it is not clear whether this can be generalized to either clinical milk fever or subclinical hypocalcaemia.

The rapid increase in energy requirements at the onset of lactation results in NEB that may begin a few days before calving and usually reaches its most negative level (nadir) about 2-3 weeks later and used to extend 10-12 weeks until the beginning of the usual breeding period (Bell, 1995; Butler and Smith, 1989).

The NEB that develops spontaneously in dairy cows, represents a physiological state of undernutrition. The severity and duration of NEB is primarily related to differences in dry matter intake and its rate of increase during early lactation. Calving in moderate condition (3 to 3.75) and maintaining feed intake during the periparturient transition period are key factors to reducing NEB and avoiding metabolic problems (milk fever, acidosis, ketosis and fat cow syndrome) that are deleterious for high milk production and fertility.

Following parturition, regardless of NEB due to elevated plasma FSH concentrations a wave of follicular development starts in 5 to 7 days after calving. Three types of follicular development have been described as follows (Beam & Butler, 1997):

1. The first dominant follicle ovulates between Days 16 to 20 after calving
2. The first dominant follicle does not ovulate, a turnover and a new follicular wave follow
3. The dominant follicle fails to ovulate and becomes cystic

The development of non-ovulatory dominant or cystic follicles prolongs the interval for

the first ovulation to 40-50 days after calving. Ovulation of a dominant follicle during early lactation depends on the re-establishment of pulsatile LH secretion (Butler, 2001). The NEB as physiological state of undernutrition may suppress pulsatile LH secretion and reduce ovarian responsiveness to LH stimulation and by this way deters ovulation (Butler, 2001; Jolly et al., 1995).

It is worth mentioning that prolonged anovulatory anestrous in only 30% of cows can be connected with reduced fertility caused by NEB (Rhodes et al., 1998; Staples et al., 1990). It seems that NEB can influence the timing of first postpartum ovulation, by which it can negatively affect fertility (Butler, 2001; Darwash et al., 2001). Cows remaining anovulatory for > 50 days of lactation are less likely to become pregnant during lactation and will be culled (Frajblat, 2000).

Plasma progesterone (P4) concentrations used to elevate during the first two or three postpartum ovulatory cycles (Spicer et al., 1990; Staples et al., 1990; Villa-Godoy et al., 1988). The rate of increase in P4 is reduced or moderated by NEB (Spicer et al., 1990; Villa-Godoy et al., 1988). At the same time, high dietary intake (both energy and protein) may also increase the metabolic clearance of P4 in high yielding cows (Wiltbank et al., 2001). Through the regulation of the uterine environment, P4 plays an important role in embryonic development and growth. A slower rate of increase in P4 after ovulation may decrease the embryo growth by Day 16 and is associated with low fertility (Butler et al., 1996; Mann et al., 1996; Shelton et al., 1990). Early postpartum NEB may adversely impact quality of oocytes during the 80-100 days required for follicle development and oocyte development (blastocyst formation rate), which exerts another carryover effect on fertility (Britt, 1992; Kruip et al., 2001). However, it is very difficult to reconcile the effect of NEB on follicles and oocytes with the effect of high dietary energy on oocyte quality and development to blastocysts in dairy cows (Armstrong et al., 2001; Boland et al., 2001). It appears that extremes in energy status in either direction may negatively influence fertility (Butler, 2001).

Fertility in dairy cows reflects the cumulative influence of metabolic, endocrine, and postpartum health components. Energy imbalance seems to be one of the most important factors, but the complex interactions of the aforementioned factors can be considered in order to be able to improve fertility (Butler, 2001), at the same time BCS, glucose, NEFA or IGF-1 from calving to AI cannot explain the low fertility rate (Snijders et al., 2001).

Cows should be challenge-fed during the dry-off period and early lactation to prevent the incidence of metabolic diseases of the puerperal period such as milk fever, acidosis, ketosis and fat cow syndrome. These diseases can increase the incidence of reproductive diseases and reduce

reproductive performance. Prevention is more preferable than treatment and requires close attention to nutrition and management (Radostits et al., 1994). The maintenance of good condition at calving and the provision of a high-density energy diet that does not produce a fatty liver in early lactation are also very important to minimize the detrimental effect of NEB on the return of oestrous cycle after calving.

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Effect and consequences of changing the length of the dry period in a dairy farm

A szárazonállás hosszának megváltozása és következményei egy tehenészetben

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Abstract

The conventional length of dry-off period in dairy cattle farm is 60 days, during this period absence of milking and regulated nutritional management are performed. Several studies had focused on decreasing the length of the dry period, and based on

literature data shortening the length is possible, but, due to our knowledge at the moment, at least a small dry period must be kept. The advantage of shortened dry period would be the economical advantage in longer milk production in the ongoing lactation.

In our study, which was carried out in a Hungarian large-scale dairy farm, fresh cow monitoring examinations were performed based on metabolic profiling of freshly calved animals. We hypothesized, that we would find difference in different dry-off length animals in some selected metabolic parameters, in the occurrence of peripartal clinical diseases, in some milk production parameters as well as in postpartum reproductive parameters. Our retrospective data collection showed that planned, 57-63 days dry-off period was reached in around half of the study animals. During our data collection we divided the cows either shorter or longer and equal than 58-day dry-off period.

During our study we sampled 10% of the freshly calved cows (n=164) in order to perform metabolic profile testing. Besides the blood biochemistry measurements (BUN, BHB, NEFA, TG, Ca, Na, P, K), we collected data regarding to postpartum clinical diseases (LDA, PM, mastitis, lameness, RFM, hypocalcaemia) as well as to milk production parameters, and to some selected reproductive parameters.

During data analysis we analysed 100 cows' data with a parity higher than one. Binary logistic regression revealed statistical significant result ($p<0.05$), regarding to occurrence of puerperal metritis ($p=0.0126$; OR=0.25) in case of Na ($p=0.01$; OR=0.77) and K ($p=0.04$; OR=1.07) blood concentrations. The analysis of every other selected parameter revealed a non-significant result.

As a result of our study we can conclude, that shortening the dry period of dairy cows did show some significant change in the occurrence of clinical diseases postpartum and some selected metabolic parameters in the blood. However, literature data are not consistent with our findings, further research must be carried out, mainly in accordance with the blood biochemistry parameters. As a final conclusion, shortened dry period may be advised for our dairy farms.

The use of transvaginal ultrasound guided access to ovary in dairy cattle

A petefészek transzvaginális ultrahang vezérelt vizsgálata tejhasznú szarvasmarhákban

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Background

Transvaginal ultrasound guided access to ovary in dairy cattle has been used many years in reproduction in dairy cattle. The principle of the method is to use an ultrasound transducer in a long holder to transvaginal visualization of the ovarian structures. A puncture of targeted ovarian structures has been made either by long needle or by short disposable needle attached to a long needleholder. Depending on other additional instruments this technique allows the collection of cells, fluid and tissue from the ovary as well as to inject active substances and cells into the ovary or simply to ablate ovarian follicular structures to stop their endocrinological activity.

Practical use of transvaginal ovarian puncture

Ultrasound-guided oocyte aspiration (ovum pick-up, OPU) combined with *in vitro* embryo production represents replacing procedures of traditional embryo transfer technologies in cattle. Transvaginal follicular aspiration has been performed in adult animals, prepubertal heifers and pregnant animals as well. Ovum pick-up represents the most frequent use of transvaginal ultrasonography in dairy cattle. Follicular fluid (FF) sampling (transvaginal follicular aspiration, TVFA) has been performed for the evaluation of the oocyte microenvironment. Various biochemical or endocrinological examinations of the FF collected by TVFA have been done when dominant or preovulatory follicles larger than 10 mm in diameter were primarily chosen for FF collection *in vivo*. Recovery of the FF for acid-base balance (ABB) analysis from live animals has been rarely described due to the technical difficulties involved in anaerobical follicular fluid sampling. Recently a new instrument especially designed for collection of FF for

ABB analysis was used. This instrument prevents the occurrence of preanalytical errors in FF collection. Transvaginal ultrasound-guided biopsy (TUB) has been developed and used for biopsy of corpus luteum (CL) to study gene expression profiling and the effect of hormones on CL function. Similarly , the use of TUB for collection of ovarian tissue containing follicles for in vitro culture or cryopreservation has been reported. Intrafollicular injection of different substances has been described, such as hCG, LH, phosphate-buffered saline or insulin-like growth factors Injection into ovarian stroma was also performed. The volume of the solution injected varies from 20 to 300 µl, the use of one or double channel instruments has been reported. Intraluteal injection directly into the corpus luteum (ILT) was performed to determine the minimum luteolytic dose of cloprostenol. Intraluteal treatment was performed by modified equipment for ultrasound guided transvaginal ovarian aspiration. Intrafollicular insemination (IFI) is based on direct introduction of a sperm suspension into a preovulatory follicle. IFI was used experimentally on a dairy herd with low fertility during the warm season of the year and succesful pregnancies were reported. Intrafollicular oocyte transfer (IFOT) is the promising approach that complements currently used procedures in *in vitro* embryo production in cattle. IFOT into the preovulatory follicle is followed by an timed insemination and embryos are collected seven days later.

Conclusion

Transvaginal ultrasound ovarian puncture in dairy cattle represents a traditional method used for many purposes in reproductive biotechnology and research in dairy cattle. New ways of using this method are still being developed.

Field trials for the assessment and optimization of the selected insemination day in dairy cows using a quantitative on-farm milk progesterone test

A termékenyítések optimális időpontjának meghatározása egy kvantitatív, helyszíni tej progeszteron teszt segítségével tejhasznú tehenekben

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Abstract

In order to ensure a good reproductive performance of cows in a modern and economical dairy farm, an effective heat observation and insemination management is of essential importance. However, due to the intensive breeding of high-performance animals, there is an ongoing reduction in heat duration and symptoms, which makes it much more difficult to determine and use the heat. Concerning this matter, the aim of this study was to examine the Hormonost® Farmer Test by Biolab GmbH with regard to the practicable measurement of the milk progesterone and the associated determination of the ideal insemination day. Therefore, progesterone measurements of milk samples collected from April to December 2014 from a total of 152 Holstein Friesian cows in a Saxon dairy farm were performed at three specified time points (day of insemination, six days and 20 days later). The evaluation of the collected data was carried out by determining the fertility indicators of the farm before and after the investigations. Through the use of the Hormonost® Farmer Test, the company's fertility statistics were significantly improved during the study. Here, the progesterone-controlled animals got pregnant significantly faster (service period: 80 days vs. 118 days), and already 20 days after insemination, a significant difference between later pregnant and non-pregnant cows could be determined ($P < 0.05$). In summary, this study has shown that on-farm milk progesterone measurements using the Hormonost® Farmer Test are a fast and cost-effective alternative for improving the

reproductive performance of a dairy farm. The Hormonost® Farmer Test ensures the reliable identification of animals which got an insecure heat diagnosis, which show quiet or inconclusive heat symptoms or suffer from a luteal weakness and provides reliable information for taking appropriate measures to improve the fertility of the livestock.

Relationships of management practices and reproductive parameters in Holstein cows on large commercial dairy farms

Menedzsment gyakorlat és a szaporodási mutatók közötti összefüggések holstein-fríz tehenekben egy tehenészeti telepen

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Abstract

The aim of our study was to examine the associations of management practices and reproductive performance in Holstein cows, and to explore the differences by parity. Management practices were surveyed between May and November 2015 in 34 large dairy herds in Hungary. Individual data of 23,784 cows that calved in 2014 in the surveyed herds were gathered. The associations of management, parity and parameters were analysed by mixed effects models. Regarding heat abatement we found that ventilation with sprinklers was associated with the shortest breeding interval ($p<0.01$), the shortest calving to conception interval ($p<0.01$), and the highest odds of being pregnant by 200 days in milk ($p<0.01$). The application of transrectal ultrasonography was associated with reduced days to first service ($p<0.05$), shorter breeding interval ($p<0.05$) and higher odds of pregnancy at 200 days in milk

(p<0.05). Early pregnancy diagnosis was more advantageous in primiparous than in multiparous cows (p<0.01).

Key words: dairy cattle, reproduction, management, heat stress, transrectal ultrasonography

Összefoglalás

Kutatásunk célja az volt, hogy megállapítsuk a holstein-fríz tehenek szaporodásbiológiai menedzsmentje és mutatói közötti összefüggéseket, ill. feltárjuk ezek ellésszám szerinti különbségeit. 2015 májusa és novembere között 34 magyarországi nagy létszámú tejelő tehenészet szaporodásbiológiai menedzsmentjét mértük fel. Az elemzésekhez annak a 23.784 tehénnek az adatát használtuk fel, amelyek a felmért állományokban 2014 során ellettek. A menedzsment, ellésszám és a mutatók összefüggéseit kevert modellekkel elemeztük. A vízpermetezéssel kombinált ventiláció járt együtt a legrövidebb két termékenyítés közötti (p<0,01), ill. újravemhesülésig eltelt idővel (p<0,01), valamint a 200. tejelő napon a vemhesség legnagyobb valószínűségével (p<0,01) a hőstressz elleni védekezési módszerek közül. A szaporodásbiológiai ultrahangvizsgálatokat végző állományokban rövidebb volt az elléstől újratermékenyítésig (p<0,05), valamint a két termékenyítés között eltelt idő (p<0,05), ill. nagyobb volt a vemhesség esélye a 200. tejelő napon (p<0,05). A korai vemhességvizsgálatok nagyobb előnnyel jártak egyszer ellett tehenekben a többször ellettekhez képest (p<0,01).

Introduction

Suboptimal reproductive performance is one of the most important sources of economic loss on large dairy cattle farms (Ózsvári & Kerényi, 2004). Therefore, numerous management practices have been introduced in order to improve reproduction in cows, making reproductive management increasingly complex on dairy farms (Fodor et al., 2016a). The aim of our study was to examine the associations of management practices with reproductive indices in cows, and to explore the differences by parity on large commercial dairy farms.

Materials and methods

We gathered information about the management practices applied to cows between 22 May and 6 November 2015 during personal interviews performed on farm visits in 34 large Holstein-Friesian dairy herds in Hungary. The inclusion criteria for the farms were as follows: (1) use of computerized on-farm records, (2) continuous participation in milk recording at least since 1 January 2011 and (3) herd size ≥ 250 cows. Individual data of cows that calved between 1 January 2014 and 31 December 2014 were gathered from the Livestock Performance Testing Ltd. (Gödöllő, Hungary). After data editing a total of 23,784 cows from 34 farms remained in the dataset. Data were edited in MS Excel 2013 (Microsoft Corporation, Redmond, WA, USA).

The associations of the management and fertility practices with the following reproductive indices were evaluated: days to first service (DFS), breeding interval (IBI), calving to conception interval (CCI), first-service conception risk (CR1) and pregnancy status at 200 days in milk (P200). Associations among herd characteristics and management practices were analysed by Fisher's exact test. The associations of management, parity and reproductive parameters were analysed by mixed effects models. Pairwise comparisons were performed by Tukey's post-hoc test when more than two options were examined within a given management practice. The statistical analyses were performed in R version 3. 4. 0. (R Core Team, 2017).

Results

The method of heat abatement was associated with IBI ($p<0.01$), CCI ($p<0.01$) and P200 ($p<0.01$). Ventilation with sprinklers was linked to the best reproductive performance, however, using ventilation only was not significantly better than no heat abatement (Table 1).

Table 1. The relationship of reproductive ultrasonography and the associated management practices with the reproductive indices of dairy cows

		Days to first service	Breeding interval (days)	Calving to conception interval (days)	Pregnant at 200 days in milk
			Estimate		Odds ratio
TRUS	**Heat abatement**				
Yes	No	73.7	42.6^{abc}	165.8^{ab}	Reference^{ac}
Yes	Ventilation	64.5	42.3^{ab}	136.7^{abc}	1.51^{abc}
Yes	Ventilation + sprinklers	64.6	36.0^{c}	129.4^{c}	1.88^{b}
No	No	87.9	44.3^{abc}	172.9^{a}	0.77^{a}
No	Ventilation	76.5	43.9^{a}	142.6^{abc}	1.18^{ac}
No	Ventilation + sprinklers	76.6	37.5^{bc}	135.0^{bc}	1.46^{bc}
TRUS	**Estrus synchronization**				
Yes	Yes	64.9^{a}	37.2	132.6	Reference^{a}
Yes	No	74.7^{ab}	39.2	143.9	1.66^{a}
No	Yes	72.6^{ab}	40.6	137.3	0.88^{ab}
No	No	83.9^{b}	42.8	149.0	0.56^{b}

		Days to first service	Breeding interval (days)	Calving to conception interval (days)	Pregnant at 200 days in milk
TRUS	**Pregnancy recheck**				
Yes	Yes	66.1[ac]	37.6	131.8	Reference
Yes	No	63.6[ab]	36.7	135.9	0.98
No	Yes	79.8[bd]	42.2	139.4	0.71
No	No	76.6[cd]	41.3	143.7	0.70

[a, b, c, d] Different superscripts indicate significant differences ($P < 0.05$).

Cows in those herds that did not apply a voluntary waiting period (or where its length was <50 days) showed superior reproductive performance compared to those that applied a voluntary waiting period (VWP) of at least 50 days, except for CR1, where no relationship was found (Table 2). Lack of a voluntary waiting period was linked to larger reduction in calving to conception interval ($p < 0.05$) and larger improvement in the probability of pregnancy at 200 days in milk ($p < 0.001$) in multiparous cows.

Table 2. Associations of voluntary waiting period (VWP) and pregnancy diagnosis frequency with the reproductive indices of dairy cows

	Days to first service		Breeding interval (days)		Calving to conception interval (days)		Pregnant at 200 days in milk	
	Est.	**P**	**Est.**	**P**	**Est.**	**P**	**Odds ratio**	**P**
Voluntary waiting period[1]								
Yes	73.3	<0.01	41.2	<0.01	142.1	0.01	Reference	<0.01
No	62.2		35.2		127.8		1.45	
Pregnancy diagnosis frequency								
> 1x/week	71.0[ab]	0.03	39.1	0.40	139.2	0.64	Reference	0.10
1x/week	71.0[a]		39.5		133.0		1.38	
< 1x/week	58.1[b]		35.9		138.9		1.09	

Est.: Estimate
[1] Yes: VWP was ≥ 50 days; No: VWP was < 50 days or no VWP was applied.
[a, b] Different superscripts indicate significant differences ($P < 0.05$).

Those herds that performed transrectal ultrasonography typically used ventilation combined with sprinklers for heat abatement in the milk producing barns (p=0.03), performed hormonal synchronization (p<0.01) and rechecked pregnancy status after the early pregnancy diagnosis (p=0.03). Therefore, transrectal ultrasonography was analysed together with these practices. When the different combinations of ultrasonography and heat abatement were analysed, those herds that applied ultrasound had reduced time to first insemination (p=0.02) and showed a tendency towards more pregnant cows by 200 days in milk (p=0.07). In the analysis of CR1, the interaction of heat abatement and ultrasonography was significant (p=0.04). When ultrasonography was evaluated together with hormonal synchronization, those farms that used both practices had significantly shorter DFS than those that applied neither methods (p < 0.01). Transrectal ultrasonography showed a tendency towards increased P200 (p=0.07), and the interaction of synchronization and ultrasonography was significant, as well (p=0.02). When the different combinations of ultrasonography and pregnancy recheck were analysed, ultrasonography was associated with DFS (p<0.01), IBI (p=0.04) and P200 (p=0.02), however, performing pregnancy recheck was not associated with any of the parameters. Early pregnancy diagnosis and pregnancy recheck were associated with larger reduction in primiparous compared to multiparous cows regarding breeding interval (p < 0.01 for both practices), calving to conception interval (p < 0.01 and p < 0.001, respectively) and larger gain in the probability of pregnancy at 200 days in milk (p < 0.001 for both practices). The odds of pregnancy by 200 days in milk tended to be higher when weekly pregnancy checks were performed compared to more frequent checks (p=0.09).

Discussion

In our study, the use of ventilation and sprinklers was associated with the best reproductive performance, however, applying ventilation fans performed similar to using none of the available heat abatement methods. Farms that used ventilation fans only often reported that fans with sprinklers were originally implemented, but due to technical problems or planning errors (e.g. the bedding in the milk producing barn had become very wet), sprinklers had finished working or were switched off (Fodor et al., 2018). Efficient heat abatement is important, since the metabolic rate of lactating dairy cows is very high, that makes them extremely sensitive to heat stress (Lucy, 2001).

The application of a minimum 50-day long VWP was linked to longer time to first insemination, longer breeding interval and delayed conception after calving, resulting in a lower proportion of pregnant cows by 200 DIM, but no relationship was found with the

success rate of first inseminations. When no complications occur around calving, no more than 40-day long VWP is needed for uterine involution (Stevenson, 2005). Generally every VWP above six weeks was less profitable than the six-week long VWP, and, therefore early breeding was profitable (Inchaisri et al., 2011).

The use of hormonal synchronization protocols was associated with the application of transrectal ultrasonography, but no associations between synchronization and reproductive parameters were found. Farms should use hormonal synchronization protocols that are simple and can be managed easily (Stevenson, 2005). In herds with low estrus detection efficiency the cost of a pregnancy can be decreased by the introduction of OvSynch. However, when OvSynch is introduced into a herd with more efficient estrus detection, the cost of a pregnancy may slightly increase despite the improved reproductive results (Stevenson, 2005).

Those reproductive management programs that applied ultrasonography were generally superior regarding reproductive performance compared to those that did not use this practice. Transrectal ultrasonography enables professionals to examine the status of the reproductive tract besides early pregnancy diagnosis (Fodor & Ózsvári, 2017). Early pregnancy diagnosis creates the opportunity to identify cows that fail to conceive soon after insemination. If early pregnancy check is coupled with management measures aiming at the quick reinsemination of open cows IBI can be reduced (Fricke, 2002). In a field trial that compared reproductive results using solely rectal palpation vs. transrectal ultrasonography, DFS, IBI and CCI were reduced by 7.0, 29.6 and 12.0 days, respectively, in the ultrasound group, which generated 43.89 EUR additional net income per cow per year compared to the palpation group (Fodor et al., 2016b). It is widely accepted that early pregnancy diagnosis must be followed by a recheck at the time when the rate of pregnancy loss is reduced (Fricke, 2002). Although pregnancy recheck yields unquestionable benefits, we could not detect any significant associations between recheck and reproductive parameters.

In conclusion, heat abatement with ventilation and sprinklers, lack of voluntary waiting period of minimum 50 days, and performing transrectal ultrasonography were the most related to improved reproductive performance, therefore, these practices are recommended to use on large dairy farms. Moreover, we also demonstrated that the associations of management and reproductive performance differs by parity.

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The evaluation of neutrophil function, superoxide dismutase activity and metabolic profile ın cows with retained placenta

Magzatburok-visszatartással rendelkező tehenek neutrofil funkciójának, szuperoxid-diszmutáz aktivitásának és a metabolikus profiljának értékelése

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The objective of this experiment was to determine the alterations of pre- and postpartum immun function, superoxide dismutase (SOD) activity and serum metabolite changes in Brown Swiss cows with or without retained placenta (RP).

The study was conducted on nineteen (9 with RP and 10 healthy) cows. Peripheral blood was sampled on d -10±4 antepartum and d 10±4 postpartum for assessment of neutrophil phagocytic and oxidative burst activities by using flow cytometry. Also, SOD activity, beta hydroxybutiric acid (BHB), nonesterified fatty acids (NEFA), calcium (Ca), phosphorus, aspartate aminotransferase (AST), alkaline phosphatase, alanine aminotransferase, creatinine kinase (CK), gamma-glutamyl transferase (GGT), lactate dehydrogenase, bilirubin, cholesterol, glucose, urea, albumin, total protein and triglyceride

concentrations measured from collected serum samples at d -10±4, 3±1, 10±4 and 30±4 relative to parturition.

Neutrophil phagocytic, oxidative burst activities; phagocytic and oxidative fluorescence intensity; phagocytic and oxidative index were similar in healthy and cows that develop RP (P>0.05). But, serum SOD activity was 30±4 IU/mL and 17±2 IU/mL for cows diagnosed with RP and healthy cows at d -10±4 antepartum respectively (P<0.05). All investigated serum metabolic parameters varied significantly over time (P<0.001). Cows with RP had significantly reduced serum concentration of Ca (8,06±0,3mg/dl) and increased BHB level (1027±137,9mmol/l) when compared with healthy cows at 3±1 days postpartum. Furthermore, these cows had greater AST, GGT, CK, NEFA concentration around calving (P<0.05). Body condition scores were similar in both groups during the study period (P>0.05).

In conclusion, retained placenta has no prolonged effect on measures of neutrophil function during pre-postpartum period. But increased antepartum SOD activity might be the predisposing factor and can be used as predictor biomarker for retained placenta.

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The average culling rate and major causes of infertility of professional dairy herds in Turkey

Professzionális tejelő állományok átlag selejtezési aránya és a meddőség fő okai Törökországban

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Abstract

The objective of this study was to determine the incidence of fertility problems and culling rate of dairy cows in central and western Turkey.

Dairy herds were free from disease such as burucellosis, IBR and tuberculosis and systematic vaccination done periodically and controlled health monitoring programs. Reproductive examination of 5068 cows from 9 dairy farm (farm A 286 cows, Farm B 601 cows, farm C 505 cows, farm D 247 cows, farm E 1575 cows, farm F 260 cows, farm G 223 cows, farm H 1276 cows, farm I 95 cows) were performed by transrectal ultrasonography together with endocrinologic, metabolic, cytologic and microbiologic examinations in d 35-45 postpartum from January 2015 to December 2016.

Cows were diagnosed with retained placenta, endometritis, cystic ovarian disease, ovarian and uterine adhesion and pyometra. The proportion of those diseases to the total number of cows were 17.9%, 13.59%, 5.03%, 1.57%, 1.49%, and 1%, respectively. The cows with failed treatment post examination were culled from the herds. The proportion of culled-cows from the herds were determined 9.05%. The rate of culling was greater in the first 100 days of postpartum. Infertility (40% of all culls) was the most prevalent reason of culling (Table 1). Also the ratio of cows with clinical disorders to total cows were 22.29 percent as well as 90% of the diseases were seen in a high producing dairy cattle.

Table 1. Reason of culling for high producing dairy cattle

Major Reasons for Culling	%
Infertility problems	40
Mastitis problem	30
Poor production	18
Other problems	12

In conclusion, the incidence of diseases in farms were similar. When the management strategies of farms, shelter conditions, metabolic and clinical status of cows were examined, it was found that there were inadequate in the dry period management and that cows had entered negative energy balance during the transition period. It had been found that postpartum diseases, particularly uterine infections, have significantly increased the culling rate. And also it was found that calving to pregnancy interval extended 30-50% compared to healthy cows.

Some variables of milk fat profile in different rearing system of dairy cows

Különböző tartási körülmények között tartott tejhasznú tehenek tejzsír profiljának egyes változói

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Abstract

The aim of this study was to evaluate the differences of some fatty acids (saturated, monounsaturated, polyunsaturated, omega - 3, omega-6 fatty acids and conjugated linolenic acid (CLA)), total milk fat, concentration of vitamin A and E in milk of dairy cows, reared in the stable management entire year or on the pasture during summer and autumn months of the year. The Slovak Peid Cattle were included into this experiment. Within 3 samplings: 18.04.2017 - both groups of dairy cows were in the

stable, 20.06.2017 and 17.10.2017 – in both samplings, the first group remained in the stable, while the second group of animals was on the pasture. The results of the sampling indicate that the milk of dairy cows on the pasture had higher concentrations of saturated, monounsaturated, omega-3 fatty acids, vitamin A, total milk fat and CLA. Our findings confirm the beneficial effect of pasture on the quality of dairy cows milk. These positive affects milk products and health status of human population.

Key words: dairy cattle, quality of milk, different management system

Introduction

Milk composition is economically important to milk producers and processors and nutritionally important to consumers. It has been known for years that variations in milk composition occur; however, the composition of milk marketed nationally has been rather constant over the last 15 years, averaging 3.6 percent fat, 3.2 percent protein, and 4.7 percent lactose. The predominant fat in milk is triacylglycerol, which contains fatty acids of short- (C_4-C_{10}), intermediate- (C_{12}-C_{16}), or long-chain (C_{18}) length. The short-chain acids are synthesized within the mammary gland from acetate and beta-hydroxybutyrate; long-chain acids are almost exclusively derived from blood plasma fatty acids of dietary origin; and intermediate-chain acids arise from both sources. In broad terms, about 50 percent of the fatty acids in milk are synthetized in the mammary gland and the other 50 percent are derived directly from blood. Approximately 75% of the total consumption of fat from ruminant animals comes from bovine milk fat (Livingstone et al., 2012). The composition of milk fatty acids (FA) has numerous effects on milk quality, including its physical properties (*e.g.*, melting point and the hardness of its butter, its crystallization and fractionation of the milk fat) and nutritional value (e.g., effects on human health). Different FA (short- and medium-chain saturated, branched, mono- and polyunsaturated, cis and trans, and conjugated FA) present in ruminant milk fat are potentially positive or negative factors in the health of consumers (Meremae et al., 2012). Dairy products provide 25-60% of the total saturated fat consumption in Europe, which for decades has made them a target of concern due to the negative effects of excessive consumption of saturated FAs on human health (Givens & Shingfield, 2006). Some milk FAs, such as butyric, oleic, polyunsaturated FA (PUFA) and conjugated linoleic acid (CLA) have potential anti-carcinogenic and anti-atherogenic effects. Other FA, such as some saturated (lauric, myristic and palmitic acids) and some trans FA (tFA) have potential negative effects on human health (Cicero et al., 2012). There are three major factors that influence the transfer of dietary fat to milk : 1. ruminal biohydrogenation; 2) absorption (digestibility); and 3) deposition

in adipose tissue (Vargas-Bello-Pérez & Garnsworthy, 2013). The orientation in our study was on some variables of fat profile in dairy cows milk at different rearing system

Experimental methods

In two different environmental conditions we analyzed dairy cows milk from aspects of some fatty acids (saturated, monounsaturated, polyunsaturated, omega -3, omega-6 fatty acids, CLA, % of total milk fat), concentration of vitamin A and E. The dairy cows were reared in the stable whole the year – group 1 (in the lowland) and during summer and autumn with combination on the pasture (in region situated at the foot of a mountain) group 2. The food ration for different groups of animals include dominantly group 1: 18 kg of corn silage,10 kg of alfalfa silage, 3 kg of CCM crush corn, 0,5 kg of barley straw, 1 kg of grass hay, 1 kg of molasses, and 7 kg of grain and mineral mixture for dairy cows, water ad libitum. Group 2: during the winter time in the stable: corn silage, clover-grass silage, meadow hay together cca 38-40 kg, rape grit 0.5 kg, Protamilk Sano 0.25 kg. During the spring/summer- autumn, pasturing from 10-12. May to first snow (Oktober/November) lawn green ad libitum, 5 kg corn silage, 0.25 kg Protamilk Sano and 2 kg grain and mineral mixture produced on the farm water ad libitum. Tank and individual samples of milk were collected at 18.04., 20.06. and 16.10. We used the following methods of determination: fatty acids - standard method gas chromatography with flame array detector; vitamin A and vitamin E - liquid chromatography with fluorescent detector. For the statistical analysis within the samplings, one way Anova with the Tukey post hoc was used. In the sampling 2, the Student´s t-test was used for the statistical analysis of the indices between the cows on pasture and the cows in the stable.

Results

Table 1. The milk parameters in dairy cows in the stable (Group 1) within three samplings

Parameter	Sampling 1 (18. 04.)	Sampling 2 (20.06.)	Sampling 3 (16.10.)
SFA g/100 g milk	2.1±0.18	2.42±0.21	2.57±0.2
MUFA g/100 g milk	0.82±0.12	0.99±0.25	0.98±0.2
PUFAg/100 g milk	0.09±0.03	0.12±0.03	0.1±0.02
ω3 g/100 g milk	0.02±0.00	0.02±0.01	0.02±0.01
ω6 g/100 g milk	0.07±0.01	0.09±0.02	0.08±0.01

Parameter	Sampling 1 (18. 04.)	Sampling 2 (20.06.)	Sampling 3 (16.10.)
Vit. E mg/100 g milk	0.08±0.01	0.08±0.00	0.08±0.00
Vit. A µg/100 g milk	28.5±4.5	31.4±6.9	40.5±7.1
CLA mg/100 g milk	13.4±2.2	17.8±3.55	14.5±2.75
Fat g/100 g milk	3.2±0.27	3.75±0.37	3.87±0.51

SFA – saturated fatty acids, MUFA - monounsaturated fatty acids,
PUFA – polyunsaturated fatty acids, ω3 - omega 3 fatty acids, ω6 – omega 6 fatty acids,
CLA – conjugated linolenic acid, A -vitamin A, E - vitamin E, fat - % of total milk fat

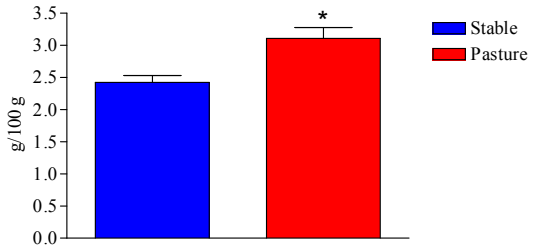
Table 2. The milk parameters in dairy cows on the pasture (Group 2) within three samplings

Parameter	Sampling 1	Sampling 2	Sampling 3
SFA	2.12±0.29[a]	3.11±0.34[b]	2.72±0.36
MUFA	0.75±0.14[a]	1.53±0.34[b]	0.84±0.38
PUFA	0.1±0.04	0.18±0.06	0.12±0.05
ω3	0.02±0.00	0.06±0.02	0.04±0.01
ω6	0.08±0.02	0.06±0.02	0.08±0.01
E	0.08±0.02	0.10±0.03	0.08±0.02
A	70.0±9.4	75.1±12.6	53.6±8.12
CLA	13.4±3.2[a]	38.9±10.8[b]	17.76±5.73[a]
Fat	3.14±0.51[a]	5.11±0.75[b]	3.91±0.3[a]

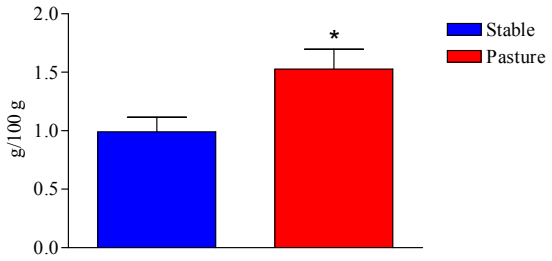
SFA – saturated fatty acids, MUFA - monounsaturated fatty acids,
PUFA – polyunsaturated fatty acids,
ω3 - omega 3 fatty acids, ω6 – omega 6 fatty acids,
CLA – conjugated linolenic acid,
A -vitamin A, E - vitamin E, fat - % of total milk fat,
Different superscripts in the row = statistical significance $p<0.05$

Figures present the results of sampling 2, in this sampling the most significant differences in milk profile between the cows in the stable and on the pasture were found.

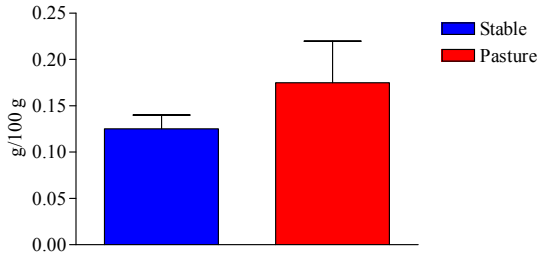
Saturated fatty acids in milk of cows



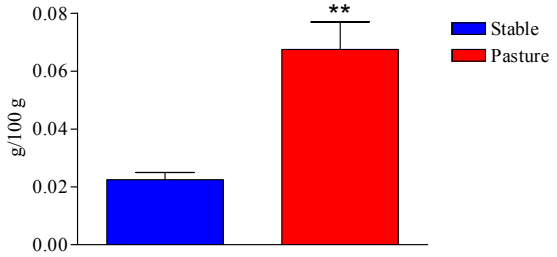
Monounsaturated fatty acids in milk of cows



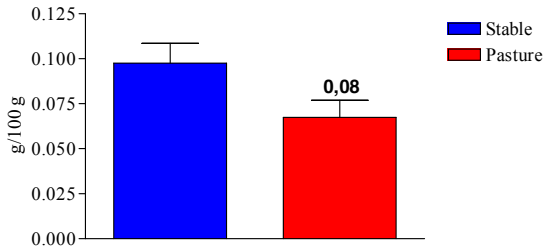
Polyunsaturated fatty acids in milk of cows



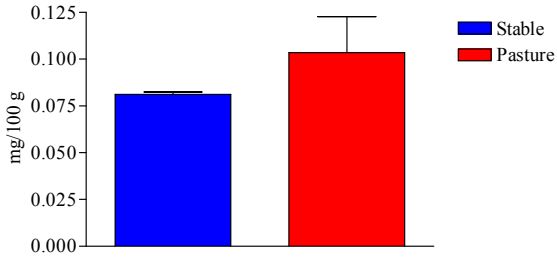
Omega-3 fatty acids in milk of cows



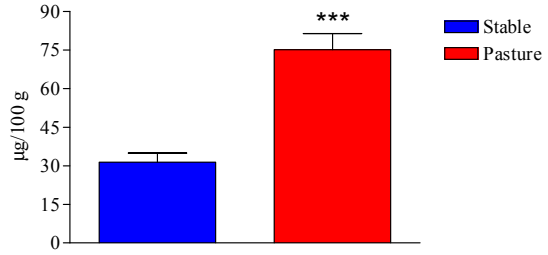
Omega-6 fatty acids in milk of cows



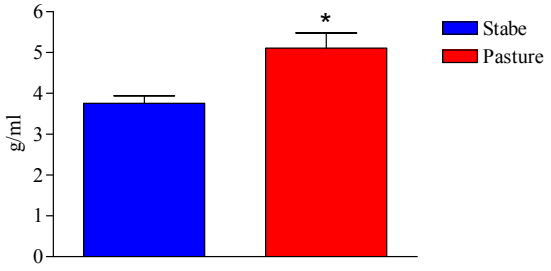
Vitamin E in milk of cows



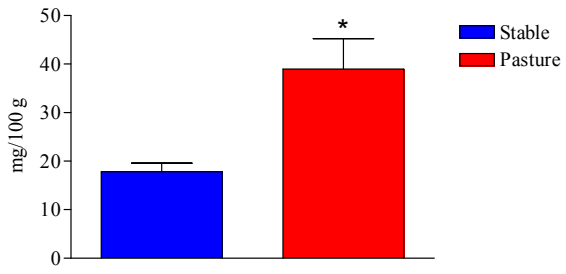
Vitamin A in milk of cows



Total milk fat in milk of cows



CLA in milk of cows



The results of the sampling indicate that the milk of dairy cows on the pasture had higher concentrations of saturated, monounsaturated, omega-3 fatty acids, vitamin A, total milk fat and CLA.

Discussion

The grazing of dairy cows has long tradition in Slovakia but now is considered unprofitable in order to achieve the high milk yields. Currently, the grazing of cattle at higher altitudes is almost unimaginable in Slovakia. Conversely, in Switzerland, France, Austria are regions with the production of milk for some famous cheeses at the altitude over 1000 m. The production of cheese in these countries is limited to a specific area, vegetation period when the cows are grazed on mountain pastures even at an altitude of 2000 m. The animals are supplemented with hay, because the use of silage for the production of long ripened cheeses is prohibited. In recent years, increased attention has been paid to the conjugated linoleic acid (CLA) in many countries of the world. A healthy person should receive at least 3 g of CLA per day. The enhancement of CLA in cattle products, such as meat and milk, through dietary management is an important factor to achieve the recommended intake of 3 g CLA *per* day (Roche et al., 2001). The CLA has anti-carcinogenic and anti-atherogenic potential, reduces the risk factors for ischemic heart disease, stroke and incident diabetes, plays an important role in the immune response, physiological bone marrow function and antioxidant capacity of the living organism. In animals, a higher intake of CLA reduced the proportion of fat and increase muscle tissue content. The consumer is more interested to organic food. It is well understood that the CLA content in milk increases with the increasing altitude of pasture. Alpine milk from an altitude of 600-650 m had an average CLA content of 8.7 $mg.g^{-1}$ of milk fat, from 900-1200 m is 16.1 $mg.g^{-1}$ of milk fat and from an altitude of 1210-2100 m is 23.6 $mg.g^{-1}$ of milk fat. In comparison, Dutch milk had only 5.1 $mg.g^{-1}$ of milk fat. A strong negative impact on CLA content in milk fat has been demonstrated due to the feeding of maize and grass silage or concentrated feeds (Martincová et al., 2016). Dairy products and meat from ruminants are the significant natural sources of CLA. Our result confirm the positive effect of the pasture on monitored milk parameters of fat metabolism in dairy cows.

Acknowledgments

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Field trial of the organic treatment of bovine digital dermatitis lesions with bovine colostrum

A szarvasmarha digitális dermatitisz elváltozások kolosztrummal való organikus kezelésének gyakorlati kipróbálása

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Abstract

Digital Dermatitis (DD) is a painful and economically important disease in cattle and one of the most important facts in our century is antibiotic resistance. This study was performed to treat DD more economically and organic with colostrum instead of antibiotics. Due to the lack of cattle with DD in the farm only 1 group has been described in this article. Total 14 active digital dermatitis lesions on rear foots from 11 Holstein dairy cows were bandaged using colostrum and all lesions have been treated in day 28. According to the results of this study, bovine colostrum has a positive effect on treatment of bovine DD lesions and it can be a cheaper and valuable treatment option especially for organic dairy farms.

Key words: digital dermatitis, organic treatment, bovine colostrum

Introduction

Digital dermatitis (DD) is a painful and infectious cause of dairy cattle lameness (Döpfer et al., 1997). Topical applications (antibiotic or non-antibiotic) have an important role on control and treatment of DD (Berry et al., 2012). Colostrum is the first mammary secretion which is being secreted during the first 24-36 h after birth, is suitable for wound healing through some immune factors (Pakkanen et al., 1997; Tanideh et al., 2016). In accordance with the study (Tanideh et al., 2016) that says colostrum can be used on wound healing, “bovine colostrum is able to cure bovine DD lesions” was

hypothesized. This article is a part of the study, performed to test this hypothesis.

Materials and Methods

This study will be carried out on at least 31 Holstein dairy cattle [in 3 groups (Group 1: colostrum, n=11; Group 2: control, n=10 and Group 3: antibiotic, n=10)] with lameness (Lameness Score /LS/: ≥3 by Sprecher et al., 1997) and active DD lesions among 231 milking cows from an organic commercial dairy farm in Aydin, TR.

Due to the lack of the cattle with active DD lesions, the study was divided into 2 years. Therefore, only **Group 1 (colostrum group, n=11)** have been described in this manuscript.

Raw/simple bovine colostrum was topically administered directly on DD lesions in Group 1 (Figure 1 b) and colostrum impregnated gauze was covered on the lesion (Figures 1c and d), afterwards bandage (Akin et al., 2013, 2015) was applied (Figures 1d, e, f, g, h, i and j). If deemed necessary, hooves were trimmed. Colostrum was obtained from recently calved cows from same farm and kept in fridge (+4°C). Bandages were changed two times a week (every Saturday and Tuesday). Meanwhile (during bandage changing), cleaning of the lesion was performed using only water from a hose. LS 1 (Sprecher et al., 1997) and skin covered lesion area with no pain during palpation were healing criterias of the DD lesions.

Figure 1. Case no 10: M2 scored DD lesion (a), and application of bovine colostrum with bandage [administration of bovine colostrum on the lesion (b, c, d), cotton (d, e), gauze (e), vet-flex (f), silver tape (g) and tar tape (h, i, j) applications].

Results

Total 14 active digital dermatitis lesions on rear foots from 11 Holstein dairy cows recorded in Group 1 and described in this abstract.

All cows were moderately lame (LS=3), and local severe pain was evident in all lesions. During bandage changing, lesions were prone to bleeding and specific bad smell was notable in all lesions. Until day 10 (third bandage), severe pain was found on 7 lesions and moderate others. Only one lesion was recovered on day 14 and others were beginning to close with skin. Meanwhile, 6 mild and 7 moderate pain and LS=2 were recorded for the rest of the cows. Recovering number of 4, 2, 4 and 3 DD lesions (Figure 2) were determined on day 17, 21, 24 and 28, respectively (Table 1).

Figure 2. First view (Day 0) of the DD lesions (Case no 2 and 4) and recovered DD lesions (Case no 2/Day 17, Case no 4/Day 24).

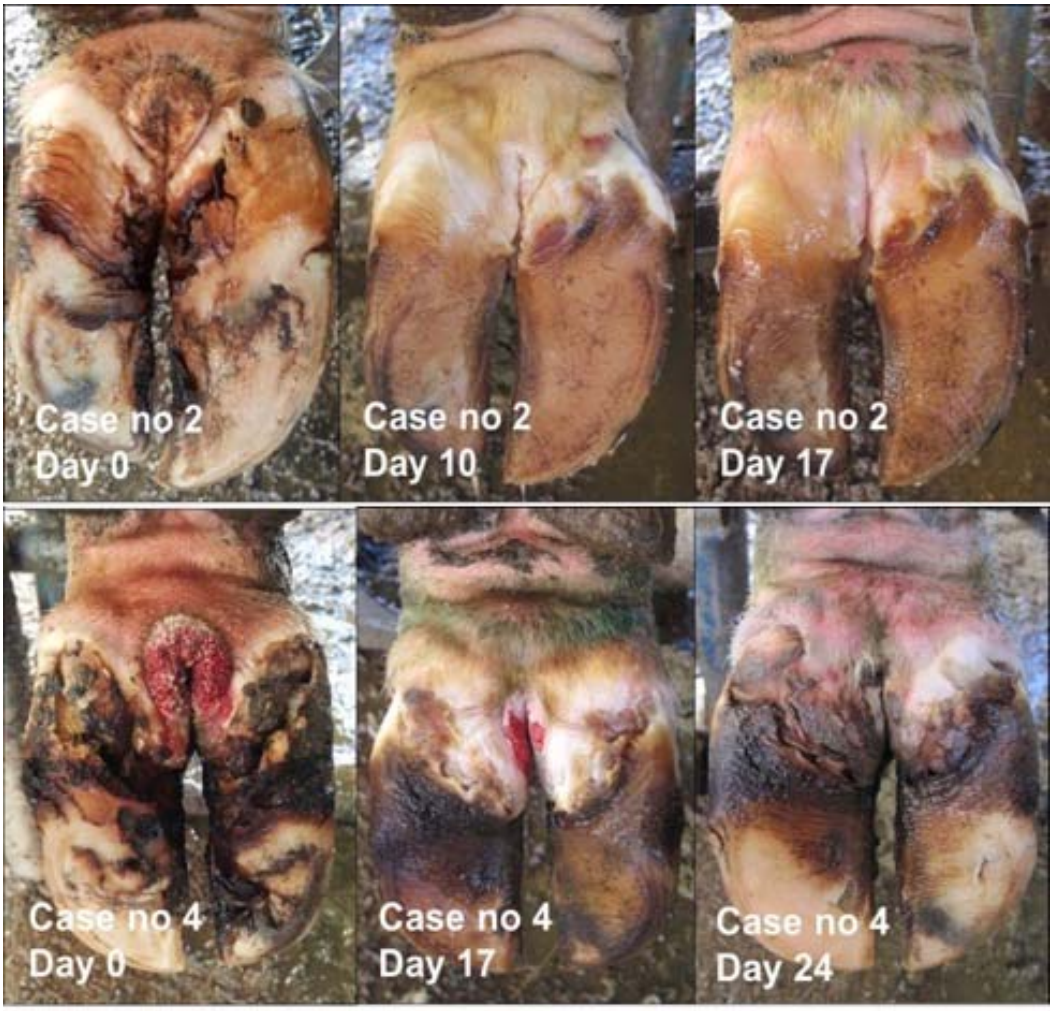

Table 1. Number of lesions recovered by bandages and days

Number of Bandage changing	-	1	2	3	4	5	6	7	8
Day	0	3	7	10	14	17	21	24	28
Number of recovering DD lesions	-	-	-	-	1	4	2	4	3

Discussion

Despite the suggestion of hoof bandages should be changed ones a week, bandages were changed twice a week in this study, due to the potential agar effect and unknown results of bovine colostrum applications on DD lesions. All lesions in this study were scored as M2 because of the severe local pain. It is unknown when the lesion has started and how long it has been there. All lesions in this study were recovered between 14 and 28 days after initiation of treatment. Individual wound healing responses would be different in each organism. In conclusion, according to the results of this study, bovine colostrum has a positive effect on treatment of bovine DD lesions and it can be a cheaper and valuable treatment option especially for organic dairy farms.

Acknowledgments

Authors wish to thank Arif Gürdal Dairy Farm. The study was approved by the Animal Ethics Committee of Adnan Menderes University (with no: 64583101/2016/202).

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Factors affecting embryonic losses after embryo transfer in dromedary camels

Embrió átültetést követő embrionális veszteségeket befolyásoló tényezők dromedári tevékben

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Abstract

The aim of the present study was to estimate the incidence of embryonic /early fetal mortalities (LEM/EFM) after embryo transfer (ET). Additionally, to find out the risk factors which could affect the incidence of LEM/EFM in the dromedary camels.

In the present study, 1904 embryos were recovered and transferred non-surgically into 1614 recipients during the period between 2014 and 2016. Pregnancy diagnosis was carried out at Day 10 after ET (Days 18-20 after mating of the donors) by using progesterone ELISA test. At Day 60, pregnancy was confirmed by transrectal ultrasonography of the recipients.

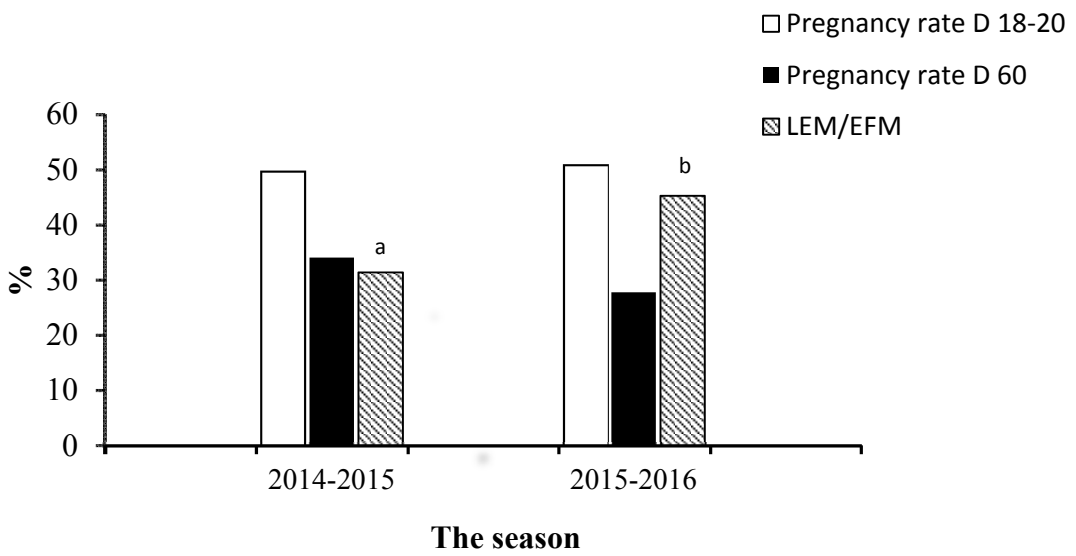
Pregnancy rates at Days 18-20 and Day 60 and the rate of LEM/EFM were 50.5%,

30.1%, and 40.5%, respectively. A significantly higher rate of LEM/EFM (P< 0.01) was observed in season 2015-2016 than that in season 2014-2015. Transferring embryos recovered from donors yielded ≥5 embryos significantly (P<0.05) increased the pregnancy rate at Day 18-20, while it significantly (P<0.05) increased the rate of LEM/EFM when compared with those recovered from donors yielded < 5 embryos. The rate of LEM/EFM was significantly (P< 0.005 and 0.0001, respectively) lower after transferring small-sized embryos than those obtained after transferring medium- and large-sized embryos. Pregnancy rates at Days 18-20 and Day 60 after transferring spherical-shaped embryos were significantly higher (P<0.0001) than that obtained after transferring folded embryos. Transferring two or more embryos into a recipient significantly (P<0.05) increased the pregnancy rate at Day 18-20 and non-significantly increased (46.2% vs. 39.2%, respectively) the rate of LEM/EFM when compared with transferring one embryo to a recipient.

In conclusion, the incidence of LEM/EFM between Days 18-20 and Day 60 after ET in the dromedary camel is high. The rate of LEM/EFM after ET might be affected by the year, size and shape of the embryos and number of the recovered and transferred embryos (Figures 1–4).

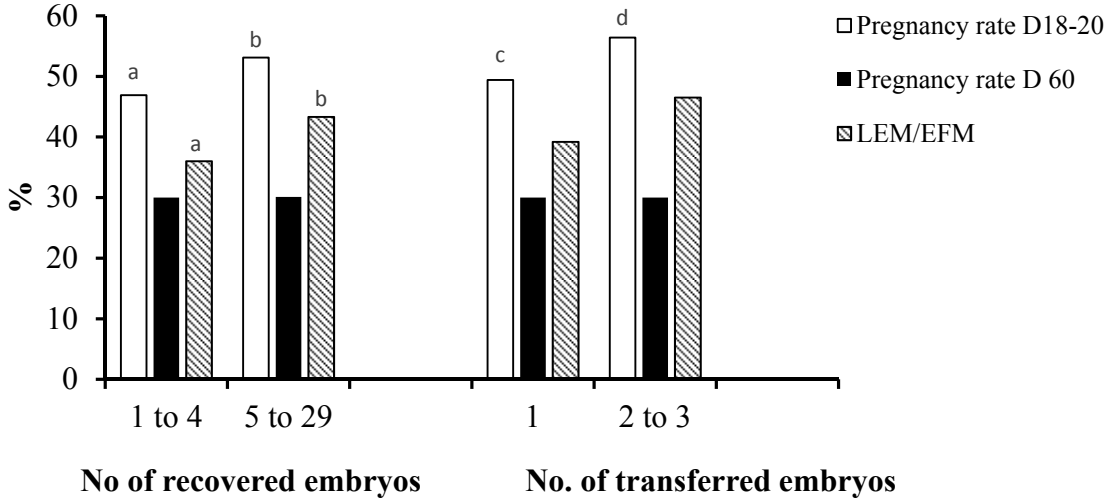
Key words: embryo transfer, embryonic/fetal mortalities, and camel

Figure 1. Effect of the year on the pregnancy rates at Days18 to 20 and Day 60 and the rate of late embryonic/fetal mortalities (LEM/EFM) after embryo transfer in the dromedary camel



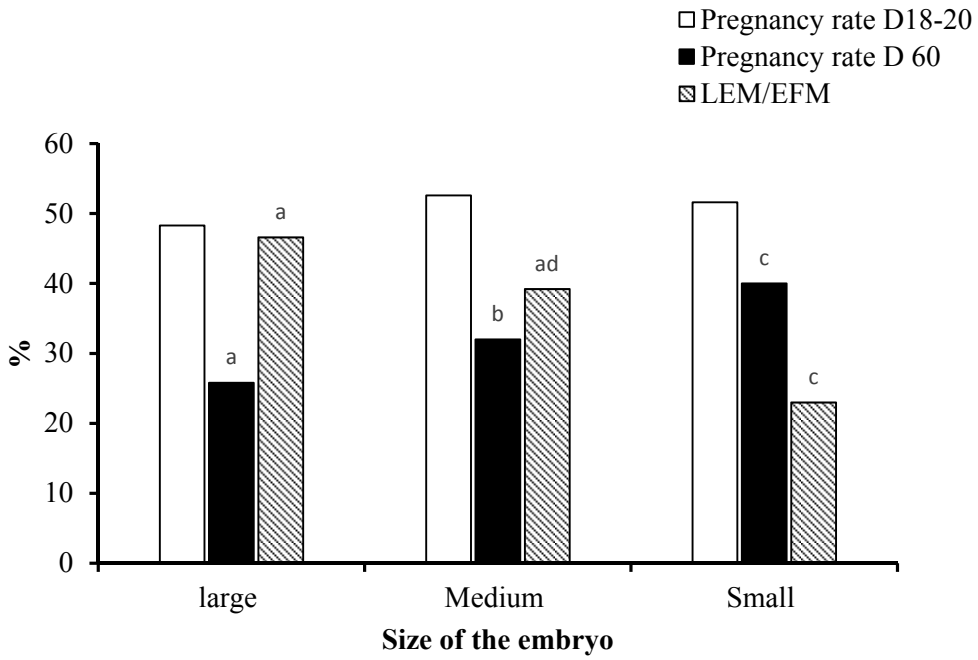
[a,b] P< 0.01

Figure 2. Effect of number of recovered and transferred embryos on the pregnancy rates at Days18 to 20 and Day 60 and the rate of late embryonic/fetal mortalities (LEM/EFM) after embryo transfer in the dromedary camel



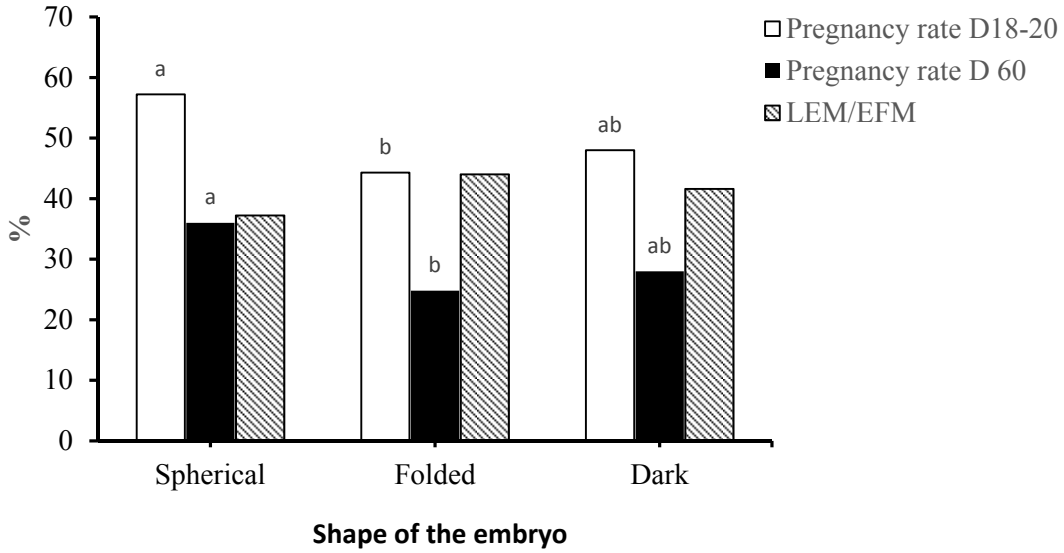
[a,b]P< 0.01; [c,d]P< 0.05

Figure 3. Effect of size of the embryo on the pregnancy rates at Days18 to 20 and Day 60 and the rate of late embryonic/fetal mortalities (LEM/EFM) after embryo transfer in the dromedary camel



[a,b]P< 0.05; [a,c]P< 0.001; [c,d]P< 0.005

Figure 4. Effect of shape of the embryo on the pregnancy rates at Days18 to 20 and Day 60 and the rate of late embryonic/fetal mortalities (LEM/EFM) after embryo transfer in the dromedary camel



[a,b]P< 0.001

Acknowledgments

The authors wish to thank Dr. Mohamoud Salama, Dr. Mohamed El-Shazlee and Mr. Mohsen Azeem for their technical assistance during the study.

Intercellular adhesion molecule-1 (ICAM-1) and C-Reactive Protein (C-RP) levels in calves infected naturally with *Mycoplasma bovis*

Intercelluláris adhéziós molekula-1 (ICAM-1) és C-reaktív fehérje (C-RP) szintje a *Mycoplasma bovis*-val természetesen fertőződött borjakban

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Abstract

Mycoplasma bovis is one of the most important pathogens causing pneumonia worldwide. In this study, it was aimed to determine intercellular adhesion molecule-1 (ICAM-1) and C-reactive protein (C-RP) levels in calves infected *Mycoplasma bovis*. The animal material of the study consisted of 30 calves including 20 simental calves with pneumonia due to *Mycoplasma bovis* at 2-4 months of age and 10 healthy calves at the same age and the race. Serum levels of intercellular adhesion molecule-1 (ICAM-1) and C-reactive protein (C-RP) were determined in calves infected *Mycoplasma bovis* and control group. Serum levels of intercellular adhesion molecule-1 (ICAM-1) and C-reactive protein (C-RP) were found to be statistically higher in the calves with pneumonia than in the control group ($P < 0.01$). A positive correlation between serum ICAM-1 concentrations and *Mycoplasma bovis* titers (increases in coefficient ratios) was statistically significant ($r = 0.664$), indicating a positive correlation between serum ICAM-1 and CRP levels and titers of *Mycoplasma bovis* in the calves with pneumonia

As a result, it was observed that serum intercellular adhesion molecule-1 (ICAM-1) and C-reactive protein (C-RP) concentrations were increased in infected pneumonia with *Mycoplasma bovis*. It is considered useful to consider these parameters in evaluating the prognosis of pneumonia cases of *Mycoplasma bovis* origin and the efficacy of applied treatment.

Key words: calf, *Mycoplasma bovis*, intercellular adhesion molecule-1 (ICAM-1), C-reactive protein (C-RP)

POSZTER BEMUTATÓK * POSTER PRESENTATIONS

Klinikai Megbetegedések * Clinical Diseases

Anyagforgalmi Rendellenességek * Metabolic Disorders

Reprodukció * Reproduction

Tőgygyulladás * Mastitis

Borjak * Calves

Kiskérődzők * Small Ruminants

Paratuberculosis, mint lehetséges közegészségügyi veszélyforrás

Paratuberculosis, as a possible public health risk

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Összefoglalás

A *Mycobacterium avium subs. paratuberculosis* (MAP) bakérium a kérődzők Johne-féle betegségének, a paratuberculosisnak a kórokozója. A humán Crohn-betegség klinikai tünetei sok hasonlóságot mutatnak a kérődzők paratuberculosis fertőzöttségével. A MAP baktériumot Chiodoni és mtsai. 1986-ban izoláltak először Crohn-betegségben szenvedő betegekből. Több tanulmányban is leírták, hogy MAP ellen termelődött ellenanyag nagyobb arányban mutatható ki a Crohn-betegséggel érintett személyeknél, mint a kontroll csoportnál. A közel 30 éves kutatások ellenére a MAP zoonotikus szerepe a mai napig nem tisztázott, nem sikerült egyértelmű összefüggést kimutatni a MAP fertőzöttség és a Crohn-betegség kialakulása között. A MAP lehetséges zoonotikus szerepe miatt sok ország, így Ausztrália, az Egyesült Államok, Hollandia, Dánia védekezési programokat indított a paratuberculosis előfordulásának csökkentése érdekében.

Abstract

Mycobacterium avium subs. paratuberculosis is a causative agent of the paratuberculosis of ruminants. Clinical symptoms of human Crohn's disease show many similarities with ruminants paratuberculosis. The MAP bacterium was first isolated by Chiodoni et al. in 1986, from patients with Crohn's disease. It has been reported in several studies that the antibody produced against MAP may be shown to be more pronounced in Crohn's disease than in the control group. Despite the nearly 30 years of research, the zoonotic role of MAP has not been clarified yet, there was no clear correlation between MAP infection and Crohn's disease. Due to the possible zoonotic role of MAP, many countries

such as Australia, the United States, the Netherlands and Denmark have launched defense programs to reduce the prevalence of paratuberculosis.

Key words: paratuberculosis, Crohn, zoonozis

Paratuberculosis

A szarvasmarhák paratuberculosisának vagy más néven Johne-betegségének (JD) a kórokozója egy sav - és alkoholló baktérium, a *Mycobacterium avium subsp. paratuberculosis* (MAP) (Johne & Frothingham, 1985). A szarvasmarhák 6 hónapos korukig a legfogékonyabbak a fertőzésre, azonban a klinikai tünetek megjelenéséig eltelt idő meglehetősen hosszú, és kiszámíthatatlan, akár 2-10 év is lehet. Az állatok a baktériumot tejjel, illetve bélsárral ürítik, ennek megfelelően a fertőződés történhet szájon át föcstejjel, a fertőzött anya bélsarával és intrauterinálisan is (Whittington & Windsor, 2009). A klinikai tüneteket az idült lesoványodás, bűzös, profúz hasmenés és fehérjevesztés következményeként kialakuló áll alatti oedema jellemzi. A betegség gyógyíthatatlan, a klinikai tüneteket megjelenésétől számított 1-2 héten belül az állatok elpusztulnak (Collins, 1996)

A Crohn-betegség

A Crohn-betegség (regionális bélgyulladás, granulomatosus ileitis) az emésztőrendszer ismeretlen eredetű krónikus, idiopátiás gyulladásos betegsége, amely a bélfal minden egyes rétegét érintheti. A betegség irreverzibilis bélkárosodással, valamint bélműködés leállásával is járhat (Lee, 2018). Az emésztőrendszer bármely szakaszát érintheti, de leggyakrabban a vékony- és vastagbelet együttesen érintő fekélyekkel járó gyulladásos folyamat (Li et al., 2018). A betegség klinikai tünetei a hasi fájdalom, hasmenés, vérzés, bélelzáródás, valamint számos szisztémás tünet. Korábbi tanulmányok szerint a Crohn-betegséget egy autoimmun betegségnek tartották, de ma már a kutatók úgy vélik, hogy környezeti és genetikai tényezők és tartós antigének közötti kölcsönhatások is szerepet játszanak a kialakulásában (McNee et al., 2015). A betegség kialakulásának genetikai vonatkozása is lehet. Az autofágia azonosítása, az IL-17/ IL-23 és a veleszületett limfoid sejtek a kulcs a Crohn-betegség patogenezisében. Ezen betegség genetikai felépítésének fokozatos megismerése arra is rávilágít, hogy az aberráns immunválaszok elfojtásának sikertelensége hozzájárulhat a betegség kialakulásához. Ezen felismerés tudatában már új kezelések tervezését szorgalmazták (Verstockt et al., 2018), azonban napjainkban egyelőre csak a tüneteket mérséklését segítő terápia áll rendelkezésre.

A szarvasmarhák paratuberculosisa és a Crohn

Az állatok széles körét, beleértve a főemlősöket is meg lehet fertőzni MAP-al, így esetlegesen ez embereknél is lehetséges. (Collins, 2011). A két betegség közös

klinikai jellemzői közé tartoznak a szakaszos hasmenés, a súlycsökkenés, az ileocoecalis területen megjelenő nyálkahártya fekélyek és granulómák (McNees et al., 2015). A JD kórszövettani képe hasonlít a Crohn-betegségéhez (Atreya et al., 2014). Számos epidemiológiai vizsgálat bizonyította a MAP DNS jelenlétét Crohn-betegek szöveteiben, így feltételezhető, hogy ezen ágensek és a betegség között erős kapcsolat áll fenn (Behr & Kapur, 2008). A közelmúltban felülvizsgálták a MAP zoonózikus szerepét, beleértve az epidemiológiáját, az emberekre való átviteli útvonalakat, a hasonló pathobiológiát és az emberi betegségekkel való kapcsolatokat. Genetika bizonyítéka lehet a zoonotikus transzmissziónak, hogy MAP genom szekvenciát izoláltak humán és állati szövetből. Egyes vizsgálatok azt mutatják, hogy az emberek ugyanúgy érzékenyek a MAP fertőzésre, mint a szarvasmarhák (Davis, 2015).

A MAP kimutathatósága az élelmiszerekben

A MAP valószínűleg fertőzött víz vagy táplálék fogyasztása után kerülhet az emberek szervezetébe (McNees et al., 2015). A kérődzők a MAP baktérimot üríthetik tejjel is, így közegészségügyi szempontból fontos lehet a nyers tej MAP-al való terheltsége. A baktérium túlélheti a pasztőrözés hőmérsékletét, mivel bizonyítottan hőrezisztensebb, mint a Mycobacterium bovis vagy a Coxiella burnetti, így a csupán pasztőrözéssel készült termékek is kockázatot jelenthetnek (Sung & Collins, 1998). Nyers húsból, illetve tejtermékekből is izoláltak MAP baktériumot tenyésztéssel, azonban a főzés és fagyasztás hatásaira elérő eredmények vannak a szakirodalomban, a nem megfelelően átsütött hús tartalmazhat MAP-t (Mutharia et. al., 2010). A MAP 16-20 hétig is képes fertőzőképes maradni a vizekben, így a természetes vizek is jelentős fertőzési forrást képviselhetnek, amennyiben a hígtrágyakezelés hiányosságai miatt a baktérium a vizekbe juthat (Whittington et al., 2015).

Az állatorvosok szerepe

Mivel az orvostudománynak még időre van szüksége paratuberculosis és a Crohn-betegség közötti kapcsolat tisztázásához, ezért az állatorvosoknak körültekintőnek kell lenni, ha a MAP-al érintett állományban dolgoznak és állományszintű védekezési programok kidolgozásával és végrehajtásával a zoonotikus kockázatot a lehetőségekhez képest minimálisra kell szorítani (Collins, 2011).

Köszönetnyilvánítás

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Serum proteins in dairy cows suffering from clinical signs of paratuberculosis

Paratuberkulózis klinikai tüneteit mutató tejelő tehenek szérumfehérje értékei

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Abstract

The objective of this study was to evaluate the serum protein pattern in dairy cows with chronic diarrhoea associated with seropositivity to *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Blood samples from 44 dairy cows with intermittent diarrhoea, loss of body weight, general wasting were included into the study. Nineteen clinically healthy dairy cows were taken as a control group. The values of total proteins, albumin and A/G ratio were significantly lower in cow with diarrhoea compared with healthy animals ($P < 0.001$). In the globulin fractions significantly higher concentrations of α_1-, β_1- and γ-globulins ($P < 0.001$), and non-significantly higher values of α_2- and β_2-globulins were observed in the diarrhoic cows. Furthermore we found β-γ bridging in 32 from 44 cows with chronic diarrhoea. Marked differences in the evaluated parameters were obtained also between high and weaker MAP-positive cows. The results indicate marked effect of chronic diarrhoea in cows seropositive for MAP on the protein profile of animals.

Key words: dairy cows, paratuberculosis, diarrhoea, serum proteins, protein fractions

Introduction

Chronic diarrhoea associated with malabsorption represents a great problem on the affected farms causing high economic losses (Smith et al., 2009; Wittek et al., 2016). Besides nutritional factors, there are several pathogens that may cause chronic diarrhoea in adult cattle Paratuberculosis, or Johne´s disease, caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is one of the most important intestinal chronic progressive granulomatous infections of ruminants with major economic impacts (Pillars et al., 2009). The infection develops

into granulomatous lymphadenitis, thickening and oedema of the intestinal mucosa resulting in intermittent treatment-resistant diarrhoea (Clarke, 1997). Consequently, the intestinal absorption of nutrients decreases resulting in malabsorption associated with protein losing enteropathy, and in decreased concentrations of blood proteins in the advanced stages of the disease (Bendixen, 1978). This study was aimed to evaluate the electrophoretic pattern of serum proteins in chronic diarrhoea associated with paratuberculosis in dairy cows seropositive for MAP-antibodies.

Experimental methods

Samples from 44 dairy cows affected by chronic diarrhoea manifesting for more than two weeks, loss of body weight, milk production and general wasting were included into this study. The sampled animals were of a low land black spotted breed, Slovak spotted breed and their crossbreeds at the age of 3.5 to 8 years, and were from four conventional dairy farms. The cows were positive for *Mycobacterium avium* subsp. *paratuberculosis* (MAP) antibodies. According to the results of MAP-Ab status, the cows were classified as highly (titre > 200, n=16) or weaker positive (titre 60 – 200, n=28). Out of these animals, 19 clinically healthy MAP-negative cows without any signs of diseases were selected as control animals.

Blood samples were taken from these animals by private veterinarians of the farms from *v. jugularis* into serum gel separator tubes. The harvested serum after centrifugation was dispensed into plastic tubes, and stored at -20 °C until it was analysed.

Immunoenzymatic ruminant MAP-Ab test was used for specific detection of anti-*Mycobacterium avium* ssp. *paratuberculosis* antibodies in blood serum (Prionics Lelystad, Lelystad, The Netherlands). To evaluate the protein profile, serum samples were analysed for the concentrations of total proteins (TP, g/l) and main protein fractions (albumin, α_1- and α_2-globulins, β_1- and β_2-globulins, and γ-globulins; %, g/l). The TP were assessed using an automated biochemical analyser Alizé (Lisabio, France) according to the biuret method with commercially available diagnostic kits (Randox, Crumlin, United Kingdom). The serum protein fractions were separated by zone electrophoresis on an agarose gel using an automated electrophoresis system Hydrasys with commercial diagnostic kits Hydragel 7 Proteine (Sebia Corporate, France). The ratios of albumin to globulins (A/G) were calculated also.

The statistical analyses were done with the programme GraphPad Prism V5.02 (GraphPad Software Inc., California, USA) using descriptive statistical procedures. The significances of differences in values between sick cows and healthy animals, as well as between highly and weaker MAP-positive cows were examined by unpaired t-test.

Results

The relative concentrations of albumin and the mean values of A/G ratios were significantly lower in diarrhoeic cows (P<0.001), with significantly lower values in high MAP-positive animals (P<0.01 and P<0.001, resp.). The α_1-globulins were significantly higher in sick cows than in healthy ones (P<0.001), while the relative values of α_2-globulins were non-significantly higher in diarrhoeic cows. Significantly higher α_2-globulins (P<0.05) were found in high MAP-positive cows. The diarrhoeic cows were found to have significantly higher relative concentrations of β_1-globulins (P<0.001) and non-significantly higher β_2-globulins. The β_2-globulins were significantly higher in high MAP-positive animals (P<0.05). The relative concentrations of γ-globulins showed significantly higher values in sick cows (P<0.001). The differences between the groups of MAP-positive cows were not significant.

The sick cows had significantly lower concentrations of TP than the clinically healthy animals (P<0.001). Lower values were found in high MAP-positive cows when compared with weaker MAP-positive. The absolute concentrations of albumin between sick and healthy animals revealed significantly lower values in diarrhoeic cows (P<0.001), being significantly lower in high MAP-positive cows (P<0.01). The absolute concentrations of α_1-globulins were significantly higher in cows suffering from diarrhoea (P<0.001). Their mean values in high and weaker positive animals were approximately similar. On the other hand, the absolute concentrations of α_2-globulins were slightly higher in healthy animals (P<0.05), no marked differences were observed between the two groups of MAP-positive cows. No significant differences were found in the concentrations of β_1- and β_2-globulins between sick and healthy cows, as well as between the two groups of MAP-positive cows. The absolute concentrations obtained for γ-globulins were significantly higher in cows with diarrhoea (P<0.01), but their mean values in high and weaker MAP-positive cows were approximately similar.

Discussion

The concentration of serum proteins reflects the balance between their synthesis, metabolism and loss. Protein losing enteropathy are characterised by increased loss of proteins into the gastrointestinal tract compared to synthesis (Braamskamp et al., 2010). The results of our study showed lower concentrations of TPs in cows with chronic diarrhoea associated with seropositivity to *Mycobacterium avium* subsp. *paratuberculosis* infection, which was more marked in high MAP-positive animals. Lower TP concentrations in MAP-positive dairy cows compared with MAP-negative animals were observed also in German Holstein dairy herds and in young bulls with paratuberculosis (Szilágyi et al., 1989; Donat et al., 2014). In the early or subclinical stages of the disease the body

tries to compensate the protein loss by increasing protein synthesis or degradation of muscle proteins to assure the synthesis of functional, as well as milk proteins in cows (Donat et al., 2014). In the study conducted by Donat et al. (2014), a decrease of TP concentrations was observed in relation to the MAP status and increase of shedding. Similar pattern was seen in our study, when we in highly MAP-positive dairy cows found lower TP values compared to weaker seropositive animals. The findings in albumin values indicate that its concentrations may reflect the magnitude of changes and predict adverse clinical outcome. Vincent et al. (2003) suggested that low concentrations of albumin in the blood serum serve as important prognostic indicator that is associated with increased mortality and morbidity.

The results of the presented study suggest a significant effect of chronic diarrhoea in cows seropositive for MAP on the protein metabolism characterised by alterations in the electrophoretic pattern of serum proteins. The values of TP, albumin and A/G ratio were lower in sick cows compared with healthy ones, while the concentrations of α_1, α_2-, β_1-, β_2- and γ-globulins were higher. Marked differences were observed between high and weaker MAP-positive cows suggesting possible diagnostic significance of some biomarkers from the protein profile in the evaluation of the severity of the disease and the magnitude of changes. However, further investigations are needed to establish the diagnostic accuracy of serum protein electrophoresis in the diagnosis of chronic diarrhoea in cattle.

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Isotachophoresis – powerful tool in the large animal laboratory

Izotachoforézis – hatékony eszköz a nagyállat laboratóriumban

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Abstract

Isotachophoresis (ITP) is a separation analytical method belonging to the group of electromigration methods. For example, the main advantages are low operating costs, minimal chemical consumption, high sensitivity, non-toxic chemicals and simple sample preparation.

In contrast to classical electrophoresis, ITP uses two types of buffers - leading and terminating. It is allowing us to analyze required group of analytes.

ITP is most commonly used in practice for the analysis of ions and organic acids. This can be used in the large animal laboratory to check various technical processes or animal health. In our lab we have been using ITP for 12 years.

We perform analyzes

• *ruminal fluid*

It is the most common application in our laboratory. In one run, lactic acid, acetic, propionic, butyric and valeric acid can be determined. These values are an important

parameter for assessing the health status and the balance of the feed ration. We can avoid subsequent serious problems by early intervention.

• *silages and feeds*

The above-mentioned acids are the products of silage fermentation processes, so their quantity is important for assessing the quality of silage.

• *milk*

Important parameter for assessing milk quality is the concentration of citric acid which allows the assessment of energy metabolism and also affects the properties of the milk during its processing.

• *blood*

Lactate, pyruvate, acetoacetate, citrate, β-hydroxybutyrate etc. - important parameters of energy metabolism can be determined by ITP.

Other important substances e.g. proteins can be analysed by UV detector. It can be seen that this technique provides valuable informations for both clinical practice and research.

The financial evaluation of BVD vaccination on a commercial Hungarian dairy farm

A BVD elleni vakcinás védekezés gazdasági elemzése egy hazai nagy létszámú holstein-fríz tehenészetben

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Abstract

The aim of our study was to quantify the production impact of an acute bovine viral diarrhea (BVD) outbreak and to perform the economic evaluation of BVD vaccination in a 1070-cow Holstein-Friesian dairy herd in Hungary. In 2010 the number of abortions

and calves' early culling and mortality rate increased on the farm unexpectedly and significantly. The BVDV infection was confirmed in summer 2011 and in autumn 2011 the BVD vaccination was started. In the cost-benefit analysis the reduction of losses due to the improvement of the BVD-affected production parameters was considered as benefit and the costs of vaccination were taken into account as cost. The annual loss caused by the acute BVD outbreak was 24.3 € per cow in 2010. The BVD vaccination yielded a profit of 7.7 € per cow in 2012. The BVD vaccination as an investment had 1.8 benefit-cost ratio and 80.35% ROI.

Key words: dairy cattle, bovine viral diarrhoea, vaccination, economics, cost-benefit analysis

Összefoglalás

Kutatásunk célja az volt, hogy számszerűsítsük egy heveny BVD járványkitörés termelési mutatókra gyakorolt hatását, és elvégezzük a BVD elleni vakcinás védekezés gazdasági elemzését egy 1070 tehenes hazai holstein-fríz állományban. A 2010-es évben váratlanul és jelentősen megnőtt a vetélések, ill. a borjak idő előtti selejtezésének és elhullásának száma. A BVDV fertőzöttséget 2011 nyarán mutatták ki, és 2011 őszén megtörtént a teljes állomány BVD elleni vakcinázása. A költség-haszon elemzés során a BVD által érintett termelési mutatók javulása révén elkerült veszteséget tekintettük a vakcinázási program hasznának, míg vakcinázás miatti kiadások jelentették a program költségét. A heveny BVD kitörés okozta éves veszteség 7,3 ezer Ft volt tehenenként 2010-ben. A BVD elleni vakcinázás hatására a telepen tehenenként 2,3 ezer Ft jövedelem keletkezett 2012-ben. A BVD vakcinázás mint befektetés költség-haszon aránya 1,8, megtérülése (ROI) 80,35% volt.

Introduction

Bovine viral diarrhoea (BVD) virus has a prominent importance among the infectious agents of cattle, due to its direct (production losses) and indirect (trade restrictions) effects on the industry (Ózsvári et al., 2001; Szabára et al., 2016a; Szabára et al., 2016b; Szabára & Ózsvári, 2013). The aim of our study was to present the production impact of an acute BVD outbreak and the economic evaluation of BVD vaccination in a large-scale Holstein-Friesian dairy herd.

Materials and methods

The study was carried out in a 1070-cow Hungarian dairy herd with an average 305-day milk yield of 8,593 kg between 2010 and 2012. In 2010 the number of abortions and calves' culling and mortality rate increased on the farm unexpectedly and significantly. The BVDV infection was confirmed in summer 2011 and in autumn 2011 the BVD vaccination was started in the herd. The major production and animal

health parameters on the farm were surveyed between 2010 and 2012. Thus, we compared the production indices influenced by the BVD outbreak (abortion rate, calves' mortality and culling rate) in the year before the vaccination (2010) with those in the year when the entire herd was vaccinated against BVD (2012) in order to conduct the cost-benefit analysis of the BVD vaccination. In the economic analysis the reduction of losses due to the improvement of the BVD-affected production parameters was considered as benefit and the costs of vaccination were taken into account as cost.

Results and Discussion

The results show that the acute BVD outbreak had a strong detrimental impact on many production and herd health parameters, but after vaccination significant improvement was observed (Table 1).

Table 1. The production data and animal health indices influenced by BVD in the dairy herd between 2010 and 2012

Parameter	2010	2011	2012
Stillbirth rate (%)	3.18	3.44	6.62
Hydrocephalus rate (%)	0.60	0.27	0
Cow mortality rate (%)	0.90	0.76	0.69
Abortion rate (%)	4.52	3.02	1.55
Suckling calf mortality rate (%)	7.19	4.78	3.76
3–6 months old calf disposal rate (%)	3.49	1.50	1.92
7 months –1 year old heifer disposal rate (%)	2.36	0.47	0.52
Rate of calf and heifer curative treatments (%)	57.16	51.44	25.75

The annual loss caused by the acute BVD outbreak was calculated to be 24.3 € per cow in 2010. The impact of BVD on milk production was excluded from the loss calculation, because it could largely be influenced by many other production factors. In the herd the annual cost of BVD vaccination was € 11,223, but the decrease in the loss owing to an acute BVD outbreak amounted to € 20,240, which means € 9,017 extra profit yearly. The BVD vaccination yielded a profit of 7.7 € per cow in 2012, so as an investment it had 1.8 benefit-cost ratio and 80.35% ROI (Table 2).

Table 2. The economic analysis of the BVD vaccination in the surveyed herd

Parameter	Before vaccination (2010)	After initiating vaccination (2012)	Difference
Cost of abortion (€/herd/year)	3,000	1,200	–1,800
Cost of calf and heifer mortality (€/herd/year)	40,533	24,747	–15,786
Culling cost of calves and heifers (€/herd/year)	4,469	1,816	–2,653
Total loss (€/herd/year)	48,002	27,762	–20,240
Loss per cow (€/year)	48.2	23.9	–24.3
Total cost of BVD vaccination (€/herd/year)	0	11,223	+11,223
Total profit of BVD vaccination (€/herd/year)		9,017	+9,017
Profit of BVD vaccination (€/cow/year)		7.7	+7.7
Benefit-cost ratio			1.80
Return on investment (%)			80.35

The findings of the case study show that an acute BVD outbreak can bring about significant economic losses, especially in BVD naïve herds. According to the result of the economic analysis, the BVD vaccination was highly profitable, since the benefits stemming from the improved herd production and health parameters exceeded the vaccination cost by far. In herds experiencing substantial losses due to BVDV infection, a vaccination program is worth considering from an economic point of view.

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Radiographic study of hind limb digits in Simmental stud bulls

Hátúlsó végtag csülkeinek radiológiai vizsgálata Simmentáli bikákban

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Abstract

Lameness is a well recognised health problem in bovine pratice mostly related to claw diseases. Radiographic findings mainly include traumatic, infective or degenerative changes of bones and joints located in the digital limb region. Most of the changes are also connected with soft tissue patologies. The aim of the study was to compare the radiographic changes on phalangeal bones and joints of medial and lateral hind limb digits due to the differences in their weight-bearing and size. The study was carried out on 30 Simmental breed stud bulls during a bigger project that included veterinary health check. Mean age of the bulls was 5.73 years and mean body mass was 1 094 kilograms.

All the animals were in optimal body condition score without clinical evidence of lameness.

The x-ray imaging was done immediately after the routine claw trimming in two standrand orthogonal, lateromedial and dorsoplantar projections. For statistical analyses Statistica v. 12.5 software was used (Statistica, StatSoft, USA, 2014).

Twenty out of 30 bulls examined (66.67%) had some digital bone and joint changes. Due to anatomic location and radiographic characteristics the patologies were divided into three groups. The most common findings were degenerative joint disease that affected metatarsophalangeal and interphalangeal joints (62.51%), periostal bone reactions (30.35%) and primary bone lesions (7.14%) of the bulls with pathological changes. There is no significant difference between incidence and anatomic distribution of radiographic changes between medial and lateral hind limb digits ($p \leq 0.05$).

In addition, there is no difference in the incidence of secondary bone and joints leasions in lateral compared to medial digits although a higher weight-bearing is on the lateral digit. These results could be explained by the fact that this study included stud bulls with no clinical manifestation of lameness, and thus, all of the changes were reported as incidental findings. The contribution of this study is the fact that the stud bulls are a rear bovine population with greater body measurements compared to the others and their locomotion problems of hind limbs can higly infulence their reproduction performances.

Two years of the national collection of health disorders in Czech dairy cows

A cseh tejelő tehenek egészségügyi rendellenességeinek értékelése az utóbbi két év adatai alapján

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Abstract

Health data in dairy cattle have been continuously collected for many decades in Scandinavia and for many years in other countries such as Austria, France and Canada but not in Czechia. This contribution provides basic analysis of such health data from the first two national studies (Q CZ) on Czech dairy producers.

In both studies (first 2016/second 2017) the data were gathered retrospectively from farmers via on-line survey for the period from July through June. Besides other information, the studies collected data on the treatment (2016) / occurrence (2017) of 20/27 common health disorders in dairy cows. The studies covered 289,802 / 281,914 cows (78/76% of Czech dairy cows) of which about 50% were Holstein. To identify the farms with incomplete data, we used a condition of minimum occurrence. Each farm with more than 20 evaluated lactations had to report at least 1 record of particular health disorder to be included in the evaluation of the disorder. The reported health events were expressed as lactational incidence rate *LIR* (number of affected lactations divided by number of lactations at risk) x 100. For editing the database and calculations we used the SAS 9.4 software.

At least one disorder was reported in 45/55% of cows. Clinical mastitis was the most common disease (*LIR* 18.6/17.9%) followed by metritis (11.3/10.9%), cystic ovary disease (7.0/10.9%) and retained placenta (5.0/5.5%) or endometritis (4.3/6.5%). Digital dermatitis (2.8/5.1%) and interdigital phlegmon (1.9/3.5%) were the frequently reported foot diseases. Primary ketosis (2.7/2.2%) and milk fever (1.7/1.8%) were the most frequently reported clinical metabolic diseases. Many

farms failed to report some events. In fact, 87/98% of them sent data on mastitis, over 75% reported on orthopedic disorders and less than 25/30% on metabolic diseases, except for milk fever, which was reported by 49/55% of farms.

Despite certain limitations, and after cautious editing, the data from Czech farmers are usable for further processing, comparisons and genetic evaluation of cattle health.

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Concentration of citric acid and osteocalcin in blood serum for osteodystrophy of cows

A vérszérum citromsav és az oszteokalcin koncentrációja tehenek osteodystrophiája esetén

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Abstract

The article presents the results of citric acid and osteocalcin serum concentration measurement in cows with osteodystrophy. The possibility of using citric acid and osteocalcin as informative test for preclinical diagnostics of osteodystrophy is substantiated. The concentration of citric acid in the blood serum of cows with subclinical course and patients with osteodystrophy was reduced in 100% of animals. Its average content was 157.7±3.30 and 146.8±1.20 μmol/l. The level of

osteocalcin was reduced in 60.0% of the cows both in the subclinical course and in 100% of patients with osteodystrophy, which was associated with a decrease in the concentration of citric acid. This is due to violated processes of calcification in the bone tissue.

Key words: cows, osteodystrophy, citric acid, osteocalcin.

Introduction

Diseases that occur with metabolic disorders, in particular mineral, despite preventive measures, which are used in cattle breeding, remain widespread and cause significant economic losses, cause lower productivity of cattle and impede the efficient work of the industry (Borsberry, 1994; Fedorovych, 2011; Slivinska & Fedorovych, 2018).

Osteodystrophy takes one of the first places among metabolic diseases of cows. The disease often has a subclinical course, in which various metabolic disturbances are developing, which are still reversible in the stage of pathognomic symptoms (Doubek et al., 1989; Liesegang et al., 1998; Fedorovych, 2011).

For preclinical diagnosis of osteodystrophy in cows hematological and biochemical studies of blood and urine are carried out, where the concentration of total calcium, inorganic phosphorus, total alkaline phosphatase and alkaline reserve are determined (Moreira et al., 2009). In the early stages of diagnosis of the disease, these indicators are not always sufficiently informative due to the ability of homeostasis to counterbalance. The imperfection of these tests requires the development of sensitive biochemical criteria for bone metabolism. Therefore, today it is relevant to identify those metabolites that are specific components of the mineral and organic bone matrix or ingredients that play a role in its formation or destruction (Abribat et al., 1992; Zeyland et al., 2006). Unfortunately, their use in domestic veterinary diagnostics is limited, which is associated with the high cost of reagents, the need for special equipment, skilled personnel (Slivinska & Fedorovych, 2018). Along with this there is a number of markers that can be a reliable criterion for assessing the state of bone tissue and do not require costly and complex manipulations (Liesegang et al., 1998-2003; Allen, 2003; Carstanjen, 2006; Dzierzecka & Charuta, 2006; Kim et al., 2010)

Literary data on the role of citric acid in the pathogenesis of osteodystrophy of cows remain controversial. There are separate reports on the metabolism of citric acid in cows (Allen, 1986), lambs (Allen, 1991) and pigs (Falkowski, 1984). Citric acid (citrate) is a highly informative indicator of bone tissue, a key metabolite in the processes of its mineralization through the formation of complex compounds with phosphorus and calcium, which helps to increase their content in the bone to the level at which the

processes of crystallization and mineralization begin (Karakashov, 1962; Beatti, 1992).

Osteocalcin is a non-collagen protein, the synthesis of which is specifically induced by vitamin D (Davicco et al., 1992; Taylor et al., 2008; Ferreri et al., 2001). There is also a link between osteocalcin and citric acid with hydroxyapatite crystals and they probably play an important role in its formation and bone resorption as a whole (Allen, 1986; Fedorovych, 2011).

The purpose of the research. To determine the level of citric acid and osteocalcin in the blood of cows with osteodystrophy and their diagnostic information in different forms of its course.

Material and methods

The research was carried out on three farms of the Lviv region on Black – Spotted cows breed aged 3-6 years in late dry period. In previous works, we have found that animal rations are not balanced by the content of macro- and trace elements and vitamins (Fedorovych, 2011).

For biochemical researches in cows, samples of blood were taken, in which determined: citric acid – by Beutler & Veh (1959) in the modification of At. Karakashova, E. Vicheva (Karakashov, 1968), osteocalcin - by the chemiluminescence method.

Results

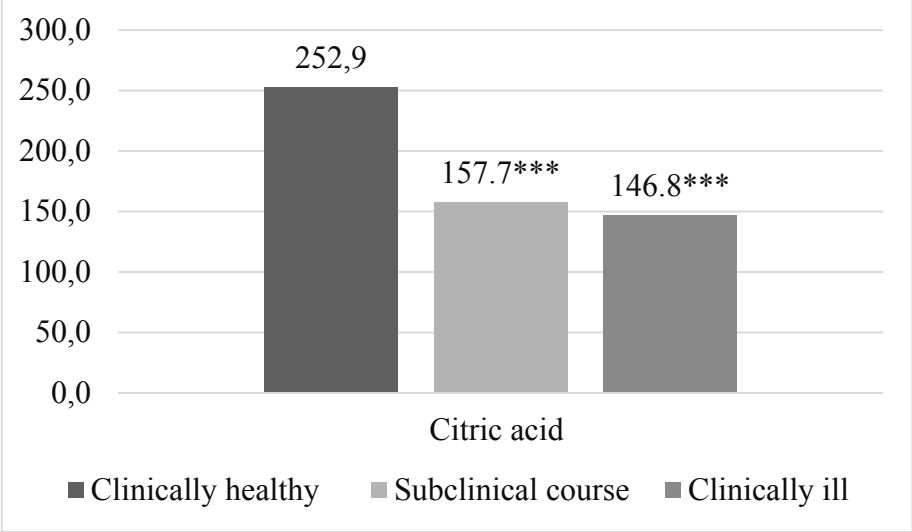
According to the results of a clinical examination of cows, it was found that the average body temperature was 38.2±0.3° C, respiration 29.0±0.9 and pulse 62±1.4 per minute. Among the clinical signs of the disease were observed: disturbance of appetite, squatting of wool, moult detention, excessive growth and deformation of hoof, thinning and partial lysis of the last pair of edges, resorption of the last 2-3 caudal vertebrae, partial deformation of the spine, spasticity of teeth, decreased fattening and productivity.

Based on the revealed symptoms and the results of laboratory tests, three groups of cows were created: 10 - clinically healthy, 10 - with subclinical course of osteodystrophy without expressed pathognomonic clinical signs and 10 - patients with clinical symptoms of osteodystrophy.

The results of the research showed (Figure 1) that the content of citric acid in the blood of cows with subclinical course of osteodystrophy ranged from 147.6 to 178.5 μmol/l and an average was 157.7±3.30 μmol/l and was probably lower (37.6%) compared to clinically healthy cows (252.9 ±2.97 μmol/l).

In clinically diseased cows, the concentration of citric acid in serum was reduced. Its average content was 146.8±1.20 μmol/l and was probably ($p < 0.001$) lower by 41.9% compared with clinically healthy ones.

Figure 1. The content of citric acid in blood serum for osteodystrophy of cows, µmol/l



Note: *** - p <0,001 compared with clinically healthy.

According to literature data, there are no reports of similar research. However, there are few literary sources which indicate that the concentration of citric acid in clinically healthy cows is 109.0 µmol/l. There is also data on the content of citric acid in lambs - 156.1-270.7 µmol/l (Allen, 1991).

According to the results of our research, it has been found that in cows with subclinical course of osteodystrophy there is a decrease in the content of citric acid in blood serum compared with the clinically healthy ones.

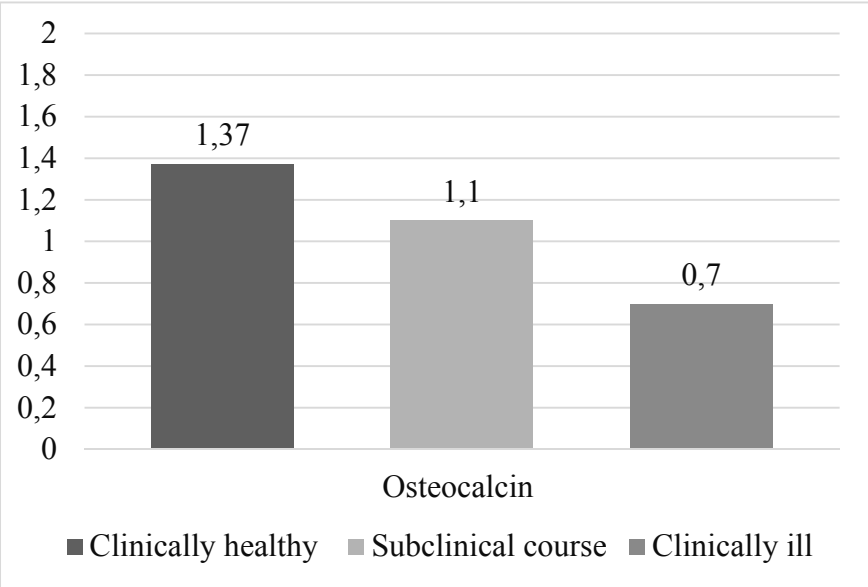
According to literature data (Taylor et al., 2008), the amount of osteocalcin in the blood of cows in the precocious period is 25.4 ng/ml. After parturition, it was noted the increase in its concentration to 32.4 ng/ml and return to output quantities within a month. The growth of osteocalcin concentration in cows after parturition is due to increased bone turnover (Moreira et al., 2009). The authors (Moreira et al., 2009) have also noted an increase in the concentration of osteocalcin before parturition, which is associated with placental synthesis. In the same research, it is noted that during the dry period the concentration of osteocalcin was significantly lower than the previous data and ranged from 19.7 to 21.4 ng/ml. The authors note that its concentration depended on the amount of phosphorus in the ration, the increase of which in feed contributes to the increase in the concentration of ostecalcin two weeks earlier, compared with rations in which the content of phosphorus was low.

In the research of other authors (Peterson et al., 2005) who had studied the effect of different levels of ration phosphorus on productivity, calcium metabolism and

predictability of hypocalcemia development, the dependence of osteocalcin concentration on the amount of phosphorus in the ration was found. The authors have established the lower the phosphorus availability of cows, the lower the concentration of osteocalcin in the blood. Therefore, it was suggested that phosphorus deficiency is one of the causes of bone resorption as it is mobilized from bone tissue into the bloodstream to maintain phosphatemia and calcemia. But, according to the authors themselves of this research, the obtained data were unlikely, it is assumed that more time is needed to establish a clear correlation (Moreira et al., 2009).

Our research has established that the concentration of osteocalcin in the blood of cows under the subclinical course of osteodystrophy varied within the range of 0.93-1.28 ng/ml, and clinically diseased - 0.58-1.05 ng/ml (Figure 2). On average, it was 1.10±0.03 and 0.7±0.04 ng / ml and was probably ($p < 0.001$) lower by 19.7 and 49.0% respectively, compared to clinically healthy cows (rice 2). The concentration of osteocalcin was lower than in the clinically healthy group, with a subclinical course of osteodystrophy in 60%, and in clinically diseased - 100% of cows.

Figure 2. The content of osteocalcin in blood serum for osteodystrophy of cows, ng/ml



It should be noted that our data differs from the received by Taylor (2008), according to which the content of osteocalcin in the blood of cows during parturition period is 25.4 ng/ml and according to Liesegang (2000) - 34.6 ng/ml.

The decrease in the concentration of osteocalcin in the blood serum of cows in the subclinical course of osteodystrophy is due to the fact that, in bone remodeling processes of resorption of bone tissue are dominated, since the provision of the

element was 49.4-57.4% of the need. Thus, our researches are consistent with the data indicated in other papers (Peterson et al., 2005; Moreira et al., 2009)

As a result of the conducted researches and the obtained results, the informative nature of the studied indices for the early diagnosis of osteodystrophy of cows was analyzed.

The concentration of citric acid in serum in 100% of cows with subclinical course and patients with osteodystrophy was reduced. Its average content was 157.7±3.30 and 146.8±1.20 µmol/l. Reduction of the concentration of citric acid occurs by suppressing the Krebs cycle in osteoblasts under the influence of parathormone, as the main regulator of calcium-phosphorus metabolism, which initially launches resorption of bone, and then suppresses its mineralization due to lower levels of citric acid in bone tissue. In general, lowering its content in the blood serum occurs faster, then developing hypocalcemia.

Reduction of citric acid in serum of cows with subclinical course of osteodystrophy is in parallel association with hypocalcemia and hypophosphataemia in 18.9 and 5.7% of cows.

According to literature, osteocalcin is a marker for the formation of bone tissue, it reflects the activity of osteoblasts. Calcium deficiency in the ration is accompanied by a decrease in its concentration in blood serum. When restoring the optimum amount of calcium in the ration, the content of osteocalcin increases in the blood, indicating a decrease in the course of calcium from the bone to the bloodstream. The level of osteocalcin was reduced in 60.0% of cows with subclinical course and in 100% of patients with osteodystrophy, which was combined with a decrease in the concentration of citric acid. That is, in the bone tissue in general, the processes of calcification were violated.

So, summing up the results of our research on the study of the concentration of citric acid and osteocalcin in the blood of cows with osteodystrophy, we can conclude that these markers are highly sensitive and specific indicators of bone tissue resorption, since they make it possible to detect changes in the bone even in cows with the subclinical course of the disease, while the level of calcium and phosphorus remain unchanged.

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Changes in milk composition indicating metabolic disorders in dairy cows

A tej összetételének változásai a tejelő tehenek anyagcserezavarait jelzik

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Abstract

The results of Slovenian official milk recording scheme from 19 farms with fertility problems were analysed with regard to the stage of lactation. The cows in the early lactation produced in average more milk with lower fat, protein and urea content in comparison to the cows in mid lactation. In one third of bovine fat content in milk deviated from normal values in early lactation. In 50.8% of early lactation bovine milk protein level was below 3.2%. Milk fat to protein ratio decreased from early to mid-lactation. Low values of milk fat and protein in many cows especially in early lactation indicating that subclinical rumen acidosis can be present in these herds. Deviations like high content of milk fat or high fat to protein ratio were not so often found. Deviations in milk composition indicate metabolic problems in some cows which may contribute to health and fertility problems reported in these herds.

Key words: cattle, milk recording, metabolic diseases, health management

Introduction

Milk yield, health and reproduction are main factors affecting profitability of dairy herd as well longevity of the cow and number of replacements needed to maintain a constant herd size. Many dairy farms face with poor health and fertility of cows. The most of the metabolic disorders occur within the first two weeks of lactation (LeBlanc, 2010). The energy demand of high yielding dairy cows at the peak of lactation exceeds the amount of net energy of the diet. This is a result of a decreased appetite after parturition and an increase in metabolic processes, which requires large amounts of substrates for synthesis of milk constituents (Mulligan et al., 2006). Metabolic disorders are mainly consequence of an imbalance between nutrient intake and needs of the dairy cow for optimal production and reproduction. Basic milk components (fat, protein, lactose) are analysed routinely as a part of milk recording system. Results of variation in milk composition may be useful for early detection of health problems. Changes in some milk variables are specifically related to the metabolic status of cow (Klinkon et al., 2004; Arnould et al., 2013). Monitoring of urea in milk is an indicator of metabolic nitrogen balance of cows (Jilek et al., 2006). The fat/protein ratio in milk is important indicator of animal health and energy balance (Richardt, 2004; Gantner et al., 2016).

The present study intended to analyse the results of milk recording that indicate metabolic problems in dairy cows. For this purpose we analysed the results of milk recording in some herds with reproductive disorders, which are often consequence of metabolic imbalances.

Material and methods

In the study the results of Slovenian official milk recording scheme from 19 farms with fertility problems (delayed cyclicity, ovarian cysts) were analysed. The results were analysed with regard to the stage of lactation; the 1st stage include the data from cows (n=177) from 0-100days in lactation and the 2nd stage the data from cows (n=219) from 101-200 days in lactation. The results from milk records; milk quantity, per cent of milk fat and milk protein, fat to protein ratio and urea concentration were analysed.

The data were processed using the statistical program SPSS (Ver 20.0). For the investigated variables descriptive statistics were calculated. The individual data were compared to the reference intervals and the percentage of cows (samples) deviating from normal values for the investigated variables were calculated.

Results and Discussion

The mean number of dairy cows in herds investigated in our study was 41.5±28.9 (range 13-120). The average milk yield per cow in herds was from 4413-9348 kg. The cows in the 1st stage of lactation produced in

average more milk with lower fat, protein and urea content in comparison to the cows in 2nd stage of lactation (Table 1). What is in agreement with results of Jozwik et al. (2012) performed on Polish Holstein-Friesian cows. At the onset of lactation the nutrient demand due to milk production increases faster than the increase in feed intake. Thus most dairy cows face some degree of negative energy balance in early lactation and in this period metabolic disorders are the most frequent (Stengärde et al., 2008). Gross et al. (2011) found the highest content of milk fat and protein one week postpartum and the fat to protein ratio peaked at 1.51±0.04 in the 3rd week, later it declined to values around 1.3 from week 7 to 12 postpartum. They found that metabolically more stressed cows during the negative energy balance in early lactation have more health disorders.

Table 1. Milk quantity and composition regarding to the stage of lactation

Variable	Stage of lactation	Mean	Standard deviation	Minimal value	Maximal value
Milk (kg)	1st	30.27	8.05	9.00	52.10
Milk fat (%)	1st	3.91	0.85	1.73	7.23
Milk protein (%)	1st	3.19	0.36	2.28	4.36
Fat/protein	1st	1.24	0.29	0.48	2.56
Urea (mg/100ml)	1st	19.95	8.26	1	42
Milk (kg)	2nd	24.67	7.17	8.30	43.90
Milk fat (%)	2nd	4.09	0.84	1.64	8.24
Milk protein (%)	2nd	3.49	0.35	2.43	4.44
Fat/protein	2nd	1.17	0.24	0.42	2.07
Urea (mg/100ml)	2nd	20.13	9.66	1	50

In the 1st stage of lactation in 26.6% of milk samples fat content was below 3.4% indicating that dietary level of effective fibre are not adequate what can lead to subacute ruminal acidosis (Oetzel, 2000; Orešnik & Lavrenčič, 2013). Subacute ruminal acidosis is suspected if 10% of the mid-lactation cows have milk fat concentration ≤ 2.5% or milk fat concentration less than milk protein concentration by 0.4% (Mulligan et al., 2006).

In 50.8% of cows in the 1st stage of lactation in our study milk protein level was below reference value (Table 2) indicating energy deficiency and reduced protein supply (Gross et al., 2011; Mulligan et al., 2006), or disturbed rumen fermentation due to ruminal acidosis (Orešnik & Lavrenčič, 2013). Microbial protein synthesis in the rumen is dependent on the energy supply. The fat to protein ratio in milk can be used as tool

indicating negative energy balance (Gross et al., 2011; Gantner et al., 2016). Milk fat to protein ratio decreased from the 1st to the 2nd stage of lactation in our study which is consistent with findings of Koeck et al. (2013) that in general fat to protein ratio was highest at the beginning of lactation between 5 and 100 days in milk. In their study ketosis was significantly correlated with fat % (0.33) and fat to protein ratio > 1.5 (0.35). In 29.9% of cows in the 1st stage of lactation and in 36.5% of cows in the 2nd stage of lactation in our study milk fat to protein ratio was below 1.1 which indicates subclinical rumen acidosis (Orešnik & Lavrenčič, 2013).

Table 2. Percentage of cows (milk samples) deviating from the reference values (Babnik et al., 2004; Orešnik & Lavrenčič, 2013) regarding to the stage of lactation

Variable	n	Below ref. value (%)	Above ref. value (%)	Reference range
1st stage of lactation				
Milk fat	177	26.6	9.6	3.4-5.0%
Milk protein	177	50.8	6.2	3.2-3.8%
Milk fat/protein	177	29.9	12.4	1.1-1.5%
Urea	168	23.8	9.5	15-30mg/100ml
2nd stage of lactation				
Milk fat	219	18.7	10.0	3.4-5.0%
Milk protein	219	21.9	22.8	3.2-3.8%
Milk fat/ protein	219	36.5	7.8	1.1-1.5%
Urea	204	21.6	11.3	15-30mg/100ml

Low values of milk fat indicate that dietary level of effective fibre is inadequate what may lead to subacute ruminal acidosis. Low level of protein in many cows, especially in the first stage of lactation, indicates energy deficiency and inadequate protein supply. More detailed analyses in herds with suspected subacute ruminal acidosis are needed to recognize factors that caused it. Deviations like high content of milk fat or high fat to protein ratio were not so often found. Deviations in milk composition indicate metabolic problems in some cows due to inadequate supply of nutrients what could contribute to health and fertility problems reported in these herds.

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Plasma lipid spectrum of cows suffering from ketosis

Ketózisos tehenek plazma lipid spektruma

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Abstract

One of the most widespread metabolic disorders in cattle is ketosis. The aim of the work was the study of lipid metabolism disorders in high-yielding Ukrainian black-and-white milk breed cows at subclinical and clinically forms of ketosis.

The research was conducted on three groups of cows: clinically healthy (n=10), suffering of subclinical (n=15) and clinically form of ketosis (n=8). The total lipids were extracted with a chloroform-methanol mixture of 2:1 by the Folch method. Total lipids were divided into classes by one-dimensional thin layer chromatography on silica gel in hexane-diethyl ether-acetic acid system with respect to 70:30:1.

While analyzing the lipid profile of cows blood at subclinical and clinical forms of ketosis compared with healthy ones, it was found increasing content of total lipids by 43.4 ($p<0.001$) and 99.2 ($p <0.001$) %, as well as absolute content of lipids classes: triacylgliceroles – by 60.9 % ($p<0.05$) and in 2.5 times ($p<0.001$), mono- and diacylglycerols – by 88.0 % ($p<0.01$) and in 3.4 times ($p<0.001$), non-esterified fatty acids (NEFA) – by 58.1 % ($p<0.001$) and in 2.4 times ($p<0.001$), free cholesterol – by 73.3 % ($p<0.01$) and in 3.5 times ($p<0.001$), lesser extent – esterified cholesterol – by 32.9 ($p<0.05$) and 39.8 % ($p<0.05$), tendency to increase phospholipids - by 24.5 and 47.1 %. In addition, clinically sick cows have shown a reduction in esterified to total cholesterol ratio by 21.2% ($p <0.001$), compared to healthy and by 15.8% ($p<0.01$) - compared with subclinical course.

In cows with ketosis, lipolytic processes are intensified as evidenced by an increase in the content of cholesterol and phospholipids.

The diagnosis of metabolic acidosis in cows suffering from ketosis using the urine study

Metabolikus acidózis diagnózisa ketózisban szenvedő tehenekben a vizelet vizsgálatával

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Abstract

In the urine of sick cows pH ($p<0.05$) and general acid-base balance are dropping. General acid-base balance of the urine depended on the degree of ketonuria: during the mild ketonuria, it was dropping to +113.8±18.25 mmol/L, moderate – to +99.9±17.56, strong – to +78.6±34.88, in some cows a significant acidotic state was found and negative indices appeared (to – 27 mmol/L), compared with +137.5±18.25 mmol/L in clinically healthy ones. General acid-base balance, pH, amount of base and B:A (base to acids ratio) of the urine were positively correlating with blood indices – pH (r = +0.6 – +0.96), HCO_3^- (r = +0.7 – +0.9), BE (r = +0.7 – +0.8) and pCO_2 (r = +0.8 and +0.97). Between the amount of acids and ammonium salts excreted with urine there was found to be a moderate negative correlation with blood pH (r = –0.5) and very strong with blood pCO_2 (r = –0.95). The close correlation between the acid-base balance indices of blood and urine allows using simple methods of urine study for diagnosing metabolic acidosis, particularly in cows suffering from ketosis.

Key words: cows, urine, ketosis, acid-base balance.

Introduction

Ketosis in cows is diagnosed mainly in the period of intensive lactation, when high milk yield is not supported by appropriate amount of nutrients intake with feed (Stöber, 2002).

The disease occurs already in the first decade after calving, but is most often diagnosed in the period from 2 to 6 weeks after delivery (Levchenko et al., 2015). In cows suffering from ketosis, underoxidized products are accumulated, causing the development of metabolic acidosis (Vlizlo & Sukhodolska, 2004). Study of pH value and acid-base balance of urine in bovine animals showed their high informativeness at various pathological conditions (Fürll & Kirbach, 1997; Bender et al., 2003). Thus, with the help of a simple and rapid determination of pH and acid-base balance of urine, it is possible to diagnose acid-base disturbance, as compared with complex blood studies performed on expensive analyzers.

The aim of this study was to establish the relationship between values of acid-base balance of blood and urine in cows with ketosis, and to establish the possibility of using simple urine test methods for diagnosing metabolic acidosis in cows.

Experimental methods

Materials for research were cows of black-and-white breed aged from 3 to 8 years with a productivity of more than 5000 kg of milk per lactation. A control (clinically healthy) and three experimental (ill with ketosis) groups of animals were formed. According to the clinical symptoms and results of urine tests by express method (diagnostic test strips Dekaphan), cows suffering from ketosis were divided into groups according to the degree of ketonuria ("+" – mild degree, "++" – moderate, "+++" – severe). In a quantitative meaning, with a mild degree of ketonuria, number of urine ketones discharged with urine measured from 2.5 to 10 mmol/L, moderate degree demonstrated from 10 to 30 mmol/L, and severe degree showed more than 30 mmol/L of urine ketones.

Blood for study was taken from jugular vein. To determine the acid-base balance, blood was collected in special 2 ml syringes with heparin. Blood was collected from uncompressed vein, gradually, ensuring no contact with atmospheric air. Parameters of acid-base balance in blood (pH, pCO_2, pO_2, HCO_3^-, BE) were investigated on Easy Blood Gas (USA) analyzer.

Urine was taken at spontaneous urination or with the help of skin massage below pudendal lips. The state of acid-base balance in urine was determined by titration (Kutas, 1965). Determination of general acid-base balance of urine was performed by fractional and simple methods. Fractional method was used to determine the quantity of ammonium ions (NH_4^+), total acid and base content, bases to acids ratio. Simple method was used to investigate only general acid-base balance of urine.

Results and Discussion

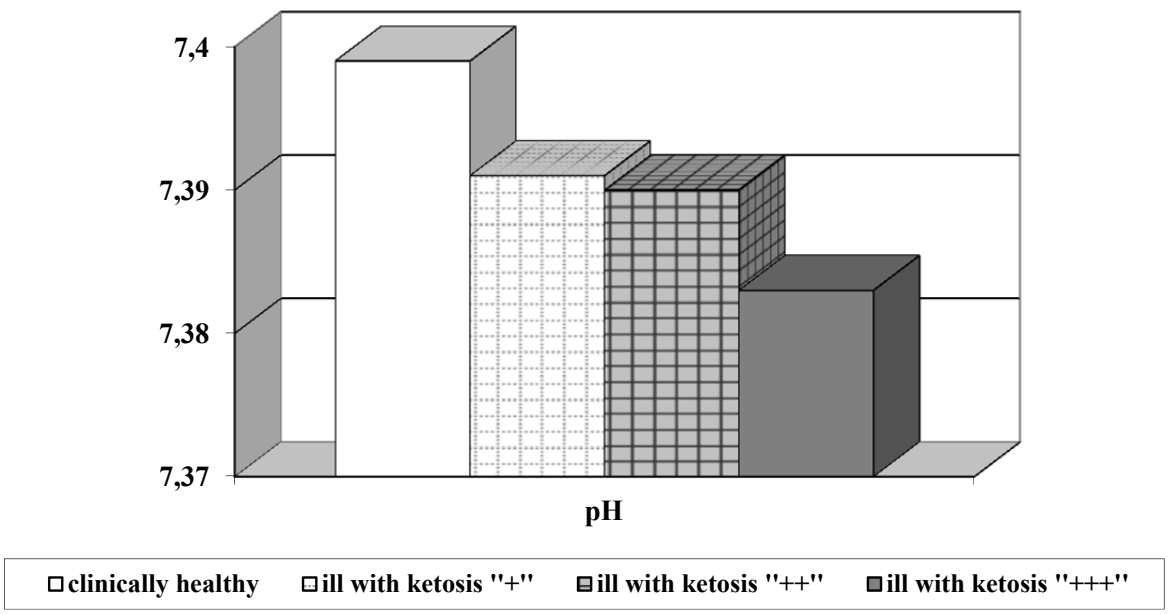
Most often, the disease was diagnosed in milk cows, who demonstrated obesity during

their dry period. In cows suffering from ketosis, clinical symptoms depended on the level of ketone bodies in the body. In most animals, distressed general condition, flaccidity, reluctant movements, sometimes long lying, odour of acetone with exhaled air and from the skin were observed. In single cows, severe depression, loss of appetite, tactile sensory impairment, and lack of response to acoustic stimuli were recorded.

In cows suffering from ketosis, ketogenesis intensified, leading to accumulation of ketone bodies in the body, therefore their total amounts in blood increased by threefold as compared with healthy ones. Of the total number of ketone bodies in clinically healthy cows, the proportion of β-hydroxybutyric acid was 84 %, acetone and acetoacetic acid made up 16 %. In blood of cows suffering from ketosis, ratio between its components varied, decreasing the proportion of β-hydroxybutyric acid (80 %), and increasing products that are more toxic to the body – acetone and acetoacetic acid (20 %). In quantitative terms, the content of acetoacetic acid and acetone increased from 0.10±0.037 to 0.34±0.041 mmol/L ($p<0.001$), and β-hydroxybutyric acid increased from 0.48±0.080 to 1.41±0.166 mmol/L ($p <0.001$).

Acid-base balance in blood of cows suffering from ketosis varied depending on the degree of ketonuria. Hydrogen index decreased with an increase in ketone bodies in urine and blood (Figure 1). In clinically healthy cows, blood pH was 7.399±0.005, in cows suffering from ketosis, with mild degree of ketonuria pH was 7.391±0.009, moderate degree of ketonuria showed 7.390±0.007, and in severe degree amounted to 7.383±0.015.

Figure 1. Change in pH in blood depending on the degree of ketonuria

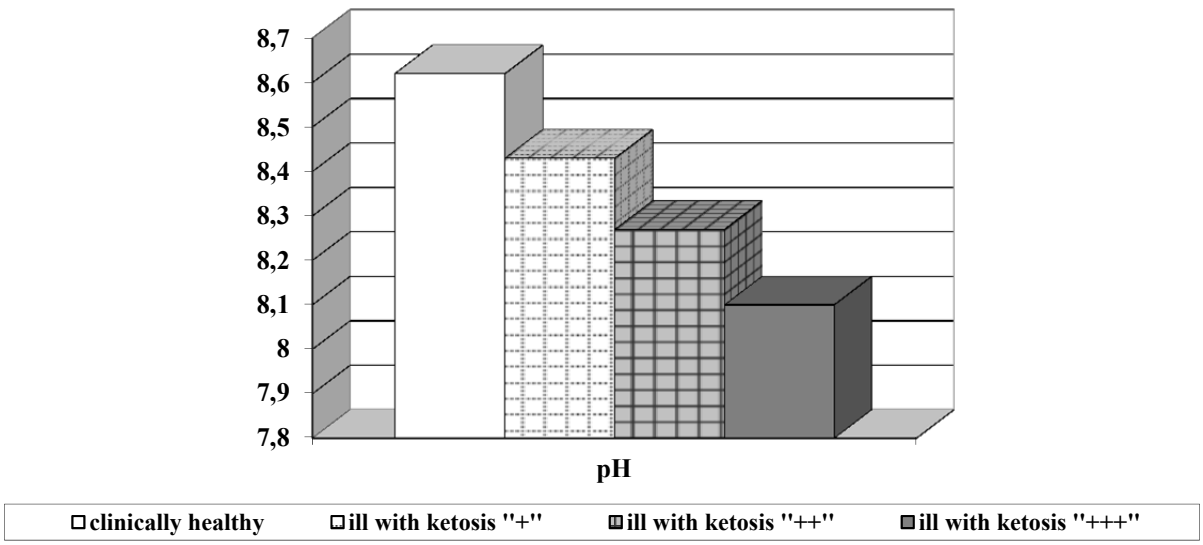


Accumulation of ketone bodies and other underoxidized products in blood causes BE deficiency. In some cases, this parameter decreased to –2.4 mmol/L. BE in blood dropped fourfold in severe degree of ketonuria, and was significantly different from clinically healthy cows (2.9±0.38 mmol/L) both in blood (0.8±0.96, $p<0.05$), and in intracellular fluid (from 3.7±0.44 to 0.9±1.06 mmol/L, $p<0.05$). With the development of ketosis in blood of diseased cows, the amount of HCO3- reduced. In cows with severely pronounced ketonuria, HCO3- concentration was on the average lower (25.8±0.91 mmol/L, $p<0.01$), compared with clinically healthy ones (28.7±0.39).

Due to compensatory mechanisms, the body is trying to maintain acid-base balance within the limits of physiological fluctuations. In cows suffering from ketosis, tachypnea was diagnosed, which led to enhanced removal of carbon dioxide through the lungs. Partial pressure of pCO_2 in blood of healthy cows was 46.4±0.59 mmHg, it constituted 45.3±0.54 – in mild degree of ketonuria, 45.0±0.62 in moderate degree, and 41.5±1.51 ($p<0.01$) in severe degree of ketonuria. Accordingly, pO_2 partial pressure in blood of cows suffering from ketosis increased from 28.3±0.74 mmHg in healthy cows up to 29.4±0.80 in cows with mild degree of ketonuria, 31.3±1.06 ($p<0.05$) in cows with moderate degree and up to 31.6±0.75 ($p<0.01$) in cows with severe degree of ketonuria.

Kidneys play an active role in compensation of metabolic acidosis, which develops with ketosis. Acid metabolic products that accumulate in blood are excreted with urine, as evidenced by pH decrease in urine ($p<0.05$). In cows with ketonuria, urine pH decreased from 8.4±0.07 to 8.1±0.29, depending on the degree of ketonuria compared with 8.6±0.05 in clinically healthy cows (Figure 2).

Figure 2. Change in pH in urine depending on the degree of ketonuria



Correspondingly, total acid-base balance in urine decreased from 137.5±18.25 mmol/L in healthy cows to 113.8±21.8 in cows with mild ketonuria, 99.9±17.56 in cows with moderate ketonuria, and 78.6±31.88 in cows with severe ketonuria. In some cases, total acid-base balance in urine decreased to –27 mmol/L, indicating obvious metabolic acidosis.

Decrease in pH and in total acid-base balance of urine takes place due to reduced excretion of bases with urine, increased excretion of acids and NH_4^+. If 176.7±25.94 mmol/L of bases and 36.6±4.55 mmol/L of acids were discharged with urine of healthy cows, then the amounts of discharged bases in cows with strongly pronounced ketonuria decreased to 101.4±14.98 mmol/L ($p<0.05$), and acid content increased to 46.0±3.48. Ratio of bases to acids decreased on the average to 3.7±0.54 in mild degree, 3.1±0.81 in moderate degree ($p<0.05$), and to 2.2±0.39 in severe ($p<0.001$) degree of ketonuria, relative to 4.9±0.51 in clinically healthy cows. NH_4^+ discharge through the kidneys increases simultaneously with an increase in the number of ketone bodies in urine. NH_4^+ increased from 10.9±0.93 mmol/L in clinically healthy cows to 20.7±8.75 in cows with strongly pronounced ketonuria, and in some animals this value reached 46.8 mmol /L.

Investigating the correlation coefficient between the level of ketone bodies in urine and acid-base balance parameters of the body in cows suffering from ketosis it was established that, depending on the degree of ketonuria, a positive correlation dependence between pH of blood and urine increases. Thus, if positive correlation between pH of blood and urine was absent in cows with mild degree of ketonuria, then in moderate degree of ketonuria, the correlation coefficient (r) was +0.5, and in severe degree it reached +0.6. The fact of active compensation of acid-base balance in blood by kidneys is confirmed by negative correlation between blood pH and the amount of urine-excreted acids (r = –0.5), as well as between blood pH and urine NH_4^+ (r = –0.5). In line with the increase of discharged acid elements from the body through the kidneys, there is a decrease in discharge of bases, which, in severe degree of ketonuria, have an extremely strong correlation relationship (r = +0.9) with blood pH. Base to acids ratio coefficient (B:A) had a very strong correlation relationship with blood pH both in moderate (r = +0.8) and in severe (r = +0.96) degrees of ketonuria. That is, this parameter qualifies the increase in discharge of acids and decrease in discharge of bases with urine. In cows suffering from ketosis with moderate and severe degrees of ketonuria, a positive correlation relationship (r = +0.6) between blood pH and total acid-base balance in urine, and a very strong correlation relationship (r = from +0.7 to +0.9) between HCO_3^- concentration in blood with bases that are excreted with urine were observed. Accordingly, the same positive correlation is observed in relation to B:A

ratio. This fact suggests that decrease in alkali reserve of blood is directly dependent on the production of additional amount of bicarbonate ions. With the development of metabolic acidosis and decrease in the hydrogen index, we observed very strong positive correlation dependence between pCO_2 and urine pH. It should be noted that this correlation increased with an increase in the accumulation of ketone bodies in the body ("+"r = +0.6, "++" – r = +0.9, "+++" – r = +0.97). Similar correlation dependence, which increased with the development of ketosis, was observed in the determination of pCO_2 and general acid-base balance of urine ("+" – r = +0.4, "++" – r = +0.7, "+++" – r = + 0.8). This fact is a sign of pulmonary compensation of metabolic acidosis. In line with the increase of underoxidized products in the body, there is an increase in discharge of acids and ammonium salts with urine, which negatively correlate (r = –0.92; –0.95) with pCO_2. Amounts of bases in urine of diseased cows decrease, therefore the correlation coefficient ranges from moderate to strong (r = from +0.4 to r = +0.7). In cows suffering from ketosis, there is a development of buffered bases deficiency in blood and a decrease in basal secretion with urine (r = from +0.7 to +0.8). In addition, it should be noted that with a decrease in blood pH, there was a strong positive correlation dependence between blood pH and blood BE (r = from +0.8 to +0.97) and bases discharged with urine (r = from +0.8 to + 0.9). Correlation between blood BE and B:A coefficient of urine in moderate degree of ketonuria was +0.7, and in severe degree it was +0.8. As far as blood pH and general acid-base balance of urine are concerned, a strong correlation relationship was found only in cows with mild degree of ketonuria (r = +0.7).

Summary

Accumulation of ketone bodies and other underoxidized products in blood of cows suffering from ketosis causes the development of metabolic acidosis, characterized by a shift of pH to the acid side, BE deficiency, decrease in pCO_2 and increase in pO_2. pH and general acid-base index decrease due to the increased acids and NH_4^+ discharge and decreased bases in urine of cows suffering from ketosis. By establishing close correlation dependence between the indices of acid-base balance of blood and urine, we can state that with the use of simple urinalysis methods it is possible to diagnose metabolic acidosis, in particular, in cows suffering from ketosis.

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Hormonal regulation of energy metabolism in ketotic cows

Az energia metabolizmus hormonális szabályozása ketotikus tehenekben

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Abstract

Analysis of examination of the level of glucose and hormones, regulating energy metabolism in ketotic cows has been performed in the paper. It was revealed that glucose level in blood of ketotic cows was by 29.3 % ($p<0.01$) lower in comparison with healthy animals, which is evidently a result of disproportion between dietary energy supply and energy consumption for metabolic processes and milk synthesis. Energy deficit in sick cows caused lowering (by 55.2 %, $p<0.001$) of insulin level, concentrations of insulin-like growth factor (by 28.2 %, $p<0.01$), leptin (by 44.7 %) and an increase (by 69.6 %, $p<0.001$) of cortisol level in blood. These changes give evidence of intensification of gluconeogenesis during period of energy deficit due to lipolysis and proteolysis. Lowering of the leptin level in blood of sick cows is associated with control of energy homeostasis by central nervous system.

Key words: cows, insulin-like growth factor, leptin, ketosis.

Introduction

Ketosis is a disease of ruminant animals, which is characterized by deep metabolic disorders (predominantly carbohydrate-lipid and protein) and is accompanied by increased formation and sharp increase of ketone bodies in blood, urine and milk. Complex symptom group, in which ketonemia, ketonuria and ketonolactia are the main signs at the beginning of disease, is typical for ketosis (Dirksen et al., 2002; Levchenko et al., 2015). Hypodynamia, excessive weight of animal, dysfunction of pituitary-adrenocortical system are the contributing factors for ketosis development. However, the main etiological factor for development of ketosis is energy deficit, primarily luck of easy-to-digest carbohydrates with a background of high milk producing ability of cows (Lee et al., 2011). Leptin, which enchains changes in the level of body fat with control of energy homeostasis by the central nervous system, is directly involved in energy metabolism. This substance is a polypeptide hormone produced by adipocytes, and its main physiological functions is to reduce the synthesis of macroergic compounds and to increase energy expenditure. Otherwise leptin is also called "hormone of appetite control", "hormone of satiety", "hormone that burns calories" (Rosicka et al., 2003). It decreases appetite, increases energy loss, changes metabolism of lipids and glucose. Another important regulator of carbohydrate metabolism is an insulin-like growth factor-1 (IGF-1), also called somatomedin C, synthesized predominantly in liver as a response to level of growth hormone and exerts potentiating influence on proliferation and differentiation of cells in all tissue types. In organism IGF-1 exhibits insulin-like activity: in the muscles it stimulates transport of amino acids and glucose, enhances cellular sensitivity to insulin, in the adipose tissue it stimulates the transport of glucose, oxidation of glucose to CO2, the inclusion of glucose in lipids (Jones et al., 1995).

Despite the already known mechanisms of action of IGF-1 and leptin, issues of their involvement in the regulation of carbohydrate metabolism in cows suffering from ketosis remain unclear. Therefore, the purpose of the study was to investigate the level of glucose and hormones, regulating energy metabolism, in cows suffering from ketosis.

Experimental methods

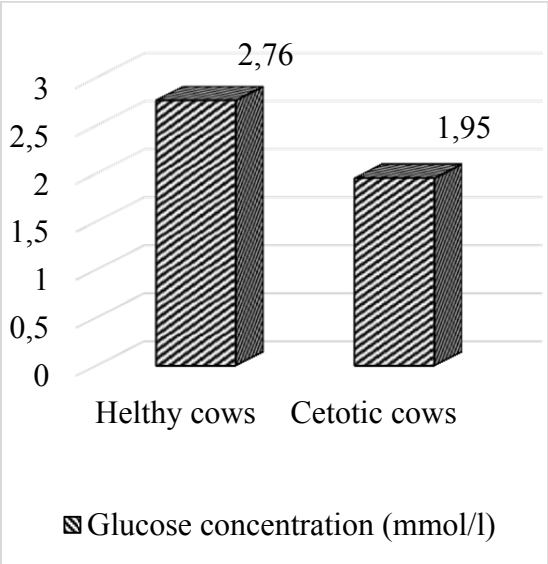
Study was performed on high-yielding milk cows in their second to sixth lactation with milk yield from 5200 to 8300 kg per lactation. Clinical examination was performed in 2 - 3 weeks after calving; level of ketone bodies in urine was determined using diagnostic strips (Ketophan, Pliva). Animals with positive result for the content of ketone bodies were selected into separate group. Blood samples were withdrawn from jugular vein before morning feeding, into sterile tubes with heparin and immediately centrifuged at 3000 rpm. Obtained plasma

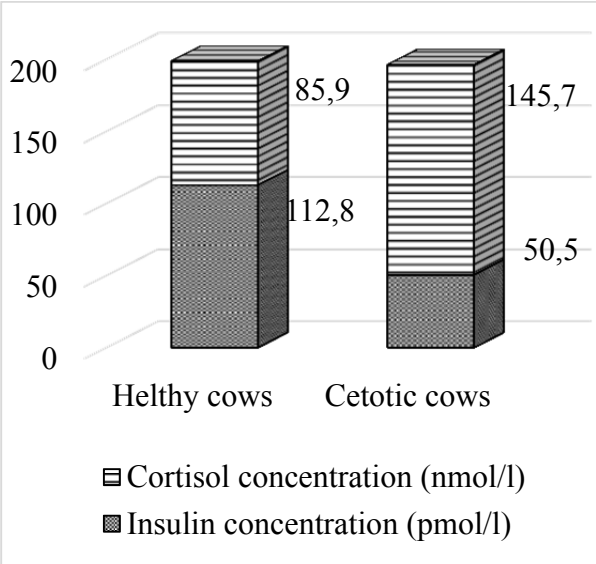
was frozen at – 20°C until analyses were done. Content of insulin, cortisol, insulin-like growth factor and leptin was determined in plasma. An immunoassay analyzer such as StatFax and a test system manufactured by "DRG" were used for investigation. Glucose concentration was determined in plasma using glucose oxidase method within 1 hour after blood collection.

Results and Discussion

During examination of ketotic cows it was revealed that some of animals were lying up, they had quick lowering of live weight and yield, and looked oppressed, some of them had muscular tremor. Ketone bodies were revealed in urine. Concentration of glucose in sick animals was lower by 29.3 %, $p<0.01$

Figure 1. Concentration of glucose, insulin and cortisol in blood of healthy and ketotic cows, n=20



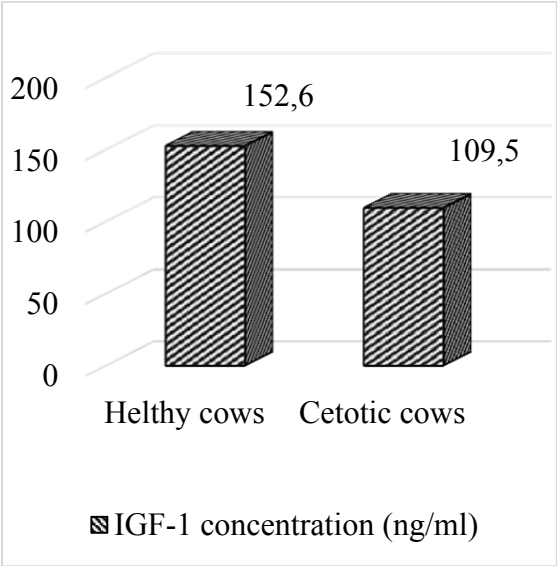


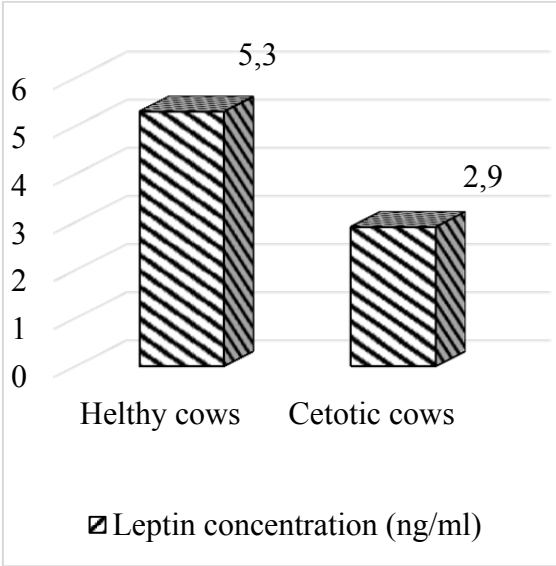
(Figure 1). Such changes might be considered as a result of disproportion between dietary energy supply and energy consumption for metabolic processes and milk synthesis. In case of energy deficit processes of gluconeogenesis are activated in the organism of ruminant animals in order to maintain blood glucose level. These processes are actively regulated by the endocrine system. We found that in cows with ketosis, the concentration of insulin in the blood was by 55.2% ($p<0.01$) lower, and the level of cortisol was by 69.6%, ($p<0.001$) higher than in healthy cows (Figure 1). Insulin activates glucokinase in hepatocytes and reduces the activity of glucose-6-phosphatase, providing the synthesis of glycogen in the liver and muscles and the flow of glucose into cells, simultaneously stimulating its oxidation and inhibiting the breakdown of glycogen (glycogenolysis). Glucocorticoids, particularly cortisol, induce all the key enzymes of

gluconeogenesis, providing this process with starting compounds and reduce the requirement of tissues in glucose, which contributes to increase of its level in blood (Abuelo et al., 2014; Marett et al., 2015). With low insulin level, an increase in the synthesis of cortisol during the period of energy shortage can enhance gluconeogenesis due to lipolysis and proteolysis.

Simultaneously, insulin is a stimulator of synthesis of IGF-1, a hormone whose metabolic effects are similar to insulin regarding mechanism of action and are realized through inhibition of adenylate cyclase activity. Obtained results showed that in plasma of ketotic cows the concentration of IGF-1 was by 28.2%, $p<0.01$ lower than in healthy animals (Figure 2).

Figure 2. Concentration of IGF-1 and leptin in blood of healthy and ketotic cows, n=20





The established decrease in IGF-1 level, associated with increased cortisol and decreased insulin concentrations confirms the existing data on the ability of IGF-1 to regulate metabolic processes, exerting endocrine, paracrine and autocrine activity (Succurro et al., 2010).

The dynamics of changes in plasma level of leptin in ketotic cows was similar to the changes in the levels of IGF-1 and insulin. Particularly, in the blood of cows suffering from ketosis, the concentration of leptin was lower by 44.7% ($p<0.05$) in comparison with such in healthy animals (Figure 2). The organism synthesizes leptin in two ways. The first and "long-term" way is conducted in adipose cells. The second "short-term" pathway for the synthesis of leptin occurs with involvement of insulin. Beta-cells of

pancreas produce insulin in response to a change in blood glucose level; they also have leptin receptors, and leptin itself acts as a negative regulator of insulin release. Insulin stimulates leptin production of in adipose cells. The blood level of leptin increases with increasing of body weight and decreases when the amount of adipose tissue is decreased. The level of leptin decreases in fasting conditions and increases with overeating. Low levels of leptin and IGF-1 in ketotic cows may also indicate poor nutritional status leading to energy deficiency in organism of these animals.

Consequently, in cows suffering from ketosis, endocrine system undergoes considerable exertion. Its compensatory mechanisms are aimed at equalizing of the energy deficit, which manifests through the lowering of blood glucose level and increasing of the activity of gluconeogenesis by reducing the concentration of insulin, IGF-1 and leptin and the growth of cortisol level.

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Comparison of monensin and hop cones effects on rumen fermentation and blood parameters in transition dairy cows

A monensin és a komlókomponensek hatásainak összehasonlítása az elléskörüli időszakban lévő tejelő tehenek bendő fermentációjára és vérparamétereire

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Abstract

Adding to the diet of both investigational supplements: monensin and hop cones decrease concentration of ammonia and increase concentration of lactate in the rumen ($p<0.05$-0.01). Monensin reduced the pH in the rumen from 7.09 to 6.86 ($p<0.05$), and hop cones to 6.99. One week after calving in the blood plasma of cows received monensin a higher concentration of glucose was observed. A decrease in the concentration of ketone bodies, urea and un-esterified fatty acids was detected in the plasma of both experimental groups ($p<0.05$-0.01).

Thus, metabolic effects of hop cones are close to the antibiotic monensin, i.e. possess properties of phyto-ionophores. Hop cones regulate rumen fermentation and metabolism in transition dairy cows, reduce the negative metabolic changes and can prevent common metabolic disorders in high-yielding cows.

Key words: cows, monensin, hop, rumen, blood

Introduction

The transition period (3 weeks before to 3 weeks after parturition) is considered as one of most critical determinant of health and productivity in dairy cows (Wankhade et al., 2017). Excessive release of fatty acids from adipose tissue, insufficient glucose synthesis in the liver, excessive ammonia formation in the rumen are major risk factor for the development of displaced abomasum,

ketosis, fatty liver and lead to poor lactation performance (Herdt, 2013; Simonov & Vlizlo, 2015).

Ionophores possess several positive aspects that are exploited in ruminant production: inhibit hyper ammonia-producing bacteria, decreasing ruminal methane emissions, reducing acetate:propionate ratio (Flythe, 2009,2017; Flythe & Aiken, 2010; Narvaez et al., 2011) and improved rumen fermentation as a whole (Wang et al., 2010). Supplementation with monensin during the transition period is reported to minimize nonesterified fatty acids (NEFA) release into systemic circulation (Contreras et al., 2018), improved energy status and feed efficiency of transition dairy cows (Drong et al., 2016). Treatment with monensin reduced the cumulative incidence of subclinical ketosis of dairy cows and can be considered as one of a range of management options for the control of subclinical ketosis in early lactation (Juchem et al., 2014; Compton et al., 2015; Raboisson & Barbier, 2017).

As the European Union disapproved the use of antibiotics to promote animal growth, there has been considerable interest in finding alternatives to monensin and other ionophore antibiotics. Bacteria make ionophores, but plants also produce antimicrobial compounds. A number of studies have demonstrated that hops possess antimicrobial activity against a range of bacterial species (Narvaez et al., 2011; Flythe, 2009,2017; Karabín et al., 2016). The hop cones consist a range of bioactive secondary metabolites, including antimicrobial prenylated phloroglucinols, which are commonly called alpha- and beta-acids. Alpha acids include humulone, cohumulone, adhumulone, posthumulone, prehumulone; beta acids include lupulone, colupulone, adlupulone as well as other analogs. Hops α- and β-acids possess antimicrobial activity against Gram positive, but not Gram-negative bacteria (Flythe, 2009). This specific activity is a property shared with monensin. Humulone, lupulone and other hop components show potent antioxidant and lipid peroxidation inhibitory activity comparable to the natural antioxidants α-tocopherol and ascorbic acid (Krofta et al., 2018; Karabín et al., 2016). Although the impact of hop extracts on fermentation has been investigated, the effect of whole hops on ruminal fermentation in cows has not been explored. Efficacy of hops on rumen fermentation and animal performance deserves further research. The aim of research was to establish the effectiveness of using hop cones supplementation to diet for the regulation of rumen fermentation and metabolism in cows at the postpartum period and during lactation.

Experimental methods

Thirty multiparous dry dairy cows were assigned to one of three treatment groups of ten cows each. The first group was control. To the diet of second group the monensin in a dose of 400 mg/head/day were added. Third group received diet with meal of dried hop cones for 10 g/head/day. The

experiment lasted during transition period. Diets were formulated to meet the nutrient requirements (NRC, 2001). Hops were supplied as type-90 pellets contained 96 g of α-acids and 58 g of ß-acids per kg dry matter (DM). Hop pellets were ground through a 1.0-mm screen and mixed with the supplement portion of the diet.

Ruminal fluid and blood were sampled one week after calving, before the morning feeding. The rumen liquid was sampled by a stomach tube and blood from jugular vein. Ammonia in rumen liquor was estimated by the method of Conway. Total lactic acid was determined color-imetrically by the method of Barker and Summerson. Plasma samples were analyzed for total protein, glucose, urea, triacylglycerols, β-hydroxybutyrate, and thiobarbituric acid reactive substances (TBARS) using special assay kits on a biochemical analyser Humalyzer 2000 (Germany). The content of nonesterified fatty acids (NEFA) was assessed colorimetrically (reaction with 1,5-diphenylcarbazide). Differences among experimental groups were analysed with one-way ANOVA. Data are reported as means ± SEM. Overall differences between treatment means were considered significant when $P \leq 0.05$.

Results and Discussion

Adding to the diet of both investigational supplements: monensin and cones of hops led to a decrease in the concentration of ammonia and increase concentrations of lactate in the rumen ($p<0.05$-0.01). Monensin reduced the pH in the rumen from 7.09 to 6.86 ($p<0.05$), and hop cones to 6.99 (Table 1).

Table 1. Effect of monensin and hop cones on characteristics of rumen fermentation (Mean±SEM, n=10)

Item	Treatment		
	Control	Monensin	Hop cones
Ammonia N, mmol/L	6.78±0.22	5.31±0.29***	5.12±0.36***
Lactate, mmol/L	5.91±0.35	6.75±0.31*	6.81±0.25*
pH	7.09±0.07	6.86±0.06*	6.99±0.05

Statistical probabilities of treatment differences, * P < 0.05; ** P < 0.01; *** P < 0.001

As was described by Flythe (2009) sodium monensin, dried hop cones or hop cones extract decreased the rate of ammonia production in rumen *in vitro*. Our results indicate that monensin and antimicrobial components of hops reduce ammonia production by ruminal bacteria *in vivo* also. On the other hand, lactic acid bacteria are

also gram-positive, but the concentration of lactate in the rumen contents has increased. Probably, the growth of lactic acid is due to the activity of amylolytic bacteria. The increase in the concentration of lactic acid has reduced the pH of the ruminal fluid, but this change was negligible.

Adding to the diet of monensin and hops cones reduced the concentration of urea in the blood plasma (Table 2), which may have been the result of less formation of ammonia in the rumen. Reducing the flow of ammonia to the blood is very important, as it weaken the metabolic loading of the liver, functioning of which in transition cows is on the limit of possibilities.

In the blood plasma of cows received monensin or hops cones, a higher concentration of glucose was observed. Most likely, it is associated with larger production of propionate in the rumen, which is the main precursor of blood glucose in ruminants.

Table 2. Effect of monensin and hop cones on blood plasma parameters (Mean±SEM, n=10)

Item	Treatment		
	Control	Monensin	Hop cones
Total protein, g/L	64.18±1.79	70.22±1.43	75.13±1.92
Urea, mmol/L	5.95±0.27	5.32±0.15*	4.73±0.21**
Glucose, mmol/L	2.78±0.07	3.34±0.05*	3.05±0.08**
Triglycerides, mmol/L	0.29±0.01	0.35±0.02	0.33±0.03
NEFA, mmol/L	0.84±0.04	0.59±0.02**	0.73±0.05
β-Hydroxybutyrate, mmol/L	0.88±0.05	0.65±0.04**	0.78±0.04*
TBARS, umol/L	1.67±0.08	1.52±0.10	1.24±0.09**

Improving the energy supply of cows is confirmed by the reduction of nonesterified fatty acids (NEFA) in the blood. It is important that the concentration of triacylglycerols in the blood did not change. NEFA are released from adipose tissue and enter the liver where esterified to triacylglycerols and are excreted in the blood as a part of very low-density lipoproteins. With excessive intake of nonesterified fatty acids, part of triacylglycerols remains in the liver and leads to steatosis. In our case, the intake of fatty acids has decreased, but triacylglycerols withdrawal has not changed what indicates the positive effect of studied additives on the liver function.

Lower concentrations of β-hydroxybutyrate, urea and nonesterified fatty acids were detected in the blood plasma of both

experimental groups ($p<0.05$-0.01). The decrease in the concentration of ketone bodies in the blood of experimental cows can be explained by changes in glucose content. Ketone bodies are formed as additional energy substrates at glucose deficiency. Since in this case the concentration of glucose increased, the need for ketone bodies was less.

Adding hops to the diet of cows has suppressed peroxidation processes. In particular, the concentration of TBARS in the blood plasma of cows fed with hops was significantly lower than in control cows. At the same time, monensin did not affect the peroxidation.

Thus, monensin and hop cones showed similar effects on the investigated parameters in the rumen and blood of transition cows. However, monensin more effectively increased the concentration of glucose and reduced the concentration of nonesterified fatty acids and ketone bodies in plasma, while hops showed antioxidant effect.

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Effects of a different body condition in dry period on energetic profile in dairy cows

Különböző kondiciók hatása a tejhasznú tehenek szárazon állás alatti energia profiljára

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Abstract

The aim of this study was to analyse the effect of different prepartal body condition on the energetic profile of dairy cows in dry period. The study was conducted on a dairy farm in Slovakia. The dairy cows were divided into two groups: first group with normal BCS 3.25 – 3.75 (n=23) and second group with an over condition BCS >4.0 (n=27). In blood serum, individual energetic metabolites: glucose (Glu), beta-hydroxybutyrate (BHB), triacyglycerides (TAG), unsterified fatty acids (NEFA) and cholesterol (Chol) were determined. Individual parameters were statistically evaluated using descriptive statistics and a non-parametric Mann-Whitney test.

Significant difference was observed only at concentrations of triacylglycerides ($p < 0.01$).

Key words: BCS, dairy cows, energetic profile, dry period

Introduction

Body condition score (BCS) is nowadays considered to be an important part of dairy cows managment. Monitoring of the BCS before partuition affect productivity, reproductive parameters and health status in postpartal period. BCS is a subjective, visual or tactile evalution of subcutaneous fat in dairy cows (Edmondson et al., 1989). Most metabolic diseases are manifested in the extreme loss of BCS, nutritional deficiency, or failure of metabolic processes occurring in the peripartal period (gestation demands turn into lactation demands). Nowadays, a condition of 3.25 to 3.5 is recommended for dry cows and it is important that these dairy cows did not gain or loose body weight in dry period. Overconditioned dairy cows or cows in a very poor condition present calving difficulties and often have postpartum complications due to immunological deficiency and metabolic disorders (Sordillo & Raphaelet, 2013).

Transient period is characterized as a period 3 weeks before and 3 weeks after parturition. High energetic demand is typical for these period of time as a result of fetal growth, lactation , negative energetic balance (NEB) and decreased apetite. Balanced energy metabolism during this period is a key factor in managing the postpartum period. Metabolites such as NEFA and BHB are commonly used as indicators of negative energy balance and ketosis in transient dairy cows. Although a slight increase in serum levels of these metabolites is normal because cows balance energy intake and outake during early lactation, but excessive increases in NEFA and BHB indicate an insufficient metabolic adaptation to NEB (Herdt, 2000). When dairy cows are unable to alter their energy metabolism quickly enough for lactation, dairy production is rapidly declining, and these dairy cows are more susceptible to metabolic disease (Herdt, 2000; LeBlanc, 2010). Serum NEFA concentration reflects the level of fat mobilization, while BHB concentration indicates complete fat oxidation in the liver (LeBlanc, 2010). Many studies have dealt with the determination of NEFA, BHB and calcium serum thresholds to predict production diseases in transient dairy cows (Ospina, 2010; Roberts et al., 2012). Increased levels of NEFA, BHB and decreased serum calcium before calving are associated with a higher risk of clinical diseases (LeBlanc et al., 2005; Seifi et al., 2011), decreased lactation (Ospina et al., 2010) and insufficient reproduction activity in the postpartal period.

Materials and Methods

The study was conducted on a dairy farm in Slovakia with approximately 400 dairy cows with average annual milk production of 8000

L. Dairy cows in dry period were fed with total mixed ration (TMR) ad libitum twice a day. 50 dairy cows were selected for the study and they were divided into two groups: first group with normal BCS 3.25 – 3.75 (n=23) and second group with an over condition BCS >4.0 (n=27).Body condition was determined in dry period by visual and tactile examination of hook bones, pin bones and tailhead. Blood samples were taken approximately 2 weeks before parturition from *v. coccygea* (Hemos H-02, Gama Group a.s, CR). In blood serum, individual energetic metabolites: glucose (Glu), beta-hydroxybutyrate (BHB), triacyglycerides (TAG) and cholesterol (Chol) were determined spectrophotometrically (Alizé, Lisanbio). Unsterified fatty acids were also analyzed spectrophotometrically (A Specord 210, Analyte Jena). Individual parameters were statistically evaluated using descriptive statistics and a non-parametric Mann-Whitney test.

Results and Discussion

Our aim was to determine how the energy profile before parturition of dairy cows with BCS> 4.00 is different compared to the cows with normal BCS. Results of the measurement the serum energy profile indicators are presented in Table 1. In the statistical evaluation, the significant difference between the groups was found only for triacylglycerides (p <0.01).

Table 1. Concentration of serum energetic metabolites in cows with different BCS (x ± SD).

	BCS 3.00 – 3.75	**BCS 4.00 – 5.00**	
BCS	3.55 ± 0.24	4.35 ± 0.34	P<0.001
NEFA	0.37 ± 0.21	0.39 ± 0.17	ns
Glu	3.79 ± 0.89	4.08 ± 0.99	ns
Chol	2.69 ± 0.75	2.40 ± 0.66	ns
TAG	0.14 ± 0.08	0.21± 0.08	p<0.01
BHB	0.45 ± 0.16	0.46 ± 0.16	ns

BCS- body condition score, NEFA – non-esterified fatty acids , Glu- glucose,
TAG- triacylglycerids, BHB- beta-hydroxybutyrate, ns- non significant

The main serum parameters which are assessed in prepartal and postpartal dairy cows are NEFA, BHB and calcium (Ospina et al., 2010; Chapinal et al., 2011; Martinez et al., 2014). These concentrations indicate the rate of adaptation to NEB, and the

occurrence of postpartum diseases can be predicted. Ospina et al. (2010) stated that if the concentration of NEFA before calving is above 0.5 mmol/l, an increased risk of retained placenta, metritis, displaced abomasum, reduced milk production and extension of service period may occur. An elevated BHB concentration (≥0.8 mmol/l) in the prepartal period is an indicator of reduced postnatal production and increased risk of abomasal dislocation (Chapinal et al., 2012). It is generally known that dairy cows with BCS> 4.00 are more susceptible to metabolic disorders in the postpartum period. Because adaptation to NEB is inadequate, lipomobilization occurs and consequently increases serum concentrations of NEFA and BHB. Overtreshold NEFA values (above 0.5 mmol/l) in our study occurred in 13.04% of dairy cows with normal body condition score and in 18.5% of overconditioned cows. Based on the study findings, we can conclude that we have not observed different concentrations of NEFA and BHB in groups of cows with a different condition.

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Effects of glucose infusion on blood potassium in cattle

Glükóz infúzió hatása a vér kálium szintre szarvasmarhákban

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Abstract

The aim of this study was to confirm whether a rapid intravenous glucose administration can lead to a significant drop in blood potassium levels in cattle. For this study seven cattle were used according to internationally recognized guidelines for animal welfare. Glucose at a dose of 1 g/kg body weight was administrated intravenously and then blood samples were taken according a time schedule of (0, 0.5, 1, 2, 4, and 6 hours for measurement of potassium and glucose blood concentrations. The results have shown a slight decrease in blood serum potassium levels at the time of maximum blood glucose concentration of (0.26 mmol/l). We have shown that glucose does have a potassium-lowering effect when administered in a single large IV dose, however, without clinical effects.

Key words: glucose, potassium, cattle

Introduction

Potassium is recognized as an essential nutrient in animal nutrition. It is the third most abundant mineral element in the animal body and the main ion of the intracellular fluid. Most of the total potassium in the

body is located in muscle tissue (Leeson et al., 1998). The most significant role of potassium is in relation to nerve innervation and muscle excitability, and it is also involved in carbohydrate metabolism (McDonald et al., 2002). Glucose requirements of the dairy cow are dominated by the necessity of the mammary gland for milk synthesis. With greater milk yield comes larger requirements for glucose, which is mainly met through glucose synthesis in the liver (Reynolds, 2005). According to research by Leonhard-Marek et al. (2012), a relationship was found between hypokalemias, insulin imbalances and ketosis in high yielding dairy cows. These changes in glucose homeostasis and hypokalemia might according to the researcher be a contributing factor to the pathogenesis of abomasal displacement resulting from decrease in abomasal tone.

The purpose of this study was to confirm whether a rapid intravenous glucose administration can lead to a significant drop in blood potassium levels in cattle.

Experimental methods

The group of the experimental animals consisted of five heifers and two Holstein-Friesian dairy cows which were admitted to the Clinic for treatment of various health disorders. They were clinically healthy at the moment of the study. Blood was collected by venepuncture of the jugular vein before and 0.5, 1, 2, 4, and 6 hours after administration of glucose. Each animal was given one gram of glucose per kg body weight, which resulted in 1000 ml of 40 % glucose solution for a 400 kg cow on maximum infusion speed. The average time of administration was 25 minutes, approximately. The plasma concentrations of glucose were assayed with the kits supplied by Randox Laboratories Ltd. on spektrophotometer Alizé (Lisabio, France). The serum potassium concentrations were determined by flame AAS method (Perkin Elmer AAnalyst 100). For statistical analysis of glucose effects on both glucose and potassium levels one way analysis of variance (ANOVA) for repeated measures was used.

Results

The glucose administration resulted in plasma increase from 4.2 mmol/l to 21.2 mmol/l within first minutes after the administration. From the peak in plasma glucose at 30 minutes, there is a steady decrease in the plasma glucose concentration to 3.36 mmol/l. ANOVA showed a strong significant effect of the glucose administration on plasma glucose levels ($p<0.001$). The serum potassium tended to decrease within the first hour after the glucose injection and then there was a steady increase of potassium serum concentration. ANOVA showed a weak significant effect ($p<0.05$) of the glucose administration on serum potassium levels (Table 1).

Table 1. Mean plasma glucose and serum K (x±sd) before and after glucose administration.

Collection times (h)	Glucose (mmol/l)	Potassium (mmol/l)
0	4.22±0.59	3.56±0.74
0.5	21.2±5.02	3.41±0.74
1	15.4±3.97	3.30±0.75
2	8.41±3.05	3.52±0.83
4	3.83±0.66	4.03±0.74
6	3.36±1.39	3.75±0.96
ANOVA	p<.001	p<0.05

Discussion

Potassium is one of the major elements and has an important role together with other elements such as sodium, chlorine and bicarbonate ions. Together they control the acid- base balance and the osmotic regulation of fluids in the body (McDonald et al., 2002) as serum potassium serves as regulator of aldosterone secretion in which high serum levels of potassium increases plasma aldosterone levels (Betancourt-Calle et al., 2001). The most abundant role of potassium is in relation to nerve and muscle excitability, and is also involved in carbohydrate metabolism (McDonald et al., 2002). Potassium deficiency is rather rare in farm animals under normal conditions, due to a high concentration of potassium in plants, for example 25 g/kg DM in grass. Symptoms of deficiency in calves given milk replacement low in potassium includes severe paralysis (McDonald et al., 2002). In general, hypokalaemia will increase the membrane potential, resulting in a hyper-polarization block causing weakness of muscles or paralysis, ileus, cardiac arrhythmias, rhabdomyolysis and renal dysfunction (Kokko & Tannen, 1986; Dow et al., 1987a,b). Hypokalaemia is commonly the result of gastrointestinal losses from either diarrhoea or vomiting or excessive renal losses due to diuretics, overload of mineral-corticoids or renal tubular acidosis (Kokko & Tannen, 1986) but also from excessive rapid bicarbonate administration, insulin with glucose administration or catecholamine release (Kaneko, 2008). As a very frequent use of glucose in the treatment of many bovine diseases it was essential to test the hypothesis of the in vivo study (Leonhard-Marek et al., 2012), as it was stated that when

glucose was administered in a single rapid dose of (500 ml, 40 %) the serum concentration of potassium strongly decreased.

The results presented in this thesis did reveal a small serum potassium-lowering effect when each of the seven animals was given 1 g/kg (500 ml, 40 %) of IV glucose within 30 minutes after glucose administration. This little drop in blood potassium was not associated with any clinical sign. However, the most of the experimental animals had the serum potassium levels slightly below the physiological range prior to the glucose administration. Thus, a slight decrease of potassium was might be too low to start some abnormal physiological processes in the organism adapted to lower potassium´s body pool.

According to the results of this study it can be concluded that a single intravenous administration of the glucose is not associated with a sever risk of metabolic and health impairment in cattle due to the strong drop in blood potassium. Thus, a use of the glucose in treatment of an energy deficiency in cattle can be further recommended.

Acknowledgments

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Antioxidant status and concentration levels of malondialdehyde (MDA) in dairy cows during periparturient period

Az antioxidáns státusz és a malondialdehid (MDA) koncentrációja a tejelő tehenekben peripartalis időszaka alatt

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Abstract

The aim of the present study was to evaluate a marker of oxidative stress – malondialdehyde (MDA), a product of lipid peroxidation, indicators of antioxidant status – glutathione peroxidase (GPx), selenium, total antioxidant status (TAS), vitamins A, E and beta-carotene and the metabolic profile in dairy cows of the Holstein breed from 6 weeks a.p. to 6 weeks p.p. The MDA concentration was significantly higher immediately after parturition compared to 6 weeks a.p. and p.p. ($P < 0.01$; $P < 0.05$). Significantly decreased GPx activity was recorded in the postpartum period until 6 weeks p.p. The TAS level ($P < 0.001$) and vitamin A concentration ($P = 0.01$) were significantly decreased immediately after parturition and the vitamin E concentration at 1 week p.p. ($P < 0.001$) compared to 6 weeks p.p. A significantly increased MDA concentration and decreased antioxidants indicate the occurrence of oxidative stress during the periparturient period in dairy cows and can contribute to increased incidence of metabolic diseases.

Key words: malondialdehyde, antioxidant, oxidative stress periparturient period, dairy cow

Introduction

Dairy cows are subjected to metabolic and physiological adaptations in the transition period between late pregnancy and early lactation (Sordillo & Aitken, 2009). The postpartal period is associated with

increased energy requirements and decreased dry matter intake (DMI) and a negative energy balance (NEB) occurs in dairy cows. The metabolic and energy demands result in the increased production of reactive oxygen species (ROS) (Sordillo, 2005; Pilarczyk et al., 2012). The concentration of ROS is controlled by the antioxidant defense system. The imbalance between their production and degradation leads to oxidative stress and oxidation of various biological substrates – macromolecules (Sies, 1993; Miller et al., 1993).

Oxidative stress, and particularly the presence of ROS, is associated with lipid peroxidation – a cause of oxidative damage. The reaction is based on oxidation of primarily unsaturated fatty acids, and their intermediates, such as malondialdehyde and 4-hydroxyalkenals, are considered markers of oxidative stress (Halliwel & Gutteridge, 1985; Niki, 2014). Oxidative stress induced during the periparturient period can be one of the reasons for the increased incidence of disease in dairy cows (Miller et al., 1993).

The aim of our study was to determine and evaluate a marker of oxidative stress and antioxidant status during the periparturient period in dairy cows using the following parameters: plasma malondialdehyde (MDA) levels, whole blood glutathione peroxidase (GPx) activity and selenium concentrations, serum total antioxidant status (TAS), vitamin A, E and beta-carotene concentrations. The basic metabolic profile was also determined in all cows – serum non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), bilirubin, glucose, urea, aspartate aminotransferase (AST), zinc, copper, calcium, phosphorus and magnesium.

Table 1. Total mixed ration composition kg/day/cow

Ingredients	*ante partum*	*post partum*
Alfalfa hay	2	1
Barley straw	2.2	0
Concentrate (DOVP)	0	6
Concentrate (DOVP – a.p.)	2.8	0
post-extraction rapeseed meal	0.8	0
Pamitate	0.5	0.15
mp iont	0	0
High moisture porn	0	3
Brewers grains	0	4
Alfalfa haylage	0	6
Maize silage	15	19

Materials and Methods

Animals and diets. The study was carried out using 10 Holstein dairy cows at a farm located in the village of Uherčice (Břeclav, South Moravia). The cows were fed a total mixed ration (TMR) according to the

antepartum and postpartum period (Table 1). All of the cows have gone through two or more lactations. The mean milk production was 9,157 liters per last lactation. The average body condition score (BCS) was 3.80 before parturition.

Sampling and analysis. A total of 60 blood samples were taken at 6 different stages of ante partum (a.p.) and post partum (p.p.) – 6 weeks, 3 weeks and 1 week a.p., immediately after parturition, and 3 and 6 weeks p.p. Blood samples were used for analysis or stored at -70 °C immediately after collection. Blood samples were collected from the vena coccygea media into Hemos sampling tubes without anticoagulant for serum determination and into Hemos sampling tubes with heparin anticoagulant for determination of whole blood and plasma. Plasma MDA concentration was determined using the high-performance liquid chromatography (HPLC) system Ultimate 3000 (Dionex, USA), as described previously (Matějčková et al., 2011). The selenium concentration in whole blood and the serum concentrations of minerals (Ca, Zn, Cu, Mg) were analyzed using atomic absorption spectrometry – AAS (SOLAAR, Thermo Scientific, USA). Vitamin A, E and beta carotene levels in serum were determined using the HPLC system Ultimate 3000 (Dionex, USA). GPx activity in whole blood was measured with a RANSEL kit (Randox Laboratories Ltd., UK) using a UV method based on that of Paglia and Valentine (1967). The serum TAS, NEFA and BHB concentrations were measured using standardized kits supplied by Randox Laboratories. The other biochemical parameters such as bilirubin, glucose, urea, AST and phosphorus in serum were determined using commercial kits (Biovendor, Czech Republic). Cobas Mira Plus (Roche, Switzerland) and Liasys (AMS, Italy) automatic biochemical analyzers were used for determination.

Statistical analysis. The results obtained were tested for the homogeneity of variances (Hartley-Cochran-Bartlett test) and the normality of distribution (Shapiro-Wilk test). The data were analyzed statistically by one-way analysis of variance (ANOVA) followed by the Fisher LSD post-hoc test. All results were expressed as mean value (x) ± standard deviation (SD).

Results and Discussion

The periparturient period is a critical phase in dairy cows and is extremely important to their health, productivity and fertility (Roche et al., 2009). This period is characterized by the depletion of antioxidants and results in an imbalance between pro-oxidants and antioxidants. The results for the oxidative stress marker and antioxidant parameters are presented in Table 2. MDA and TAS values may be considered indicators of the balance between pro-oxidants and antioxidants. In our study, the mean MDA concentration (0.276 μmol/l) was significantly higher

immediately after parturition as compared to 6 weeks a.p. ($P < 0.01$) and 6 weeks p.p. ($P < 0.05$) which indicates an insufficient amount of antioxidants and an increase of ROS, including lipid peroxidation products, and an increasing risk of oxidative stress (Castillo et al., 2005; Gong & Xiao, 2016). According to Ghiselli et al. (2000), the TAS evaluates the overall antioxidant capacity in the organism and can therefore provide information about antioxidants in serum. It could confirm the fact that the highest mean TAS level in this study was found at 6 weeks p.p., as were the highest vitamin A and vitamin E concentrations. The TAS results either from increased ROS production or antioxidant intake in the diet, and can therefore provide relevant information about the balance between pro-oxidants and antioxidants (Ghiselli et al., 2000). The results of the present study showed the lowest significant TAS level immediately after calving (0.77 mmol/l; $P < 0.001$) in dairy cows. Similar data were obtained by Gong and Xiao (2016) who found a decrease of TAS levels in the

Table 2. Marker of oxidative stress and antioxidant parameters in dairy cows from 6 weeks before parturition to 6 weeks after parturition

Group No.		MDA µmol/l	GPx µkat/l	Se µg/l	TAS mmol/l	vit A µmol/l	vit E µmol/l	β-kar µmol/l
1	x	0.231^{A}	$1168.5^{\alpha,a,b}$	162.8	0.83	0.74	7.70	5.21
6 w a.p.	SD	0.045	139.1	19.3	0.10	0.22	2.15	2.60
2	x	0.234	$1085.5^{\beta,A}$	156.1	$0.80^{\alpha,a}$	0.75^{α}	6.38^{a}	5.27
3 w a.p.	SD	0.068	106.2	16.6	0.09	0.16	1.52	2.40
3	x	$0.276^{A,\alpha}$	1046.3^{α}	169.0	$0.77^{\beta,b}$	0.65^{β}	6.45^{b}	5.34
p.p.	SD	0.066	246.5	21.1	0.11	0.34	2.15	3.30
4	x	0.265	$872.3^{a,\beta,B}$	155.9	0.86^{A}	0.79	5.37^{c}	4.56
1 w p.p.	SD	0.051	178.3	19.8	0.05	0.24	1.49	1.44
5	x	0.272	$792.7^{b,A,c}$	159.0	$0.94^{\alpha,\beta}$	0.85	7.28	4.14
3 w p.p.	SD	0.048	268.7	26.1	0.12	0.27	2.34	3.04
6	x	0.248^{α}	$1153.0^{B,c}$	169.8	$0.98^{a,b,A}$	$1.04^{\alpha,\beta}$	$12.14^{a,b,c}$	5.56
6 w p.p.	SD	0.034	158.5	24.4	0.12	0.26	3.30	3.63

x – mean value; SD – standard deviation; significant differences between groups are indicated by using the same indices in a column: α, β – $P < 0{,}05$; A, B – $P < 0{,}01$; a, b, c – $P < 0{,}001$; w – week; a.p. – *ante partum*; p.p. – *post partum*; MDA – malondialdehyde; GPx – glutathione peroxidase; GSH – glutathione; Se – selenium; TAS – total antioxidant status; vit A – vitamin A; vit E – vitamin E; β-kar – beta carotene

early lactation period. The enzyme GPx, one of the maincomponents of the antioxidant defense system, plays a key role in protecting the organism against ROS (Pilarczyk et al., 2012). In our study, decreased GPx activity was recorded in the postpartum period until 6 weeks p.p. A statistically significant difference in GPx activity was recorded immediately after parturition (1046.3 µkat/l; $P < 0.05$) and at 1 week p.p. (872.3 µkat/l; $P < 0.001$) compared to 6 weeks a.p. This finding is in agreement with the published findings of Konvičná et al. (2015) who observed a reduction in GPx activity 1 week after parturition as a reason for increasing postpartal oxidative stress. GPx activity is also used in indirect determination of selenium status and indicates long-term selenium supplementation (Pavlata et al., 2000). Our results confirm the correlation with the selenium concentration (r = 0.34) and are in line with previous studies by Pilarczyk et al. (2012) (r = 0.46) and Konvičná et al. (2015) (r = 0.52). A deficiency of antioxidants such as vitamin A and vitamin E is typical for the transition period. The main reason for their lower concentrations around parturition is an increase in vitamin levels in the colostrum (Rizzo et al., 2013). A decrease in vitamin concentrations was also observed in our study. A significantly decreased vitamin A concentration (0.65 µmol/l; $P = 0.01$) was found immediately after parturition and the mean vitamin E concentration (1.49 µmol/l; $P < 0.001$) was significantly lower at 1 week p.p. The beta carotene concentration was also decreased at 1 week after calving.

The results for the metabolic profile parameters are shown in Table 3. Insufficient energy intake in the periparturient period results in a decrease in glucose levels and an increase in NEFA levels as a response to lipomobilization (Omur et al., 2016). This could explain the findings in our study in which a decrease in glucose concentration from 1 week p.p. was observed and the highest NEFA value was found at 1 week after calving (0.48 mmol/l; $P < 0.001$). A significant but non-pathological increase in NEFA concentration may be the reason for BHB concentration stability during the periparturient period and dairy cows not being predisposed to an increased incidence of ketosis. The lowest significant urea value was found at 1 week after parturition (2.63 mmol/l; $P < 0.001$) in dairy cows. This is in line with the findings of Omur et al. (2016) who reported a decrease in urea concentration in the postpartum period as a reason for liver damage and a decrease in protein anabolism. The significant increase in serum AST levels after calving ($P < 0.01$) may be associated with increasing synthetic activity in the liver due to protein mobilization (Park et al., 2002) or with slight liver damage caused by lipomobilization (Omur et al., 2016), and is in agreement with the results observed by Castillo et al. (2006). Copper and zinc are components of enzyme Cu-Zn superoxide dismutase (SOD) in the antioxidant system. Goff & Stable (1990) found that Zn

concentrations decrease at parturition in dairy cows and that after 3 days their levels are again within physiological ranges. On the basis of this finding, we also predicted a decrease in Cu concentration at parturition in dairy cows. The lowest significant zinc (11.42 µmol/l; $P < 0.01$) and copper (10.51 µmol/l; $P < 0.05$) concentrations were determined immediately after parturition in dairy cows.

In conclusion, the occurrence of oxidative stress, particularly shortly after calving, is a long-standing problem in dairy cows. Regular monitoring of the antioxidant defense system and antioxidant supplementation can help protect against a number of diseases that occur during the periparturient period in dairy cows.

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Table 3. Biochemical parameters and minerals in dairy cows from 6 weeks before parturition to 6 weeks after parturition

Group No.		BHB mmol/l	NEMK mmol/l	Bili µmol/l	Glu mmol/l	Urea mmol/l	AST µmol/l	Ca mmol/l	P mmol/l	Zn µmol/l	Cu µmol/l	Mg mmol/l
1	x	0,57	$0,14^{a,b}$	$4,9^{A}$	$3,1^{\alpha,A}$	$4,57^{\alpha,a,b}$	$0,85^{A,\alpha}$	$2,32^{a}$	$1,61^{\alpha,A}$	$13,49^{A}$	11,96	$0,82^{A}$
6 w a.p.	SD	0,14	0,08	1,9	0,3	1,02	0,20	0,11	0,43	1,32	1,99	0,10
2	x	0,66	$0,13^{A,B,c}$	4,0	$3,2^{\beta,B}$	$5,05^{A,c,d}$	1,02	$2,31^{b}$	$1,71^{B}$	$13,00^{\alpha}$	$10,99^{\alpha}$	0,98
3 w a.p.	SD	0,10	0,14	1,8	0,3	0,97	0,31	0,09	0,39	2,19	1,29	0,11
3	x	0,53	$0,33^{a,A}$	4,0	3,1	$3,20^{\alpha,A}$	0,99	$2,08^{a,b,c,d,e}$	$1,29^{\alpha}$	$11,42^{A,B,\alpha,\beta}$	$10,51^{\beta}$	$1,05^{A,B}$
p.p.	SD	0,19	0,17	1,8	0,6	1,13	0,25	0,20	0,42	2,31	1,40	0,21
4	x	0,56	$0,48^{b,c,C}$	3,0	$2,6^{\alpha,\beta}$	$2,63^{a,c}$	$1,31^{A}$	$2,29^{c}$	$1,25^{A,B,\beta,\gamma}$	12,88	$12,51^{\alpha,\beta}$	$0,83^{B}$
1 w p.p.	SD	0,18	0,16	2,3	0,5	1,07	0,31	0,14	0,30	2,07	1,14	0,10
5	x	0,63	$0,32^{B}$	$2,4^{A,B}$	$2,5^{A,B}$	$3,08^{b,d}$	$1,13^{\alpha}$	$2,31^{d}$	$1,72^{\beta}$	$12,49^{\beta}$	12,51	0,90
3 w p.p.	SD	0,16	0,20	1,0	0,4	0,77	0,16	0,14	0,55	2,47	1,31	0,10
6	x	0,49	$0,27^{C}$	$5,0^{B}$	2,4	3,25	0,82	$2,35^{e}$	$1,49^{\gamma}$	$13,39^{B}$	11,34	0,97
6 w p.p.	SD	0,16	0,16	1,2	0,4	0,82	0,40	0,08	0,37	1,15	2,13	0,11

x – mean value; SD – standard deviation; significant differences between groups are indicated by using the same indices in a column: α, β – $P < 0,05$; A, B – $P < 0,01$; a, b, c – $P < 0,001$; w – week; a.p. – *ante partum*; p.p. – *post partum*; BHB – beta-hydroxybutyrate; NEFA – non-esterified fatty acids; Bili – bilirubin; Glu – glucose; AST – aspartate aminotransferase; Ca – calcium; P – phosphorus; Zn – zinc; Cu – copper; Mg - magnesium

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Distribution, etiology, course and diagnosis specificity of polymorbid internal pathology in cows

Polimorbid megbetegedések megoszlása, etiológiája, perspektívája és diagnózis-specifitása tehenekben

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Abstract

There is introduced the idea as to polymorbidity of internal pathology in high-producing cows, experimentally and theoretically substantiated its pathogenesis. For the first time in Ukraine there was used complex approaching as to the study of clinical and functional status of high-producing cows in different keeping and feeding systems, there were determined physiological limits of biochemical indexes that are indicative to infringement of rumen digestion, carbohydrates and lipid exchanges, functional condition of liver that are the basis for elaborating informative diagnostic methods.

Polymorbid pathology was diagnosed in 32% of cows. For the diagnosis of

polymorbid internal pathology in high-producing cows represented by ketosis, hepatodystrophy, rumen hypotony the most informative indexes are determining the ketone body in urine, the level of pH in rumen content, the content of butyric acid, general protein and albumins in blood serum, the results of mercury chloride test, hyperfermentemia of AsAT.

Key words: polymorbid internal pathology, rumen distonia, ketosis, hepathodystrophy, etiology, diagnosis, dairy cow.

Introduction

For the most part, the diseases of animals, including those of non-contagious etiology, are studied as mono-diseases. At the same time, in veterinary medicine, there may be found associated infectious diseases with two or more pathogens (for example, rotavirus enteritis and escherichiosis, escherichiosis and cryptosporidiosis, etc.) (Verbitskii et al., 2004). Internal non-infectious diseases are mostly polyetiologic, and therefore, having the same or similar causative factors, run parallel. Furthermore, because the pathology of any organ or metabolic disorders may cause complications, the pathological processes mostly extend to other organs and systems. In the national and world scientific literature there are many examples of parallel and combined course of many internal animal diseases, obstetric, surgical pathology, etc. (Levchenko et al., 1998; Sokoluk et al., 2011; Chapinal et al., 2012; Raboisson et al., 2014). Such a combination of several diseases is called a polymorbide internal pathology. Consequently, polymorbide internal pathology (Greek ***poly*** means many; Latin ***morbus*** means - a disease) is a series of diseases that have a simultaneous course and common or similar etiology and interdependent pathogenetic links, symptoms, and syndromes. At the core of the development of polymorbidity in high-yielding cows are ketosis, hepatodistrophy and forestomach hypotony (dystony) (Sakhniuk, 2006).

The aim of the present study was to study the etiology, course specificity and changes in the clinical and biochemical status in cows with polymorbid internal pathology (PIP).

Materials and Methods

Clinical and experimental studies were carried out in the winter-spring period. The Holstein cows of 1st-3rd lactation, 1–14 and 20–80 days after calving there were used in the study.

The diagnosis of PIP was based on anamnesis, clinical data, ration analysis, laboratory tests results of blood, urine and rumen content (Sakhniuk, 2006). Biochemical parameters of blood were determined by unified methods on the BioChemical analyzer Stat Fax-4500 (USA), fraction of volatile fatty acids (PFAs) – using chromatograph "Hrom-5" (Czech Republic).

Results and Discussion

The polymorbid internal pathology (PIP) is represented by the development of rumen dystonia, ketosis and hepatodistrophy. Simultaneous course of the diseases was diagnosed in 217 cows, which makes up 32.0% of the cows studied. It included 34.7% (8.4–65.7) of cows 1–14 days after delivery and 31.2% (11,254.8) of cows in early lactation. Significant fluctuations in the distribution of pathology we contributed to the different structure of feeding rations and energy supply.

The main causes of the development of PIP were: a) violation of the structure of the cows rations: the share of coarse (hay and straw) fodder by the exchange energy was 6,0-16,1%, concentrates – 48.0-61.5, succulent fodder – 32.9-48.5%; the rations often lack exchange energy and dry matter content; b) a deficit in the diet of highly-fermented carbohydrates - sugar and starch, and their ratio with digestible protein was low - 1.3-1.8:1, versus 2.0-2.5:1 normally; c) feeding a large amount of concentrated feed (3.5-4.0 kg per meal, two or three meals per day. Most of the concentrates were distributed during the first half of the day; d) an imbalance of rations with macro- and microelements and vitamins; e) obesity in the dry period; e) hypodynamia.

In the most of sick cows the pathology was manifested by inhibition of general state, decreased milk production and appetite, refusal of concentrates, taste distortion, in some animals - anorexia and forestomach hypotonia (3-6 times per 5 min). One third of cows at the beginning of the disease showed a sharp decrease in milk yield (1.5-2 times), anorexia, hyperesthesia, shaky stroke, tremor of muscles, grinding of teeth. With the development of the disease there were noted further oppression, drowsiness, the animals stood with their head lowered, the reaction to the external stimuli was minimal, difficulties while standing up. The affected cows quickly lose their body condition score (up to 60-80 kg per cow). Body temperature in sick cows was at the upper limit or slightly increased. In the vast majority of the affected cows there were found the weakening of pulse and tones of the heart, tachycardia, tachypnoe. 36.4% of the cows showed an increase in the liver size (in the 12th intercostal space, the liver dropped below the hook line by 8-13 cm, and in the 11th - much lower of the horizontal line of the middle of the shoulder blade, sometimes coinciding with the edge of the costal arch, and the width of the dull zone reached 20-30 cm. Fecal masses in affected animals were often not formed, semi-liquid and even watery consistency.

The infringement of the ration structure and the feeding regime caused the development of forestomach dystonia, the reducton of the pH of the rumen content to 5.7-6.3 (5.9 ± 0.02), the decrease of the number of infusorians in 1.83 times and lowering the reductase activity (Table 1).

Table 1. The indexes of rumen content in high-yielding cows (n=391)

Indexes	Biometric indicator	Groups		
		healthy cows	PIP cows	p <
Hydrogen Index (pH)	Lim M ± m	5,9–7,2 6,7±0,02	5,7–6,3 5,9±0,02	0,001
Number of infusorians, thsd / ml	Lim M ± m	200–1350 657,7±14,7	125–975 358,5±36,70	0,001
Reductivase activity, s	Lim M ± m	10–280 90,5±3,64	15–600 137,3±10,11	0,001

The synthesis of volatile fatty acids (VFA) decreased (p<0.001) in the rumen of affected animals. In particular, the level of acetic acid, decreased to 42.0 ± 0.48%, the propionic acid level raised to 28.8 ± 0.33 and butyric acid raised notably up to 28.0 ± 0.33%. This lead to a decrease in the ratio between propionic and butyric acids to 1.03:1 (in healthy cows - 1.3-1.4:1) and stimulated the ketogenesis (Sakhniuk, 2006). Digestive disorders in the cows forestomach deteriorates because of lack of fiber in the ration (77.1-85.7% of the need), which, in turn, suppresses their motor function, and also reduces the synthesis of acetic acid in the rumen (Table 2).

Table 2. The content of volatile fatty acids and their fractions in the rumen of high-yielding cows (n=391)

Indexes	Biometric indexes	Groups		
		healthy cows	PIP cows	p_1 <
VFA content, mmol/L	Lim M ± m	50,0–175,0 111,4±1,53	45,0–195,0 102,6±2,14	0,001
Including, acetic acid, %	Lim M ± m	31,7–66,9 47,9±0,45	27,2–55,0 42,0±0,48	0,001
Propionic acid, %	Lim M ± m	18,8–34,9 27,0±0,24	20,9–44,2 28,8±0,33	0,001
Butyric acid, %	Lim M ± m	12,6–33,4 21,4±0,44	14,2–38,0 28,0±0,33	0,001
Izo-valeric acid, %	Lim M ± m	0–7,2 1,5±0,17	0–10,1 0,67±0,13	0,001
Valeric acid, %	Lim M ± m	0–7,9 2,2±0,12	0–7,1 0,61±0,11	0,001

In 59.3-84.8% of cows with PIP develops hyperproteinemia (91.6 ± 0.71 g/l). In 20.9% of the affected cows the total protein content was greater than 100 g/l, and its average the value was 1.2 times greater compared to clinically healthy animals (p<0.001; Table 3).

Table 3. Protein exchange, functional state of the liver and kidneys in cows with PIP (n=897)

Indexes	Biometric indexes	Groups		
		Healthy cows, (n=680)	Cows with PIP, (n=217)	p <
Total protein, g/L	Lim M ± m	70.0–86.0 77.8±0.24	59.4–117.7 91.6±0.71	0.001
Albumines. %	Lim M ± m	36.0–50.0 39.2±0.19	11.0–38.0 26.7±0.73	0.001
Mercury chloride test. ml	Lim M ± m	1.52–1.74 1.63±0.004	0.8–2.0 1.28±0.009	0.001
Urea. mmol/L	Lim M ± m	3.0–5.0 4.0±0.05	0.9–9.84 3.72±0.19	0.1
Kreatinine. mkmol/L	Lim M ± m	83.0–180.0 131.3±3.80	53.4–332.0 157.2±3.24	0.001
AsAT. mmol/Lxhr	Lim M ± m	1.42–2.14 1.78±0.02	1.36–5.2 2.46±0.06	0.001
ALAT. mmol/Lxhr	Lim M ± m	0.45–1.03 0.74±0.01	0.13–3.46 0.94±0.03	0.001
GGTP. mkkat/Lxhr	Lim M ± m	0.25–0.45 0.35±0.004	0.11–1.25 0.47±0.01	0.001

The deep alterations of the exchange of simple proteins and liver pathology were confirmed by the development of hypoalbuminemia (11.0-38.0%). positive coagulation tests with mercury chloride (1.28 ± 0.009 ml). copper sulfate (1.84 ± 0.02 ml) and formaldehyde (97.2%) solutions. decreased urea (less than 3.0 mmol/l) in 39.6% of affected cows.

The increase in the activity of AsAT was established in 97.7% of with PIP (2.46±0.06 mmol/Lxhr), hyperfermantemia of AlAT - in 32.3%, which is an indicator of the membrane disruption of plasmolema and mitochondria of hepatocytes. The increase of GGTP activity was found in blood serum of 49.8% of affected cows, indicating the damage to the intrahepatic bile ducts and the development of cholestasis. In 41.4% of cases, the activity of GGTP and AsAT was combined (Table 3).

The profound nature of metabolic changes in the body of affected cows is confirmed by the results of glucose metabolism studies:

hypoglycemia was diagnosed in 35.0-62.2% of animals (2.18 ± 0.04 mmol/L), with 37.2% of them having glucose level less than 2.0 mmol/L. and in some cases - within 1.3-1.1 mmol/L. The liver damage and metabolic abnormalities are also verified by the changes of lipid metabolism indices (Table 4). In severe cases the content of total lipids and cholesterol in blood serum increased. In affected cows there was diagnosed ketonuria and in 58.6% of the animals the concentration of acetic bodies in the urine was 5-7 to 13-15 mmol/L. and in 17.2% of freshly calved cows - higher than 15 mmol/l (normally not above 1.5 mmol/L). The results attests the acute and subacute course of ketosis. In one third of the cows during severe liver disease, ketonuria is negligible (up to 1.7-3.0 mmol/L) which should be taken into account during the diagnosis of ketosis (Levchenko & Sakhniuk, 2004; LeBlanc, 2010; Chapinal et al., 2012).

Table 4. Indicators of carbohydrate-lipid metabolism in high-yielding cows (n=897)

Indexes	Biometric indexes	Groups		
		Healthy cows. (n=680)	Cows with PIP. (n=217)	p <
Glucosa. mmol/L	Lim M±m	2.04–3.32 2.7±0.02	0.64–3.66 2.2±0.04	0.001
Total lipids. g/L	Lim M±m	4.50–8.50 6.5±0.14	2.12–12.6 5.8±0.22	0.01
Cholesterol. mmol/L	Lim M±m	2.30–4.0 3.1±0.15	1.35–6.32 2.7±0.15	0.05
Triacilglicerols. mmol/L	Lim M±m	0.18–0.64 0.4±0.02	0.05–0.58 0.2±0.04	0.001
β-lipoproteins. un.exten.	Lim M±m	0.25–0.50 0.4±0.08	0.08–0.31 0.3±0.02	0.1

The PIP of high-yielding cows reduces the activity of thyroid follicular cells, which in turn reduces the secretion of thriiodothyronine and thyroxine in 1.6 and 2.1 times, respectively, compared to clinically healthy animals (p <0.001). The decrease in the concentration of T3 and T4 hormones was combined with the increase in the synthesis of thyroid stimulating hormone pituitary (TSG). The content of TSG was 4.2 times greater (100-850 nMO/ml; 330.0 ± 88.24) than in clinically healthy cows (Levchenko & Sakhniuk, 2004; Schulz, 2014).

Thus, the violation of the feeding regimen and the structure of rations for dry and lactating cows predetermine the development of multiply or polymorbide

internal pathology, which are mainly presented by forestomach dystonia, ketosis and hepatodistrophy.

The most informative laboratory tests for the diagnosis of multiply internal pathology in highly productive cows are determining the content of ketone bodies in the urine, pH values of the rumen content, the proportion of butyric acid (an increase of 66.9% of affected cows), total protein and blood serum albumin (decrease in 97.8%). mercury chloride tests (positive in 97.2 and 94.9%). increased activity of AsAT (hyper-fermentemia in 97.7% of affected cows). There was established direct correlative dependence (r = + 0.59) between a violation of the albumins exchange and a positive Mercury chloride test.

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Prophylaxis of trace minerals deficiencies (Co, Cu, Zn) in dairy cows

Nyomelemhiány (Co, Cu, Zn) megelőzése tejelő tehenekben

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Abstract

Today, organic compounds of trace elements are used for the prophylaxis of microelementosis in animals. The aim of the work is to investigate the influence of inorganic and organic compounds of scarce microelements on the state of certain biochemical parameters of blood in dairy cows.

The deficiency of microelements was compensated by the use of inorganic compounds in the form of salts (first group) and organic compounds in the form of metionates and lysinates (the second group) by dairy cows, mixing them with forage, once a day, for 60 days.

According to the results of the study of blood serum of dairy cows, a low content of Cuprum, Zinc, Cobalt, total protein, albumin and high activity of AST, ALT, and ALP was established.

In the serum of experimental groups cows the use of compounds of deficient micronutrients elevated concentration of Cobalt by 48.6 ($p<0.05$) and 88.9% ($p<0.01$), Cuprum – 60.8 ($p<0.001$) and 95.1% ($p<0.001$), Zinc – by 50.1 and 75.6% ($p<0.001$). On the background of increase of trace elements level, normalization of total protein albumin content, and activity of AST, ALT and ALP was established.

It was found that the concentration of Cuprum, in cows of the second group compared to the first, was higher by 31,3% ($p<0,05$), Zinc – 19,3% ($p<0,05$), total protein – by 8.7 ($p<0.05$), albumin – by 11.5% ($p<0.01$), and the activity of AST and ALP was lower by 14.8 ($p<0,05$) and 15.3% ($p<0.05$) respectively.

The usage of the complex of organic compounds of trace minerals for dairy cows contributes to of the best therapeutic effect compared with the use of inorganic compounds.

Indices of blood and milk of cows at action of nickel citrate in the first two months of lactation

Nikkel-citrát hatása a laktáció első két hónapjában a tehenek egyes vér és tej paramétereire

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Abstract

Feeding nickel citrate during a month in quantities 0.1 mg/kg DM of fodder contributed to a decrease in the activity of AsAT and an increase in the concentration of phenolglucuronides. Daily milk yields increased by 1.6 %, and its fat content - by 0.11 %. In the 2nd month - there was a decrease in the content of the CIC in blood, LPO and sialic acids and a decrease in the AsAT activity. Dairy productivity of cows increased by 8.1 %, and the fat content by 0.09 %. Feeding during a month nickel citrate in the amount of 0.3 mg/kg DM of fodder contributed to an increase of phenolsulfate content in the blood of cows and phenolglucuronides and a decrease of TBK-active products and increase in average daily milk yields by 4.1 %. Applying supplement during two months resulted in the CIC content decrease and the LPO. Dairy productivity of cows increased by 6.5 %, and fat content by 0.13 %.

Key words: cows, nickel citrate, biochemical parameters

Introduction

To provide complete nutrition to the organism of animals, mineral additives containing Cu, I, Co, Zn, Se, Cr and other biotic trace elements are used in world practice. The salts of organic acids containing macro- and trace elements, in particular, citrates of trace elements, which are safe for the health of animals and humans, are actively studied. Such salts of citric acid and many bioelements have antioxidant and radioprotective ability, as well as optimizing influence on the functioning of various systems of the human and animal organism. In particular the little-studied element Nickel should be noted. Its biological property is to enhance the action of insulin in a living organism, to influence the enzymatic and hematopoiesis processes, to support the

normal structure of cell membranes and nucleic acids (Skalnyj & Rudakov, 2004). In recent years, a new direction in science - elemental nanotechnology, is rapidly developing, which provides the possibility to apply nanocomposites of macro-microelements in biology, livestock and veterinary medicine (Chekman, 2009). Application of carboxylates, in particular, citrates of trace elements, obtained on the basis of nanobiotechnology, in animal feeding provides high biological and technological efficiency and ecological safety of these compounds. It is characteristic that the stimulating effect of these nanomaterials on the functions of individual organs, systems and the entire organism is achieved due to use of significantly smaller (10-20 times) doses compared with the mineral salts of these elements (Hostenek, 2006; Borysevych et al, 2010; Fedoruk et al, 2015). The research goal was to investigate the effect of various amounts of nickel citrate, produced by the nanotechnology method, on the physiological and biochemical processes in the cow organism, their productivity and biological value of milk in the first two months of lactation.

Experimental methods

The investigation was conducted on three groups of cows of Ukrainian black-and-white milk breed, 8 animals in each, 3-4 lactations, analogs by live weight (590-650 kg), physiological condition, productivity (6-6.5 thousand kg of milk per previous lactation). The I group (control) received the basic ration (BR), which was defined according to the physiological state, productivity and body weight of animals (Bogdanov & Kandyba, 2012). The II group - experimental, received daily BR on the 9th month of pregnancy and in the first two months after calving with Ni citrate in the amount of 0.1 mg/kg DM of fodder, and the III group - experimental, received BR in the indicated periods of research with addition of Ni citrate in the amount of 0.3 mg/kg DM of fodder. The mineral additive was made by the method of Kosinov & Kaplunenko (2009) using nanotechnology (Kosinov & Kaplunenko, 2009).

Blood obtained one time in the preparatory period 25-35 days prior to calving and on the 30th and 60th days of lactation was used for the laboratory tests as well as samples of milk from daily milk yield selected on a monthly basis. According to the methods described in the book by Vlizlo et al. (2012) it was defined in the blood: the number of free phenols and phenols conjugated with sulfuric and glucuronic acids, the content of circulating immune complexes (CIC), lipid hydroperoxides (LPO), TBK-active products, the content of sialic acids, immunoglobulins, the activity of aspartate aminotransferase (AsAT) and alanine aminotransferase (AlAT) using Human sets (Germany) on the biochemical analyzer "Humalyzer 2000" in accordance with the manufacturer's manuals. Fat content was determined in the milk of cows on the

apparatus "EKOMILK TOTAL". The obtained digital data was statistically processed using standard Microsoft Excel statistical software suite. The degree of likelihood of comparative data was estimated by the Student's coefficient.

Results

Feeding Ni citrate during a month in the amount of 0.1 mg/kg DM of the fodder contributed to the decrease in the activity of aminotransferases, in particular, AsAT by 21.5% (p<0,01) and not probably and AlAT, and in the second month - a decrease in the content of the CIC - by 15.9% (p<0,05) and activity of AsAT by 17.1% (p<0.05) in the blood of animals of the second experimental group (Table 1). Reduce of the activity of aminotransferases and the content of the CIC indicates the corrective effect of Ni citrate in the amount of 0.1 mg/kg DM of fodder on biochemical processes in the organism of cows. Feeding III experimental group animals Ni citrate in the amount of 0.3 mg/kg DM during the month did not have a significant effect on the indicated parameters in their blood. Prolonged application of the supplement influenced the reduction of the CIC concentration in the blood of cows by 18.9% (p<0.01), and also led to a decrease in the activity of aminotransferases.

Table 1. Physiological and biochemical indices of blood of cows (Mean±SEM, n = 5)

Parameter	Group	Investigation period		
		Preparation	experimental, month of feeding additive	
			1	2
AlAT, U/L	I	26.0±0.95	31.34±2.37	28.33±1.17
	II	232±2.34	28.00±3.85	24.58±3.38
	III	24.8±1.22	28.86±3.30	23.33±1.74
AsAT, U/L	I	61.6±4.68	85.14±1.29	72.83±1.47
	II	54.7±2.23	66.85±4.17**	60.40±3.59*
	III	59.9±10.42	74.34±4.91	66.30±8.45
CIC, unit of extinction	I	90.4±2.73	85.2±3.81	91.20±3.11
	II	91.8±2.90	83.5±4.48	76.7±3.47*
	III	91.8±2.30	81.0±5.85	74.0±3.19**
LPO, unit U/ml	I	4.59±0.18	4.64±0.90	4.54±0.012
	II	4.69±0.25	5.59+0.39	4.35+0.017**
	III	4.86+0.35	5.34±0.13	4.43±0.021*
TBK-active products, nmol/ml	I	3.22±0.13	4.40±0.29	2.78±0.18
	II	3.08±0.17	4.25±0.15	2.33+0.28
	III	3.22±0.10	3.60±0.16*	2.32±0.26

Note: in this and following tables the likelihood of differences between the control and experimental groups was taken into account * - p <0.05; ** - p <0.01; *** - p <0.001

Thus, at short-term feeding of Ni citrate, there was an unlikely decrease of TBK-active products in the blood of cows of the second group. The additive had a more significant effect at its prolonged application, which was manifested by a probable decrease in the content of LPO by 4.2% and unprobable - of TBC-active products in the 2nd month of feeding Ni citrate. Application of Ni citrate during a month in the amount of 0.3 mg/kg DM of fodder contributed to a decrease in the concentration of TBK-active products by 18.2% ($p < 0.05$), in the second month decrease of LPO by 2.4% ($p<0.05$) and unlikely - TBC-active products in the blood of the third experimental group animals.

As it's known, a decrease in the number of final LPO products and TBK-active products, indicates activation of the antioxidant system of the organism, which contributes to the reduction of hydroperoxides, which have the greatest influence on the structural and functional properties of cell membranes of the endometrium.

The investigation has established probable changes in the immunobiological parameters of blood of animals, which are more pronounced in the long-term use of a mineral additive. Thus, in the blood of cows of the II experimental group, in the second month of feeding nickel citrate the content of sialic acids decreased by 9.2% ($p<0.01$) (Table 2). It should be noted that sialic acids that are the final link of prosthetic mucoprotein groups play a special role in the mechanism of non-specific protection of the organism. Reducing their concentration indicates a positive transformation of the cow organism concerning immunobiological reactivity and enhancement of protective mechanisms of its nonspecific resistance. It should be noted that probable changes in the values of immunoglobulins in the blood of experimental group cows at the investigation period were not revealed.

Table 2. Immunobiological parameters of cows blood (Mean±SEM, n = 5)

Parameter	Group	Investigation periods		
		preparatory	experimental, month feeding additive	
			1	2
Immunoglobulins, g/L	I	17.21±1.36	18.15±1.39	12.56±1.50
	II	20.98±1.35	19.00±1.37	10.01±1.16
	III	20.81±2.25	19.76±1.36	10.43±0.49
Cialic acid, conditional units	I	142.2±2.03	94.0±8.58	136.6±3.06
	II	137.8±1.39	78.75±1.97	124.0±1.15**
	III	141.6±4.04	100.2±4.10	131.5±9.04

The mineral supplement in the abovementioned quantities positively influenced the detoxification processes in the animals organism of both II and III experimental groups, especially in the first month of its application (Table 3). Thus, feeding nickel citrate in the amount of 0.1 mg/kg DM of fodder during a month contributed to increase of binding free phenols in the organism with an increase of their conjugated compounds concentration in the blood of II experimental group animals, in particular phenol glucuronides by 20.2% ($p<0.05$). Instead, application of a mineral additive in the amount of 0.3 mg/kg DM of fodder contributed to the more pronounced activation of the detoxification function of the organism with an increase of both phenol sulfates and phenol glucuronides concentration in blood of the III experimental group animals respectively by 23.1 and 21.2% ($p<0.05$) in the 1st month compared to the similar indicators of the control group animals.

Increase of phenol sulphates concentration in the blood of II experimental group cows and phenol glucuronides in group III, against the background of slight fluctuations in the free phenols value, indicates an increase of the detoxification processes in the animals organism in experimental groups at the action of the applied nickel citrate amounts. Feeding Ni citrate during two month to experimental group animals did not have a significant effect on the researched parameters, which obviously indicates the adaptation of their organism to the applied nickel citrate supplementation doses.

Table 3. Content of phenols in the blood of cows, µmol/L (Mean±SEM, n = 5)

Parameter	Group	Investigation periods		
		preparatory	experimental, month feeding additive	
			1	2
Free phenols	I	13.57±0.88	13.24±0.63	14.65±0.14
	II	13.40±2.98	14.56±1.23	16.22±2.23
	III	16.22±1.03	13.90±0.90	15.72±2.18
Phenolsulphates	I	19.58±0.56	21.82±0.56	24.80±3.42
	II	18.83±0.90	22.19±1.40	22.19±2.34
	III	17.53±3.09	26.85±1.78*	22.94±3.37
Phenolglucuronides	I	58.08±2.34	53.57±3.32	63.48±3.99
	II	51.32±2.03	64.38±1.70*	78.79±9.75
	III	59.88±4.13	64.91±2.16*	80.59±7.66

It should be noted that the different amount of Ni citrate in the ration influenced cows milk yield (Table 4). Thus, feeding the supplement during a month in the amount of 0.1 mg/kg DM of fodder contributed to an increase in average daily milk yield of II experimental group cows by 1.6%, its fat content - by 0.11%, and in the course of two months - by 8.1 and 0.09% respectively.

Table 4. Daily yield and fat content of cow milk (Mean±SEM, n = 5)

Parameter	Group	Month of feeding additive	
		1	2
Daily yield, kg	I	24.4±0.80	24.8±1.16
	II	24.8±1.65	26.8±1.11
	III	25.4±0.55	26.4±3.93
Fat, %	I	3.65±0.24	3.70±0.41
	II	3.76±0.48	3.79±0.17
	III	3.61±0.09	3.83±0.56

Application of nickel citrate in the amount of 0.3 mg/kg DM of fodder during a month contributed to an increase in milk yield of cows by 4.1%, and in the second month by 6.5% and fat content by 0.13%. Thus, feeding of a mineral additive in the form of nickel citrate for 0.1 mg/kg DM of fodder contributed to an increase in the phenol sulphates and phenol glucuronides content and decrease in the content of CIC, LPO and sialic acids, as well as the activity of blood aminotransferases, which is more pronounced for AsAT. The average daily milk yields of cows during the first and second months of feeding the mineral supplement increased by 1.6 and 8.1%, respectively, and fat - by 0.11 and 0.09%.

Feeding of nickel citrate for 0.3 mg/kg DM of fodder contributed to the decrease of CIC, LPO and TBK-active products content in the blood of the III experimental group cows and increased detoxification processes in the blood. Under these conditions, the average daily milk yield in animals during the investigation period was higher by 4.1 and 6.5%, respectively, and fat in the second month by 0.13% compared with the corresponding rates of control group cows. The obtained results indicate the activation of physiological and biochemical processes in the blood and lipid secretion of cows milk during application of nickel citrate in doses of 0.1 and 0.3 mg/kg of dry matter of the ration.

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On-site measurement of lactate levels during transition period in dairy cows

A laktátszintek helyszíni meghatározása a tejhasznú tehenekben az ellés körüli időszakban

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Abstract

The vast majority of metabolic disease occurs in transition period in comparison to other relevant lactation stages. To the best of our knowledge rapid and point of care measurement for lactate levels in transition period of cows are lacking in the literature. Lactate evaluation might be used for prognosis of chronic inflamatory respiratory disease bronchopneumonia, abomasal disease, clinical and subclinical mastitis. Regarding diagnosis of subclinical disease among herds, it is important to build up cutoff point and referance levels for avoiding misdiagnosis and unsatisfactory therapeutic interventions. For a long while, most of the analysis could only be performed by laboratories requiring routine lab work and long time. Nowadays, on farm ancillary exams about lactate measurement are really fast and practice as well as to be performed individually or on herd level subclinic disease diagnosis. Additionally various handheld devices tried under field conditions are available for veterinarian practice. In the present study, Vet Photometer DP 700 (Diaglobal, Genartek, Germany, Turkey) was used measuring lactate levels on cow-side, able to give quick results (range 0.5-18 mmol/L), working with enzymatic - colorimetric method. A total of 12 Holstein cows, at transition period, were enrolled. Blood samples were withdrawn into heparized tubes weekly (1st week prepartum-2nd week postpartum). Lactate levels were as follows; 1st week prepartum (0.34-21.4 mmol/L), parturition (0,6-6.19 mmol/L), postpartum 1st week (0.39-3.09 mmol/L) and postpartum 2nd week (0.55-3.09 mmol/L). Available evidence suggested that there might be variation and fluctuation in lactate levels during transition period, indicating that the results must be interpreted with caution, taking into account anaerobic fermentation.

Key words: dairy cows, lactate, transition period

Introduction

The transition period, chracterized by three weeks prepartum to three weeks after parturition is the critical time related to physiological, metabolic, and nutritional challenging of dairy cattle (Grummer, 1995; Drackley, 1999).

Lactate largely used in different tissue of veterinary medicine is a useful metabolite for prognosis and evaluation of most of cattle diseases as bronchopneumonia, abomasal disease, clinical, subclinical mastitis and uterine disease (Davis et al., 2004; Nagy et al., 2006; Boulay et al., 2014; Murakami et al., 2017) and also other conditions such as inflammatory response, endotoxemia and mortality (Sorge et al., 2009).

Nowadays, on farm ancillary exams about lactate measurement are really fast and practice compare with laboratuary technics. Additionally various handheld devices tried under field conditions are available for veterinarian practices (Buczinski et al., 2014).

The present authors were unaware of finding documented reports regarding rapid and point of care measurement and for lactate levels in transition period cows. For this purpose in the present study we aimed to measure consecutive weekly cow-side lactate analysis during transition period.

Materials and Methods

In the present study totally 12 multiparous holstein dairy cows in private commercial farms located in the Aydın, Western Region of Turkey, were enrolled. All of the cows during the last two weeks before the parturition were observed and fed with total mix ration.

Blood samples were withdrawn into heparized tubes every weekly (1st week prepartum- 2nd week postpartum) in the farm. For the lactate analysis, blood samples were obtained to heparinized tubes from the *coccygeal vein* and heparinized sample transferred Eppendorf tubes, were separated plasma by the mini centrifuge device proceed with 5 minutes at 6000 rpm. Lactate levels on cow-side were measured with Vet Photometer DP 700 (Diaglobal, Genartek, Germany, Turkey), able to give quick results (range 0.5-18 mmol/L). Analysis principle based on enzymatic-colorimetric method has lactate converted to pyruvate with lactate oxidase firstly and in the next enzymatic step, the blue-purple color intensity of occured H_2O_2 dyed by quinonimine has reflected to plasma lactate concentration at 520 nm wavelength.

Results

Lactate levels were determined in the 1st week prepartum (0.34-21.4 mmol/L), parturition (0,6-6.19 mmol/L), postpartum 1st week (0.39-3.09 mmol/L) and postpartum 2nd week (0.55-3.09 mmol/L) by the new cow-side device. During the transition period the lowest lactate level and highest lactate level were measured as 0.34 mmol/L and 21.4 mmol/L,

respectively. We obtained variable and fluctuate lactate results during this period.

Discussion

Given the transition period diseases, both farmer and country might be affected economically (Fetrow, 2017). Nowadays several metabolic biomaakers are available in association with increased animal product and milk yield despite increased metabolical diseases in the transtion period (Buczinski et al., 2014). However upto date solely one study evaluated lactate levels as determined in transition cows with fatty liver. In the latter research lactate was determined steady concentration during 4 days prepartum to 36th days postpartum but, astonishingly, control group lactate level was found higher before parturition and 3th days after parturion compared with experimental group (Ametaj et al., 2005). Our data indicated compatible lactate results in this study.

Available evidence suggested that there might be variation and fluctuation in lactate levels during transition period, indicating that the results must be interpreted with caution, taking into account anaerobic fermentation.

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Evaluation of the sperm fertilization status by flow cytometry

A spermium fertilitási státuszának értékelése áramlási citometriával

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Abstract

Flow cytometry is a modern method allowing analysis of semen based on light scattering and fluorescence, recording and evaluation of computer technology, making it an objective assessment. The assessment of the semen quality by this method allows to minimalize the deficiencies and inaccuracies of other techniques. With the possibility to assess the quality of the semen using this method, deficiencies and inaccuracies of other techniques are minimalized. Flow cytometry in combination with the use of fluorescent probes provide rapid, objective, accurate and comprehensive analysis of the semen. It allows evaluation of thousands of sperm for their structure and properties in a short time, even from a minimal amount of semen. Using a suitable technique, it is also possible to use several fluorescent dyes simultaneously, and analyse a number of parameters in one sample. Flow cytometry has become an important technique in analysis of the morphological and functional properties of sperm and is used for research in veterinary science as well as for routine assessment of the semen quality.

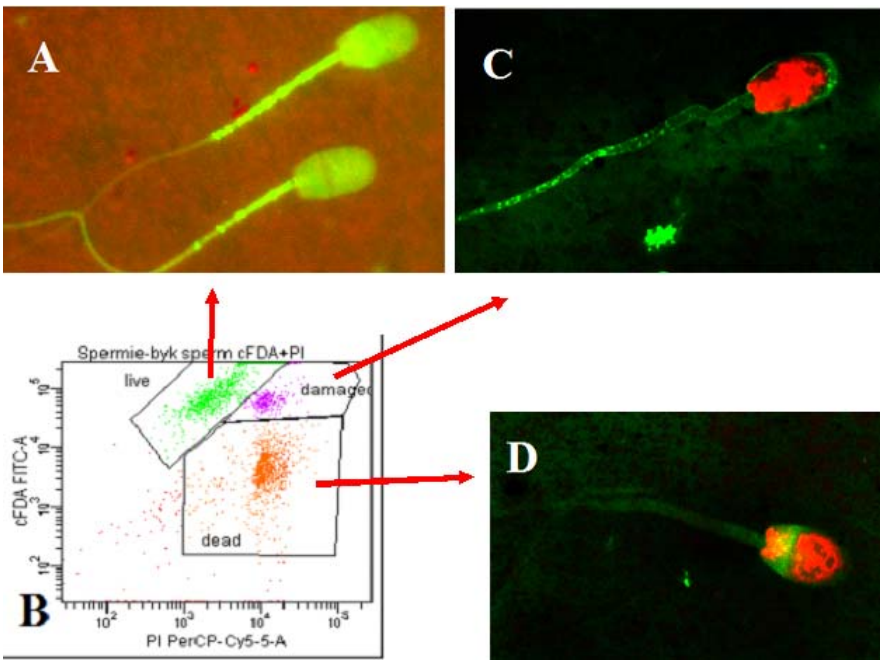
Key words: Sperm, flow cytometry, fluorescent dyes, multicolour analysis

Introduction

The aim of all examinations of the semen quality is to quickly, objectively, accurately, and inexpensively determine the fertilizing ability of spermatozoa in the sample. Flow cytometry allows evaluation of several thousand spermatozoa, which can be examined at the same time, even from a minimal amount of semen, with each sperm cell analysed separately. Combination of fluorescent dyes enables simultaneous examination of several attributes relating to one sperm like DNA content, activity of the

mitochondrial section, membrane integrity and others. Each attribute describes a different sperm characteristic affecting fertilizing ability or resistance to stress (Peňa, 2015). At the same time, flow cytometry allows to analyze the semen as a whole, so it is possible to distinguish different sperm populations. For example, spermatozoa with undamaged cytoplasmic membranes from spermatozoa with damaged cytoplasmic membranes (Celeghini et al., 2010). Fluorescent dyes are markers that can absorb light energy (e.g. laser) of a certain wavelength and subsequently to emit the light with a longer wavelength. These two processes are called excitation and emission. The emission is known as fluorescence, which can be recorded and analysed (Aghaeepour et al., 2013). Fluorochromes can bind to specific cell molecules or components, or they can be accumulated in certain parts of cells. After the light beam passes, they show part of the sperm, where they are bound (Silva et al., 2012). The discovery of various fluorescence dyes has allowed broader analyses of semen quality at functional, biochemical and ultrastructural levels (Gillan et al., 2005).

Figure 1. Sperm viability analysis evaluated by flow cytometry and fluorescence microscopy using fluorescent dyes CFDA and PI. (source: own)



A: Illustration of living sperm by a fluorescence microscope. Spermatozoa are stained green with CFDA.
B: Dot plot from a flow cytometer. Green colour represents live sperm, orange represents dead sperm and dying sperm are represented by purple colouration.
C: Illustration of dying sperm whose mitochondrial section is still stained by CFDA, but the heads are stained by PI.
D: Illustration of dead sperm after staining with PI. The head is stained by red dye PI and mitochondrial section is without colour.

Sperm viability assessment

Within this analysis, the integrity of the plasma membrane serves as an indirect indicator of sperm viability. We can perform the analysis using fluorescent dyes that stain living or dead sperm cells or a combination of both dyes (Gillan et al., 2005). The most commonly used dye for staining dead spermatozoa is propidium iodide (PI). It can pass through damaged membranes and colour the DNA to red (Oldenhof et al., 2011). It is often combined with some vital dyes such as carboxyfluoresceindiacetate (CFDA), which emits a green fluorescence in living cells *(Figure 1)* (Magistrini et al., 1997). With flow cytometry, it is possible to assess the integrity of the entire cytoplasmic membrane, which is necessary to maintain the stability of the internal environment, or only the acrosome, which is necessary for egg fertilization (Rodríguez-Martínez, 2003).

Assessing mitochondrial activity

Mitochondria are irreplaceable organelles for sperm movement, because they provide energy substrates. Fluorescent probes which are using for their visualization, penetrate into living sperm cells and staining active mitochondria (Garner et al., 1997). The intensity of fluorescence depends on the number of functional mitochondria. The most popular probes are 5,5,6,6-tetrachloro-1,1,3,3-tetraethylbenzimidazolylcarbocyanine iodide (JC-1), Rhodamine 123 (R123) or MitoTracker dyes (Hossain et al., 2011).

Sperm chromatin analysis

Spermatozoa are very susceptible to exogenous and endogenous stressors during the spermatogenesis and during sperm maturation in the epididymis. Many factors can affect DNA integrity which is extremely important for normal embryo development. Due to the importance of genetic information for offspring, several methods have been developed to detect damaged DNA in spermatozoa (Cordelli et al., 2005). The sperm chromatin structure assay (SCSA) uses the acridine orange dye and is based on high sensitivity of abnormal chromatin to induced DNA denaturation (Evenson & Wixon, 2006). TUNEL (terminal transferased UTP nick-end labelling) test allows determination of sperm DNA fragmentation. Using this test, it is possible to identify breaks in the DNA chain by fluorescently labelled nucleotides, which can be detected in the sperm using flow cytometry (Petrunkina & Harrison, 2011).

Detection of oxidative stress and lipoperoxidation by flow cytometry

Various fluorochromes can be used that respond specifically to a particular type of ROS or non-specific probes that are oxidized by multiple types of ROS. Subsequently, emitted fluorescence indicates the level of oxidative stress. The examples of specific dyes are dihydroethidium (DHE) and MitoSOXTM^{TM} which react with the

superoxide anion (O_2) (Hossain et al., 2011). One non-specific dye is 2,7-dichlorodihydrofluorescein diacetate (H2DCFDA) serving as an indicator of intracellular presence of ROS in spermatozoa and emitting green fluorescence as a result of oxidation (Kim et al., 2010). Other non-specific dye is C11-BODIPY 581/591. This probe can be incorporated into the sperm cell membrane. Its reactivity to peroxidation is comparable to the reactivity of unsaturated fatty acids, which are abundant in the spermatozoa membrane. This dye emits green fluorescence after peroxidation (Drummen et al., 2004).

Conclusion

Spermatozoa are complicated cells that must possess many characteristics to fertilize an egg. Flow cytometry is a modern and objective method which allows, using the suitable probes, analyses of many sperm characteristics simultaneously in short time. Moreover, in case of the poor fertilization ability of the sperm it is possible to determine which part of the spermatozoa is damaged. Flow cytometry is useful not only for quality examination of the morphological and functional attributes of spermatozoa, but also helps to understand better the biology of fertilization.

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Correlation between of antioxidant defence enzymes activity and malate-aspartate shuttle in ejaculates

Az antioxidáns védekezési enzim aktivitás és malát-aszpartát transzfer korrelációja az ejakulátumokban

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Abstract

Connection between activity of malate-aspartate shuttle enzymes and antioxidant defense enzymes in freshly obtained bull ejaculates were studied. It was registered that there was positive correlation (η = 0.367) between activity of malate dehydrogenase (MDH) and superoxide dismutase (SOD). Glutathione peroxidase maximum activity (GPO; 1.01±0.09 μmol/min×mg of protein) manifested when activity of MDH was 0.20 – 0.30 nmol/min×mg of protein (η = 0.407). MDH activity negatively correlated with catalase activity (CAT; η = 0.566). Aspartate transaminase activity (AsAT) weakly correlates with SOD (η = 0.281). When AsAT activity was high so was the activity of GPO (η = 0.425) and CAT activity was low (η = 0.458).

Key words: malate-aspartate shuttle, antioxidant defense system, oxygen active forms, spermatozoa, bulls

Introduction

Metabolism of spermatozoon is provided by glycolysis and respiration. Functioning of malate-aspartate shuttle is closely connected with electron transport chain. This shuttle consists of cytoplasmic and mitochondrial isoforms of AsAT and MDH (Mitchell et al., 2009). However, the process of generating energy is accompanied by the formation of active forms of Oxygen (ROS), whose role in the metabolism of semen is ambiguous. On the one hand, they damage the

membrane structures of the spermatozoa (lipid and protein components), and on the other hand, their formation is a necessary condition for the capacitation and activation of the movement of the germ cells when the fertilization of the oocyte begins (Ford, 2004). The level of ROS is regulated by the antioxidant defense system, in particular its enzymatic link: SOD, GPO and CAT (Fernandez-Santos et al., 2009). In this regard, the correlations between the activity of the malate-aspartate shunt and the activity of enzymatic link of antioxidant defense in the semen of the bulls were investigated to determine possible ways of adjusting metabolic processes and to ensure high physiological characteristics and fertility of spermatozoa.

Experimental methods

In freshly obtained bull (n = 60) ejaculates activities of SOD (IU/mg of protein) – by quantity of formed nitroformazane, CAT (µmol/min×mg of protein) – with ammonium molybdate, GPO (µmol/min×mg of protein) – with Ellman's reagent, MDH (nmol/min×mg of protein) – by speed of NADH oxidation, AsAT (nmol/min×mg of protein) – with dinitrophenyl hydrazine were studied.

Results

Bull sperm could be characterized by activity of SOD – 4.2±0.26 IU/mg of protein, GPO and CAT activities were, correspondingly, 0.79±0.092 and 0.30±0.048 µmol/min× mg of protein, activity of malate-aspartate shuttle: MDH and AST, correspondingly, were 0.31±0.07 and 63.2±6.30 nmol/min× mg of protein. Enzymes of malate-aspartate shuttle had a correlation with the activity of the enzymatic level of antioxidant defense (Table 1).

Table 1. Correlation between activity of malate dehydrogenase and activity of antioxidant defense enzymes

Enzyme activity, ×mg of protein	MDH activity, nmol/min×mg of protein						η
	0.20 <		0.20 – 0.30		> 0.30		
	n	Mean±SEM	n	Mean±SEM	n	Mean±SEM	
SOD, IU^{-1}	14	3.2±0.16	18	4.7±0.18***	15	4.4±0.17***	0.367
CAT, µmol/min	14	0.44±0.045	18	0.29±0.013***	15	0.23±0.036	0.566
GPO, µmol/min	14	0.69±0.06**	13	1.01±0.09	12	0.80±0.06*	0.472

* Note: The difference is statistically significant compared to the minimum value of the indicator
* - $p < 0.05$; ** - $p < 0.01$ *** - $p < 0.001$

In ejaculates with low MDH activity (<0.20 nmol/min×mg of protein) SOD activity was also on low levels (3.2±0.16 IU/mg of protein). When MDH activity was 0.20–0.30 nmol/min×mg of protein activity of SOD increased on 32.0% ($p < 0.001$). When MDH activity was more than 0.30 nmol/min×mg of protein i SOD activity raised on 27.3 % ($p < 0.001$). Correlation ratio between activities of MDH and SOD was medium strength ($\eta = 0.367$).

MDH activity correlated diversely with enzymes that utilize H_2O_2. For example, when MDH activity was from 0.20 to 0.30 nmol/min×mg of protein CAT activity lowered on 35.1 % ($p < 0.001$), compared to maximal value. When MDH activity was more than 0.30 nmol/min×mg of protein – CAT activity was lower on 52.3 %. Correlation ratio between MDH and CAT was negative with medium strength ($\eta = 0.566$). Maximal activity of GPO (1.01±0.09 µmol/min×mg of protein) was when MDH activity was 0.20 – 0.30 nmol/min×mg of protein, when MDH activity was lower than 0.20 nmol/min×mg of protein or more than 0.30 nmol/min×mg of protein GPO activity was lower on, correspondingly, 31.7 % ($p < 0.01$) and 20.8 % ($p < 0.05$). Correlation ratio between MDH and GPO was positive with medium strength ($\eta = 0.472$). AsAT activity had correlation with activities of antioxidant defense system enzymes (Table 2).

Table 2. Correlation between activity of aspartate transaminase and activity of antioxidant defense enzymes

Enzyme activity, ×mg of protein	AsAT activity, nmol/min×mg of protein						η
	50.0 <		50.0 – 70.0		> 70.0		
	n	Mean±SEM	n	Mean±SEM	n	Mean±SEM	
SOD, IU	7	3.0±0.20	27	4.6±0.12***	21	4.1±0.15***	0.281
CAT, µmol/min	7	0.43±0.048	25	0.25±0.038	18	0.25±0.032	0.458
GPO, µmol/min	15	0.66±0.10	14	0.93±0.09	11	0.96±0.08*	0.425

When AsAT activity was lower than 50.0 nmol/min×mg of protein SOD activity was low (3.0±0.20 IU/mg of protein), and when AsAT activity raised (more than 50.0 mg of protein) SOD activity also increased (on 26.9 – 34.8 %; $p < 0.001$) and was 4.1 – 4.6 IU/mg of protein. Correlation ratio between AsAT and SOD was weak ($\eta = 0.281$).

AsAT affects activity CAT in bull sperm. When AsAT activity was less than 50.0 nmol/min×mg of protein, CAT activity

was the highest – 0.43±0.048 µmol/ min×mg of protein, and when AsAT activity was more than 50.0 nmol/min×mg of protein CAT activity was lower on 41.9 % and was 0.25 µmol/min×mg of protein. Correlation ratio between AsAT and CAT activities was negative with medium strength ($\eta = 0.458$). AsAT activity also correlates with GPO activity. In ejaculates with AsAT activity less than 50.0 nmol/min×mg of protein activity of GPO was 0.66±0.10 µmol/min×mg of protein. When AsAT activity was 70.0 nmol/min×mg of protein GPO activity was higher on 29.1 % and when AsAT activity was higher than 70.0 nmol/min×mg of protein GPO activity was 0.96±0.08 µmol/min×mg of protein, that was on 31.7 % higher than minimal value ($p < 0.05$). Correlation ratio between AsAT and GPO activities was positive with medium strength ($\eta = 0.425$).

Discussion

As it follows from the results of research, the enzymes of malate-aspartate shuttle and antioxidant protection in ejaculates and sperm cells are interacting with each other. Spermatozoa in the process of aerobic oxidation of carbohydrates consume oxygen, which provides the formation and use of pyruvate in the Krebs cycle. At the same time, the effective functioning of Krebs cycle and resynthesis of ATP is provided in spermatozoa with malate-aspartate shuttle and intake of glutamate and malate. Oxidation, supply and utilization of substrates in the Krebs cycle and the transport of electrons in the respiratory chain of mitochondria, including with the involvement of the malate-aspartate shuttle, causes the formation of $O_2^{\bullet-}$ and H_2O_2 and, accordingly, the activation of the AOD enzymatic link. In the bovine semen, SOD converts $O_2^{\bullet-}$ into H_2O_2, and the formed H_2O_2 is destroyed by the participation of the GPO and CAT. So, the activation of AsAT and MDG is accompanied by increased formation of $O_2^{\bullet-}$, which stimulates the activity of GPO and reduces the activity of CAT. Violation of the functioning of the malate-aspartate sperm shuttle (low activity of AsAT and MDG) characterizes the excess of the formation of H_2O_2 and the activation of CAT.

Conclusions

Thus, in semen, result of transformation of $O_2^{\bullet -}$ by SOD is low formation of H_2O_2 and activation of GPO. Activation of malate-aspartate shuttle enzymes is characterized by low intensity formation of H_2O_2 and increased activity GPO and CAT decrease.

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A szaporodásbiológiai mutatókat javítandó asszisztált reprodukciós technika bevezetésének eredményei Holstein-fríz tejelő szarvasmarha telepen

The results of introducing assisted reproduction techniques to improve reproductive performances in Holstein-Friesian dairy cow farm

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Összefoglalás

Vizsgálatunkban az Ovsynch programmal elért eredményeket és a szakmai tudás fontosságát szerettük volna bemutatni egy holstein-fríz tejelő szarvasmarha telepen. Az általunk elemzett időszakban (2013-2017 évek) a vizsgált telepen növekedett a nőivarú egyedek létszáma üszők vásárlása miatt. A program bevezetésének sikerességét, a protokoll alkalmazása előtti és utáni időszak termékenyítési indexei alapján szemléltettük. A tehenek termékenyítési indexében statisztikailag pozitív változás mutatható ki a program bevezetése után (8,8-ról 4,4-re csökkent), az üszőknél nem volt eltérés (3,9, illetve 3,3). Az emberi tényezőt is vizsgáltuk a termékenyítés eredményességére. A prog-

ram alkalmazása előtt az inszeminátorok munkájának teljesítménye eltérő volt Személy_1 és Személy_3 esetén (9,4, ill. 5,1). A protokoll bevezetése után a vizsgált 3 személy munkájának teljesítményében szignifikáns különbség nem mutatható ki, tehát az Ovsynch program bevezetése az inszeminátorok termékenyítésének eredményeit azonos szintre hozta.

Abstract

Analysis and maintain good reproductive performances in dairy herds has economic importance. In progressive animal husbandry the use of assisted-reproductive technics is prosperous. On the examined Holstein-Friesian dairy farm the Ovsynch protocol was induced in order to manage the increasing population size and improve the reproductive performances. We analyzed the results of the program evaluating the fertility indexes before and after the introduction. The cow's fertility index was 8.8 in 2016. After introducing Ovsynch protocol it decreased to 4.4, which is statistically significant result. There were no significant differences in heifer fertility indexes (3.9 vs. 3.3). We analyzed the human-factor effect on the reproductive performances and fertility indexes too. The fertility index of inseminations done by Person_1 was 9.4 vs. Person_3's was 5.1. Evaluating the work-efficiency after introduction of Ovsynch protocol, there were no significant differences between the three inseminator's indexes. The introduction of Ovsynch program improved the cow's reproductive performances and equalized the inseminators' efficiency as well.

Bevezetés

Ha nincs borjú, nincs tej!

Borjúszaporulat nélkül sem tejtermelés, sem hústermelés nem képzelhető el. A szaporítás szervezése ugyanakkor a legnagyobb gondosságot igénylő feladat. A szarvasmarha-állomány 2010 óta tartó növekedése tovább folytatódott, 2017. június 1-jén számuk elérte a 864 ezret. Az állománylétszám és a laktációs tejtermelés növekedése miatt azonban egyre több telepen szaporodásbiológiai problémák jelentkeztek, gyakoribbá vált a csendes- és tünetszegény ivarzások száma, nehezebbé vált az ivarzók kiválogatása, nőtt a két ellés közti idő. Asszisztált reprodukciónak (AR) a termékenyülés elősegítése céljából alkalmazott eljárásokat nevezzük. A modern, 21. századi állattenyésztés már nem képzelhető el az AR módszerek alkalmazása nélkül. Ezen technikáknak meghatározó szerepük van és lesz a jövőben is az állattenyésztés termelékenységének javításában (Pálfyné, 2014).

Az Ovsynch programban gonadotrop releasing hormont (GnRH) és prosztaglandin F2α (PGf2α) hormont használnak az ivarzás elősegítésére, illetve több egyed ivarzásának szinkronizálásra. A kezelés végén nem kell az ivarzókat válogatni, időhöz kötött, programozható termékenyítést tesz lehetővé (Gábor, 2000; Gábor et al., 2004; Krjanecz, 2014).

Az Ovsynch-eljárás 3 kezelést jelent:

- első alkalommal GnRH,
- 7 nap múlva egy PGF2α kezelés, majd
- 30-48 órával később ismételt GnRH kezelés és 24 órán belül termékenyítés.

Anyag és módszer

Az általunk vizsgált holstein-fríz tejelő szarvasmarha állományban a nőivarú egyedek száma 2013-ban kevesebb, mint 950 db, majd 2017-re már 1034 db volt. Az állománylétszám növekedés volt a legfőbb oka az ivarzás-szinkronizálás bevezetésének. Az Ovsynch protokollt 2016 novemberében kezdték el alkalmazni az üszőknél és a teheneknél egyaránt, ezért a bevezetés előtti (2016 év január-október) és bevezetés utáni (2016 november - 2017 január) időszakok tehén és üsző termékenyítési indexét vizsgáltuk.

Az ellés utáni 27-28, 42 és 60 napos teheneket vizsgálják meg rektálisan és ha szükséges méhkezelést kapnak. Ha folyat 60 nap előtt, akkor fejés után kiválogatják és inszeminálják. Az Ovsynch programot a 60 naposak vizsgálata után 1 héttel indítják, ha a tehén és üsző nem ivarzott vagy a vemhességvizsgálat alapján a tehén üresen maradt. Az egyedek lépésszámlálóval vagy chippel vannak ellátva, így a fejőházba belépve a rendszer azonosítja őket. Az adatokat a Crystal és a TALP programot programokból gyűjtöttük. Az ivarzás-szinkronizálási program bevezetésének vizsgálatára (valamint amikor két inszeminátor teljesítményét vizsgáltuk) a termékenyítési indexek összehasonlításakor az F-próbát és a két független mintás t-próbát alkalmaztuk. Abban az esetben, amikor három személy teljesítményét hasonlítottuk össze az egytényezős varianciaanalízist használtuk.

Eredmények

A tehenek átlagos termékenyítési indexének szórása a program alkalmazásának hatására szignifikánsan csökkent, mivel 2016-ban 8,8±2,5, a program bevezetése utáni időszakban pedig 4,4±1,1 ($F_{(9,8)}$=5,1; P=0,015) volt. A t-érték 5,1 (P<0,0001) volt, ami azt jelenti, hogy a program bevezetése után szignifikánsan javult a tehenek termékenyítési indexeinek átlaga.

Az üszők átlagos termékenyítési indexe a program bevezetése előtti időszakban 3,9±0,7, az Ovsynch program bevezetése után pedig 3,3±0,7 ($F_{(9,8)}$=1,0; P=0,489) volt. A t-érték 1,9 (P=0,065) volt, tehát az üszőknél nem volt statisztikailag kimutatható különbség a két vizsgált időszak átlagos termékenyítési indexeinek eredményében.

Eredményeink szerint tehát az Ovsynch protokoll bevezetésével a tehenek termékenyítési indexében statisztikailag szignifikáns pozitív változás mutatható ki, az üszőknél nem volt eltérés.

Vizsgálataink során az inszeminátorok teljesítményét is elemeztük. Négy személy által végzett termékenyítések eredményeit vizsgáltuk, külön a tehenek és külön az üszők

termékenyítési indexei alapján. Az első vizsgálatban *Személy_1 és Személy_3* munkáját hasonlítottuk össze 2016. január 1- 2017. január 1. időtartam közt.

A második vizsgálatban *Személy_2 - 3 - 4* munkáját elemeztük 2017. februártól - júliusig.

A S*zemély_3* teljesítményének szórása a tehenek átlagos termékenyítési indexe alapján 5,1±4,5 nagyobb volt, mint a *Személy_1*-nek 9,4±2,1 ($F_{(12,12)}$=4,7; P=0,006). A t-érték 3,0 (P=0,008) volt, ebből arra következtethetünk, hogy a *Személy_1* termékenyítési eredményei kimutathatóan gyengébbek voltak a vizsgált időszakban.

Az üszők termékenyítési indexe alapján értékelve a *Személy_3*-nak a teljesítménye 2,93±0,71, míg a *Személy_1*-nek 4,18±0,72 ($F_{(12,\ 9)}$=1,1; P=0,498) volt, ami azt jelentette, hogy a szórásokban nem volt különbség. A t-próba értéke 4,09 (P<0,001), ami azt mutatja, hogy *Személy_1* munkája az üszők termékenyítésénél is gyengébb volt, mint *Személy_3*-é.

Személy_2-3-4 munkája teljesítményének alakulása a féléves időtartamban:

A három személy esetében a tehenek átlagos termékenyítési indexeinek a szórása homogén, mivel 4,8±2,5; 4,6±1,7 és 3,5±0,5 ($F_{(2,18)}$=2,1; P=0,145) volt. A variancia-analízis pedig nem mutatott ki különbséget az átlagok között ($F_{(2,18)}$=1,1; P=0,343).

Az üszők esetében az átlagos termékenyítési indexek szórása homogén, 2,3±1,7; 2,8±3,0 és 3,0±0,9 ($F_{(2,18)}$=1,3; P=0,303) volt. A varianciaanalízis szintén nem mutatott ki különbséget az átlagok között ($F_{(2,18)}$=0,3; P=0,781).

Az Ovsynch protokoll bevezetése után vizsgált 3 személy munkájának teljesítményében szignifikáns különbség nem mutatható ki, tehát a program bevezetése az inszeminátorok termékenyítésének eredményeit azonos szintre hozta.

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A keresztezés hatása a tejhasznosítású tehenek termékenységére és állategészségügyi állapotára

The influence of crossbreeding on prolificacy and health condition of dairy cows

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Összefoglalás

Amíg a sertés- és a baromfitenyésztésben évtizedek óta a keresztezés a legeredményesebb módszer, addig a tejhasznosítású szarvasmarháknál a fajtatiszta tenyésztés a jellemző. A gazdaságosságot leginkább veszélyeztető termékenyülési és egészségügyi gondok arra fogják kényszeríteni a tenyésztőket, hogy korábbi tenyésztési stratégiájukat átgondolják és a heterózist generációról-generációra magas szinten tartó keresztezést válasszák. Magyarországon a keresztezés a holstein-fríz fajtán alapul és a párosításhoz a svéd vörös és a montbéliarde fajtát javasolják. A heterózistenyésztés előnyei jellemzően a termékenységben és az állategészségügyi helyzetben jelentkeznek. Megállapítottuk, hogy szinte minden termékenységgel kapcsolatos paraméterben (termékenyítési index, két ellés közötti idő, szervizperiódus, stb.) a keresztezett egyedek felülmúlják fajtatiszta anyjukat. Az állategészségügyi helyzet megítéléséhez az állományban végzett egészségügyi beavatkozások számát vettük alapul. Eredményeink szerint mindkét keresztezési konstrukció esetében csökken az egy egyedre jutó egészségügyi beavatkozások száma.

Kulcsszavak: tejhasznosítás, keresztezés, ProCross

Abstract

While in swine-and poultry breeding crossbreeding has been the most effective method for decades, in dairy cattle breeding we use pure breeding. The problems related to fertility and health, which have a great negative economic impact will force breeding strategy to be reconsidered by breeders and crossbreeding which keep heterosis on a high level from generation to generation to be

chosen by them. Holstein-Friesian is the basis of crossbreeding and studies suggest Swedish red and Montbéliarde to be crossbred with that. The advantages of crossbreeding characteristically occur in fertility and health condition. If we compare the results of fertility of crossbred animals with their purebred mothers', it can be said that almost in every parameter (such as fertility index, calving interval, service period, etc.) crossbred animals are superior to their purebred mothers. To define health condition, we determined the number of interventions executed in the population. According to our results, the number of interventions is dramatically lower in both crossbred groups than in the purebred one.

Key words: dairy cow, crossbreeding, ProCross

Bevezetés

A tejhasznosítású szarvasmarhatartásban a múlt század közepétől tart a vita, hogy milyen típusú tehénnel lehet gazdaságosan tejet előállítani és milyen legyen az alkalmazott tenyésztési módszer. Mivel a tejből származó árbevételt jellemzően a termelt tej mennyisége és nem a tej összetétele döntötte el, így a típust, valamint a megválasztott tenyésztési eljárást is a nagy mennyiségű tej termelésére irányuló törekvés határozta meg. Míg a sertés- és baromfitenyésztésben évtizedek óta a keresztezés a legeredményesebb módszer, addig a tejhasznosítású szarvasmarháknál a fajtatiszta tenyésztés a jellemző. A beltenyésztés eredményeként jelentkező hátrányokat, különösen a termékenységgel kapcsolatos paraméterekben tapasztalhatjuk.

Ezt igazolják Bezdicek et al. (2015), akik a beltenyésztési együttható növekedésével az első elléskori életkor növekedését tapasztalták. Hasonló eredményre jutottak Thompson et al. (2000), valamint Smith et al. (1998), akik a beltenyésztési együttható százalékonkénti növekedésével az első elléskori életkor fél nappal történő emelkedését regisztrálták. Beltenyésztés hatására csökken a tehenek termékenysége, a beltenyésztett egyedeknél az ellés körülményesebben zajlott le. Azoknál az állatoknál, amelyeknél az együttható 6,25- és 12,5 % között volt, a termékenyítési index közel 2 %-kal romlott (Gutiérrez et al., 2007).

A fajtatiszta tenyésztés hátrányainak kiküszöbölésére, valamint a tej összetételének javítása miatt kell tehát beszélnünk a keresztezésről és ezáltal a heterózistenyésztés lehetőségeiről.

A keresztezéssel kapcsolatos nemzetközi tapasztalatok kedvezőek. Heins (2014) montbéliarde, jersey és holstein-fríz fajtatiszta tehenek, valamint keresztezett állományok termékenységét hasonlította össze és megállapította, hogy a keresztezett tehenek termékenyítési indexe 23 %-kal jobb volt és a selejtezési arány is kedvezőbbnek bizonyult. A nehéz ellést kutatva Hansen et al. (2015) azt tapasztalták, hogy montbéliarde-dal keresztezett holstein-fríz teheneknél 12 %-kal, a brown swiss-szel

keresztezett holstein-fríz teheneknél pedig 11,9 %-kal kevesebb volt a nehéz ellés. Kutatásuk során elemezték a két ellés közötti időt, amiben a keresztezett tehenek 20-27 nappal jobb eredményt értek el.

A lehetséges tenyésztési módszerek közül a háromfajtás rotációs keresztezés tudja a heterózist generációról-generációra magas szinten tartani. A szakirodalom több keresztezési kombináció eredményeiről számol be, de úgy gondoljuk, hazánkban a háromfajtás rotációs keresztezésen alapuló ProCross lehet a leginkább célravezető. Magyarországon a holstein-fríz fajtán alapul a keresztezés és a párosításhoz a svéd vörös és a montbéliarde fajtát javasolják.

A kísérlet leírás

Elemzéseinkhez az adatokat egy dunántúli tejtermelő nagyüzem szolgáltatta. A több évtizede holstein-fríz fajtával dolgozó tehenészet 2013-ban kezdte meg a rotációs keresztezést, így jelenleg már az F_1-es generációknak a lezárt laktációit értékelhetjük. A termékenységgel és állategészségügyi helyzettel kapcsolatos legfontosabb mutatókat 305 napos standard laktációkra adjuk meg. A RISKA rendszerben feljegyzett eredményeket az országos ALI program segítségével ellenőriztük. A statisztikai kiértékeléshez az SPSS programcsomagot használtunk.

Eredmények

A ProCross keresztezés sémájának megfelelően a holstein-fríz teheneket svéd vörös bikákkal termékenyítették. Az F_1-es egyedek értékmérőit fajtatiszta anyjukkal vetettük össze (1. táblázat).

A svéd vörössel keresztezett tehenek több paraméterben is lényegesen meghaladták holstein-fríz anyjuk teljesítményét. Bár a tenyésztésbevétel és az első elléskori életkor nem tért el egymástól, de a hatékonyság szempontjából jelentős különbséget tapasztaltunk. Ezek közül kiemelhető a több, mint 50 nappal lerövidült két ellés közötti idő, a jobb termékenyítési index, valamint az egy állatra jutó kezelések számában mérhető javulás. A heterózishatás tehát több értékmérőben is igazolódott.

A holstein-fríz üszőket a programnak megfelelően mondbéliarde bikákkal termékenyítették. Az eredményeket a 2. táblázat tartalmazza.

A svéd vörös keresztezett egyedekhez képest talán még szembetűnőbb a keresztezés termékenységi és állategészségügyi paraméterekre gyakorolt pozitív hatása. Az F_1-eseknél a termékenyítési index kivételével minden paraméter szignifikánsan előnyösebben alakult. Több mint egy hónappal hamarabb ellettek az állatok, a szervizperiódus és a két ellés közötti idő is több mint egy hónappal volt rövidebb a keresztezetteknél. Az egy egyedre jutó kezelések száma közel negyedére csökkent.

Az eddigi elemzések igazolják a keresztezés előnyeit, de a további konstrukció kiértékelése is szükséges ahhoz, hogy még megbízhatóbb következtetésekre jussunk.

1. táblázat. A svéd vörös keresztezett tehenek értékmérőinek összehasonlítása fajtatiszta anyjukkal
Table 1. The traits of crossbred cows compared to their purebred mothers

Értékmérők (1)	**Holstein-fríz x Svéd vörös F_1 (n=184)** (2)			**Holstein-fríz anyák (n=184)** (3)			**Szignifikancia - szint** (4)
	Átlag (5)	**Szórás** (6)	**CV %** (7)	**Átlag** (8)	**Szórás** (9)	**CV %** (10)	
Tenyésztésbevétel (nap) (11)	435	34,06	7,82	439	61,58	14,02	NS
Első elléskori életkor (nap) (12)	747	96,81	12,95	745	58,59	7,85	NS
Szervizperiódus (nap) (13)	54,24	35,47	65,68	90,12	41,25	45,83	**
Termékenyítési index (14)	2,66	1,11	41,72	3,33	1,82	54,65	*
Két ellés közötti idő (nap) (15)	387	69,81	18,02	443	116,09	26,20	***
Láb (16)	2,14	0,66	30,84	5,72	1,82	31,82	***
Tőgy (17)	1,60	0,75	46,87	4,08	2,11	51,71	***
Méh (18)	2,51	0,80	31,37	3,51	2,01	57,26	NS
Összes kezelés (19)	6,25	2,14	34,24	13,31	6,11	45,90	***

Traits (1); Holstein-Friesian x Swedish Red crossbreds (2); Holstein-Friesian mothers (3);
Level of significance (4); Average (5); Standard deviation (6); Coefficient of variation (7); Average (8); Standard deviation (9); Coefficient of variation (10); Age at first insemination (11); Age at first calving (12); Service period (13); Fertility index (14); Calving interval (15); Interventions: Foot (16); Udder (17); Womb (18); Interventions altogether (19)

2. táblázat. Monbéliarde keresztezett tehenek értékmérőinek összehasonlítása fajtatiszta anyjukkal
Table 2. The traits of Montbéliarde crossbred cows compared to their purebred mother's

Értékmérők (1)	**Holstein-fríz x Montbéliarde F_1 (n=80)** (2)			**Holstein-fríz anyák (n=80)** (3)			**Szignifikancia - szint** (4)
	Átlag	**Szórás**	**CV %**	**Átlag**	**Szórás**	**CV %**	
Tenyésztésbevétel (nap) (5)	417	29,95	7,17	456	59,23	12,96	***
Első elléskori életkor (nap) (6)	722	47,28	6,55	779	111,63	14,32	***
Szervizperiódus (nap) (7)	33,01	14,25	43,18	74,95	28,50	38,51	**
Termékenyítési index (8)	2,23	1,41	63,22	2,55	1,11	43,52	NS
Két ellés közötti idő (nap) (9)	373	73,32	19,64	438	103,60	23,60	***
Láb (10)	2,04	0,79	38,73	6,32	2,45	38,77	***
Tőgy (11)	1,21	0,51	42,15	4,14	1,28	30,91	*
Méh (12)	0,58	0,11	18,96	2,15	0,98	45,58	**
Összes kezelés (13)	3,83	1,15	30,02	12,61	5,30	42,03	***

Traits (1); Holstein-Friesian x Montbéliarde crossbreds (2); Holstein-Friesian mothers (3); Level of significance (4); Average (5); Standard deviation (6); Coefficient of variation (7); Average (8); Standard deviation (9); Coefficient of variation (10); Age at first insemination (11); Age at first calving (12); Service period (13); Fertility index (14); Calving interval (15); Interventions: Foot (16); Udder (17); Womb (18); Interventions altogether (19)

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Associations of management practices and reproductive performance in Holstein-Friesian replacement heifers

Holstein-fríz tenyészüszők szaporodásbiológiai menedzsmentje és mutatói közötti összefüggések elemzése

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Abstract

The aim of our study was to identify reproductive management practices related to improved performance of Holstein-Friesian replacement heifers on large commercial farms. Management practices and the individual data of 14,763 heifers first inseminated in 2014 were analysed from 33 dairy herds in Hungary. The associations between management practices and major reproductive parameters were examined by mixed effects models. Those herds that used estrus detection aids experienced reduced age at first service ($p<0.001$) and age at first calving ($p=0.001$). Observing estrus in short (≤ 40 minutes long) periods (vs. observing all day long) showed a tendency towards lower first calving age ($p=0.057$) and was associated with higher odds of pregnancy at 20 months of age ($p=0.02$). Frequent pregnancy diagnosis (i.e. more than once per

week) was associated with younger first calving age (p=0.023). The uptake of more intensive reproductive management practices is suggested in heifers on large dairy farms.

Key words: dairy heifer, reproduction, management, age at first calving

Összefoglalás

Kutatásunk célja az volt, hogy meghatározzuk a jobb szaporodási eredményekkel járó menedzsment intézkedéseket a nagy létszámú tehenészetek holstein-fríz tenyészüszőinél. Elemzéseinkhez 33 nagy létszámú magyarországi tehenészet üszőknél alkalmazott szaporodásbiológiai menedzsment intézkedéseit mértük fel, valamint 14.763 tenyészüszőjének egyedi adatait használtuk, amelyek első termékenyítése 2014-ben történt. A menedzsment intézkedések és a főbb szaporodási mutatók közötti összefüggéseket kevert modellek segítségével elemeztük. Az ivarzókeresési segédeszközöket használó állományokban alacsonyabb volt az üszők első termékenyítési (p<0,001) és első ellési kora (p=0,001). Azokban a gazdaságokban, amelyekben az ivarzás-megfigyelés rövid (legfeljebb 40 perces) időszakokra korlátozódott (egész napos ivarzás-megfigyelés helyett), alacsonyabb volt az üszők életkora első elléskor (p=0,057), valamint nagyobb eséllyel voltak vemhesek 20 hónapos korban (p=0,02). Azokban a tehenészetekben, ahol gyakrabban vizsgálták az üszők vemhességi állapotát, mint hetente, alacsonyabb volt az első ellési kor (p=0,023). Eredményeink alapján javasoljuk az intenzív szaporodásbiológiai menedzsment intézkedések bevezetését a nagy létszámú tehenészetek üszőinél.

Introduction

Raising replacement heifers is the second largest expense in milk production, representing 15-20% of the total operating costs of a dairy farm (Heinrichs, 1993). Each additional day beyond the economically optimal age at first calving increases the costs of rearing by 2.87 GBP (Boulton et al., 2017). The aim of our study was to identify those reproductive management practices that are associated with the improvement of the major reproductive parameters in replacement dairy heifers.

Materials and methods

We conducted a survey between May and November 2015 in 34 large Holstein-Friesian dairy herds in Hungary. The following inclusion criteria were set up for the herds: (1) use of computerized on-farm records, (2) continuous participation in milk recording dated from at least January 1, 2011 and (3) herd size ≥250 cows. At least two dairy herds were surveyed from each of the seven regions of Hungary. Farm managers and veterinarians of each herd were personally interviewed about the management practices applied to heifers in 2014. Individual heifer data from

the participating farms were gathered from Livestock Performance Testing Ltd. (Gödöllő, Hungary). Data of heifers first inseminated between January 1, 2014, and December 31, 2014, were analysed retrospectively. One farm had only 12 complete heifer records, and therefore, was excluded from the study. After data editing, a total of 14,763 heifers from 33 farms remained in the dataset. Data were edited in MS Excel (Microsoft Corporation, Redmond, WA, USA). The following reproductive indices were evaluated: age at first service (AFS), age at first calving (AFC), conception risk to first insemination (CR1) and pregnancy status at 20 months of age. When calculating the probability of pregnancy at 20 months of age, heifers already culled by this age were excluded. The associations of the management practices and parameters were assessed by linear and logistic mixed effects models. When three options were examined within a given management practice, pairwise comparisons of the options were performed by Tukey's post-hoc test. The statistical analyses were performed in R version 3. 3. 2. (R Core Team, 2016). The level of significance was set to 0.05.

Results

Mean (± SD) herd size of the participating farms was 702 ± 359 cows, with a 305-day milk yield of 9,993 ± 972 kg on average.

Median AFS was 15.16 months. The use of estrus detection aids (i.e. activity meters, tail chalking) was associated with lower age at first service compared to solely visual observation ($p<0.001$). Those herds that performed visual estrus observation only at certain parts of the day or in short periods (<40 minutes) had lower AFS compared to herds where continuous estrus observation was performed ($p=0.077$). The exclusive use of conventional non-sexed semen was associated with higher AFS compared to the farms which use sexed semen ($p<0.001$).

Median AFC was 24.97 months. The application of estrus detection aids was associated with reduced first calving age compared to visual observation exclusively ($p=0.001$). Discontinuous estrus observation showed a tendency towards lower AFC compared to continuous observation ($p=0.057$); however, the lowest AFC was achieved when no visual observation was performed. On farms where conventional semen was used exclusively, AFC was higher than that on farms that used sexed semen only for the first AI ($p=0.048$). AFC tended to be lower when pregnancy rechecks were performed ($p=0.070$). Checking pregnancy status more than once per week was related to lower AFC, compared to weekly or less frequent pregnancy checks ($p=0.023$) (Table 1).

The average CR1 was 49.6%. Employing a reproduction advisor was associated with higher success rates for first inseminations ($p=0.028$). CR1 was higher in heifers inseminated with conventional semen only,

compared to those inseminated with sexed semen ($p<0.05$).

In the studied herds, 89.6% of the heifers were pregnant by 20 months of age. The use of estrus detection aids showed a tendency toward higher probability of pregnancy ($p=0.074$). A larger number of 20-month-old heifers were pregnant in herds with discontinuous estrus observation compared to those with visual estrus detection throughout the day ($p=0.020$). The probability of pregnancy at 20 months was lower in heifers from herds which had less experience (1-3 years), compared to those who had at least 4 years of experience, in sexed semen use ($p=0.020$). The probability of pregnancy in 20-month-old heifers was higher when a pregnancy recheck was performed ($p=0.009$) (Table 2).

Discussion

The use of estrus detection aids was associated with better reproductive indices in our study. We studied large commercial farms, where the large number of replacement heifers could potentially limit the efficiency of visual observation; therefore, the use of estrus detection aids was advantageous (Fodor et al., 2018b). Following the adoption of activity monitoring systems, the improvement in insemination risk (42 vs. 50%) led to an increase in pregnancy risk (15 vs. 17%) (Neves & LeBlanc, 2015). The authors believe that visual observation was more efficient when performed only at certain parts of the day, which in most cases meant short periods (<40 minutes) dedicated to observation, probably due to the responsible employee's higher level of commitment to this activity.

Higher AFS among exclusive conventional semen users was presumably related to some other management decision, not to the semen type itself (Fodor et al., 2018a). Possibly, herds not inseminated with sexed semen experienced poor heifer fertility, and farmers tried to compensate for this by using exclusively conventional semen, which generally yields higher success rates compared to sexed semen; however, it is also possible that CR1 was reduced due to the use of the sexed semen itself. In a study of more than 1,300 Holstein heifers, lower pregnancy per AI for first service was found in heifers inseminated with sexed semen (40.2 vs. 51.8%), and time from first AI to calving was longer, as well (10.2 vs. 9.9 months) (Chebel et al., 2010).

Performing pregnancy checks frequently (more than once a week) was linked to reduced AFC, possibly because the time lost until open heifers were found was reduced. Recheck of pregnancy status tended to be associated with reduced AFC, and was related to a greater chance of pregnancy at 20 months of age. This suggests the need for pregnancy rechecks, despite pregnancy wastage being more common in cows than in heifers (Gábor et al., 2008).

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Table 1. Associations of management practices with age at first service (AFS) and age at first calving (AFC)

	Mean AFS	95% confidence interval	P
Method of estrus detection			
Visual observation only	15.38	15.02-15.77	<0.001
Estrus detection aids	14.33	13.79-14.92	
Duration of visual observation			
Continuously during the day	15.58	15.16-16.03	0.055
Not continuous	14.79	14.15-15.51	
No observation	14.73	13.62-16.12	
Number of services with sexed semen			
The first AI only	14.76[a]	14.05-15.58	<0.001
More than one AI	15.35[a]	14.50-16.34	
No sexed semen	16.51[b]	15.51-17.69	
	Mean AFC	**95% confidence interval**	**P**
Method of estrus detection			
Visual observation only	25.32	24.93-25.72	0.001
Estrus detection aids	24.44	23.96-24.95	
Duration of visual observation			
Continuously during the day	25.57	25.51-25.57	0.025
Not continuous	24.76	24.68-24.77	
No observation	24.43	24.34-24.44	
Number of services with sexed semen			
The first AI only	24.54[a]	24.45-25.36	0.048
More than one AI	25.40[ab]	24.51-26.38	
No sexed semen	26.07[b]	24.70-27.70	
Pregnancy recheck			
No	25.30	25.28-25.30	0.07
Yes	24.72	24.69-24.72	
Frequency of pregnancy diagnosis			
Weekly or less frequently	25.25	25.23-25.25	0.023
More than once per week	24.63	24.60-24.63	

[a, b] Different superscripts within a management practice with three options indicate a significant ($p<0.05$) difference.

Table 2. Associations of management practices with first-service conception risk (CR1) and pregnancy status at 20 months of age

	CR1 (Odds ratio)	95% confidence interval	P
Reproduction advisor			
Yes		Reference	0.028
No	0.67	0.47 – 0.96	
Number of services with sexed semen			
The first AI only		Reference[a]	0.003
More than one AI	0.92[a]	0.62 – 1.37	
No sexed semen use	3.27[b]	1.52 – 7.04	
Years of sexed semen use			
≥ 4 years		Reference[a]	0.001
1-3 years	0.77[a]	0.52 – 1.14	
No sexed semen use	2.50[b]	1.40 – 4.49	
	Pregnancy at 20 months of age (Odds ratio)	**95% confidence interval**	**P**
Method of estrus detection			
Visual observation only		Reference	0.074
Estrus detection aids	2.88	0.90 – 9.17	
Duration of visual observation			
Continuously during the day		Reference[a]	0.017
Not continuous	3.03[b]	1.34 – 6.88	
No observation	3.85[ab]	0.75 – 19.70	
Years of sexed semen use			
≥ 4 years		Reference[a]	0.019
1-3 years	0.28[b]	0.11 – 0.71	
No sexed semen use	1.27[ab]	0.30 – 5.38	
Pregnancy recheck			
No		Reference	0.009
Yes	3.60	1.37 – 9.43	

[a, b] Different superscripts within a management practice with three options indicate a significant ($p<0.05$) difference.

Evaluation of the reproductive performance on large Hungarian dairy farms

A nagy létszámú magyarországi tejelő tehenészetek szaporodási eredményeinek értékelése

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Abstract

The aim of this survey was to assess the average values of the major reproductive indices in the Hungarian dairies. The authors surveyed the major reproductive indices on 21 large commercial dairy farms from six Hungarian counties between February and May 2015. Altogether the individual data of 12,723 cows were collected from the farm management software RISKA (Systo Ltd., Budapest, Hungary). Associations between the major reproductive parameters were analysed by Spearman's rank correlation. Average calving-to-conception interval, services per conception and first service conception rate were 160.1 days, 4.3 and 22.3%, respectively. It was proven that pregnancy rate (PR) and corrected pregnancy rate (cPR – pregnancy rate within 200 days in milk) are applicable on Hungarian dairy farms due to their strong correlation ($p < 0.05$) with other reproductive parameters. PR and cPR were 9.6% and 17.3%, on average, respectively.

Key words: dairy cattle, reproductive performance, management, pregnancy rate

Összefoglalás

Kutatásunk célja az volt, hogy felmérjük a hazai tejelő tehenészetek főbb szaporodási mutatóit. Magyarország hat megyéjének 21 nagy létszámú tehenészetében mértük fel a főbb szaporodási mutatókat 2015 februárja és májusa között. Összesen 12.723 tehén egyedi adatát gyűjtöttük ki a RISKA telep-irányítási szoftverből (Systo Kft., Budapest). A főbb szaporodási mutatók közötti össze-függéseket Spearman-féle rangkorrelációs teszttel elemeztük. Az elléstől újravemhesü-

lésig eltelt idő, a termékenyítési index, ill. az első termékenyítésre vemhesültek aránya 160,1 nap, 4,3, ill. 22,3% volt átlagosan. Igazoltuk, hogy a vemhességi ráta (PR), valamint a korrigált vemhességi ráta (cPR – 200. tejelő napon belül számított vemhességi ráta) jól alkalmazhatóak a hazai tejtermelő tehenészetekben, mivel értékük szorosan ($p<0,05$) összefügg a többi szaporodási mutató értékével. A PR, ill. a cPR átlagértéke 9,6%, ill. 17,3% volt.

Introduction

Profitability is fundamentally influenced by the reproductive success of the herd (Ózsvári & Kerényi, 2004; Ózsvári, 2013). However, the evaluation of reproductive performance varies greatly among dairy farms in Hungary (Kranjec et al., 2016). The authors aimed to assess the average values of the major reproductive indices in the Hungarian dairy herds and to define more relevant reproductive indices. Moreover, corrected PR (cPR), as a new parameter for measuring reproductive success, was developed and its suitability for routine use was tested. Corrected PR was developed to tailor PR to the Hungarian circumstances, thus, its advantages can be pushed.

Materials and methods

The authors surveyed production and reproduction indices and the reproductive management in large-scale Hungarian dairy herds. The data of 12,723 cows were collected in 21 herds in six Hungarian counties between February and May 2015. However, the optimal VWP varies among dairies to a limited extent, 60 days was uniformly used in this work. Production and reproductive parameters were analysed in Microsoft Excel 2013 (Microsoft Corporation, Redmond, WA, USA) and in R software.

Parameters considering all cows were readily available in the farm management software, and included the following indices:

- *% of culled cows* (%CULL): the % of cull candidates and already culled cows.
- *Open cows > 60 DIM* (O60): the % of not inseminated, or inseminated, but open and not re-inseminated cows. The denominator is the number of non-cull cows beyond the voluntary waiting period (VWP).
- *Service period* (SP): the average time it takes for the cows to conceive. SP = Σ DIM to conception (pregnant cows) / number of pregnant cows.
- *Interbreeding interval* (IBI): the average number of days between two subsequent inseminations.
- *Re-insemination at 12-21 days* (R21): the % of re-inseminations between 12-21 days after the previous insemination within the total number of re-inseminations.
- *Services per conception* (SPC): the total number of inseminations in a period over the number of conceived cows in the same period.

- *Days to successful first insemination* (DS1I): the average service period of the cows that conceived for the first insemination.
- *Conception rate of first inseminations* (CR1): the % of cows in a period that conceived for the first insemination.
- *% pregnant* (%Preg): the % of pregnant cows as a % of the total number of cows in the herd.

Cows beyond VWP and not culled were regarded as the optimal reproductive population ("ORP cows"). Since open cows beyond 200 DIM are usually culled in the US, it was used as a reference here, as well. Only ORP cows were taken into account at the calculation of the following indices. On the one hand, these indices describe reproductive performance in a more realistic way, on the other hand, they facilitate the comparison of the results of the dairies internationally.

- *% pregnant in ORP* (%PORP): cull cows are not taken into account, because they should not be inseminated. Similarly, if VWP is strictly applied (here 60 days), then pregnant cows should only be searched for among the cows that calved before this date.
- *Service period within 200 DIM* (SP200): SP was quantified for every single pregnant cow, and the average SP was calculated for those conceiving within 200 DIM.
- *% pregnant within 200 DIM* (%Preg200): the % of pregnant cows with an SP < 200 days.
- *Services per conception of pregnant cows within 200 DIM* (SPCP200): the average number of inseminations needed for those cows conceiving within 200 DIM.
- *Open cows beyond 285 DIM* (O285): the % of cows within the ORP, which have been milking for more than 285 days, produce less than 25 kg milk daily and are open.
- *Pregnancy Rate* (PR): the theoretically possible number of oestrus cycles of the pregnant cows was calculated by subtracting VWP from the SP, and this was divided by 21. The result was rounded upwards (e.g. if the quotient was 1.24, then it should be rounded up to 2). The way of calculation is similar in the case of the nonpregnant cows, as well, but VWP was subtracted from DIM. The possible number of oestrus cycles was summed up for all the ORP cows and the number of pregnant cows was divided by the sum.
- *Corrected Pregnancy Rate* (cPR): for those cows conceiving within 200 DIM, the number of pregnant cows was divided by the total number of possible oestrus cycles until conception, as described at PR. Actually, this way the pregnancy rate of pregnant cows within 200 DIM was calculated (PRP200). Assuming that during the same period (i.e. average number of days to conception of the pregnant cows) equal number of oestrus cycles occurred in the nonpregnant population, as well, cPR was calculated as follows: cPR = PRP200/2.

Results and Discussion

The major production and reproduction parameters of the surveyed herds are shown in Table 1.

Table 1. Production and reproductive parameters of the herds (n=21)

Parameter	Average	Minimum	Maximum
Number of cows	606	169	1,269
%CULL	5	0	17
Lactational milk yield (kg)	9,851	8,200	12,174
O60 (%)	12	2.2	34
SP (days)	160	125	205
IBI (days)	29	23	38
R21 (%)	46	26	61
SPC	4.27	2.8	7.2
DS1I (days)	76	52	107
CR1 (%)	22	14	36
%Preg	43	28.8	60.5
%PORP	53	37	67
SP200 (days)	109	95	121
%Preg200	72	56	86
SPCP200	2.06	1.49	2.41
PR (%)	10	4.4	16
cPR (%)	18	14.6	23.11

Table 1. shows that the performance of the dairies do not comply with the former – but commonly used – reference values. Suggested values for SP used to be below 115 days, >60% for CR1 and 1.5-2.2 for SPC (Radostits et al., 1994; Szenci, 1999). Even the best herds could not reach these goals which were set up in the '90s. We also evaluated the correlation between reproductive indices: strong negative correlation existed between two parameters, when the Spearman's rank correlation coefficient was between -0.5 and -1, whereas strong positive correlation was found when this coefficient was between 0.5 and 1. In case of SP, SPC, CR1, productivity, PR and cPR we found 6, 7, 5, 4, 8 and 9 strong correlations with other parameters, respectively. Therefore, PR and cPR showed the largest number of significant correlations with other reproductive parameters.

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Changes in the endometrial expression of growth factors associated with the estrus cycle in grazing dairy cows

A legelő tejhasznú tehenek nemi ciklusával kapcsolatos növekedési faktorok endometrialis expressziójának változása

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Abstract

The aim of this study was to evaluate the effect of milk production on estradiol (E2) and progesterone (P4) serum concentrations, and on endometrial expression of vascular endothelial growth factor (VEGF) and their receptors (VEGFR1 and VEGFR2), nodal growth differentiation factor (NODAL), epidermal growth factor receptor (EGFR), estradiol receptor (E2R) and progesterone receptor (P4R) on days 0, 4 and 9 of estrus cycle in grazing dairy cows.

High milk producer cows (HP), medium milk producer cows (MP) and anestrous cows were blood and endometrium sampled on days 0, 4 and 9. The mRNA expression was performed using RT-qPCR.

MP cows had higher concentrations of serum P4 concentration that HP (P=0.003). Time had an effect on P4 (P<0.001) and E2 (P=0.019) serum concentrations and on the endometrial expression of E2R (P=0.049) and VEGFR2 (P<0.001). No differences in growth factors and receptors endometrial expression were found between HP and MP cows.

Key words: endometrial expression, qPCR, growth factors, dairy cows, estrus cycle

Introduction

There is an ample debate among researchers about possible antagonism between high milk production and reproductive performance. One reasonable explanation seems that increasing milk production is associated with higher metabolism of the steroid hormones. Lactating dairy cows have higher energy requirements than non-lactating cows and higher feed intake to achieve these energy requirements. The rise in feed intake leads to a dramatic increase in liver blood flow, which tends to elevate the metabolism of Estradiol (E2) and Progesterone (P4) with subsequent lower circulating concentrations of them. It might alter many aspects of normal reproductive physiology due to the central roles of steroid hormones in almost every aspect of normal reproduction (Wiltbank et al., 2006). In this line, some studies support the hypothesis that high producing dairy cows present some level of subfertility (Lucy, 2001; Thatcher et al., 2006).

Studies have shown evidence that ovarian steroid hormones regulate uterine function by stimulating the local production of growth factors and cytokines. Therefore, alterations in endometrial expression of local growth factors could reflect disorders of the endocrine environment including blood concentrations of P4 and E2 (Katagiri & Moriyoshi, 2013; Sagsoz & Saruhan, 2011).

Epidermal Growth Factor (EGF), Vascular Endothelial Growth Factor (VEGF) and some Transforming Growth factors (Katagiri & Takahashi, 2004; Kliem et al., 1998; Sagsoz and Saruhan, 2011) were described as important growth factors during early stages of pregnancy, being its expression sensitive to changes in peripheral concentrations of P4 and E2.

EGFR and their main ligands were postulated as an important part of the early embryo-maternal talk during the preimplantation stage and were associated with uterine regulation during this period (Kliem et al., 1998) while VEGF is usually related to vascular permeability and proliferation, and survival and cellular migration (Ortega Serrano et al., 2016). Nodal growth differentiation factor (NODAL) was described as a factor involved in endometrial remodelation during preimplantation stage and in the establishment of a new pregnancy.

The aim of this study was to evaluate the effect of milk production on E2 and P4 serum concentrations, and on endometrial expression of VEGF and their receptors (VEGFR1 and VEGFR2), NODAL, EGFR, estradiol receptor (E2R) and progesterone receptor (P4R) on days 0, 4 and 9 of estrus cycle in grazing dairy cows.

Materials and Methods

The study was conducted in grazing dairy farms in Buenos Aires province, Argentina. Holstein cows (n=10) were randomly allocated in groups: high milk producer

cows (HP, ~10.000 kg/305d), medium milk producer cows (MP, ~7.500 kg/305d) and anestrous cows. HP and MP cows were estrus synchronized with a Presynch-Ovsynch protocol. Endometrial cells samples were obtained from endometrium of the synchronized cows on days 0, 4 and 9 post-estrus (representing estrus, metestrus and diestrus, respectively) by using cytobrush technique (Kasimanickam et al., 2005; Madoz et al., 2014).

Cells obtained from brushes were used to evaluate and discard cows with subclinical endometritis using a cutoff point of 5% polymorphonuclear cells (Madoz et al., 2013) and for RNA isolation and subsequent RT-qPCR analysis. The same procedure was carried out with anestrous cows, but they were sampled on hazard days.

From all cows, on days 0, 4 and 9 of the estrus cycle, a blood sample from tail vessels was obtained to measure P4 and E2 by quimioluminescence.

RNA isolation was performed using Trizol. After complementary DNA (cDNA) was synthesized, reactions of qPCR for bovine genes (VEGF, VEGFR1, VEGFR2, Nodal, EGFR, P4R and E2R) were carried out using specific primers. Beta-actin was used as housekeeping and samples of anestrus cows as calibrators. Quantitative analysis of the mRNA expression of the selected factors in endometrial epithelial cells was performed using real-time PCR (Rotor-Gene Q, Quiagen, Germany).

To analyze the relative level of expression, double-Δ CT method was used. Data were analyzed with Proc GLIMMIX (SAS, 2016) by using a Gamma distribution and a log link function. Models included the fixed effect of time (0 d vs. 4 d vs. 9 d), treatment (HP vs. MP) and their interaction, and the random effect of the cow. Statistical significance was set at $P<0.05$ and a tendency at $P<0.1$.

The Graduate School and the Laboratory Animal Care and Use Committees of the Faculty of Veterinary Sciences at the National University of La Plata have approved this study.

Results

Results are showed as LSM±SE. Treatment group only had an effect on serum P4 concentration given that MP had higher concentrations that HP (2.23±1.13 and 1.28±1.11; P=0.003). Time had an effect on P4, showing that cows were estrus synchronized, (d0: 0.42±1.17; d4: 1.87±1.14 and d9: 6.129±1.14; P<0.001) and E2 serum concentrations (d0: 6.19±1.14; d4: 7.49±1.14 and d9: 9,18±1,14 ; P=0.019).

Time also had an effect on the expression of E2R (d0: 1.97±1.15; d4: 2.91± 1.13; d9: 2.01±1.14; P=0.049) and VEGFR2 (d0: 0.81 ±1.27; d4: 0.67±1.22 and d9: 0.69± 1.24; P<0.001). Time had a tendency to affect nodal expression (P=0.092). Time*

Treatment had a tendency to affect EGFR expression (P=0.066). The remaining effects had a P>0.1.

Conclusions

In agreement with our hypothesis and other studies, serum concentration of P4 was reduced in HP cows in comparison with MP cows; in addition, there were significance differences in serum concentration of E2 and P4 according to days of estrus cycle showing they were correctly estrus synchronized. Conversely, in disagreement with our hypothesis, there were no differences between HP and MP in endometrial mRNA expression of any growth factor or receptor studied. It results was not expected given that steroids hormones (E2 and P4) are responsible for regulating the endometrial expression of a wide variety of genes related to fertility, and significance variations in these hormones concentrations were found. These results could probably be associated with a reduced number of samples processed.

Finally, the time had an effect on the expression of E2R, VEGFR2, and Nodal factor showing a variation according to the estrus cycle.

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Effect of milk production on endometrial gene expression in grazing dairy cows

A tejtermelés hatása a legelő tejhasznú tehenek endometriális génexpressziójára

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Abstract

Declining fertility in lactating dairy cows has been associated with the metabolic stress of milk production. The aim of this study was to analyze the endometrial global gene expression in high milk production (HMP) and medium milk production (MMP) dairy cows on diestrous. Holstein cows (~50 DIM) without any clinical disease were included

and allocated in two groups: HMP (~10.000kg/305d) and MMP (~7.500kg/305d). Biopsy uterine samples were obtained from all estrus synchronized cows on day 9 post-estrus.

Gene expression analysis of the endometrial biopsies was performed by RNA sequencing using the HiSeq 1500 platform 2x150pb (Illumina Inc. CA, USA). More than 300 differentially expressed genes (DEG) between groups were found. Up-regulated DEG in HMP cows compared to MMP cows were related with the immune system, while down-regulated DEG were related with Epidermal Growth Factor and cell adhesion molecules which may be associated with the reduced fertility observed on these animals.

Key words: RNA sequencing, milk production, fertility, diestrus, uterus

Introduction

Good fertility of dairy cows is the key to economically successful dairy farming. However, during the last two decades, fertility measured as conception rates and days from calving to successful breeding is declining. There is no doubt that production per cow has increased, but it is unclear how much of this increase can explain the apparent decrease in fertility (LeBlanc, 2010).

Cows with high milk production (HMP) have greater energy requirements and higher feed consumption than medium milk production cows (MMP). This leads to a dramatic increase in liver blood flow, which tend to elevate de metabolism of Estradiol (E2) and Progesterone (P4) affecting many aspects of normal reproductive physiology (Wiltbank et al., 2006).

Global gene expression analyses have demonstrated that alterations in energy balance can modify endometrial gene expression (Cerri et al., 2012; Thompson et al., 2012; Forde et al., 2017). Altered endometrial gene expression induced by low P4 was associated with a reduced capacity of the uterus to support conceptus development (Forde et al., 2011). However, the effect of low P4 concentrations on endometrial expression is not yet well known.

The aim of this study was to analyze the endometrial global gene expression in HMP and MMP on diestrous using RNA sequencing technology.

Materials and Methods

This study was conducted in Buenos Aires province, Argentina. Grazing Holstein dairy cows (~50 DIM) without any clinical disease and BCS >2.50 were included and allocated in two groups: HMP (~10.000kg/305d, n=3) and MMP (~7.500kg/305d, n=3). All cows were estrus synchronized with a Presynch-Ovsynch protocol. Blood was obtained on days 0, 4 and 9 to measure concentrations of P4 by quimioluminescence (Immuno-analyzer Elecsys and Cobas e, Roche®, Mannheim, Germany).

Endometrial samples were collected using a biopsy instrument on day 9 post-estrus, immediately transferred into cryotubes, placed in liquid nitrogen and then stored at -80ºC. RNA isolation was performed using TRIzol reagent according to the manufacturer´s protocol. The cDNA libraries for RNA-seq were constructed using the TruSeq Stranded mRNA and sequenced using the HiSeq 1500 platform 2x150pb (Illumina Inc. CA, USA). Resulting sequence reads were aligned to the bovine reference genome (bosTau8), using the Rsubread package (R-software). Differentially expressed genes (DEG, $p<0.05$) between HMP and MMP cows were determined by genewise statistical analysis (EdgeR package). Functional analysis for DEG was performed with the functional annotation clustering tool of the DAVID database.

The Graduate School and the Laboratory Animal Care and Use Committees of the Faculty of Veterinary Sciences at the National University of La Plata have approved this study.

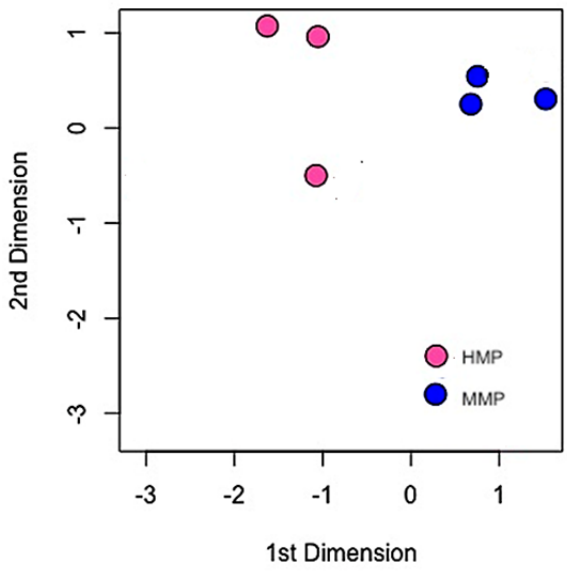
Results

There were over 300 DEG between HMP and MMP cows. The MDS analysis showed that samples from MMP cows clustered apart from HMP cows' samples (Figure 1).

The main clusters significantly enriched with the DEG up-regulated in HMP cows compared to MMP cows were related with the immune system, while the clusters of down-regulated DEG were related with Epidermal Growth Factor (EGF) and cell adhesion molecules.

Blood concentrations of P4 on day 9 averaged 4.5±0.437 ng/ml and 8.54±1.42 ng/ml in HMP and MMP, respectively.

Figure 1. Principal component analysis. A plot of the factors contributing to the overall transcriptional profile of uterine biopsy samples of individual cows. Blue dots represent MMP cows and pink dots represent HMP cows on day 9 of the estrus cycle



Discussion and Conclusion

HMP and MMP on diestrus showed differences in their endometrial transcriptomics that are related with higher expression of the immune system and the inhibition of the EGF system and cell adhesion molecules in the endometrium of HMP. These results are in agreement with several links found between fat metabolism,

inflammation and immune function (LeBlanc, 2012) and alteration in ovarian steroid hormone and endometrial EGF profile described in repeated breeder cows and HMP cows (Katagiri et al., 2013). In conclusion, there are differences in endometrial gene expression between HMP and MMP probably associated with the reduced fertility observed on HMP cows.

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Preliminary results of the use of a calving detector in Holstein-Friesian heifers and cows

Ellés kezdetét jelző készülék alkalmazása során nyert előzetes eredmények Holstein-fríz üszőkön és teheneken

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Abstract

The aim of this work was to examine the efficiency of the calving monitoring system Moocall at a cattle farm in Lower Saxony, Germany. Moocall is an electronic motion sensor that is attached manually to the base of the tail of prepartum cattle 4 days prior to the predicted calving date (PCD). It registers specific tail movement, and returns an alarm in form of short message services (SMS) in case of an imminent calving. First high activity alert means an enhanced activity over the preceding 1 hour and second high activity alert if it continues for the consecutive hour.

Twenty-four animals equipped with Moocall sensors were enrolled in this study, 16 heifers and 8 cows after their 1st (n=5) and 2nd lactation (n=3). To determine the exact point of the initiation of calving, clinical examinations were carried out every 6 hours during 7 days before pcd. The heifers of this study calved 276.6 ± 9.1 days following AI (dpai) and the cows 283.9 ± 4.2 dpai (mean±SD). Seventeen animals calved spontaneously. One cow had slight, 6 heifers mild (n=3) or severe (n=3) difficulties at calving and needed various obstetric assistance, but caesarean section was not necessary in any cases. While twenty-two vital calves were born, one was weak and a further one died at birth, both of these from heifers. Moocall sent 6.1 ± 3.9 SMS (max: 14; min: 2) in heifers and 6.5 ± 4.3 SMS (max: 13; min: 2) in cows. In heifers, 61 SMS

contained class 1 alarms and 36 class 2 alarms. In cows, 35 and 17 SMS contained class 1 and 2 alarms, respectively. The time lag between the first SMS of Moocall and the clinical recognition of labor was 36.9 ± 32.1 h (max: 95 h; min: 1 h) for heifers and 61.1 ± 66.5 h (max: 177 h; min: 0 h) for cows. The time elapsed between the last SMS of Moocall and clinical recognition of labor amounted to -1.2 ± 7.3 h (max: 23.2 h; min: -14.2 h) for heifers and -2.1 ±1.9 h (max: -1.3 h; min: -5.2 h) for cows. The period between the first SMS of Moocall and the completion of calving (calf being completely expelled) lasted 40.7 ± 32.8 h (max: 97.2 h; min: 2 h) for heifers, and 63.4 ± 66.1 h (max: 179.1 h; min: 2.5 h) for cows. Ten out of the 24 animals kept the device permanently attached during the study. We registered a mean number of loss of the device of 1.4 ± 1.9 (max: 6; min: 0) for heifers and 1.0 ± 1.9 (max: 4; min: 0) for cows. In 18 cases the device was detached or lost by the animals, or it was necessary to remove it temporarily because of tail swelling (n=3) or because the device was attached for longer than 4 days (n=2). Moocall informed consistently about the loss of the device in one case, often in 3 cases and in 4 cases it did not inform at all.

Our preliminary results indicate that Moocall could be one possible method to predict due calvings in dairy cattle, however, further calculations are necessary to determine its efficiency. We consider that clinical examination remains the gold standard for the detection of calving animals but any additional automatized system could support the achievement of best calving detection, especially in cases where continuous observations with trained personnel cannot be provided.

Acknowledgment

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Összefoglalás

A Moocall ellés kezdetét jelző készülék kipróbálását egy alsó-szászországi (Németország) tehenészetben végeztük. Vizsgálatunk célja a készülék használhatóságának, pontosságának felmérése volt az ellések kellő időben történő előrejelzéséhez. Alkalmazásával kapcsolatos előzetes eredményeinket az alábbaikban foglaljuk össze:

A Moocall ellésjelző készüléket a várható ellés előtt 4 nappal helyeztük fel a péranyílás magasságában a farokra 16 üsző és 8 tehén esetében, melyek közül 5 korábban már egy, 3 pedig két lezárt laktációval rendelkezett. A készülék a farokmozgások változása alapján érzékeli a közeledő ellés jeleit, és rövid szöveges üzenetet küld a megadott mobil-

telefonszámokra, ha a megelőző 1, vagy az azt követő órában ilyen folyamatokat kellő számban érzékel.

Az ellés pontos kezdetének magállapítására a várható ellések előtti 7. naptól kezdődően 6 óránkénti klinikai vizsgálatokat végeztünk. Az üszők 276,6 ± 9,1 napos a tehenek pedig 283,9 ± 4,2 napos vemhesség végén ellettek. Míg 17 állat magától ellett, egy tehénnél kis, három-három üszőnél pedig közepes, illetve jelentős mértékű ellési segélynyújtásra volt szükség, de császármetszésre nem volt szükség. Az üszőktől egy gyenge életképességű és egy holtmagzat született. A Moocall 6,1 ± 3,9 SMS-t küldött (max: 14; min: 2) üszők esetében, és 6,5 ± 4,3-at (max: 13; min: 2) tehenek esetében. Az üszőknél 61 SMS volt első és 36 második értesítés, ez teheneknél 35 és 17 volt összesen. Az első SMS és az ellés kezdete között üszőknél 6,9 ± 32,1 óra (max: 95, min: 1 óra), teheneknél 61,1 ± 66,5 óra (max: 177, min: 0 óra) telt el. Az utolsó SMS és az ellés kezdete között üszők nél -1,2 ± 7,3 óra (max: 23,2, min: -14,2 óra), a teheneknél pedig -2,1 ± 1,9 óra (max: -1,3, min: -5,2 óra) telt el. A 24 állatból 10-nél a készülék végig a farkon maradt, összesen 18-szor azonban leesett, vagy a farkon kialakuló duzzanat miatt (3 állatnál), vagy a farkon tarthatóság maximálisan javasolt 4 napjának elérése miatt (2 esetben) időszakosan le kellett venni.

A Moocall alkalmazásával szerzett előzetes tapasztalataink tejhasznú szarvasmarhákban alapján elmondható, hogy a módszer egy az ellés-előrejelzések új lehetőségei közül, hatékonyságát azonban várhatóan csak további elemzéseket követően tudjuk pontosan megállapítani. Véleményünk szerint a klinikai vizsgálat továbbra is a legmegbízhatóbb módszer, de bármely kiegészítő, önálló eljárás is segítheti az ellés pontos előrejelzését, különösen ott, ahol a folyamatos, szakképzett helyszíni ellési felügyelet nem biztosítható.

Susceptibility and resistance of selected pathogens of the mammary gland of cattle from Austria and Czech Republic in 2017

Ausztriai és csehországi szarvasmarha tőgypatogén kórokozóinak érzékenysége és rezisztenciája 2017-ben

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Abstract

The aim of this study was to evaluate the *in-vitro* susceptibility of bovine mastitis pathogens to penicillin, to a combination of cephalexin and kanamycin and to a combination of penicillin and framycetin. Field isolates from Austria and Czech Republic were tested by using the agar disc diffusion test according to CLSI guidelines. Evaluation of susceptibility was based on the recommendations of CLSI Vet01-S2 (2015) and on the literature (Pillar et al., 2009; Silley et al., 2012; Pillar et al., 2014). The results points at the good susceptibility to non-critical antimicrobial combinations of cephalexin-kanamycin and penicillin-framycetin in both Gram-positive and Gram-negative groups of bacteria.

Key words: non-critical antibiotic, antimicrobial susceptibility, disc diffusion method, synergy

Introduction

Cephalosporins and penicillins are of immense importance in mastitis therapies. Concurrently, the World Health Organization (WHO) classified systemic third and fourth generation cephalosporins and fluoroquinolones as the Highest Priority Critically Important Antibiotics (HPCIA) with the recommendation to restrict the use in animal husbandry in order to minimize the risk of resistances. With the aim of prudent use of antibiotics, there is a need for alternatives for using in the field containing non-critical antibiotics as active substances. First generation cephalosporins and penicillins have a good activity against a wide spectrum of

Gram-positive bacteria. In combination with aminoglycosides a broader spectrum of pathogens could be covered. Studies additionally demonstrated that combinations had a synergistic and additive effect resulting in larger and faster killing rates of mastitis pathogens (Lohuis et al., 1995; Ganière & Denuault, 2009). However, a high in vitro susceptibility against bovine mastitis pathogens is a prerequisite for treatment success. Therefore, the aim of this study was to assess the in vitro susceptibility and resistance situation of prevalent mastitis pathogens to penicillin and to combinations of non-critical antibiotics cephalexin and kanamycin (Ubrolexin®, Boehringer Ingelheim) as well as penicillin and framycetin (Benestermycin®/ Ubrostar®, Boehringer Ingelheim).

Material and Methods

Boehringer Ingelheim RCV GmbH & Co KG (Austria), the Diagnostic laboratory of the Clinic for Ruminants at the University of Veterinary Medicine Vienna (Austria), and the Special Microbiology Department of the State Veterinary Institute Olomouc (Czech Republic) participated in this research.

Isolation and identification of bacteria, bacteriological confirmation

A total of 225 quarter bovine milk samples were examined in the Czech Republic between July and November 2017. All samples were cultivated on the Meat Peptone Blood Agar (MPBA) (Trios Ltd. Prague, Czech Republic) and incubation was carried out at 37 ± 1 °C for 42 - 48 hours. In parallel, milk samples were incubated after inoculation on MPBA in the culture tubes at 37 ± 1 °C for 18 - 24 hours. Subsequently, incubated milk samples were inoculated on the Edward´s Agar (EDW) (Trios Ltd. Prague, Czech Republic). Inoculated plates with EDW were again incubated at 37 ± 1 °C for a further 18 - 24 hours (Bzdil, 2004). The Austrian milk samples were processed according to National Mastitis Council guidelines (1999). Milk samples were plated onto Columbia agar (OXOID, Basingstoke, UK) with addition of sheep blood (5%) and incubated at 37 °C. A total of 418 quarter bovine milk samples were examined in Austria in the same period. All isolates originated from single-quarter milk samples of cows with clinical or subclinical mastitis. Despite the anamnesis of mastitis, growing in pure culture on Columbia agar was a prerequisite for inclusion in the study. Suspicious colonies captured in the Czech Republic were isolated and subsequently confirmed by the phenotypic molecular mass spectrometry method, MALDI TOF MS, based on the proteomics analysis, and MALDI Biotyper software (Bruker Daltonik GmbH, Bremen, Germany). The Austrian strains were identified to the genus or species level by conventional methods such as colony morphology, haemolytic pattern on blood agar, Lancefield serogrouping, esculin reaction, growing on a modified Rambach agar (streptococci), coagulase tube test (differentiation between *S. aureus* and CNS), KOH test, growing pattern on McConkey agar and oxidase reaction. In case of doubt,

bacterial species were confirmed by the MALDI TOF MS system and MALDI Biotyper software (Bruker Daltonik GmbH, Bremen, Germany). All collected strains were stored at -80 °C until further analysis.

Antimicrobial susceptibility testing

Following pathogens were selected for the susceptibility testing: *Staphylococcus aureus*, Coagulase-negative staphylococci (CNS), *Streptococcus uberis*, *Streptococcus dysgalactiae*, and *Escherichia coli*.

The disc diffusion test according to the guidelines from CLSI (CLSI 2008) was performed for the antimicrobial susceptibility/resistance assessment. Müller Hinton agar was used for susceptibility testing of coliforms and staphylococci, for streptococci Müller Hinton agar supplemented with 5 % sheep blood was used. For all pathogens commercially manufactured discs containing 10 IU of penicillin G combined with 100 µg framycetin and discs with combinations of cephalexin and kanamycin at 30 and 15 µg, were tested. Additionally, commercially manufactured discs containing 10 IU penicillin G were assessed (all discs from Mast, Merseyside, UK). Disc interpretative criteria, as published by Pillar et al. (2009) and Silley et al. (2012), were used for combinations of kanamycin and cephalexin. For combinations of penicillin and framycetin, interpretative criteria published by Pillar et al. (2014) were used. Diameters for penicillin G were evaluated as published by CLSI (2015). With regard to *Sc. uberis* and penicillin, no interpretative criteria are available, thus the criteria for ß-hemolytic streptococci were applied.

Results

The tables below (Tables 1 and 2) show the susceptibility of staphylococci and streptococci to penicillin, and the susceptibility of staphylococci, streptococci and *Escherichia coli* to the combinations of cephalexin and kanamycin as well as penicillin and framycetin. The results for the isolates of each country are summarized in separate tables. (Legend: S - susceptible, I - intermediate, R - resistant)

Table 1. The susceptibility and resistance of the isolates panel from Austria

Susceptibility to:	penicillin			cephalexin-kanamycin			penicillin-framycetin		
	S	I	R	S	I	R	S	I	R
S. aureus (n=90)	89%		11%	99%	0%	1%	97%	3%	0%
CNS (n=63)	87%		13%	100%	0%	0%	100%	0%	0%
Sc. dysgalactiae (n=35)	100%		0%	100%	0%	0%	100%	0%	0%
Sc. uberis (n=124)	98%		2%	99%	1%	0%	100%	0%	0%
E. coli (n=50)				92%	6%	2%	94%	2%	4%

Table 2. The susceptibility and resistance of the isolates panel from Czech Republic

Susceptibility to:	penicillin			cephalexin-kanamycin			penicillin-framycetin		
	S	I	R	S	I	R	S	I	R
S. aureus (n=46)	35%		65%	89%	2%	9%	100%	2%	0%
CNS (n=68)	57%		43%	100%	0%	0%	100%	0%	0%
Sc. dysgalactiae (n=10)	100%		0%	100%	0%	0%	100%	0%	0%
Sc. uberis (n=82)	100%		0%	100%	0%	0%	100%	0%	0%
E. coli (n=13)				77%	8%	15%	100%	0%	0%

Discussion

Synergistic and additive effects resulting in higher and faster bactericidal activity have been found when narrow spectrum antibiotics such as cephalexin and kanamycin are combined (Ganière & Denuault, 2009). For combinations of penicillin and framycetin similar interactions against *S. aureus* were confirmed (Lohuis et al., 1995). However, high in-vitro sensitivities against most prevalent mastitis pathogens are a decisive criterion for success in mastitis therapies, although other parameters such as pharmacological properties (distribution of the antibiotic in the mammary tissue) or pathogen specific virulence factors (formation of biofilms or abscesses) may be closely related with bacterial cure too (Poutrel et al., 2018). In-vitro resistance is associated with treatment failure. In this study the most prevalent mastitis pathogens, such as *Staphylococcus aureus*, CNS, *Streptococcus dysgalactiae, Streptococcus uberis* and *Escherichia coli* were tested against combinations of cephalexin/kanamycin and penicillin/ framycetin, the two intramammary (IMM) formulations that cover a broad spectrum of Gram-positive and Gram-negative bacteria.

Our results show low overall resistance levels of both IMM antibiotic combinations to prevalent pathogens. This is very important because acute clinical mastitis therapy often has to be initiated without the results of antimicrobial susceptibility testing. While the susceptibility of staphylococci towards penicillin is not as good as to the penicillin/framycetin combination, the susceptibility of streptococci is comparable.

Very interesting is the fact that *S. aureus* and CNS strains susceptibility to penicillin G differs in the Czech Republic and in Austria. Quite similar situation could be seen in cephalexin-kanamycin combination in the case of *S. aureus* and *E. coli*.

Conclusion

These results point at the good susceptibility to non-critical antimicrobial combinations of cephalexin-kanamycin and penicillin-framycetin in both Gram-positive and Gram-negative groups of bacteria. Penicillin should also be considered as an option for the treatment of udder infections caused by Gram-positive bacteria.

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Susceptibility of Gram positive udder pathogens to penicillin and to combinations of cephalexin-kanamycin and penicillin-framycetin in Hungarian dairy herds

Gram-pozitív tőgypatogén kórokozók érzékenysége penicillinnel, ill. cefalexin-kanamicin és penicillin-framicetin kombinációjával szemben magyar tejelő állományokban

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Abstract

The aim of this study was to investigate the relevance of non-critical antibiotic penicillin and antibiotic combinations of cephalexin-kanamycin and penicillin-framycetin to Gram-positive udder pathogens isolated from mastitis milk in problem herds, and besides the general resistance situation to assess differences between herds. Gram-positive bacteria isolated from bovine mastitis milk originating from 24 dairy herds were tested for their antimicrobial susceptibility to penicillin, and to cephalexin-kanamycin and penicillin-framycetin combinations, by agar disc diffusion tests. The results show the good susceptibility to non-critical antimicrobial combinations of cephalexin-kanamycin and penicillin-framycetin of Gram-positive udder pathogens in general, but there were differences between the herds.

Key words: Gram-positive, non-critical antibiotic, antimicrobial susceptibility, disc diffusion method, synergy.

Introduction

Prudent use of antimicrobials aims at changes in the handling of antibiotics by veterinary practitioners. It includes less use of antibiotics in general, and in case of use, the use of non-critical ones whenever it is possible. In case of mastitis in dairy cattle due to Gram-negative bacteria, the benefit of

antibiotics in itself is challenged. In order to use non-critical antibiotics in a proper way for the treatment of mastitis due to Gram-positive bacteria, there is a need to assess the susceptibility to such antibiotics available as drugs and according to recognized and validated methods. This study investigates the relevance of non-critical antibiotic penicillin and antibiotic combinations of cephalexin-kanamycin (Ubrolexin®, Boehringer Ingelheim) and penicillin-framycetin (Ubrostar®, Boehringer Ingelheim) to Gram-positive udder pathogens isolated from mastitis milk in problem herds. Besides the general resistance situation, differences between herds are pointed out.

Materials and Methods

Microbiological examination

The milk samples were taken from clinically or subclinically sick udder quarters before milking, after the disinfection of the teat end. The samples were processed on the same day after transportation in an electronic cool box, or it was done the next morning. In this case the samples were kept in a deep freezer on -20°C for one night. The samples were cultivated on four different agar plates: Columbia agar with aesculin; MacConkey agar; Sabouraud agar; Edwards agar (Bak-Teszt Ltd., Budapest, Hungary). Before the culturing, the surface of the media was dried in an incubator on 37°C. The culturing was done by metallic loops, which were sterilized with flame (over a Bunsen burner). With this method 10 µl of milk was examined from each sample. The incubation time was 45-48 hours on 37°C. The aspects of the identification were the following:

- colony morphology (size; form; elevation; margin; colour; surface, etc.),
- appearance and type of haemolysis (α-, β- , γ-),
- Catalase test,
- Coagulase test (Prolex Staph Kit, Pro-Lab Diagnostics, Toronto, Canada),
- aesculin splitting ability (*Str. uberis* and E. *faecalis*) – examined under an UV light,
- Lancfield grouping with a quick latex test. (Prolex™ Strep Grouping Kit; Pro-Lab Diagnostics, Toronto, Canada).

Antibiogram

The antibiotic susceptibility testing was done by disc diffusion method on Mueller Hinton agar with blood (in case of *Streptococcus spp.*) or without blood (in case of *Staphylococcus spp.*) (Bak-Teszt Ltd., Budapest, Hungary). For that 2-3 identical colonies were diluted in a broth, and this broth was spread on the surface of the MH agar. The examined antibiotics and antibiotic combinations were the following: penicillin G 10 IU, cephalexin-kanamycin 15+30 µg, penicillin-framycetin 100+10 µg. (Biolab Ltd., Budapest, Hungary and Mast Group Ltd., Merseyside, UK) The discs were placed on the agar plates with a

dedicated dispenser (Oxoid™ Antimicrobial Susceptibility Disk Dispenser). After 24h of incubation on 37°C the inhibition zone diameters were measured with a digital calliper. Disc interpretative criteria, as published by Pillar et al. (2009) and Silley et al. (2012), were used for combinations of kanamycin and cephalexin. For combinations of penicillin and framycetin, interpretative criteria published by Pillar et al. (2014) were used. Diameters for penicillin G were evaluated as published by CLSI (2015). With regard to *Sc. uberis* and penicillin, no interpretative criteria are available, thus the criteria for ß-hemolytic streptococci were used.

Results

The table below (Table 1) show the susceptibility of staphylococci and streptococci to penicillin, and to the combinations of cephalexin and kanamycin as well as penicillin and framycetin.

Table 1. The susceptibility of staphylococci and streptococci to the examined antibiotics

Susceptibility to:	penicillin			cephalexin-kanamycin			penicillin-framycetin		
	S	I	R	S	I	R	S	I	R
S. aureus (n=66)	82%		18%	86%	11%	3%	95%	2%	3%
CNS (n=90)	63%		37%	97%	0%	3%	96%	1%	3%
Sc. dysgalactiae (n=19)	84%		16%	84%	5%	11%	89%	0%	11%
Sc. uberis (n=157)	46%		54%	62%	4%	33%	62%	11%	27%

Discussion

This study focuses on the Gram-positive bacteria that cause mastitis in cattle. The use of antibiotics for the treatment of mastitis caused by Gram-negative pathogens is more and more challenged, whereas the use of NSAID (non-steroidal anti-inflammatory drugs) is emphasized not only for the most severe cases, but also within the routine treatment of mild cases (McDougal et al., 2009; McDougal et al., 2017).

The overall results show the good susceptibility to the antimicrobials tested, the susceptibility to the combinations being always better than to penicillin. This general situation regarding the bacterial

susceptibility and resistance to antibiotics does not reflect the particular case of each and every herd. In different herds multiple pathogens were isolated and tested, and it appears that the distribution of the susceptibility and resistance to antibiotics, taken at herd level, does not correspond to the general case, and thus cannot be deducted from it. So in certain herds we observe a very strong proportion of resistant strains to one or even to several antibiotics, while on the contrary in other herds few or no resistances were found. In other herds the situation is more diverse.

In the case that all the bacteria isolated within a given herd belong to the same species and are considered as contagious, it is highly probable that the same strain has been isolated several times. With regard to *Streptococcus uberis*, and its environmental reservoir, the probability of the uniqueness of the strain is rather low. From 16 herds *Sc. uberis* was isolated between 6 and 12 times. The susceptibility of each *Sc. uberis* isolate was tested towards 3 antimicrobials which are penicillin and the 2 combinations. It appears that in 8% of the cases all the strains isolated within a given herd are resistant to the same antimicrobial among the three antimicrobials tested. In 15% of the cases all the strains are susceptible to the same antimicrobial, and in 77% of the cases both susceptible and resistant strains to the same antimicrobial coexist. It is worth to note that within the same herd all strains might behave similarly towards one given antimicrobial or combination, and at the same time not all of these strains behave similarly towards another antimicrobial, hence some strains being susceptible and other strains resistant.

Whatever is the reason of this situation, it underlines the necessity to perform sensitivity testing in every herd, because it is not possible to derive the particular case from the general situation. The general situation, of course, gives a good indication for the choice of antibiotics to test, but the treatment is based on the herd or individual case.

Conclusion

The results show the good susceptibility of Gram-positive udder pathogens to non-critical antimicrobial combinations of cephalexin-kanamycin and penicillin-framycetin in general. The susceptibility and resistance situation at the single herd level differs from the general situation, thus in practice it is necessary to test the susceptibility to antimicrobials for pathogens isolated in each herd.

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Susceptibility of major udder pathogens to penicillin and to combinations of cephalexin-kanamycin and penicillin-framycetin in Poland and in Romania

Főbb tőgypatogén kórokozók érzékenysége penicillinre, ill. cephalexin-kanamicin és penicillin-framycetin kombinációjára Lengyelországban és Romániában

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Abstract

The aim of the study was to assess the relevance of non-critical antibiotic penicillin and antibiotic combinations cephalexin-kanamycin and penicillin-framycetin to udder pathogens in field isolates in Poland and Romania. Bacteria isolated from bovine mastitis milk during 2017 by two accredited (ISO 17025) diagnostic labs, one in Poland and one in Romania, were tested for their antimicrobial susceptibility to penicillin, and to cephalexin-kanamycin and penicillin-framycetin combinations, by agar disc diffusion tests. The results show the good susceptibility to non-critical antimicrobials in both Gram-positive and Gram-negative isolates sets in Poland as well as for Gram positive isolates in Romania, whereas Gram negative pathogens isolated in Romania show some more resistance.

Key words: non-critical antibiotic, antimicrobial susceptibility, disc diffusion method, synergy

Introduction

Prudent use of antimicrobials aims at less use of antibiotics in general, and in case of use, at the use of non-critical ones whenever it is possible. This study assesses the

relevance of non-critical antibiotics or their combinations to udder pathogens in field isolates from Poland and Romania. Major mastitis pathogens isolated during 2017 in two accredited (ISO 17025) diagnostic labs, one in each country, were tested by disc diffusion method for their susceptibility to penicillin in case of Gram-positive bacteria, and to combinations of cephalexin-kanamycin and penicillin-framycetin for both, Gram-positive and Gram-negative bacteria. Pharmaceutical products for the treatment of mastitis in lactation (Ubrolexin®, Boehringer Ingelheim) and for dry cow therapy (Ubrostar®, Boehringer Ingelheim) containing the two combinations of antibiotics are available in both countries.

Materials and methods

The study was conducted in the Department of Microbiology of Synevovet Laboratory Bucharest (Romania), and by Biolab SLW in Ostroda (Poland), together with Boehringer Ingelheim RCV (Austria). The samples were collected in sterile recipients and transported in maximum 24 hours to the laboratory. The following culture media were used to inoculate the pus culture: Columbia Agar with 5% sheep blood, Chocolate Agar (in a 5% CO2 enriched atmosphere), MacConkey Agar and Edwards agar medium (OXOID). Bacterial identifications were carried out mainly by using API biochemical tests (Poland) or by mass spectrometry (Romania). Antibiotic susceptibility testing was performed using classical disc diffusion method, while respecting the CLSI VET01S recommendations.

Results

The tables below (Tables 1 and 2) show the susceptibility of staphylococci and streptococci to penicillin, and the susceptibility of staphylococci, streptococci and *Escherichia coli* to the combinations of cephalexin and kanamycin as well as penicillin and framycetin. The results for the isolates of each country are summarized in separate tables. (S: susceptible, I: intermediate, R: resistant)

Isolates panel from Poland

The number of bacterial isolates per species is as follows: *Staphylococcus aureus* (n=13), CNS (n=31), *Streptococcus dysgalactiae* (n=4), *Streptococcus uberis* (n=32), *Escherichia coli* (n=12).

Isolates panel from Romania

The number of bacterial isolates per species is as follows: *Staphylococcus aureus* (n=24), CNS (n=6), *Streptococcus dysgalactiae* (n=30), *Streptococcus uberis* (n=45), *Escherichia coli* (n=167).

Table 1. The susceptibility of the isolates from Poland to the examined antibiotics

Susceptibility to:	penicillin			cephalexin-kanamycin			penicillin - framycetin		
	S	I	R	S	I	R	S	I	R
Staphylococci (n=44)	100%		0%	100%	0%	0%	100%	0%	0%
Streptococci (n=36)	100%		0%	95%	0%	5%	100%	0%	0%
E. coli (n=12)				100%	0%	0%	100%	0%	0%

Table 2. The susceptibility of the isolates from Romania to the examined antibiotics

Susceptibility to:	penicillin			cephalexin-kanamycin			penicillin - framycetin		
	S	I	R	S	I	R	S	I	R
Staphylococci (n=30)	37%		63%	100%	0%	0%	67%	0%	33%
Streptococci (n=75)	100%		0%	97%	3%	0%	97%	3%	0%
E. coli (n=167)				47%	32%	22%	83%	2%	15%

Discussion

Bacterial species were included in the scope of the study when there were criteria available for the interpretation of the inhibition diameters for the antimicrobial sensitivity testing by disc diffusion method according to the CLSI standard, for both antimicrobial combinations tested. These bacteria are the following ones: *Staphylococcus aureus*, CNS (coagulase negative staphylococci*), Streptococcus dysgalactiae, Streptococcus uberis*, and *Escherichia coli*. Except for the latter, all isolates were also tested for their susceptibility to penicillin. With regard to *Sc. uberis* and penicillin, no interpretative criteria are available, thus the criteria for ß-hemolytic streptococci were applied.

When more than one antibiotic is used at the same time, as it is the case when using combinations of cephalexin and kanamycin or of penicillin and framycetin, the efficacy of the combination cannot be deducted from the efficacy of each compound taken

separately. It is necessary to evaluate their combined efficacy. In fact, there are three different modes of interaction possible for antibiotic combinations: antagonism, indifference, and synergy. (Ganière & Denuault, 2009). It should be noted that in the case of the combination of cephalexin and kanamycin a synergistic interaction against *Sc. uberis, S. aureus* and *E. coli* has been demonstrated (Ganière & Denuault, 2009), as well as for the combination of penicillin and framycetin against *S. aureus* (Lohuis et al., 1995).

In order to evaluate the efficacy of antibiotic combinations, a recognized and standardized method is a prerequisite. Consequently, interpretative criteria for the disc diffusion method are needed according to the same standard, CLSI in the present case, in order to evaluate properly the *in vitro* susceptibility. For both combinations of antibiotics tested in this study, these criteria were established in accordance with the CLSI standard, and published (Pillar et al., 2009; Silley et al., 2012; Pillar et al., 2014).

These requirements being well established, the isolates were tested accordingly. The results point out the overall good susceptibility to both combinations tested. That is the last necessary requisite to comply with the rules of proper and prudent use of the combinations of cephalexin and kanamycin as well as of penicillin and framycetin.

With regard to penicillin the results show its good *in vitro* efficacy especially on pathogens isolated in Poland, and a lesser efficacy against the staphylococci isolated in Romania. Hence this molecule is still and always stays an option to be considered for the treatment of infections due Gram-positive bacteria.

Conclusion

The results show the good susceptibility to non-critical antimicrobial combinations cephalexin and kanamycin as well as penicillin and framycetin in both Gram positive and Gram negative isolates sets in Poland as well as for Gram positive isolates in Romania, whereas Gram negative pathogens isolated in Romania show some more resistance. Penicillin is also always to be considered, as an option for the treatment of udder infections due to Gram-positive bacteria.

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Influence of diseases on milk yield and composition in a herd of Holstein-Friesian cows

Megbetegedések hatása a tejtermelésre és a tejösszetételre egy Holstein-fríz állományban

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Abstract

Diseases in dairy cows negatively influence milk production and profitability of the herd. The occurrence of certain diseases and their influence on milk yield and composition in a herd of 230 Holstein-Friesian dairy cows was studied. Data for six years were statistically analysed by means of the statistical program SAS/STAT. The most frequent disease was

mastitis, followed by endometritis, retained placenta, hypocalcaemia, and locomotor disorders. The average daily milk yield in a herd was 24.30 kg and the average milk composition was as follows: 4.09% fat, 3.48% protein, 4.60% lactose, and 526,000 somatic cells/ml. Correlations between milk yield and composition of milk were highly statistically significant (P<0.0001). Lower milk yield was observed in cows with mastitis (21.44 kg) compared to the herd average (24.30 kg). Cows with endometritis had higher milk yield (28.89 kg), but lower fat (3.85 %) and protein (3.27%) content. Cows with retained placenta had slightly higher daily milk yield (25.87 kg) and a lower protein and lactose content than the herd average. Milk yield was a little lower than average in cows with locomotor diseases. Animals with ketosis had higher milk yield (26.35 kg) and higher fat content (4.26%). High milk yield (25.08 kg) was observed in cows with hypocalcaemia as well, with high percentage of fat (4.57%) and protein (3.42%) content. All diseases were associated with an increased number of somatic cells/ml of milk. It has been established that diseases have a statistically significant effect on milk yield and milk composition, and on the somatic cells count in milk (P<0.0001).

Key words: dairy cows, Holstein-Friesian breed, milk yield, milk composition, diseases

Introduction

The economy of milk production depends on milk yield, fertility and health of dairy cows in the herd, as well as on the age at culling. Various factors affect the occurrence of diseases in dairy cows. Health and fertility disorders are more common in cows with higher milk yields (Fürll, 2000; Constable et al., 2017). Most metabolic diseases occur in high producing dairy cows during late pregnancy and in the postpartum period when the metabolism is most heavily burdened. The main causes of metabolic diseases include disorders in the intake of nutrients, disorders in the activity of metabolic regulators, and congenital disorders in enzymatic processes. Metabolic diseases are reduced with an appropriate change in diet and breeding (Klinkon et al., 2000; Constable et al., 2017). Ketosis is a disease of high producing dairy cows. The disease usually occurs a few weeks after calving and lasts for several days or weeks. Due to a breakdown of body fat and digestive disorders, ketosis is accompanied by rapid weight loss. Postpartum paresis is an acute afebrile metabolic disease that occurs upon calving and at the onset of lactation (Constable et al., 2017). For the locomotion and well-being of cows, it is essential that they have no leg problems. Hoof diseases are the third most important loss factor, right after infertility and mastitis. There is a correlation between the occurrence of leg and hoof diseases and other diseases (Rowlands et al., 1986). Fertility disorders also cause considerable economic loss, as the best cows are often culled due to poor fertility (Orešnik, 1999).

Endometritis occurs as a result of unprofessional interventions during calving, poor hygienic conditions in the stable, insemination errors, inadequate breeding conditions, and inadequate meals. It can result in prolonged uterus involution and embryonic mortality. It has been reported that 90% of cows have endometritis in the first 10 days after calving, but it is usually eliminated by the animals themselves. A higher risk of endometritis is detected in cows with retained placenta (Bartlett et al., 1986; Opsomer, 2015; Constable et al., 2017). Mastitis causes milk producers greater losses than any other disease. It is attributed to several factors. Disease susceptibility and/or resistance can be hereditary or acquired. Environmental factors include stress as a result of breeding methods, teat and udder injuries, changes in milking process and parlour, faulty milking machine, non-hygienic milking, adverse weather conditions, sudden changes in meals, digestive disorders, irregular or inadequate hoof corrections, and poor hygiene conditions in the environment. Mastitis usually results in lower protein content and changed composition thereof (Pengov & Klinkon, 2001; Constable et al., 2017). Subclinical mastitis reduces milk production by 10 to 12%, while clinical mastitis reduces milk production by 15 to 20% and even more (Arsov, 1986). In case of ketosis, milk yield and protein content are greatly reduced, while somatic cells count in milk is below 250,000/ml (Fourichon et al., 1999). Postpartum paresis affects milk yield and composition indirectly through other diseases. Klinkon & Nemec (2002) state that hypocalcaemia reduces the quantity of milk proteins. Hoof diseases cause pain and discomfort, thus reducing feed intake and, indirectly, milk yield. Milk production decreases by 20 to 80%. Hoof diseases also affect the health of the mammary gland (Zemljič, 1998; Warnick et al., 2001; Greene et al., 2002). Uterus inflammation and retained placenta have a negative effect on fertility and hence on milk yield (Klinkon et al., 2000; Constable et al., 2017).

Experimental methods

Data on the health and milk yield of controlled cows on a farm of 230 dairy cows over a period of six years were collected and analysed. Over this period, the incidence and effect of mastitis, postpartum paresis, ketosis, retained placenta, endometritis, hoof diseases and phlegmon on daily milk yield were studied. The collected data were processed with the SAS/STAT statistical program. The following calculations were made:

1. Basic statistical parameters ($\bar{x}$, SD, KV, minimum and maximum) for: milk yield and composition at monthly controls in sick and healthy dairy cows.
2. Variance analysis; the characteristics of individual effects were assessed by means of an F-test to show the share of explained variance.

The following statistical model was used to calculate the variance analysis:

$y_{ijklmn} = \mu + M_i + L_j + B_k + Z_l + K_m + e_{ijklmn}$

y_{ijklmn} = characteristic considered

μ = mean model value

M_i = month effect i (i = 1….12)

L_j = year effect j (j = 1…..6)

B_k = disease effect (k = mastitis, postpartum paresis, ketosis, retained placenta, endometritis, hoof disease, phlegmon)

Z_l = effect of consecutive lactation l (l = 1…5)

K_m = effect of consecutive control m (m = 1….10)

e_{ijklmn} = accidental model error

Results and discussion

Table 1 presents the basic statistics on milk yield and composition for sick and healthy dairy cows. The number of sick cows was considerably lower than the number of healthy cows. On average, healthy cows had slightly less milk than sick cows. We found that the best dairy cows were susceptible to disease at the onset of lactation, but nevertheless produced a lot of milk. The milk of sick cows contained more fat and less lactose than the milk of healthy cows. As expected, the average count of somatic cells in the milk of sick cows was higher.

Table 1. Basic statistics on milk yield and composition on control day – sick and healthy dairy cows

		n	$\bar{x}$	SD	KV (%)	Minimum	Maximum
Milk yield (kg)	Sick	593	24.57	8.54	34.78	2.60	46.40
	Healthy	10.897	24.29	8.01	32.96	2.00	52.30
Fat content (%)	Sick	568	4.36	1.13	25.86	1.53	7.83
	Healthy	10.819	4.07	0.79	19.48	1.50	7.97
Protein content (%)	Sick	587	3.43	0.54	15.84	2.24	6.95
	Healthy	10.841	3.48	0.40	11.61	2.20	6.47
Lactose content (%)	Sick	585	4.49	0.29	6.57	3.22	5.16
	Healthy	10.833	4.61	0.24	5.21	3.13	5.35
Somatic cells count (x 1000/ml)	Sick	465	1,175	1,653	140,61	1	9,591
	Healthy	8,487	491	867	176,58	5	9,997

Table 2 presents the average milk yield and composition per dairy cow at monthly controls in case of disease.

Table 2. Average milk yield and composition at monthly control in case of disease

Disease	n	Milk yield (kg)	Fat content (%)	Protein content (%)	Lactose content (%)	Somatic cells count (x 1000/ml)
		$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD
Mastitis	1.156	21.44 ± 8.08	4.72 ± 1.15	3.62 ± 0.60	4.41 ± 0.31	1,885 ± 1,937
Postpartum paresis	115	25.08 ± 12.85	4.57 ± 1.32	3.42 ± 0.58	4.37 ± 0.21	1,518 ± 2,285
Ketosis	22	26.53 ± 8.23	4.26 ± 1.17	2.69 ± 0.35	4.52 ± 0.20	1,024 ± 1,637
Retained placenta	237	25.87 ± 7.95	4.31 ± 1.08	3.39 ± 0.47	4.46 ± 0.30	887 ± 1,425
Endometritis	854	28.89 ± 7.66	3.85 ± 0.91	3.27 ± 0.39	4.63 ± 0.23	566 ± 1,139
Hoof disease	76	21.78 ± 8.58	4.49 ± 1.12	3.39 ± 0.48	4.51 ± 0.29	478 ± 678
Phlegmon	53	23.12 ± 4.64	4.57 ± 0.88	3.22 ± 0.60	4.58 ± 0.18	601 ± 575

In case of mastitis, hoof disease and phlegmon, the average milk yield was lower than the herd average listed in Table 2. In case of postpartum paresis, ketosis, retained placenta and endometritis, the average milk yield was higher. As these diseases usually occur at the onset of lactation, milk yield – although reduced as a result of the disease – was still above the herd average. Fat content was lower than the herd average in endometritis but higher in cases of other diseases. The milk of dairy cows affected by postpartum paresis, ketosis, retained placenta, endometritis, hoof disease and phlegmon contained less protein than the herd average. The most noticeable decrease in protein was in the case of ketosis (2.69%), whereas in the case of mastitis protein content was higher than the average in the herd. Similar findings were reported by Klinkon et al. (2000). In all cases but one (endometritis), lactose content was lower than the herd average (4.60%). In case of mastitis, lactose content in milk decreased, as indicated also by Klinkon et al. (2000). In most diseases, somatic cells count/ml of milk was higher than the herd average.

Table 3 shows p-values for the effects included in the statistical model: month and year of treatment/sampling of milk, presence of disease (yes/no), consecutive lactation and consecutive control. The table reveals that all effects included in the model were

highly statistically significant for milk yield. Similarly, they had a statistically significant effect on fat content, with the exception of the control month. Protein content was statistically significantly affected only by the presence/absence of disease and consecutive control/stage of lactation. All effects included in the model, with the exception of year, were statistically significant for lactose content. Somatic cells count was statistically significantly affected only by disease and consecutive lactation. Table 3 also shows the coefficients of determination, i.e. the shares of the explained variance. Most variability was explained for milk yield (33.51%), while for other characteristics the share of explained variance was lower.

Table 3. P-values, coefficients of determination (R^2) and residual standard deviation (σ_e) for the characteristics of milked milk

Variability source	DF	Milk yield	Fat	Protein	Lactose	SCC
Month	1	0.0003	0.8003	0.1880	0.0078	0.5736
Year	1	<0.0001	<0.0001	0.1438	0.1816	0.3646
Disease	1	0.0004	<0.0001	<0.0001	<0.0001	<0.0001
Cons. lact.	4	<0.0001	0.0299	0.3555	<0.0001	0.0228
Cons. control	9	<0,0001	0.0006	<0.0001	<0.0001	0.5493
R^2	16	33.51	14.37	17.96	15.31	14.74
σ_e		7.06	1.06	0.50	0.28	1553

Conclusions

Based on the analysis of health and of the results of monthly controls of milk yield monitored over a period of six years, it can be concluded as follows:

- Sick dairy cows had a slightly higher milk yield and fat content and a lower protein and lactose content than healthy dairy cows. The somatic cells count in sick cows was considerably high.
- Compared to the herd average, dairy cows affected by mastitis produced less milk (21.44 kg) with higher fat (4.72%) and protein (3.62%) content and lower lactose content (4.41%). The somatic cells count/ml of milk was considerably high.
- Dairy cows affected by endometritis had a high milk yield (28.89 kg) low on fat (3.85%) and protein (3.27%).
- Dairy cows with retained placenta had a high daily milk yield (25.87 kg) with high fat content (4.31%). Protein content (3.39%)

and lactose content (4.46%) was lower than the herd average, while the somatic cells count was high (887,000/ml of milk).

- In case of hoof disease and phlegmon, milk yield was slightly lower (21.78 and 23.12 kg) than the herd average; fat content (4.49 and 4.57%) was higher, while protein content (3.39 and 3.22%) and lactose content (4.51 and 4.58%) were below the average. The somatic cells count was slightly increased.
- Dairy cows affected by ketosis had a high milk yield (26.53 kg) with high fat content (4.26%). Protein (2.69%) and lactose (4.52%) content was low. The somatic cells count was high.
- Dairy cows affected by postpartum paresis had a high milk yield (25.08 kg) with high fat (4.57%) and protein (3.42%) content. Lactose content was low (4.37%). The somatic cells count was high.
- Diseases have a statistically significant effect on milk yield, milk composition (fat, protein, lactose), and somatic cells count ($P = 0.0004$; $P < 0.0001$).

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The importance of colostrum management in newborn calves

Kolosztrum menedzsment fontossága újszülött borjakban

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Abstract

Colostrum management is the most important factor determining calf health and survival. Colostrum origin is one of the main reasons for calf deaths in our country. Proper provision of cauliflower and insufficient colostrum result in passive transfer failure. In passive transfer failure, the newborn calves cannot get enough levels of antibodies. This contributes to the excessive increase in calf death rates and other short- and long-term losses associated with animal health, welfare and productivity. A successful colostrum management program requires producers to continuously deliver the correct amount of clean, quality colostrum in the first few hours of life. In this review, the colostrogenesis process is highlighted and detailed information on the important components of the colostrum will be given. At the same time, the key components of ensuring and monitoring a successful colostrum management program will be discussed.

Key words: colostrum, newborn, calf

Effects of short term clinoptilolite supplementation on weight gain in Holstein calves

Rövid távú klinoptilolit kiegészítés hatása a Holstein borjak súlygyarapodására

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Abstract

The aim of the present study was to determine whether short term supplemantation at two levels (1 or 2 g/kg) of clinoptilolite (CLNP) in the colostrum of dairy calves had any effect on total weight gain (TWG) and mean daily gain of weight (mdwg).

A total of 24 clinically healthy calves without diarrhea were assigned in a subset of three major groups of calves were studied: group I (n=8) received colostrum with clnp at the rate of 1/kg started immediately following calving at 12 and 24 hours, for a total of 2 doses. Group II consisted of calves (n=8) receiving colostrum with clnp at the rate of 2/kg within the same interval as the first experimental group. The last group III, which served as control, received solely colostrum.

Group had significant effect on weight (kg) of Holstein calves [twg (mean±SE)- control: 12.66±0.349, group I: 14.73±0.414, group II: 14.19±0.468, $p<0.01$; mdwg (mean±SE)- control: 0.338±0.0155, group I: 0.396±0.0189, group II: 0.397±0.0196, $p<0.05$]. There was a significant difference observed for twg ($p<0.01$) and mdwg ($p<0.05$) among control group and both clnp treatment groups.

The results showed that the addition of both levels of clinoptilolite administration via colostrum appeared to enhance twg and mdwg in newborn calves without having any observable adverse effect.

Key words: calves, clinoptilolite, efficacy, health, weight

Alternative phytotherapeutic treatment approach in calves with pneumonia: *Pelargonium sidoides* (Preliminary results)

Tüdőgyulladásos borjak alternatív fitoterápiás kezelése: Pelargonium sidoides (Előzetes eredmények)

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Abstract

Pelargonium sidoides (*P. sidoides*) extract herbal remedy is used to the treatment of human respiratory infections with effectively. The objective of this study was evaluated efficiency of *P. sidoides* for clinically amelioration of pneumonia in weaning calves. For this purpose, eight calves up to 4 months age diagnosed with pneumonia following clinical examination were added to study. In clinical examination attitude, appetency, nasal and lacrimal discharge, ear drop, coughing, mucus membrane, fecal score, temperature, pulsation and respiration rate were evaluated. All calves were treated with 20 drops (3 ml) *P. sidoides* extract twice daily for 5 days. These calves were treated before with antibiotics (Enrofloxacin) by farm Veterinary Surgeon. The clinical signs included respiratory rates, heart rates, lacrimal and nasal discharge, body temperature and coughing were recorded during the treatment period. In six of the eight calves the respiratory and heart rate, body temperature and coughing were reduced significantly. The other two calves had a lameness via the antibiotics application. In all calves fecal consistency score were extended and appetency were increased. According to the preliminary clinical findings of this study was concluded that in calves with pneumonia the *P. sidoides* extract might be an alternative natural phytotherapeutic treatment approach and further studies related to the subject might be warranted.

Key words: natural treatment, *Pelargonium sidoides*, pneumonia, weaning calves.

Introduction

Given bovine respiratory disease (BRD) therapy based on nonsteroidal anti-inflammatory drugs/ or antibiotics, corticosteroids are contraindicated about their suppressive effects on immune system (Barragry, 1997; Van De Weerdt & Lekeux, 1997). Plant based medicines were used in Asia for animal health care for prophylactic and therapeutic properties. In addition, herbal medicines have no negative consequences (e.g. toxic metabolites residues in meat and milk, resistance), as synthetic drugs (Rastogi et al., 2015). In medicine, *Pelargonium sidoides* (*P. sidoides)* extract were used in different countries (e.g. Germany, Turkey, Brazil, Mexico) for an alternative treatment of acute bronchitis markeded as Umckaloabo or Umca. Latest researches have provided some description of the mechanism of action of extract, which is thought to be related to immune-modulatory (Kayser et al., 2001; Kolodziej et al., 2003), and antimicrobial (Kayser & Kolodziej, 1997) properties. *Pelargonium reniforme* and *P. sidoides* extracts has antifungal and antitubercular activities in variant minimum inhibitory concentrations (Mativandlela et al. 2006). In this study it was aimed to evaluate the efficiency of *P. sidoides* for clinically amelioration of pneumonia in weaning calves.

Experimental methods

A total of 8 calves up to 4 months of age with pneumonia were enrolled to the study. These calves were treated before with antibiotics (Enrofloxacin) by farm Veterinary Surgeon and then was referred to the faculty clinics (Adnan Menderes University, Faculty of Veterinary Medicine) when there was no clinical improvement after antibiotic therapy.

All calves were treated with 20 drops (3 ml) *P. sidoides* extract (Umca sol., Abdi İbrahim) twice daily for 5 days. Calves were evaluated by same researcher. Respiratory rates, heart rates, lacrimal and nasal discharge, body temperature and coughing were examined throughout the study period.

Results

In six of the eight calves the respiratory and heart rate, body temperature and coughing were reduced significantly (Table 1). The other two calves had a lameness in relation to previous antibiotic application by the referring veterinary. In all calves fecal consistency score were extended (data not shown) and appetency were increased.

Table 1. Changes of certain clinical parameters after treatment

	day 1	day 2	day 3	day 4	day 5	*p value*
			Mean scores			
Lacrimal discharge	3	3	2	0	0	0.001
Nasal discharge	3	2	1	0	0	0.001
T °C	39.8 °C	39.5 °C	39.4 °C	39.4 °C	39.1 °C	0.05

Discussion

The outcomes of the trial approve that bovine respiratory disease can be treated with *P. sidoides* extract. The decreases in lacrimal/nasal discharge scores and body temperature were significant to those of calves treated with the extract. Comparatively, the efficiency and side effects of antibiotic usage for patients with a clinical diagnosis of respiratory disease complex has been well known both in humans and cattle (Smucny et al., 2002; Elitok & Elitok, 2004). Furthermore, antibiotics are known to be risky with regard to occurrence of adverse events, whereas they are recommended for up to 80% of patients with respiratory problems (Smucny et al., 2002). This study might highlight alternative treatment modality other than antibiotic usage. Furthermore it might be suggested that the latter herbal extract might substitute/alternate exceeding/overwhelming antibiotic usage in calves with respiratory disease.

There have been some limitations on this trial. One is the diagnosis of respiratory disease complex made on only clinical baseline. Another limitation of this trial is calves with respiratory symptoms were treated before with antibiotics.

Nevertheless, preliminary results from this research suggest that *P. sidoides* extract is also efficient in patients with longer onset of symptoms prior to enrolment. In conclusion, this extract might be an alternative phytotherapeutic agent for bovine respiratory disease complex and further studies might be designed to compare the effects with placebo and antibiotic groups.

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The use of thermography for monitoring the heat developing during hot iron dehorning of calves

A borjak forró vassal történő szarvtalanitásakor keletkezett hő ellenőrzése termogáffal

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Abstract

Hot iron dehorning of calves is a safe, but painful procedure during which high temperatures take effect on the skull. However, those temperatures have not been measured so far. Infrared thermography makes it possible to detect superficial temperature changes. The aim of this study was to determine, whether it is possible to measure changes in the local temperature during and after hot iron dehorning.

The study was performed in 149 female Holstein calves. 135 of these calves were dehorned using a hot iron dehorner. The remaining 14 calves were sham dehorned, meaning that only the cold dehorner was pressed on their hornbuds. The thermal images were recorded with the high end camera ThermoPro TP8 (DIAS Infrared GmbH). Images were taken from both horn buds, respectively dehorning sites, as well as from both eyes before, during and after the dehorning at certain time intervals.

After applying the hot iron dehorner, the superficial temperature in the area of the former hornbuds rose significantly ($P<0.05$). The maximum temperature that was collected directly after removing the dehorner from the hornbuds, was 87 °C. Thirty minutes after dehorning, the superficial temperatures were already reduced by 50%. For the following two hours, where the thermographic measurements were conducted in half-hourly intervals, the thermographic temperatures were unchanged. Four hours after dehorning the superficial temperatures returned to the initial temperature value collected before dehorning. A similar development of the temperature curve was observed at the eyes, except for the temperature measurement directly after dehorning. On the contrary to the temperature profile seen at the dehorning sites, the superficial temperatures around the nasal corner of the eyes, rose not already 5 minutes after dehorning, but half an hour later. Interestingly the sham dehorned calves showed a similar development of the thermographic eye temperatures, which allows us to assume that there is no influence of the hot iron dehorner on the superficial temperature of the eye.

In conclusion, the infrared thermography is a suitable method for recording the local temperature development during and after the thermal dehorning. It can be hypothesized that the development of the superficial temperatures at the eyes and the dehorning sites are based on the perioperative stress response.

Influence of Alpine pasturing in the epidemiology of Toxoplasma gondii and Neospora caninum in sheep in Austria

Az alpesi legelő hatása a Toxoplasma gondii és a Neospora caninum epidemiológiájára ausztriai juhokban

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Abstract

Toxoplasma (T.) gondii and *Neospora (N.) caninum* are both protozoan parasites which can affect sheep, causing reproductive disorders. The aim of this study is the detection of the seroprevalence of *T. gondii* and *N. caninum* in sheep originated from a local district in Austria. In addition, point of interest is the influence of alpine pasturing of the seroprevalence over the summer before and after alpine pasturing. 237 sheep were tested before and after alpine pasturing. The serum-samples from the sheep were tested on specific antibodies against *T. gondii* and *N. caninum* by enzyme-linked immunosorbent assay (ELISA). The seroprevalence for *N. caninum* in all sheep before and after summer were 0%. The sheep probably never had contact with *N. caninum*. Seroprevalence for *T. gondii* was 82% before summer and 83% after the alpine pasturing period in summer. For *T. gondii* a high seroprevalence in sheep was found in this region. Over alpine pasturing period 11 sheep seroconverted to positive; 10 to lower titres, means from positive to negative/questionable status. Alpine pasturing may play a role in the spread of the disease. A protection with high antibody-titres may be time limited because of sinking titres.

Introduction

Toxoplasma (T.) gondii and *Neospora (N.) caninum* are both protozoan parasites which can affect sheep, causing reproductive disorders like embryonic death and resorption, mummification, abortion and

stillbirth (Buxton & Finlayson, 1986; Dubey & Lindsay, 1990; Buxton et al., 1998). Thus, infections with these pathogens are followed by often unnoticed high economic losses.

The aim of the present study was the detection of the seroprevalence of *T. gondii* and *N. caninum* in a local district in sheep in Austria. Moreover the influence of alpine pasturing, which is a part of farming in many regions in Austria on the spread of the disease was investigated. The study included adult sheep originated from 16 different farms pasturing their animals during summertime together on one communal alpine pasture. The communal pasture is located on Hauser Kaibling, which is a popular region for skiing and hiking in Styria, a local district from Austria.

Material and Methods

237 serum samples (16 flocks) collected before alpine pasturing and after alpine pasturing were investigated. The investigated samples originated from a further study. Serum samples were evaluated by an enzyme-linked immunosorbent assay (ELISA – from IDEXX laboratory) detecting specific antibodies against *T. gondii* and *N. caninum*. The ELISA-Test kit defined four different categories depending on the titre-height: negative, questionable, weak positive and positive. “Weak positive” and “positive” were summed up here to category “positive” for interpretation.

Results

Specific antibodies against *T. gondii* were detected in 82.3% (195/237) before and 82.7% (196/237) of sheep after alpine pasturing. Serum samples of 5 sheep antibody negative and 6 questionable before alpine pasturing showed a definite positive result after pasturing. In 10 tested serum samples being positive before pasturing, 6 negative and 4 questionable (lower) titres were revealed after pasturing. In total 10.1% (24/237) had higher titres after alpine pasturing period, 16.5% (39/237) had lower titres and the major part with 73.4% (174/237) stayed on the same antibody-titre level. Specific antibodies against *N. caninum* were found in 0.0% (0/237) of sheep before and after alpine pasturing.

Discussion

Reproductive disorders were not reported from the flocks described here. Antibodies against *N. caninum* were not found in any sheep investigated here. The sheep probably never had contact with *N. caninum*. However, to find out, if *N. caninum* is involved as an agent causing reproductive disorders and abortion in sheep in Austria, suspicious cases/material should be tested on it.

According *T. gondii* a seroprevalence of 83% in sheep was investigated. The increasing number of antibody positive animals during pasturing showed that alpine

pasturing may play a role in the spread of the disease. An infection shortly before alpine pasturing with detection of higher antibody-titres after pasturing-period cannot be ruled out. However, further studies are needed to get more information according the importance of alpine pasturing in the epidemiology of the disease. Sinking antibody-titres over the pasturing-period in summer may indicate that the time of antibody-protection after an infection is limited.

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Effect of corpus luteum and age of sheep on the embryo survival

A corpus luteum és a kor hatása a juh embriók túlélésére

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Abstract

The aim of the study was to evaluate the - influence of number of corpus luteum and age of recipients on embryo survival after the MOET method. In the study MOET method was performed on dairy sheep breeds (Improved Wallachian, Merino and

Cigája). The study looked at sheep aged between 1 and 5 years. Embryos were obtained on a 5-6 day after fertilisation. We used for obtaining the embryos surgical retrograde method. The amount of corpus luteum was evaluated in recipients during transfers. The embryos were evaluated in 4 degrees according to International Embryo Transfer Society (ITES). Pregnancy was determined by ultrasonography between 30th- 50th day after transmission.

Key words: corpus luteum, embryo survival, sheep pregnancy

Introduction

Factors specific to embryos and recipients may affect the survival of transferred embryos in cattle, sheep and goats. Among them, embryo transfer method, stage of embryo development, embryo quality, number of corpora lutea (CL), and age of the recipients have been reported to be significant (Armstrong & Evans, 1983; Thompson et al., 1995). Basically, the embryo collection in small ruminants can be performed using surgical, laparoscopic (Flores-Foxworth et al., 1992) or transcervical methods (Fonseca et al., 2013).

Experimental methods

The preparation of the donors we did according to the synchronization program. The program started with the application of intravaginal progesterone swabs for 12 days (Chronogest CR vag. Spong A.U.V.). From the 10th day of the synchronization program, for the purpose of superovulation, donors were given a foliculostimulation hormone (Folicotropin Spofa). Hormonal injection preparation was performed at a dose of 15 - 24 mg twice a day. After superovulation, sheep were admitted. There were 5 sheep for 1 ram. The synchronization program of recipients started similarly with intravaginal progesterone tampons, which were left for 12 days. By choosing the tampon continued the hormonal preparation of PMSG (Sergon inj. Sicc. A.U.V.) at 250-350 IU. The embryo obtaining was performed on the 5-6 day after admission by the semilaparoscopic method. The method of obtaining involved general anaesthesia, laparotomy in the line of the linea alba, uterine isolation, embryo flushing out, and the suture of uterus and abdominal wall. Uterus and ovary were selected from the abdominal cavity. The paediatric Foley catheter was inserted through the uterine wall near the bifurcation. We introduced a small soft catheter into the oviduct, which was clamped in this place. A small amount of medium was used to flush embryos from the uterus, and then we applied 25 ml of medium near the top of the uterine corner. The embryos were flushed through the Foley catheter into a collecting bowl and categorized into 6 stages according development and very early morula, early morula, morula, blastocyst, expanded blastocyst, and collapsed blastocyst. In

addition to the stages of development, we classified embryos according quality to 4 degrees of quality according to the IETS Agreements on the basis of compactness and uniformity of cellular structure. For optimal and standard gain, we considered the 5 to 6 embryo from the donor. After evaluation and assessment of the embryos, the best quality was transferred first and the weakest but still capable transplanted embryos at the end. We used the LABOVAL 4 – stereomicroscope (M292581, Carl Zeiss Jena, Jena, Germany) for the evaluation and classification of the embryos. The evaluation of the number of yellow bodies was performed during the transmission and all transmissions were carried out in the ipsilateral corner of the uterus with a larger number of yellow bodies. Heat in recipients was detected by vasectomized rams. Pregnancy was determined by ultrasonography between 30th and 50th day after transmission. Statistical analysis was performed with Student t- test.

Results and Discussion

Armstrong et al. (1983) reported that embryo survival in the goat is positively correlated with the number of ovulations in the recipients' ovaries. Tervit (1987) observed that, in unilaterally ovulating recipients, embryo survival was not affected by transferring one embryo to each uterine horn or by transferring both embryos to the uterine horn ipsilateral to the ovulations. Goats appear to be more tolerant than sheep to variations in donor recipient synchrony (Armstrong & Evans, 1983). In our work 520 embryos were transported from which 398 survived. The higher survival rates of embryo after transfer were confirmed in one year old sheep (77.1%), which was also confirmed statistically ($P < 0.05$). The lowest embryo survival was recorded in 5 year old sheep (68.5%). Bari et al. (2003) confirmed the higher percentage of pregnant sheep at the age of 2 to 3 year than in 1 to 4 year old sheep. Inadequate CL function may be a cause of low survival rate in ewe recipients. Studies in cattle indicated no better survival in maiden heifers compared with those calved once, when maintained under identical management (Broadbent & Stewart, 1991). Heifers are preferred as recipients, since they are less likely to be under nutritional stress, or to have a history of health problems. In general, these results coincide with those published in dairy cows (Callesen et al., 1994). The stage of development and quality of embryos has significant effects on survival rate after embryo transfer (Hasler, 1998; Lamb, 2005). Selection of the best embryos at the best stage of development for transfer improves the pregnancy rate. In the present study the survival of embryos according to ITES classification was in group with 1 corpus luteum highest at 2nd degree (74.7%).The best embryo survival was in group with 2 corpus luteum according ITES classification at 1 degree (78.7%). In group of sheep with 3 corpus luteum the survival of embryos was

highest at 3 (82.3%) degrees of ITES clarification. The worst result was demonstrated at the 4th degree of ITES in all 3 groups of sheep.

Conclusion

The embryo transfer techniques used in small ruminants worldwide are mostly based on surgical procedures. These actions are performed under general anaesthesia which needs a combination of animal fasting and drugs for secure animal handling and surgery manipulations. In conclusion, the results of the present study have identified a number of factors that may contribute to success of embryo transfer program in sheep.

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Effect of development, quality and GnRH agonist administration on embryo survival in sheep

Fejlődés, minőség, valamint a GnRH agonista adás hatása a juh embrió túlélésére

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Abstract

The aim of this study was to evaluate the stadium of development, quality of transferred embryos and the effect of the used GnRH agonist hormonal preparation on embryo survival in sheep. We found out that the day of embryo collection had no significant impact on embryo survival and that the degree of embryo survival was similar after transfer of morulas and blastocysts. Significantly lower survival rate was recorded at the stage of very early morula and at the early morula stage was survival rate median. Advanced embryos (with accelerated development - blastocysts obtained on day 5) showed significantly higher survival versus delayed embryos (morulas obtained on day 6). We compared the embryo survival rate on the basis of quality and it showed that the 1st and 2nd degree of embryo quality had significantly better survival rate than the 3rd and 4th level of quality. The treatment of sheep with Supergestran (GnRH agonist) for 6 – 7 days after embryo transfer did not increase the survival rate of the embryos.

Key words: sheep, embryo transfer, survival rate, synchronisation

Introduction

The choice of methods and procedures of assisted reproduction should be made on the basis of knowledge of estrus physiology,

folliculogenesis and ovulation. This requirement is especially true for the Easter lamb production, which dates from year to year and therefore requires co-operation with marketing. Multiple ovulation and embryo transfer (MOET) of sheep enhance genetic and breeding value of material after quality parental pairs. The specific factors affecting the survival of transferred embryos are embryonic development stages, embryonic quality, superovulation response, age and parity.

Experimental methods

The experiment included sheep dairy breeds (Improved Wallachian, Cigaja and Merino), which were divided into 2 groups: donors and recipients. The animals in the experiment had to be clinically healthy, without reproductive disorders and with the corresponding breeding standard.

At the start of this study, the female donor and recipient ovulation cycles were synchronized, to which the intravaginal progesterone sponges Chronogest CR vag. spong. a. u. v. were used, which field left in situ for 12 days. The donor group of sheep Folicotropin (FSH) at a dose of 15-24 mg was administered twice daily for 4 days after the introduction of the superovulatory swabs. The group of recipients was given PMSG in Sergon sicc. inj. a.u.v. after withdrawal of intravaginal swabs (day 12), at a dose of 250-350 IU. Subsequently, during the fertile period, the donors were admitted with ram (5 sheep for 1 ram). Intrauterine insemination was carried out with fresh semen of the respective barbs 44-46 hours after sponge extraction. The embryos were flushed on the 5th and 6th day after insemination by the semilaparoscopic method in total anaesthesia via the Foley catheter. Donor sheep were divided into two groups. From first group, embryos were obtained on day 5 and from second group on day 6. After flushing embryos were categorized into sixth stages of development (very early morula, early morula, morula, blastocyst, expanded blastocyst, collapsed blastocyst) and categorized to 4 degrees of quality (according to IETS) using a stereomicroscope.

After the evaluation, the embryos were transferred to recipients in approximately 5 pieces. The recipients were divided into two groups and regularly monitored by ram after vasectomy. The first recipients with heat symptoms were transferred embryos obtained on day 5, other embryos obtained on day 6. Pregnancy was diagnosed ultrasonographically using a 5 MHz transabdominal probe between 30th and 50th day after transmission.

Results

The number of obtained embryos (transmissible and incompetent) over the monitored period was 520. From this number 398 embryos were capable for transmission and thus the transmission was done. The aim of this work was to find out the influence of the stage of development

and quality of transferred embryos on the success of their survival after transfer to recipients and the effect of administration agonist GnRH recipient embryo. Based on our results, we found that day of embryo obtaining had no significant impact on embryo survival. The survival rate was similar after transfer of morulas (75.5%) and blastocysts (75.9%). A lower survival rate was recorded at the very early morula stage (55.4%) and the median survival rate at the early morula stage (69.8%). Advanced embryos (with accelerated development - blastocysts obtained on Day 5) showed significantly higher (91.7%, $P < 0.05$) survival rate versus delayed embryos (morulas obtained on day 6, 68.9%).

After comparing of the embryo survival rate on the basis of quality, it was shown that the 1st and 2nd degree of embryo quality showed significantly better survival rate (75.6% and 73.8%, respectively, $P < 0.05$) than the 3rd and 4th quality level (61.4% and 37.5% respectively). Supergestran (GnRH agonist) sheep treatment for 6 – 7 days after embryo transfer did not increase embryo survival rate.

Discussion

In recipients, the synchronized heat appeared within ± 4 hours after heat in donors. This synchronization protocol has largely contributed to a high rate of embryo survival. The impact of embryo development and their subsequent gain D - 5, D - 6 did not show differences in embryo survival and their survival was very similar. McKelvey et al. (1985), prefer the gain and transfer of embryos to D-6 or D-7 (blastocyst stage). Other research works performed on sheep and dairy cows (Pope et al., 1986; Breuel et al., 1991) confirm our results. Under the conditions of agricultural primary production, MOET is normally performed in D - 6 (stage of early blastocyst), which also prefers our University workplace in Košice. If embryo cohorts (D - 6) are morulas stages, delayed embryonic development or damage to the retardation, which affects the ability to survive after transmission. When embryos were gained during our experiment (D-5), we expected embryos in the morula stage. In the case of very early and early morulas gains, there was significantly worse survival than in properly evolving embryos, as confirmed by Bari et al. (2003). In the development of delayed embryos, they have better survival if there is a mild asynchrony between the heat of donors and recipients. Heat of donors in this case should avoid heat of recipients for about 12 hours. The higher survival rate of embryos in D - 5 with accelerated development - blastocysts as compared to delayed D - 6 morulas, is reported by several authors and was confirmed in other species of farm animals (Pope et al., 1986). These factors can very well reflect differences in maternal recognition timing that is critical for retardation of oxytocin receptors and prostaglandin production (Beard & Lamming, 1994). Embryo survival was

significantly higher in 1st and 2nd degree embryos, which imparts better adaptive abilities compared to 3rd and 4th grade embryos. 4th degree embryo transfer is not practiced, it was performed only for experimental purpose to assess survival. Several authors point out the significant effects of the internal environment of the organism, nutrition, energy balance of the organism on embryo survival (Donaldson, 1985; Breuel et al., 1991). Overall, we can conclude that our results are in agreement with other authors, that embryos obtained in MOET in sheep have a sufficiently high chance of survival after transfer to recipients when a high synergistic effect, standard superovulatory response, optimal gain and embryo transfer performance, as well as the physiological development of early embryos. The administration of the GnRH-Supergestran agonist was administered in order to improve the survival of the developing embryos. This methodology verified and advocated several authors in order to increase the percentage of pregnancy in other situations in sheep and cattle. The exact mechanisms of action are not fully explained. Some authors point out the fact that GnRH applied on days improves luteal functions (Bari et al., 2003; Beck et al., 1994). The further authors support the view that GnRH interferes with luteal mechanisms (Peters et al., 1992), probably by stimulating luteinization of many estrogen follicles to delay the luteinization process until the maternal organism signals that it is capable of preventing luteolysis by blocking the development of uterine oxytocin receptor (Beard & Lamming, 1994). In our work, the positive effect of GnRH on the survival of embryos after transfer to recipients was not observed and not recorded.

Conclusion

Factors influencing embryo survival after transfer to recipient sheep were studied. Based on these results, it can be confirmed that both of factors embryonic and recipient play an important role in the success of embryo transfer in sheep.

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Serum protein electrophoretic pattern and acute phase protein concentrations in sheep after induced articular cartilage defect

A szérum fehérje elektroforetikus profilja és az akut fázisú fehérje koncentrációja juhokban indukált ízületi porcelváltozást követően

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Abstract

The objective of this study was to evaluate the serum protein electrophoretic pattern and the concentrations of some acute phase proteins in sheep before and after the reconstruction of experimentally induced articular cartilage defects. Into the evaluation we included five clinically healthy female sheep of the crossbreed of merino and valachian sheep at the age of 1.5 – 2 years. The concentrations of total serum

proteins (TP), protein fractions and selected acute phase proteins - serum amyloid A (SAA), haptoglobin (Hp) and C-reactive protein (CRP) were measured before and on day 7, 14, and 30 after the creation of defect on articular cartilage and filling with a polyhydroxybutyrate/chitosan based biopolymer composite implant. The TP concentrations showed no marked differences during the evaluated period. Albumin values decreased on day 7 and 14 after surgery. In the concentrations of α_1-, α_2-, β- and γ_2-globulins a gradual significant increase was observed during the post-operative period ($P<0.05$). The γ_1-globulins decreased slightly 7 days after surgery, with subsequent gradual increase to pre-operative values. The SAA values increased 7 days after the surgical intervention ($P<0.01$) and in the concentrations of Hp a more gradual increase was found till the day 14 after surgery. The values of CRP increased significantly till the day 14 after surgery ($P<0.05$). Presented results suggest after surgical intervention marked changes in the most of serum proteins fractions and evaluated acute phase protein concentrations.

Key words: articular cartilage defect, serum amyloid A, haptoglobin, C-reactive protein, protein fractions

Introduction

The synthesis of serum proteins is strongly controlled to maintain their physiological balanced concentrations. Any pathological processes in the body may result in alterations in the serum protein concentrations (Pieper et al., 2003). At present, there is a lack of information on the possible effect of cartilage reconstruction using biopolymers on the concentrations of biochemical indicators in animals. Alterations in the biomarker profile, including some serum proteins, may potentially indicate risk for the progression of the disease process, and could be useful for early diagnosis of post-operative complications and infections, in order to early detect uncontrolled inflammatory reactions and prevent prolonged convalescence (Demura et al., 2006). Therefore, the aim of this study was to evaluate the alterations in the serum protein pattern and the concentrations of selected acute phase proteins in sheep after the reconstruction of experimentally induced articular cartilage defects using polyhydroxybutyrate/chitosan based biopolymer material, and to monitor their changes during the healing period of first 30 days after implantation.

Experimental methods

The study was carried out on five clinically healthy female sheep of the crossbreed of merino and valachian sheep. They were at the age of 1.5 – 2 years and in good nutritional condition with an average body weight of 50.7 ± 1.9 kg at arrival and were

housed at the Clinic of Ruminants of the University of Veterinary Medicine and Pharmacy in Košice. Before the inclusion into the study, the animals underwent standard pre-operative clinical examination. After surgical intervention, the health status of the animals was evaluated daily until the end of the study.

Blood samples for the determination of the concentrations of total serum proteins, selected acute phase proteins, and separation of serum protein fractions were obtained before surgery and on day 7, 14, and 30 after surgical intervention. Approval of the experimental protocol was obtained from the State Veterinary and Food Administration of the Slovak Republic No. 3508/17-221. After anesthesia, a defect in the articular cartilage of the left stifle joint was induced. The site of the created defect was then filled with a polyhydroxybutyrate/chitosan blend (PHB/CHIT) prepared acording to Medvecky et al. (2014). All sheeps received post-operative systemic broad spectrum antibiotic and non-steroidal anti-inflammatory drug.

The total protein (TP, g/l) concentrations in the blood serum were assessed on an automated biochemical analyser Alizé (Lisabio, France) using commercial diagnostic kits (Randox, UK). The serum protein fractions were separated by zone electrophoresis on an agarose gel using an automated electrophoresis system Hydrasys (Sebia Corporate, France) with commercial diagnostic kits Hydragel 7 Proteine (Sebia Corporate, France) according to the procedure described by the manufacturer. The following protein fractions were identitifed: albumin, α_1- and α_2-globulins, β-globulins, and γ_1- and γ_2-globulins (g/l).

The serum concentrations of selected inflammatory markers - serum amyloid A (SAA, μg/ml), haptoglobin (Hp, mg/ml) and C-reactive protein (CRP, μg/ml) were measured. SAA was analysed by sandwich enzyme linked immunosorbent assay (ELISA) using commercial multispecies kits (Tridelta Developmet, Ireland). Sheep CRP was measured by solid-phase ELISA assay (Life Diagnostics, Inc., USA). Haptoglobin was assessed using commercial colorimetric kits (Tridelta Development, Ireland) in microplates.

All statistical analyses were carried out using the programme GraphPad Prism V5.02 (GraphPad Software Inc., USA). Friedman test was used to examine the changes during the peri-operative period, the significance of differences between the sample collections was evaluated by Dunn`s Multiple Comparisons test.

Results

Various grade of lameness was observed in all sheep for 90 days after the surgical intervention. The surgical wound developed no signs of inflammation and was without discharge in all the evaluated sheep. The

animals have shown improvement with no serious complications and inflammatory processes in other organ systems.

In the concentrations of total proteins before surgery and in the early post-operative period no marked differences were found. The highest mean concentration of albumin was obtained prior to surgery, the values in the post-operative period were non-significantly lower. Significant alterations during the evaluated period were found in the concentrations of α_1-globulins ($P<0.05$), showing a gradual significant increase till the day 14 after surgery ($P<0.05$) and a subsequent decrease on the day 30 of the healing. Similar trends were observed in the concentrations of α_2- and β-globulins ($P<0.05$). The concentrations of γ_1-globulins decreased slightly 7 days after surgery. The absolute concentrations of γ_2-globulins a gradually significantly increased till the end of the evaluated period ($P<0.05$).

The SAA values before surgery were very low and increased significantly in response to the surgical procedure, being the highest on day 7 after surgery ($P<0.01$). In the concentrations of Hp gradual non-significant increase was found till the day 14 after surgery, with a subsequent decrease on day 30 after the surgical procedure. The mean value on day 30 after surgery was more than 20-fold higher than the pre-operative concentrations. In the concentrations of CRP, a slight gradual significant increase of values was observed being the highest on day 14 after surgery ($P<0.05$). The changes of CRP during the evaluated period were significant ($P<0.05$).

Discussion

Animal studies are fundamental to show the efficacy and safety of new cartilage defects repair before its clinical use in humans. Not only small experimental animals, but also large animal models (goats, sheep, pigs) have been succesfully used to demonstrate new implants for the treatment of osteochondral and cartilage defects (Hao et al., 2010). The profile of serum proteins after cartilage reconstruction and implantation of biomaterials was previously not described. In the presented study, major alterations were observed in the electrophoretic pattern of serum proteins and distribution of the most of serum protein fractions. While the concentrations of albumin decreased after the creation of cartilage defect and implantation of biopolymer, the concentrations of α-, as well as β-globulins increased after the surgical intervention, showing markedly higher peaks on the electrophoretogram. The concentrations of SAA and Hp in the evaluated sheep were low before surgery and increased after the induction of cartilage damage and implantation of polyhydroxybutyrate/chitosan based biopolymer. On the day 30 after implantation the values showed a tendency to decrease to those obtained prior to

surgery, suggesting uncomplicated healing process and the absence of inflammatory reactions. Differences were obtained in the rate of increase and subsequent decrease during the healing period among the measured inflammatory markers. While the concentrations of SAA in sheep increased more than 200-fold already 7 days after the surgical intervention, the values of Hp showed more gradual increase till the day 14 after surgery (approximately a 50-fold increase). These results indicate that some biomarkers from the serum protein profile may be used for the evaluation of post-operative period, progression of the disease process and uncontrolled post-operative inflammatory responses.

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Effect of non-invasive castration (banding or elastor) method on weight gain, behaviour and meat quality of ram lambs: Preliminary notice

A vértelen úton történő herélés hatásának vizsgálata a kosbárányok súlygyarapodására, viselkedésére és húsminőségére: Előzetes közlemény

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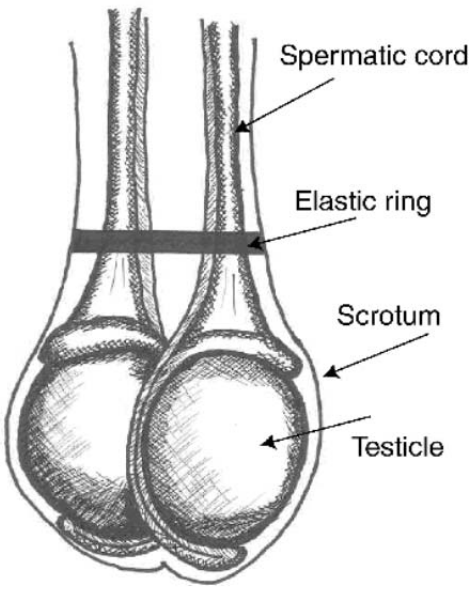
Abstract

Nowadays the most important source of income of Hungarian sheep industry is the lamb meat. The lambs can be distinguished according to their sales weight, such as milk-fed lamb (13-20 kg), weanling lamb (20-27 kg), roast-lamb (27-35 kg), and heavy weight lamb (above 35 kg). In case of producing heavy weight lambs at the end of fattening period the males and females needs to keep separately or the rams should be castrated, because they reach sexual maturity and with the sexual activity bothers the ewes. However, if farmers do not castrate animals and both males and females are allowed to run together, the inferior males could mate with females passing on undesirable pregnancy. Because of testosterone production the carcass composition and weight development debase as well.

Castration is the removal or destruction of the testes, epididymis and a portion of each spermatic cord from a ram. The surgical methods of castration (with knife) used is painful to the animals and also predispose them to infections. The banding or elastrator method involves cutting off the blood supply to the testicles with a heavy-duty rubber band or ring. An elastrator is a special applicator that stretches the rubber band and applies it to the neck of the scrotum. The rings must be strong enough to cut off blood flow in the arteries as well as the veins. The proper placements of the rings see Figure 1 (Hamito, 2008).

Figure 1. Proper placement of the rings



The scrotum and testes will fall off in two to 4 weeks, depending on the size of the testicles. This method causes some stress to the animal due to the pain and discomfort experienced by the constricting band, especially for 10 to 15 minutes after the elastrator band is applied. It is recommended to check the animals daily to be sure that the rubber band is still around the neck of the scrotum and for signs of infection. The non-surgical castration method is less stressful for the animals; in addition there is less risk of infection.

We examine the effect of binding castration method on daily weight gain, behaviour and meat quality of rams.

Preliminary we did questionnaire survey if the Hungarian farmers know and/or use this method. The results of the survey see in Table 1.

Table 1. The results of the survey, if the Hungarian sheep keepers know and/or use the non-surgical elastrator method for castration

A.	Filled the questionnaire **n= 41**		**%**
B.	from **A.**	keep lambs for slaughter now (n=24)	**58.5**
C.	from **B.**	keep the rams and ewes together (n=21)	**87.5**
D.	from **C.**	castrate the rams (n=4)	**19**
E.	from **A.**	know the elastor and/or use(d) it (n=23)	**56**
	from **E.**	have/had positive experience with the elastor-use (n=23)	**100**
	from **A.**	don't know the elastor /n=18/ but would try (n=12)	**67**

– 11 % of the elastor-user had some little problem (e.g. a minor wound arose on the skin and the fly came in).
– 75 % prefer the elastor from those who know both the surgical and the non-surgical castration method, respectively.

On the field experiment there will be three groups of ram lambs: S: surgically castrated, E: castrated with elastor and C: control (not castrated). We measure the weaning weight of the lambs, their weight in every week during five months; calculate the daily weight gain; we observe the behaviour (disquiet, sexual activity, etc.) and make organoleptic examination after slaughtering.

Összefoglalás

A vágóbárány előállításkor nagysúlyú pecsenyebárány (35 kg feletti) nevelés estén az ivarokat már külön kell tartani a hízlalás végén vagy a kosokat ki kell herélni, mert addigra már elérik az ivarérett kort, a szexuális érdeklődés elkezdődése befolyásolja a jerkékkel való viselkedésüket, súlygyarapodásukat, tesztoszterontermelésük a húsminőségüket. A gyűrűzéssel való herélés kevesebb stresszt okoz az állat életében, mindemellett kisebb fertőzésnek vannak kitéve a kosbárányok. Vizsgálatainkban kérdőíves felméréssel tájékozódtunk arról, hogy a magyarországi juhtartók ismerik vagy használják-e a heregyűrűzéses kasztrációt. Eredményeink szerint a gyűrűzést használók 100%-a pozitív tapasztalatokkal rendelkezik. Akik mind a két herélési módszert ismerik, azoknak 75%a szívesebben használja a gyűrűzést. Telepi kísérleteinkben 3 csoportot alakítunk ki, melyben a kosokat: 1. a választás napján gyűrűzzük 2: hagyományos úton heréljük 3: kontrol, a kosok gyűrűzetlenül maradnak és nem is heréljük ki őket. A csoportok beállítása után mérjük a választási súlyt, majd az állatok súlyát hetente. A kutatás körülbelül 5 hónapja alatt az állatok viselkedését figyeljük, a takarmány felvételt, a hetenkénti súlygyarapodást mérjük, majd vágás után a hús minőségét vizsgáljuk organoleptikus módszerrel.

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Serum 25-hydroxyvitamin D levels in association with Giardiasis in lambs

Szérum 25-hidroxi-D-vitamin szintje Giardiasis esetén bárányokban

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Abstract

The purpose of the present study was to analyze 25-hydroxyvitamin D3 levels in diarrheic lambs without known etiology and to determine the relation with disease activity. Enrolled research groups were as follows; I. group of diarrheic lambs (infected with giardiasis) at the age of 11 to 37 days of age and II. group involved healthy lambs age matched. In all groups of lambs, 1 mL of blood samples were withdrawn from *vena cephalica antebrachii* which were forwarded to laboratory for 25-hydroxyvitamin D3 level analysis as detected by radioimmunoassay method. 25-hydroxy-vitamin D3 levels represented low [11–32 ng of 25(OH)D3/mL of plasma in all diarrheic lambs] and high vitamin D status [67–95 ng of 25(OH) D3/mL of plasma in healthy calves]. Vitamin D deficiency strikingly related to giardiasis should promptly change therapeutic protocoles to those of diarrheic lambs.

Key words: giardiasis, lambs, vitamin D

Quality of spermatozoa after addition of nanocitrates microelements in diluted ram ejaculates

A spermiumok minősége nanocitrát-mikroelem hozzáadását követően hígított kos ejakulátumokban

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Abstracts

Microelements play an important role in regulation of spermatozoa metabolism. Cu^{2+}, Zn^{2+} and Mn^{2+} are included in active centers of enzymes glycolysis, respiratory chain and antioxidant defense and have influence on physiological parameters of spermatozoa. Thus, influence of Cu^{2+}-, Zn^{2+}- and Mn^{2+}-nanocitrate on the quality of spermatozoa in diluted ram ejaculates. Based on the studies of oxygen consumption, activity of the enzymes of the mitochondrial respiration chain and the survival of spermatozoa, it has been established that optimal doses that normalize oxidative processes and maintain the survival of sperm at a high level are 0.06 mg/l Zn^{2+}-, 0.01 mg/l Mn^{2+}- and 0.004 mg/l Cu^{2+}- nanocitrates in diluted by phosphate-saline buffer ram ejaculates. It is not advisable to use higher doses of nanocitrates because of the violation of the oxidation processes intensity and the survival of sperm.

Key words: nanocitrates microelements, spermatozoa, ejaculates, ram.

Abbreviations: SDH – succinate dehydrogenase, CO – cytochrome oxidase, ROS – reactive oxygen species

Introduction

To microelements that play an important role in securing energy needs and utilizing cytotoxic products of sperm metabolism belong Cu^{2+}, Zn^{2+} and Mn^{2+} (Pal et al., 2017). It is proved that content of these microelements correlates positively with the morphology, activity and duration of survival of spermatozoa (Massányi et al.,

2004; Eghbali et al., 2008; Tvrdá et al., 2013).

However, when diluting and technological processing and preparation of ejaculates for cryopreservation, the natural content of microelements changes, affecting not only the structural components of the spermatozoa, but also violates the use of substrates and the resynthesis of ATP. One of the ways to preserve high physiological characteristics and fertility of spermatozoa is to balance the composition of diluent of ejaculates with microelements. However, the use of microelements in form of inorganic salts is less effective in comparison with organic forms (Rowe et al., 2014).

Objectives. To research the influence of microelements (Cu^{2+}, Zn^{2+} and Mn^{2+}) in form of nanocitrates on spermatozoa quality in diluted ram ejaculates.

Experimental methods

Studies were conducted at the Institute of Animal Biology NAAS. Ram ejaculates (n=8) received an artificial vagina with the regimen of the use of the rams twice a week. For researches, ejaculates with volume – 1.89 ± 0.20 ml, concentration – 2.90 ± 0.23×10^9 cells/ml and activity – 7.5 − 8.0 points were selected. Ejaculates were diluted by phosphate-saline buffer (NaCl–0.8 g, KCl–0.02 g, Na_2HPO_4–0.11 g, KH_2PO_4–0.02 g, $MgCl_2$–0.01 g, H_2O to 100 ml) and divided into several parts: control – without addition and experimental –with addition of microelements in form of citrates in doses: Zn^{2+} - 0.06, 0.6 and 3.0; Mn^{2+} – 0.01, 0.1 and 1.0; Cu^{2+} – 0.004, 0.04 and 0.4 mg/l. Such parameters were determined: spermatozoa survival (h) at temperature 2−4°C till suspension of rectilinear motion, oxygen consumption − by polarography (ng-atom O_2/min×0.1 ml of sperm) at temperature 38.5°C, succinate dehydrogenase activity (SDH, UI/h×0.1 ml of sperm) with help of 2,3,5-tri nitrotetrazolium, cytochrome oxidase activity (CO, UI/h×0.1 ml of sperm) with "Nadi" reagent. The analysis of the digital material was carried out by the method of variation statistics. Results are quoted as arithmetic mean ± standard error of mean.

Results

Addition of Zn^{2+}- and Cu^{2+}-nanocitrates in doses 0.06 and 0.004 mg/l lowers respiration activity of sperm on, correspondingly, 40.3 %, a 0.01 mg/l, addition of Mn^{2+}-nanocitrate – on 38.4 %, comparing to control (Table 1).

Table 1. The intensity of oxidative processes and quality of sperm of rams when adding nanocitrates of microelements to ejaculate, Mean±SEM

Nanocitrate	Dose, mg/l	Oxygen consumtion, ng-atom O_2/0.1 ml×min; n=3	Enzymatic activity, UI/ 0.1 ml×h; n=6		Survival at 0-4 °C, h; n=8
			SDH	CO	
Zn^{2+}-	3.0	0.18±0.012**	20.0±1.77	33.7±2.07	84.6±4.90
	0.60	0.24±0.015**	17.5±2.17	32.5±2.17	96.0±8.00
	0.06	0.40±0.058	10.0±1.77	35.0±2.50	96.0±8.00
	η	0.418	0.354	0.395	0.093
Mn^{2+}-	1.0	0.15±0.035**	27.5±2.83**	43.3±5.61	84.0±4.24
	0.10	0.21±0.050**	28.3±3.04**	38.3±4.51	96.0±7.35
	0.01	0.48±0.029	24.1±1.83*	46.7±3.04	102.0±7.04
	η	0.432	0.379	0.078	0.124
Cu^{2+}-	0.40	0.08±0.015***	6.7±0.96**	73.3±5.61**	63.0±5.91**
	0.04	0.08±0.010***	10.0±1.18	70.0±2.36***	75.0±7.87*
	0.004	0.40±0.050*	15.0±2.36	56.7±3.04*	93.0±8.94
	η	0.524	0400	0.579	0.312
Control	-	0.67±0.090	15.8±2.48	45.0±3.91	100.2±8.20

* Note. The difference is statistically significant compared to control – *p <0.05; **p <0.01; ***p <0.001

With an increase in the content of nanocitrates of microelements, the peculiarities of their influence on the respiratory activity of the sperm are manifested. In particular, addition of Cu^{2+}-nanocitrate more than 0.04 mg/l decreased respiratory activity of sperm to minimal value (0.08 ng-atom O_2/min×0.1 ml of sperm). Zn^{2+}- and Mn^{2+}- nanocitrates in doses 0.6 and 0.1 mg/l inhibited on 64.2 and 68.7 % Oxygen consumption. When adding maximal doses of Zn^{2+}- and Mn^{2+}-nanocitrates (3.0 and 1.0 mg/l) spermatozoa respiration activity was on 73.2 and 77.7 % lower than in control. Nanocitrates in ram diluted ejaculates had an ambiguous affect the mitochondrial respiration chain enzymes activity. Addition of increasing doses of Zn^{2+}- nanocitrate statistically did not change the SDH activity – the value was within the range of the mean statistical error (10.0–20.0 UI/hour×0.1 ml of sperm). Addition of Mn^{2+}- nanocitrate stimulated SDH activity: when adding 0.01 mg/l it increased on 34.5

% ($p < 0.05$), and after addition of more than 0.1 mg/l – on 42.4–46.0 % ($p < 0.01$). Addition of 0.004 mg/l Cu^{2+}-nanocitrate did not have influence on SDH activity, and doses 0.04 and 0.4 mg/l inhibit on 36.8 and 57.6 % ($p < 0.01$) enzymatic activity. CO activity decreased on 22.8–27.8 % after addition of Zn^{2+}-nanocitrate, comparing to control (32.5–35.0 UI/hour×0.1 ml of sperm). Similarly, when increasing content of Mn^{2+}- nanocitrate in sperm the enzyme activity is almost unchanged and is within the range of control samples (38.3 – 45.0 UI/hour×0.1 ml of sperm). After addition of Cu^{2+}- nanocitrate more than 0.004 mg/l CO activity increased on 20.7%, when adding more than 0.04 mg/l higher on 35.8 – 38.7 %, comparing to control ($p<0.05$ – 0.001).

Survival of spermatozoa when using nanocitrates: Zn^{2+}- and Mn^{2+}- in doses to, correspondingly, 0.6 and 0.1 mg/l, Cu^{2+}– 0.004 mg/l was within the control levels (96 – 102 h). Content increase of Zn^{2+}- and Mn^{2+}- nanocitrates in ram semen lead to a decrease at 16 hours of spermatozoa survival. Similarly, addition of 0.04 mg/l Cu^{2+}- nanocitrate lowered on 25 hours ($p<0.05$) spermatozoa survival, and when adding 0.4 mg/l – on 37 hours ($p<0.01$), comparing to control.

Discussion

Nanocitrates of microelements in ram semen had a mixed effect on the intensity of oxidative processes and the survival of spermatozoa. Since citrate (a component of the nanoparticle) is present in all used compound with microelements, its role as a factor of influence on the metabolism of spermatozoa is offset. Therefore, changes in biochemical parameters and survival of spermatozoa are due to the content and role of microelements in the oxidative processes of spermatozoa and ejaculates. It is known, that Zn^{2+}, Mn^{2+} and Cu^{2+} are included in the active centers of glycolysis enzymes, the respiratory chain of mitochondria and enzymes of antioxidant defense system. In particular, Cu^{2+}, Zn^{2+} and Mn^{2+} are SOD cofactors – enzyme that transforms $O^{\bullet-}$ and inhibits ROS formation. Probably, the minimal doses of nanocitrates of microelements normalize the course of oxidative processes, which is accompanied by inhibition of free radical formation processes and oxidation of structural components of the spermatozoa (lipids and plasma proteins and spermatozoa membranes). Increase the content of nanocitrates Cu^{2+}, Zn^{2+} and Mn^{2+} higher than optimal limits inhibits respiratory activity, causes imbalance in the links of the respiratory chain of mitochondria and reduces the survival of spermatozoa. The obtained results are consistent with the statement on the negative influence of excess microelements on the physiological characteristics and fertility of spermatozoa (Wirth et al., 2010; Sengupta et al., 2013).

Conclusions

Optimal doses that normalize oxidative processes and maintain the survival of sperm at a high level are 0.06 mg/l Zn^{2+}-, 0.01 mg/l Mn^{2+}- and 0.004 mg/l Cu^{2+}- nanocitrates in diluted by phosphate-saline buffer ram ejaculates. It is not advisable to use higher doses of nanocitrates because of the violation of the oxidation processes intensity and the survival of sperm.

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Study of tetracycline administration effects on ruminal activity in sheep with foot rot

Tetraciklin adás hatásának vizsgálata a bendő aktivitásra büdös sántaság esetén juhokban

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Abstract

The objective of the study was undertaken to assess effects of tetracycline administration on the number of ruminal contractions. Rumen contractions were monitored in 23 sheep prior to administration of oxytetracycline and recorded every 12 hours for 84 hours after intramuscular injection of the antibiotic. Analysis of variance (ANOVA) was used to analyse the time effect of tetracycline treatment on the rumen contractions. Our results showed a significant decrease ($p<0.01$) in ruminal contractions following application of oxytetracycline, with a maximum decrease at 24 hours following oxytetracycline application and a return to the initial rumen contraction frequency by 60 – 72 hours following oxytetracycline application. This decline in ruminal motility can cause production losses, particularly in lactating ewes, causing a fall in milk production and decreased gains for suckling lambs. Despite the resulting transient production decreases, oxytetracycline remains the antibiotic drug of choice for the treatment of bacterial infections in small ruminants, foot rot especially.

Key words: tetracycline, rumen activity, sheep

Introduction

Belonging to broad-spectrum antibiotics, the group of tetracyclines is composed: tetracycline, oxytetracycline, doxycycline and minocycline. They are actively transported into prokaryotic cells and inhibit protein synthesis by competing with tRNA for the A site of the ribosome. They have a

bacteriostatic effect. Tetracyclines chelate metal ions such as calcium, magnesium, iron and aluminum, forming non-absorbable complexes. Therefore if given orally, the presence of milk, antacids or iron preparations can decrease absorption. Undesirable effects of the drug include gastrointestinal disturbances due to direct irritation and modification of the normal gut flora, and vitamin B complex deficiency can occur as a consequence. Since they chelate with Ca^{2+} ions, tetracyclines are deposited in bones and teeth, causing staining and sometimes dental hypoplasia and bone deformities (Neuvonen, 1976). Rumen and abomasal motilities are reduced in hypocalcaemia due to the general effects of a depression of levels of ionised calcium on smooth muscle contractility and on neuromuscular transmission.

Tetracycline are widely used in the treatment of the foot rot in ruminants. In a study of 209 sheep farmers, those which treated sheep with foot rot using parenteral antibiotics and foot sprays had a peak prevalence of 2 %, as opposed to a peak prevalence of 9 % in farmers who treated foot rot by hoof trimming and topical spraying (Wassink et al., 2003). In other study, treatment of foot rot with parenteral oxytetracycline reduced clinical lameness in sheep significantly (Strobel et al., 2014).

The aim of the study was undertaken to assess effects of long acting tetracycline administration on the number of ruminal contractions.

Experimental methods

Rumen contractions were monitored in 23 sheep prior to administration of oxytetracycline and recorded every 12 hours for 84 hours after intramuscular injection of the antibiotic. The sheep were admitted to the clinic for treatment of the foot rot. After the diagnosis of foot rot, patients were treated with a single intramuscular injection of a long acting oxytetracycline. The drug was administered into the neck muscles. Feeding of the experimental animals consisted of 0.2 kg of concentrates, and free access to hay and water. Rumen contractions were counted by placing a stethoscope in the para-lumbar fossa on the left side of the animal and counting the number of ruminal contractions over a 5 minute period. In addition, intensity of the rumen contractions were assessed by auscultation in the same time.

Analysis of variance (ANOVA) was used to analyse the time effect of tetracycline treatment on the rumen contractions.

Results

ANOVA revealed a significant decrease ($p<0.01$) in ruminal contractions following application of oxytetracycline, with a maximum decrease at 24 hours following

oxytetracycline application and a return to the initial rumen contraction frequency by 60 – 72 hours following oxytetracycline application (Table 1).

As well as a reduction in the frequency of ruminal contractions, there was a decrease in the intensity of the contractions. In general the contractions became quieter and sometimes difficult to distinguish effectively. Typically around 24 hours after antibiotic administration the intensity was at its lowest, coinciding with the greatest decrease in frequency. The strength of contractions gradually returned to normal over the same time period as contraction frequency.

Table 1. The rumen contractions prior to and after oxytetracycline administration

RC/time	0 hrs	12 hrs	24 hrs	36 hrs	48 hrs	60 hrs	72 hrs	84 hrs
x	7.3	5.17	4.09	5.00	5.96	7.17	7.65	7.57
s	1.55	1.30	1.16	1.21	1.19	1.34	1.37	1.34

RC: number of rumen contractions (5 min); ANOVA: $p<0.01$

Discussion

Rumen motility has a direct effect on the productivity levels of livestock, particularly on milk production. In sheep used for milking or ewes nursing lambs, a decreased rumen motility can cause a significant drop in milk quantity or decreased weight gains in lambs.

According to the pharmacodynamic study, intramuscular administration of oxytetracycline resulted in plateau-shaped concentration-time curves in serum and synovial fluid. Peak levels in serum (1.68 ± 0.47 µg/ml) occurred at 3-8 hours post injection (Bengtsson et al., 1989). As oxytetracycline chelates Ca^{2+} ions (Neuvonen, 1976), once the antibiotic reaches the bloodstream it will chelate with calcium in the serum, resulting in hypocalcaemia. Rumen and abomasal motilities are reduced in hypocalcaemia due to the general effects of a depression of levels of ionised calcium on smooth muscle contractility and on neuromuscular transmission (Ebashi, 1972).

In our study of 23 sheep, on average the rumen contractions decreased by 44 %, with the lowest number of contractions recorded 24 hours after application of long acting oxytetracycline. However our study did not take into account other factors affecting

rumen contractions, such as feeding regimen, composition of the dietary ration, underlying disease processes and environmental temperature (Attebery & Johnson, 1969).

This decline in ruminal motility can cause production losses, particularly in lactating ewes, causing a fall in milk production and decreased gains for suckling lambs. Despite the resulting transient production decreases, oxytetracycline remains the antibiotic drug of choice for the treatment of bacterial infections in small ruminants, foot rot especially.

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The influence of technological factors on caprine milk production

Technológiai tényezők hatása a kecskék tejtermelésére

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Abstract

The breeding of goats is an important sector in the process of development and modernization. By introducing support measures for farmers for the breeding of goats, there have appeared in different regions of Romania farms that apply modern and breeding technologies, but also to improve the production of milk. The purpose of the research was to identify the period during which the highest levels of milk production are obtained in relation to lactation age and month under conditions of optimization of maintenance and nutrition technology. Against this background, although the total number increased by more than 55% in 2015 compared to 2005, milk production registered only a 35% increase over the same period, due only to the increase of the milked effectives. Therefore, starting from the fact that out of over 1,400 thousand heads of goats in Romania over 57% belong to the local Carpathian breed, we considered it appropriate to carry out some research to identify effective solutions for improving the milk production in this breed. In this respect, a group consisting of females of different ages was constituted but the same experimental treatment was applied to limit the influence of different factors.

Key words: Carpathian goat, goat milk, technological factors

Introduction

For breeding goats, it is particularly important for the selection to be intensified so that this species is also systematically

evaluated on the basis of productive performance. By doing so, we can find within the different goat populations some high-yielding females that could be nominated as goat-moms and used to produce valuable breeders. Subsequently, by promoting them in the breeding group, a greater influence can be exerted on the process of improving the production of milk for this species.

The role and importance of controlling milk production results from the fact that the data obtained can also serve for:

- the nomination of farms and the formation of elite kernels;
- conducting mattings based on objective criteria;
- prompt and accurate information of farmers on the results of control to improve farm results;
- implementing an effective improvement plan.

In Romania, goat breeding is in full development and relatively similar concerns have been made on goat herds in other breeding areas (Chetroiu & Călin, 2013). In the research, the objectives included in the experimental protocol aimed at assessing some technological factors that could be used to increase the productive performance of local goats. There were included in the groups of actual goats in the area represented by the Central Plateau of Moldova that were in different lactations. The control activities were carried out according to a previously elaborated scheme, which aimed at observing all the norms and specific technical activities.

Materials and Methods

The biological material was represented by adult goats of the Carpathian breed, a breed which holds over 45% of the total goat population in Romania. In order to limit the influence of different factors, the consignments set up benefit from the same conditions of maintenance and feeding. Also, in order to diminish the influence of age on the lactogenic potential in the experimental groups, we included, in equal proportions, goats from the first to the seventh lactation.

The performance evaluation for milk yielded during the lactation submitted to the control was based on the application of successive productive checking, using for the lactation period the Nica method (this process takes into account the fact that 1 kg gain achieved by lambs in the lactating period is obtained with 4.5 kg milk until the age of 60 days), while for the period in which the females were exclusively milked the applied method has been AT4 respecting the technical specifications suggested by the International Committee for Animal Recording.

Under the applied system, the total duration of lactation was 205 days. For the period of lactation the first control was planned at 28

days from the moment of lambing and the second one during the 58th day of lactation. For the milking period after lamb weaning, controls were placed at regular intervals of 28 days.

Individual milk yields have been recorded alternated, during the morning milking and in the evening milking at the next control to, and so on.

Estimation of the average total production of milk was carried out using the Fleischmann method.

$$\text{Milk yield [kg]} = \mathrm{L}_1.\mathrm{int}_1 + \sum_{i=2an} \left(\frac{\mathrm{L}_i + \mathrm{L}_{i-1}}{2}.\mathrm{int}_i\right) + \mathrm{L}_n.14$$

where:

L1 = milk yield of the 1st monthly test;
Li = milk yield of the ith monthly test (i = 1,..., n);
Ln = milk yield of the last test;
int1 = number of days from kidding to 1st monthly test;
inti= number of days between monthly tests (i-1) and i (i = 1,...,n);
n = total number of monthly test for a specific animal.

The achieved results have been input into the data base, used to run statistical analysis with the algorithm REML (Restricted Maximum Likelihood), which provides the achievements of the statistical parametric estimators within the normal range. The REML estimator is the maximum likelihood estimate of the parameters which uses only the information not contained in the estimate of the regression vector, and thereby automatically corrects for the degrees of freedom which are lost in estimating the regression vector.

Results and Discussion

Evaluation of the production of milking milk in relation to the lactation month. The researches revealed that during the lactation period considered, the daily milk production exceeded 500 g in May, June and July and were very close to this value in the August-October period. This indicates the superior lactogenic potential of the Carpathian breeds, but the rather high values determined for the coefficient of variability indicate a strong heterogeneity of the character.

The reason for this situation is, first of all, the unsustainable selection for the milk product and secondly the high variability may be due to the consequent inappropriate application of various inadequate feeding and maintenance techniques. Analysing the technologies applied on each holding where

the research was carried out, a variety of them was found, but in most of them they applied technologies specific to the traditional systems, and the food applied during the stabling was done without taking into account those elements that could maintain an increase in individual performance.

Analysis of the milk quantity obtained during the same lactation period shows that the highest daily average production was 680.75 ± 33.714 g and was obtained at the evening milking of the control day applied in June (Table 1). Most of the results obtained are superior to other values published in the speciality literature (Taftă, 1994; Pascal et al., 1999; Pascal et al., 2006; Taftă & Machidon, 2006; Zaharia, 2011; Pascal et al., 2013). This allows us to affirm that the optimization of technological factors and the application of nutritionally balanced rations can create favourable conditions for the increase of milk production in goats with minimal additional costs.

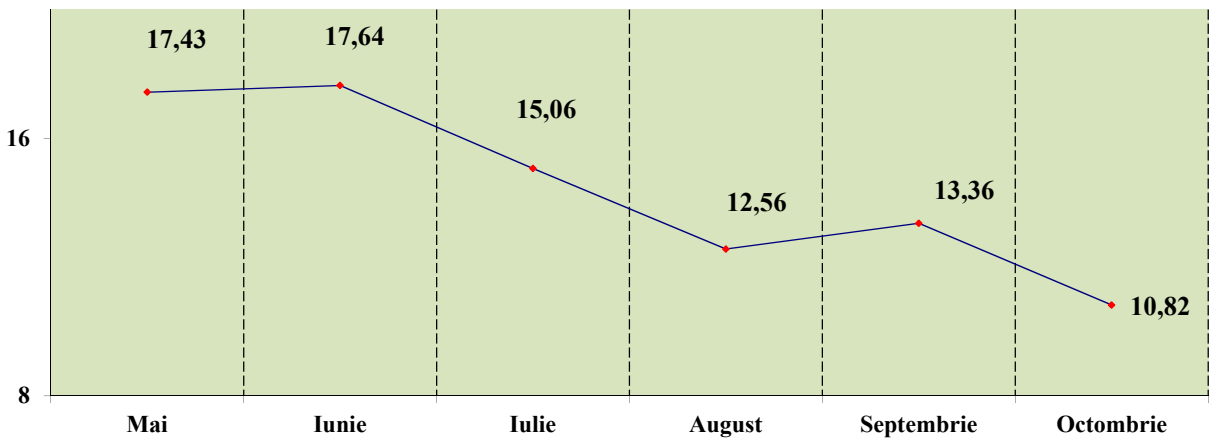
Analysing the lactation curve we can see that its tip is placed in the first two months after weaning. In August, due to extremely high heat, the average monthly individual milk quantity was with 16.60% lower than in July and with 6.37% compared to September. Referring to the monthly production of the total individual milk quantity obtained between May and October, we can see that in May and June we obtained 20.07% and respectively 20.31%.

Table 1. Monthly dynamics of milk production (g)

Month	Milking	n	$\bar{X} \pm s_{\bar{X}}$	V%	Limits	
					Min	Max
May	Evening	35	634.64± 22.879	32,15	255	1200
	Morning	35	589.45 ± 31,170	25.41	310	855
	Total day	70	612.25± 31.415	22.45	255	1200
	Total month May (l)	**70**	**17.43**	**-**	**-**	**-**
June	Evening	35	680.75 ± 33,714	33.58	300	1050
	Morning	35	598.52 ± 28.783	40.22	350	1100
	Total day	70	630.05 ± 23.541	30,54	300	1100
	Total month June (l)	**70**	**17.64**	**-**	**-**	**-**
July	Evening	35	553.64 ± 72.879	43.66	110	840
	Morning	35	518.89 ± 59.359	34.32	270	720
	Total day	70	538.00 ± 47.126	39.17	110	840
	Total month July (l)	**70**	**15.06**	**-**	**-**	**-**

Month	Milking	n	$\bar{X} \pm s_{\bar{X}}$	V%	Limits	
					Min	Max
August	Evening	35	421.36 ± 34.241	38.12	200	800
	Morning	35	475.91 ± 28.457	28.05	300	730
	Total day	70	448.64 ± 22.390	33.11	200	800
	Total month August (I)	**70**	**12.56**			
September	Evening	35	482.61 ± 28.702	28.52	280	880
	Morning	35	471.82 ± 39.873	39.64	300	960
	Total day	70	477.33 ± 24.128	33.91	280	960
	Total month Sept. (l)	**70**	**13.36**	**-**	**-**	**-**
October	Evening	35	402.16 ± 18. 270	22.45	175	630
	Morning	35	371.32 ± 27.303	35.14	185	665
	Total day	70	387.67 ± 15. 813	28.25	175	665
	Total month Oct. (I)	**70**	**10.82**	**-**	**-**	**-**

Figure 1. Lactation curve relative to the lactation month in the Carpathian breed (kg)



The evaluation of total milk production obtained from goats in different lactations, but benefiting from the same experimental treatment, was a first objective of the research carried out. For the purpose of determining the main attributes on which milk quantity depends, a representative number of goats found in different lactations were studied.

In order to assess the productive level in relation to age, the investigated effective was structured into seven age groups depending on the livestock generation to which it belongs. The study shows that the largest quantities of milk were obtained from goats that were in the third and fourth lactation and belonged to the age group of five to six years.

This is of particular practical significance because, through technical control measures carried out in the first lactations, we can obtain early enough information on the productive level characteristic of the goats, which also allows for a more efficient selection of the breeding biological material.

Compared with the average milk production obtained from the whole analysed effective, only the production obtained in the first lactation and in the last two have absolute average values below the average of the performance of the whole effective.

Table 2. Milk production specific to goats kept in stabbing in relation to age and lactation number

Lactation	n	Level of production (l)		Difference from first lactation		Age of goats (years)
		$\overline{X} \pm s_{\overline{X}}$	V%	**absolute (l)**	**relative (%)**	
I	5	157.534 ± 0.654	11.87	-	-	2.5
a -II[a]	5	183.974 ± 0.327	12.34	26.440	14.38	4
a - III[a]	5	232.098 ± 0.341	9.41	74.564	32.12	5
a - IV[a]	5	218.987 ± 0.401	11.63	61.453	28.06	6
a - V[a]	5	188.896 ± 0.243	12.13	31.362	16.60	7
a - VI[a]	5	163.988 ± 0.189	9.20	6.454	3.93	8
a - VII[a]	5	148.977 ± 0.274	9.31	- 8.573	- 5.43	9
Total	**35**	**181.438 ± 0.322**	**10.33**	**23.904**	**13.17**	**-**

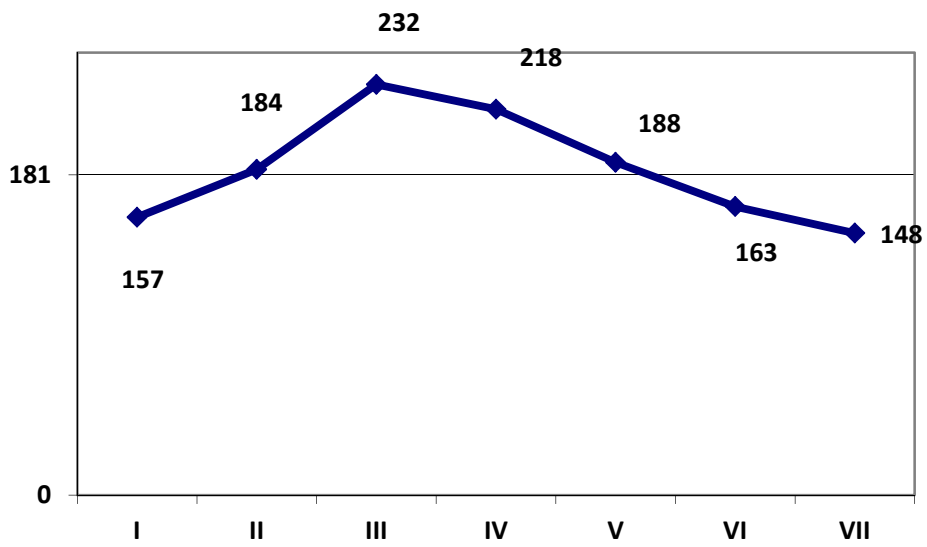
!

The carried out research has confirmed that there are kernels and individuals with superior productive potential in the local goat populations. Under these conditions, if these populations are subject to an intense selection process, it can be anticipated that in a not too long time, a radical change in the quality of the biological material will occur as well as a particular increase in productive performance.

From the analysis of the lactation curve in goats, we notice that the maximum yields are obtained from goats that are at the third lactation. This aspect is very important because in the case of goats, the maximum

milk yield is obtained when the age is of 3-4 years and we can intensify the selection by removing those with modest production from the circuit and using as buck mothers of reproduction goats from those with superior productions.

Figure 2. Milk production curve in relation to lactation number and average of population (l)



Success can be complete if all these activities are combined with others which aim at improving the conditions of maintenance, nutrition in accordance with the nutritional requirements specific to each physiological state, the directed breeding of the young goat, the improvement of the pastures, the extension of the maintenance in the permanent setting, the optimal dimensioning of the age structure, etc.

Conclusions

1. The assessment of productive performance in relation to the number of lactations shows that the highest level was obtained in the third lactation, the level reached being with 32% superior to the amount of milk obtained from the females which were at the first lactation.
2. The processing of the data obtained from the application of the productive control also highlights the fact that from the females at the seventh lactation a lower milk production is obtained by more than 8% compared to those at the beginning of the milk exploitation.
3. Analysis of the quantity of milk obtained from goats in the milking situation exclusively shows that the highest average daily yield was 680.75 ± 33.714 g and was obtained at the evening meal of the control day applied in June.
4. Since August due to the extremely high heat, the individual monthly average

milk quantity was with 16.60% lower than in July and with 6.37% compared to September.

5. Referring to the monthly production of the total individual milk quantity obtained between May and October we can see that in May and June we obtained 20.07% and respectively 20.31%.
6. The milk quality analysis shows that an average of 86.65% water and 13.35% SU was recorded on the total period of lactation.
7. In the first month under control it was found that the average milk protein level was 2.62%, rising to 3.23% in August and reaching the maximum value of 3.75% in October.
8. The analysis of economic efficiency based on incomes achieved through the capitalization of the base production indicates a reduction of more than 25% in August and 38% in October.

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The investigations with Pearson correlations of relationships among some testicular and body measurements of Alpine male goats

Az alpesi hímivarú kecskék egyes testicularis és testméretei közötti kapcsolat vizsgálata Pearson-korrelációval

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Abstract

This study was conducted to investigate the relationships among some testicular and body measurements of 21 head Alpine male goats at the same age raised between 2016 July to 2017 January in a private farm in Aydin, Turkey. In this study body length (BL), chest girth (CG), wither height (WH) and testicular width (TW) were measured by a tape. Body weight (BW) was determined by weighing scale. Testicular diameter (TD) from testicular measurements was measured by a caliper. Testicular length (TL) was detected by a tape. Testis weight (TWT) was calculated from testis length and testicular width. The relations among traits were investigated by Pearson correlation. The positive and significant correlations were among BL, CG and BW (respectively, 0.512 and 0.790) (P<0.01). Besides, the correlations between TWT and TL (0.660), and TWT and TW (0.944), were determined statistically significant (P<0.01). These correlations showed that BW could be estimated relatively rightly from BL and CG than other body measurements in Alpine male goats. Similarly, TW could be estimated from TL and TWH. In conclusion, BL, CG, TL and TWH could be used as selection criterions.

Key words: Alpine male goat, body traits, testicular traits, correlations

Introduction

The Turkey's goat population reached 11 million 10 thousand 590 head as an obtained statistical data in 2017 (Anonymous, 2017).

The share of goat is constituted to 18.4 % in total animal existence of Turkey and goat population is present 101 thousand 085 head at Aydin province (TUIK, 2017). The goat breeding is carried out base on mostly natural pasture and 80-90% feed requirements of animals are supplied with natural grazing lands as pasture and highland in most of the regions in Turkey (Ceyhan et al., 2017). In Turkey, the indigenous goat breeds are usually raised for milk, meat and mohair yield.

Alpine goats are typically short haired; their hairs have all color combinations (endemic brown, dull grey). They have medium head and ears, erect ears. The most characteristic feature is that no matter what the body color, there is a dark line from wither to rump in back (Oklohoma State University, 1998). Alpine goat is originally effective for milk with an efficiency of 550-600 l, possessing goat efficiency as 1.5. Alpine's adaptability is high at severe climatic and farm conditions. They are used as especially breeding material in hybridization studies with hair goat in Turkey (Keskin et al., 2012).

In different goat breeds as detected by relationships among body weight and some testicular measurements were investigated several authors (Németh et al., 2005; Turk et al., 2005; Kabiraj et al., 2011; Waheed et al., 2011; Akpa et al., 2013a; Akpa et al., 2013b; Ferreira et al., 2013; Jahan et al., 2013; Okere et al., 2014; Maksimović et al., 2015; Elmaz et al., 2016; Gemeda & Workalemahu, 2017). The objective of this study was to determine of relationships among some testicular and body measurements of Alpine male goats.

Materials and Methods

This investigation was carried out on a livestock farm of the Aydin, Turkey. A total of twenty one male Alpine goats (1.5 years old) were enrolled in the study. The animals were released grazing at 08.30 and 16:00 at pasturage. The potable water of animal material was supplied ad libitum. In this study, body weight (BW), body length (BL), chest girth (CG), wither height (WH) were considered as some body measurements of male goats in study. The testicular traits were evaluated as testicular width (TW), left testicular diameter (TDL), right testicular diameter (TDR), testicular length (TL) and testis weight (TWT). BL, CG, WH and TW were measured by a tape. BW was determined by weighing scale. TD from testicular measurements was measured by a caliper. Testicular length (TL) was detected by a tape. Testis weight (TW) was calculated from testis length and testicular width.

This formulae was determined by Bailey et al. (1996), it was given below;

$TWT = 0.5533 \times TL \times TW^2$, where; TWT = Testicular weight, TL = Testicular length, TW = Testicular width. The correlations among body weight, other body

measurement and some testicular traits were calculated by using Pearson's correlations analyses (SPSS, 2009).

Results

Least square means and standard errors of some body and testicular traits of Alpine goats were given in Table 1. The overall mean of BW, BL, CG and WH were found 28.72 ± 0.777 kg, 74.09 ± 1.314 cm, 80.82 ± 1.002 cm and 64.74 ± 0.779 cm respectively. The overall mean of TW, RTD, LTD, TL and TWT were determined 7.49 ± 0.203 kg, 3.79 ± 0.074 cm, 3.65 ± 0.121 cm, 7.99 ± 0.169 cm and 254.63 ± 16.506 kg, respectively.

Table 1. Least squares means and standard errors body and testicular traits of Alpine goats

Traits	N	$\overline{X} \pm S_{\overline{X}}$	Min	Max
Body weight (BW) (kg)	21	28.72 ± 0.777	22.50	36.10
Body length (BL) (cm)	21	74.09 ± 1.314	63.00	83.00
Chest girth (CG) (cm)	21	80.82 ± 1.002	75.00	89.00
Wither height (WH) (cm)	21	64.74 ± 0.779	60.00	71.50
Testicular width (TW)(cm)	21	7.49 ± 0.203	5.90	9.00
Right testicular diameter (RTD) (cm)	21	3.79 ± 0.074	3.30	4.51
Left testicular diameter (LTD) (cm)	21	3.65 ± 0.121	3.00	5.01
Testis length (TL) (cm)	21	7.99 ± 0.169	6.49	9.50
Testis weight (TWT) (kg)	21	254.63 ± 16.506	150.08	403.36

The relationships among body and testicular measurements were summarized in Table 2. The highest correlations were found between CG-BW and TWT-TWH (respectively, 0.790 and 0.994) and were significant (P<0.01). The positive and significant correlations were among BL-BW (P<0.05) and TWT-TL (P<0.01) (respectively, 0.512 and 0.660). The moderate, low and negative correlations were found among some measurements (such as WH-RTD and TL-BL) and were insignificant (P>0.05).

Table 2. The correlations among measurements

	BW	BL	CG	WH	RTD	LTD	TW	TL	TWT
BW	1								
BL	0.512*	1							
CG	0.790**	0.358	1						
WH	0.108	0.137	-0.017	1					
RTD	0.289	0.039	-0.193	0.267	1				
LTD	0.025	0.282	0.022	-0.017	0.198	1			
TW	0.283	0.057	0.228	0.115	0.118	0.108	1		
TL	0.346	-0.041	0.315	0.082	0.279	0.183	0.390	1	
TWT	0.364	0.015	0.317	0.082	0.181	0.141	0.994**	0.660**	1

*: P<0.05; **: P<0.01; BW: Body Weight; BL: Body Length; CG: C hest Girth; WH: Wither Height; RTD: Right testicular diameter; LTD: Left testicular diameter; TW: Testicular width ;TL: Testis length; TWT:Testis weight

Discussions

The main objective of this study was to detect the relationships among some testicular and body measurements of Alpine male goats. Physical analyses have been frequently enrolled for characterization of different animals regarding several previous researchers. Hence, external body measurements were preferred to predict body weight, as well as to predict carcass characteristics (Khan et al., 2006; Pesmen & Yardimci, 2008; Abd-Alla 2014). Body size and shape analysis might be of beneficial for selecting growth comprising the breeder to select early maturing and late maturing animals of different sizes (Akpa et al., 2013a). Even if genetic interpretation aroused limited usage, describing some identificative linear traits could be useful and farmers' friendly instruments for selecting goats with desirable characters (Haldar et al., 2014).

Least squares mean of BW in the present study was found as 28.72 kg. This value was lower than mean values of Alpine goats reported by Németh et al. (2005), Maksimović et al.(2015) and Beetal goats reported by Waheed et al. (2011) and Hair goats reported by (Elmaz et al. (2016), similar to findings of Black Bengal goats

detected by Kabiraj et al. (2011) and Boer goats found by Okere et al. (2014) and higher than Afar, Long-eared Somali and Woyto-Guji goats denoted by Gemeda & Workalemahu (2017).

Overall mean of BL (74.09 cm) was found similar to those reported for Alpine goats (Németh et al. 2005; Maksimović et al. 2015) and Beetal maole goats (Waheed et al., 2011). This value was higher than findings reported for Boer goats (Okere et al., 2014) and lower than results detected for Alpine goats (Ferreira et al. 2013) and Hair goats (Elmaz et al. 2016).

The mean of CG (80.82 cm) was found similar to findings of Alpine goats reported by Maksimović et al. (2015). This value was higher than findings reported for Boer goats (Okere et al., 2014), lower than findings reported for Alpine goats (Ferreira et al., 2013), Beetal male goats reported by Waheed et al. (2011) and Hair goats reported by Elmaz et al. (2016).

The present results on the overall mean of WH (67.74 cm) was found similar to the results of Németh et al. (2005) and Maksimović et al. (2015). This value was found higher than those detected by Akpa et al. (2013); Okere et al. (2014), lower than findings of Waheed et al. (2011), Ferreira et al. (2013) and Elmaz et al. (2016).

Least square mean of the TW was found as 7.49 cm. This result was lower than findings of Akpa et al. (2013a) and higher than findings of Kabiraj et al. (2011).

The mean of right testicular diameter was found as 3.79 cm. This value was found lower than results of Turk et al. (2005), Elmaz et al. (2016) and Gemeda & Workalemahu (2017). The mean of left testicular diameter was found as 3.65 cm. This finding was found lower than results of Turk et al. (2005), Elmaz et al. (2016) and Gemeda & Workalemahu (2017).

The overall mean of TL (7.99 cm) was lower than results of Turk et al. (2005), Akpa et al. (2013a), Elmaz et al. (2016), higher than findings of Kabiraj et al. (2011).

Finally, the mean of TWT (254.63 kg) detected in the present study was lower than the findings of some researchers (Turk et al. 2005, Akpa et al. 2013a).

In the present study, high correlations between CG-BW and TWT-TWH (respectively, 0.790 and 0.994) were found statistically significant ($P<0.01$). Similarly, positive and significant correlations were found among BL-BW ($P<0.05$) and TWT-TL ($P<0.01$) (respectively, 0.512 and 0.660). According to the report of Gemeda and Workalemahu (2017) for Afar and Long-eared Somali breeds, correlations among BW-TL (0.96), BW-TD (0.96), TW-TL (0.90) and TW-TD (0.88) were significant ($P<0.01$). Besides, the correlations among BW-TW (0.93) and BW-TL (0.78) for Black Bengal goats were

found statistically significant (P<0.01) (Kabiraj et al. 2011). Accordingly, the findings of this study were similar to results that were obtained for Red Sokoto goats (Akpa et al. 2013b) and male Boer goats (Okere et al., 2014).

Conclusions

The results of this study are conforming with the indications of some research, whereas showed divergent to some previous literatures. The monitored differences in body weight, body and testicular measurements could have related to environmental conditions, failure management and feeding arrangement in a farm. According to findings of the present study for welfare of animals at this farm in Aydin, management and environmental conditions must be improved. These relationships showed that BW could be estimated relatively directly from BL and CG than other body measurements in Alpine male goats. Similarly, TWT could be estimated from TL and TW. In conclusion, BL, CG, TL and TW could be used as selection criterion.

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Acute ruminal acidosis in goats: A case study

Akut bendő-acidózis kecskékben: Esettanulmány

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Abstract

Study investigates the case of four goats contracting the acute ruminal acidosis and subsequent way of treatment. Significant changes in the acid-base parameters were detected following the treatment using bicarbonate, which suggests fast improvement towards the physiological state. With no animal dying despite the high mortality of this condition signifies that quick and proper treatment is crucial.

Key words: acute ruminal acidosis, goats, acid-base balance, treatment

Introduction

Acute ruminal acidosis is becoming increasingly common among domestic small ruminants. However, little attention has been given to the evaluation of the true incidence and impact of acidosis in these species. This condition can be responsible for considerable morbidity (10-50 %) and mortality up to 90 % in untreated cases (Ullah et al. 2013; Snyder et al. 2017).

The aim of this study is to present a clinical case of four goats which, following the excessive consumption of dry beet sugar, contracted severe acute ruminal acidosis.

Results and Discussion

All four goats were slightly depressed and had profuse diarrhoea at the time of presentation. There were no other clinical findings. Based on the information from the owner, acute ruminal acidosis was suspected and samples of ruminal fluid and blood were taken.

Notable low values of pH of the ruminal fluid were caused by high concentration of lactate (Table 1).

Table 1. Results from ruminal fluid analysis taken by ororuminal probe before treatment

Patient ID	**1**	**2**	**3**	**4**	***Overall***
pH	4.44	4.24	4.74	4.31	*4.43*
Total acidity	100.63	111.24	82.1	95.4	*97.34*
Protozoa [10^3/ml]	16	16	32	64	*32*
Lactate [mmol/L]	127.1	119.6	106.6	111.8	*116.3*
VFA [mmol/L]	19.1	22.3	39.6	38.7	*29.9*

Based on venous blood examination (Table 2), there was severe acidemia (pH) in all four cases prior to treatment with visible negative Standard Base Excess (SBE) which together with low bicarbonate concentration ($cHCO_3$-) stands for severe base deficit within the blood.

Immediate therapy was conducted with evacuation of the rumen content via ororumial tube and ruminal application of sodium bicarbonate (1g/kg BW), brewer's yeast and camomile and linseed extract. The therapy was complemented by intravenous infusion of 8.4% bicarbonate solution in the dose based on calculation: mEq = Base Excess x Body Weight [kg] x 0.3 and intravenous application of *Flunixinum megluminicum* (2.2 mg/kg BW), *acidum methylphenoxypropionicum* (10 mg/kg BW) and glucose (0.5-1 g/kg BW). The same procedure was repeated the next day with addition of plasma-lyte infusion (1000 ml pro toto). For goat ID 2 the therapy was prolonged by one more day.

Table 2. Results from acid-base balance of venous blood (v. juguaris)

Patient ID	**1**	**2**	**3**	**4**	***Overall***
		pH			
Before treatment	7.083	7.125	7.066	7.120	*7.099*
1st Day	7.367	7.350	7.377	7.332	*7.357*
2nd Day	7.442	7.379	7.446	7.451	*7.430*

Patient ID	**1**	**2**	**3**	**4**	***Overall***
			pO_2 [kPa]		
Before treatment	8.84	10.50	11.40	10.90	*10.41*
1st Day	7.41	6.35	7.23	6.98	*6.99*
2nd Day	7.94	6.83	5.61	5.54	*6.48*
			pCO_2 [kPa]		
Before treatment	4.44	3.73	4.01	4.01	*4.05*
1st Day	7.78	4.83	4.46	4.54	*5.40*
2nd Day	5.05	4.67	6.00	5.34	*5.27*
			SBE [mmol/L]		
Before treatment	-18.5	-18.7	-19.9	-18.1	*-18.8*
1st Day	-4.3	-5.1	-5.0	-7.2	*-5.4*
2nd Day	1.7	-4.1	6.4	3.7	*1.9*
			$cHCO_3$- [mmol/L]		
Before treatment	10.0	10.2	9.4	10.5	*10.0*
1st Day	20.6	19.7	20.2	18.2	*19.7*
2nd Day	25.7	20.7	29.1	26.9	*25.6*

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The effect of maternal age on the quality of colostrum and changes of hematological and biochemical parameters in neonatal goats after colostrum intake

Az anyai életkor hatása a kolosztrum minőségére és a hematológiai és biokémiai paraméterek változásaira újszülött kecskékben kolosztrum felvétele után

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Abstract

The research was focused on the quality of colostrum in goats and the influence of maternal age on changes in the haematologic profile in kids after colostrum intake.

Total samples were taken from 14 goats and 20 kids of Goat white shorthair breed. Goats were divided into two groups. The first group included 1.5 – 2 years old goats and the second group included 3 – 10 years old goats. The colostrum samples were taken immediately after birth. IgG and specific gravity were determined in colostrum samples. Blood samples from the kids were collected immediately after birth and 2 hours after first intake of colostrum. Basic haematological profile and biochemical profile such as total protein, albumin, urea, creatinine, gamma glutamyltransferase, glutathione peroxidase, aspartate aminotransferase, Vitamins A and E, thyroxine, calcium, magnesenium, phosphorus and selenium were determined in blood. The results obtained were tested for the homogenity of variances (Hartley-Cochran-Bartlett test) and the normality of distribution (Shapiro-Wilk test). The data were analyzed statistically by one-way analysis of variance (ANOVA) followed by the Fisher LSD post-hoc test. All results were expressed as average and ± standard deviation.

The influence of the mother's age on the specific gravity of colostrum was confirmed (Table 1). The effect of maternal age on IgG concentration has not been showed. From biochemical parameters in kid, the influence of maternal age was confirmed on total protein (TP), creatinine (CREA), gamma glutamyltransferase (GMT), aspartate aminotransferase (AST) and vitamin A concentration in serum. From haematological parameters the effect of maternal age was demonstrated only in leukocytes (WBC) count in kids (Table 2). All newborn kids benefit and were without any health problems after colostrum intake.

Table 1. Influence of the mother´s age on the specific gravity of colostrum and IgG

	IgG in colostrum (mg/ml)	specific gravity of colostrum (Brix %)
Goats 1.5 – 2 years old	40.48 ± 13.75	24.47 ± 4.00*
Goats 3-10 years old	34.04 ± 11.61	19.85 ± 3.91

Table 2. Influence of mother´s age on the biochemical and haematological parameteres after intake colostrum

	TP g/l	CREA umol/l	GMT ukat/l	AST µmol/l	Vit A µmol/l	WBC $*10^9$/l
Kids from goats 1.5 – 2 years	58.7±9.91**	96.7±27.62**	11.03±6.36**	1.52±0.43***	0.38±0.04***	11.13±1.08**
Kids from goats 3-10 years	43.9±9.00	164.1±28.42	3.24±2.90	0.62±0.20	0.19±0.10	6.15±2.46

Total protein (TP), creatinine (CREA), gamma glutamyltransferase (GMT), aspartate aminotransferase (AST), leukocytes (WBC)
Results were expressed as average and ± standard deviation
* P < 0.05. ** P < 0.01. *** P < 0.001 – significant differences

Cytological evaluation of erythroblastic cell line in bone marrow smears of young fallow deer (*Dama dama)*

Erythroblastic sejtvonal citológiai értékelése a fiatal dámvad csontvelő kenetjeiben (Dama dama)

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Abstract

The aim of this study was to evaluate erythroblastic cell line in bone marrow smears of young *Dama dama*.

Ten, 3 months old fallow deer, were chosen to participate in this study. Bone marrow samples were obtained using 90mm, 13G biopsy needle. The animals were premedicated using xylazine prior to sampling. The biopsy site was prepared in accordance with standard surgical procedures. Bone marrow was collected into test tubes without anticoagulant. Smears were performed immediately after collection on labeled slides. Bone marrow smears were stained using MGG method. Staining time was 80 seconds for the May-Grünwald solution and 4-5 minutes for the Giemsa solution. Bone marrow cells were counted in the SH-96/24D cell counter.

Polychromatic erythroblasts were the largest cell group in the erythroblastic line (17.1%). The smallest group were proerythroblasts (5.3%). Cytological evaluation of erythroblastic cell line of bone marrow of young *Dama dama* indicates a higher erythroblastic activity of the bone marrow in comparison to hematopoietic activity in adult fallow deer.

Table 1. Determination of erythroblastic activity of the bone marrow

Number of animal	1	2	3	4	5	6	7	8	9	10	Mean	SD
PROERBL %	5.2	4.3	6.1	4.3	4.0	6.0	7.9	5.3	4.8	5.1	5.3	1.15
BASO ERBL %	9.2	5.7	5.8	9.3	6.5	9.1	9.4	8.1	8.3	10.1	8.15	1.60
POLY ERBL %	13.9	15.1	15.8	16.8	15.2	20.6	23	16.8	15.4	18.5	17.11	2.82
ORTO ERBL%	16	8.7	10.6	9.8	8.7	9.0	7.8	8.2	8.2	6.1	9.31	2.64

PROERBL – proerythroblast
BASO ERBL – basophilic erythroblast
POLY ERBL – polychromatic erythroblast
ORTO ERBL – orthochromatic erythroblast

Effects of some biotic and abiotic factors on gastrointestinal parasite infections of sheep

Egyes biotikus és abiotikus tényezők hatása a juhok belső parazita fertőzésére

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Abstract

Gastrointestinal parasite can cause serious infection in sheep all around the world and reduce the production and increase the cost of production. There are different dominant parasites under different climate and technology circumtance that can appear and cause serious health problem in sheep. Due to the climate change and the changing technology (grasing managemant, indoor-outdoor breeding) the major effective parasites and immunity against the parasite of sheep can be changed. In two Hungarian Merino sheep groups on seperated pastures (two groups of a treated and a nontreated with anthemintic) there were examed the parasite infection, condition change and production in different production period. Tere was examed the correlation between the infection intesity and the temperature, the changing of intesity and periodicity of the precipitation that also can affect the worm infection significanty.

Összefoglalás

A világ juhtenyésztésében jelentős gazdasági és állategészségügyi kárt okoz a juhok belső parazitái okozta megbetegedések. Eltérő időjárási és technológiai viszonyok hatására a súlyos fertőzéseket okozó paraziták is eltérnek a juh populációkban. A klímaváltozás és a változó tartástechnológia (legeltetés, zárt vagy szabad tartás) módosíthatja a paraziták virulenciáját és a juhok immunválaszát a különböző parazitás fertőzésekkel szemben. Két magyar merinó juh csoportnak (egy belső élősködők ellen kezelt és egy kezeletlen csoport) vizsgáltuk a parazita fertőzöttségét, a kondíció változását és a termelési eredmé-

nyeit eltérő termelési periódusban. Továbbá megvizsgáltuk a hőmérséklet és a csapadékeloszlás változásának kapcsolatát a juhok fertőzöttségével, amelyek jelentősen befolyásolhatják a paraziták jelenlétét és virulenciáját.

Az Intézményközi Kiskérődző Biotechnológiai Kutatócsoport (IKBK) megalakulása és eddigi eredményei

Foundation and results of the Interinstitutional Small Ruminant Biotechnology Research Group

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Abstract

Interinstitutional Small Ruminant Biotechnology Research Group has been set up in 2015, when the Embryo Transfer Center has been established in the University of Debrecen. The research group performed several Multiple Ovulation and Embryo Transfer program in cooperation with the Breeding Association of the BMC (Blanche du Massif Central, France) in the same year. Since then, the scientists fulfilled laparoscopic artificial insemination, embryo flushing, embryo freezing projects: among

others the biotechnological program of importing white suffolk sheep breed (one of Australia's most important and producting breeds') into the European Union.

Összefoglalás

Az IKBK működését 2015-ben kezdte meg, amikor is elkezdődött a Debreceni Egyetemen a stabil embrióátültető állomás megtervezése és engedélyeztetése. Ugyanezen év őszén a francia BMC (Blanche du Massif Central) juhfajta tenyésztőszervezetével hajtott végre közös Multiple Ovulation and Embryo Transfer programsorozatot a kutatócsoport. Az azóta eltelt időszakban számos, kiskérődző laparoszkópos termékenyítéssel, embriókinyeréssel, embriófagyasztással kapcsolatos kutatást és programot jegyeznek: többek között Ausztrália legkiválóbb termelési tulajdonságokkal rendelkező fajtájának, a fehér suffolknak az Európai Unióban történő honosítását, az ezzel kapcsolatos biotechnológiai program végrehajtását.

EFOP-3.6.2-16-2017-00001 Komplex vidékgazdasági és fenntarthatósági fejlesztések kutatása, szolgáltatási hálózatának kidolgozása a Kárpát-medencében

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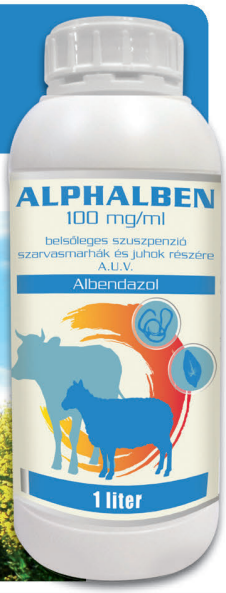

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Javallatok:

Szarvasmarhák és juhok albendazolra érzékeny gasztrointesztinális orsóférgek, tüdőférgek, galandférgek és kifejlett mételyek által okozott fertőzéseinek kezelésére.

Gasztrointesztinális orsóférgek: Haemonchus spp., Ostertagia spp., Trichostrongylus spp., Bunostomum spp., Cooperia spp., Nematodirus spp., Chabertia spp., Oesophagostomum spp., Toxocara spp.

Tüdőférgek: Dictyocaulus spp.

Galandférgek: Moniezia spp.

Kifejlett mételyek: Fasciola hepatica, Dicrocoelium dendriticum

Célállat fajok: Szarvasmarha és juh

Hatóanyag:
Egy ml tartalmaz:
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Adagolás és alkalmazási mód:

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Szarvasmarha: Gasztrointesztinális orsóférgek, tüdőférgek és galandférgek által okozott fertőzések kezelésére: 7,5 mg albendazol / ttkg (7,5 ml készítmény / 100 ttkg).

Fasciola hepatica és Dicrocoelium dendriticum által okozott fertőzések vagy 2. típusú ostertagiosis kezelésére: 10 mg albendazol / ttkg (10 ml készítmény / 100 ttkg).

Juh: Gasztrointesztinális orsóférgek, tüdőférgek és galandférgek által okozott fertőzések kezelésére:

5 mg albendazol / ttkg (0,5 ml készítmény / 10 ttkg).

Fasciola hepatica és Dicrocoelium dendriticum által okozott fertőzések kezelésére: 7,5 mg albendazol / ttkg (0,75 ml készítmény / 10 ttkg).

Élelmezés-egészségügyi várakozási idő(k)

Szarvasmarha: Hús és egyéb ehető szövetek: 14 nap. Tej: 5 nap

Juh: Hús és egyéb ehető szövetek: 14 nap. A készítmény alkalmazása emberi fogyasztásra szánt tejet termelő juhoknál nem engedélyezett.

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Célállat faj: szarvasmarha.

Javallatok: Oxiklozanidra érzékeny kifejlett stádiumú Fasciola hepatica által okozott krónikus májmételykór (fascioliasis) kezelésére. Fertőző galandféregízek (Moniezia spp.) eliminálására. Alkalmazható tejelő szarvasmarhákban.

Adagolás: Szájon át alkalmazandó. Felhasználás előtt alaposan fel kell rázni. A pontos adagolás érdekében az állatok testtömegét a lehető legpontosabban kell meghatározni és az adagoló eszköz pontosságát ellenőrizni kell. Ha az állatokat inkább csoportosan kezelik, és nem egyenként, akkor a csoportokat a testtömegeknek megfelelően kell kialakítani és az adagolást úgy végezni, hogy az aluldozírozás vagy a túladagolás elkerülhetővé váljon. A testtömeg szerinti adagolás 10 mg oxiklozanid testtömeg-kilogrammonként. Szarvasmarha: 3 ml készítmény 10 ttkg-onként.

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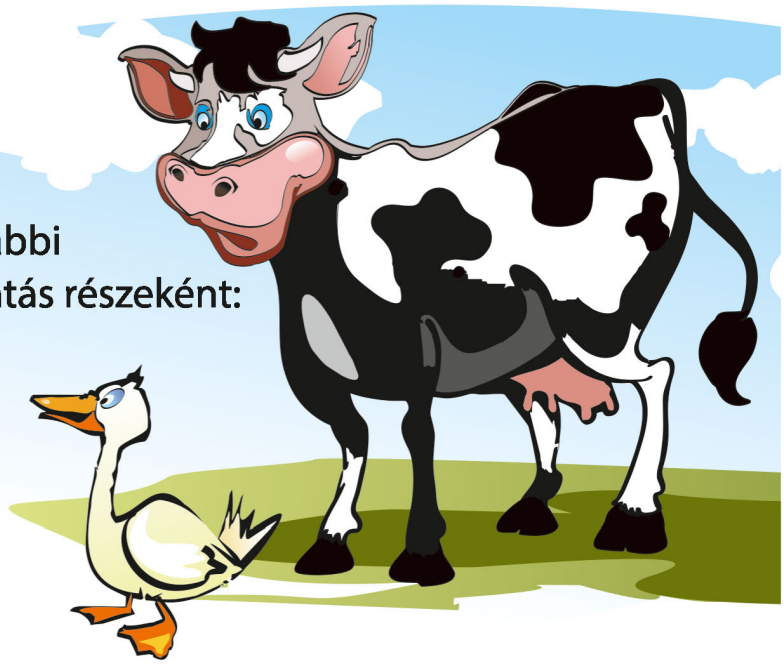

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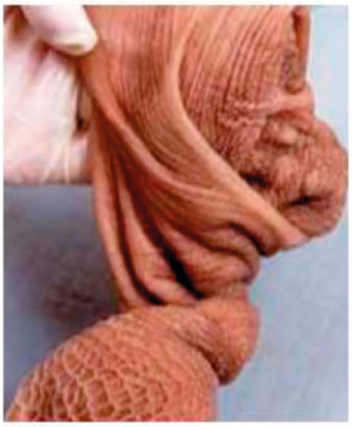
A telepi felmérés két részből áll: Borjúnevelési Program és Növendéküsző

I. Borjúnevelési Program

A borjak vitalitásának növelését komplexen kell kezelni. Jelen esetben nem csak az egyedi ketreces takarmányozásukat vizsgáljuk meg, hanem már születésük előtt az előkészítő és elletői környezetet is górcső alá vesszük. Erre azért van szükség, mert a kolosztrum minőségét (IgG) az itt etetett TMR mikroelem és táplálóanyag összetétele fogja befolyásolni. Kutatások bizonyítják, hogy a borjak antibiotikumos kezelésének és elhullásának a legfőbb oka az úgynevezett FPT (failure of passive transfer), azaz a gyenge passzív immunitás. Az ilyen jellegű problémák kiszűréséhez különböző műszerek és eszközök állnak a rendelkezésünkre, melyek segítségével fel tudjuk kutatni a telepen található **immunitási problémák** okát és ezáltal **technológiai és takarmányozási javaslatot** tudunk adni azok megoldására. Ezen felül javaslatot tudunk tenni a **teljes tartástechnológiai és takarmányozási protokoll** kialakítására. A piacon található borjútápok döntő hányada nagy bekeverési arányú és gyenge emészthetőségű ipari melléktermékeket tartalmaz. Ennek megoldására került kifejlesztésre a **VitaCalf Junior**, amely a **kiváló emészthetőség** mellett olyan kiegészítő anyagokat is tartalmaz melyek hatására növelni tudjuk a **borjak immunitását**. Célunk a borjak bendőpapillázottságát olyannyira megnövelni, hogy a választás után lényegesen hatékonyabban tudják a takarmányokat emészteni. Erre azért van szükség, mert általában ebben a korosztályban jelentkeznek először a fejlődési problémák (szétnövések), amik már jelentősebb gazdasági károkat tudnak okozni a telepeken. A papillák fejlődését az emésztőrendszerbe bekerült illózsírsavak növelésével tudjuk elérni, amit a naponta megetetett abrakhányad befolyásol.

Diet: Milk only
6 weeks



Diet: Milk and hay
6 weeks

Diet: Milk and grain
6 weeks

A célzott etetési kísérletek bizonyítják, hogy az új fejlesztésű VitaCalf Junior borjúindító táp etetésével nagymértékben tudjuk növelni a borjak fajlagos súlygyarapodása mellett azok immunitását is.

II. Növendéküsző Program

A Vitafort Zrt. növendéküsző program a választástól egészen a problémamentes ellésig tart. Célunk a növendékek **optimális takarmányozása korosztálynak megfelelően**. A tejelő tehenészetek takarmányozásában talán ez a szegmens a legelhanyagoltabb. A tehenészetek versenyképességének növelésében viszont meghatározó szerepet játszik. Jelenleg a gazdaságokban átlagosan az első sikeres termékenyítés 17 hónapos korra tehető és az első ellés 26 hónapos korban következik be. **Ez a szám azt bizonyítja, hogy két-három hónappal van lemaradva a magyar átlag a legjobban teljesítő telepekkel szemben és ezáltal a tartási és takarmányozási költségek lényegesen, akár több mint 10%-al magasabbak.**

A **Vitafort VitaCalf Program négyfázisú, korcsoportos takarmányozási rendszerét** azért fejlesztettük ki, mert így optimalizálni tudjuk a növendékek TMR adagjait mind mikroelem mind pedig táplálóanyag tartalom tekintetében. A **VitaCalf Grow koncentrátum** segítségével képesek vagyunk az eddig problémát okozó **kis létszámú csoportok precíz takarmányozására**, ami segítségével ki tudjuk alakítani az általunk javasolt korosztályos takarmányozási rendszert a telepeken. A program részét képezi egy **monitoring rendszer** is, ami segítségével folyamatosan figyelemmel kísérjük a tenyészüszők ideális növekedését, ezáltal bármilyen probléma felmerülése esetén pontosan meg tudjuk határozni, hogy hol és milyen módon kell korrigálnunk

Összegzésül elmondhatjuk, hogy a Vitafort szarvasmarha takarmányozási szakemberei által kidolgozott, s a piacon egyedülálló VitaCalf Program (korcsoportos borjú és növendéküsző takarmányozási és monitoring program) segítségével felügyelni tudjuk a telepen lévő állatok egészségi és fejlődési állapotát, ezáltal optimalizáljuk a korcsoportok takarmányozását és kiegyensúlyozott növekedését. Célunk a telepen lévő növendékek felkészítése a jövőbeni hasznos élettartamra és a majdani tejtermelés növelésére.

Amennyiben a **VitaCalf Program** felkeltette érdeklődését és új alapokon szeretné állománya utánpótlását felkészíteni a tejtermelésre, keresse a Vitafort Zrt. szarvasmarha szakspecialistáit valamint területi értékesítési szaktanácsadót.

Paulicsek János
értékesítési szaktanácsadó
Vitafort Zrt.

VALÓDI MEGOLDÁS

Draxxin

NAXCEL
DEVELOPED FOR CATTLE, DESIGNED FOR YOU

Synulox™ RTU

DRAXXIN INJEKCIÓ:

légzőszervi komplex megbetegedések esetén az elsőként választandó antibiotikum

- Széleskörű indikációval rendelkezik
- Könnyen adagolható: 1ml / 40 ttkg., magas tüdőkoncentráció
- Rövid várakozási idő (hús: 22 nap)
  - Kísérletes vizsgálatokban a tulathromycin az antibakteriális hatás mellett immunmoduláns és gyulladáscsökkentő hatást is mutatott

NAXCEL CATTLE INJEKCIÓ:

egydózisos, 7 napos hatástartamú ceftiofur terápia

- Megbízhatóan legyőzi a méhgyulladást és a panaritiumot
  - Méhgyulladás és panaritium betegségek bakteriológiai profiljának megfelelő antibiotikum
    - Könnyebb munkaszervezés és kevesebb stressz
      - 0 nap várakozási idő tejre

SYNULOX RTU INJEKCIÓ:

széles hatásspektrum Gram-pozitív és Gram-negatív baktériumok ellen

- Légzőszervi, lágyszöveti, ízületi és köldök fertőzések, tőgygyulladás, méhgyulladás
- Rövid várakozási idő tejre (48 óra)
- Kombinálható Synulox LC-vel
- Napi egyszeri alkalmazás intramuszkulárisan vagy szubkután

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Lezárás dátuma: 2018. május 17.

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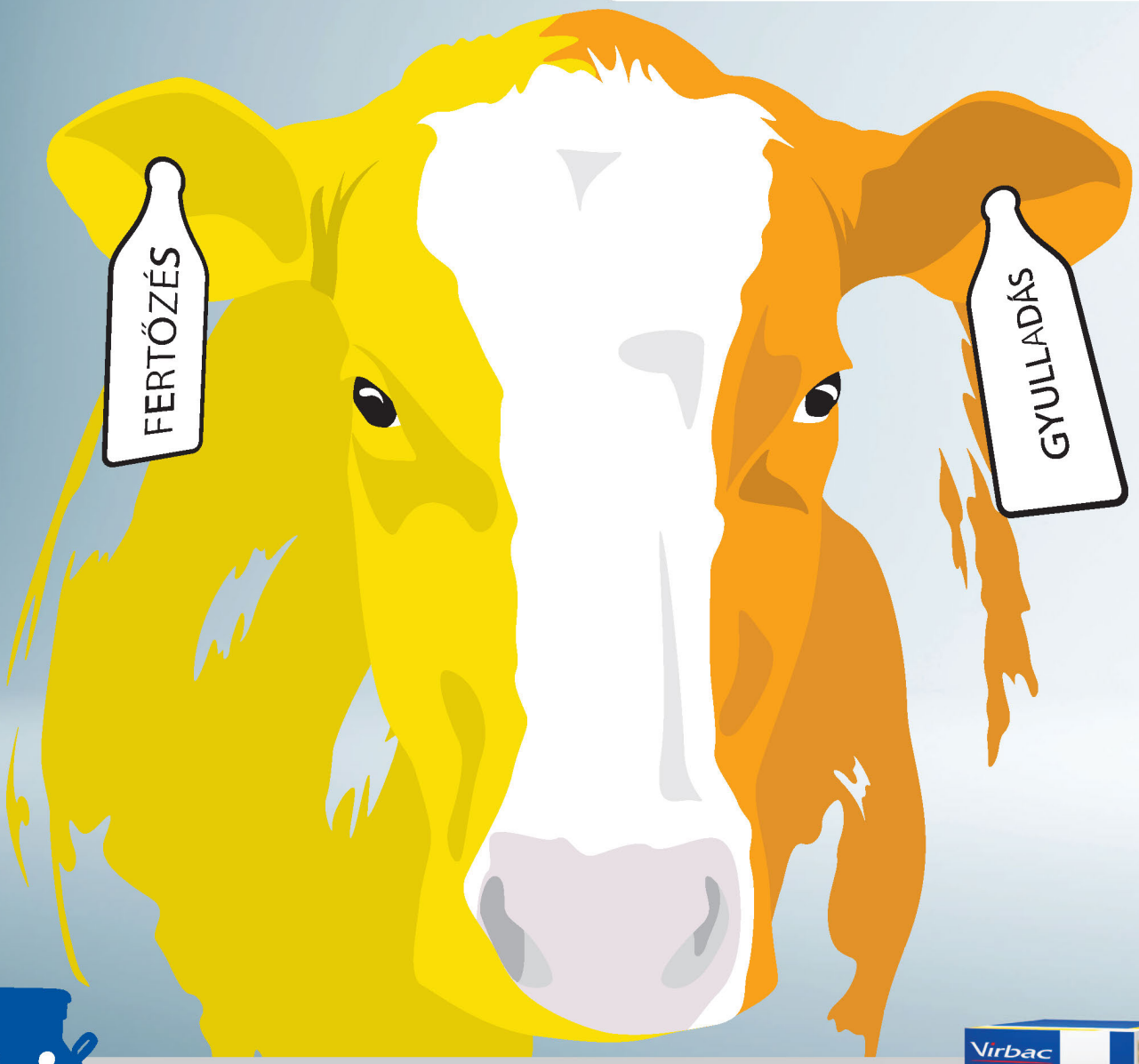
Curacef® DUO

CEFTIOFUR 50 mg/ml + KETOPROFEN 150 mg/ml



TEJ ÉEVI NULLA ÓRA

Ahol van fertőzés, ott van gyulladás is.

MINDKETTŐT KEZELI & FENNTARTJA A TERMELÉST

Egyedülálló antibiotikum és nem szteroid gyulladáscsökkentő kombináció tejelő tehenek és tehenészetek számára.

- Az állatok számára: gyorsabb gyógyulás, kevesebb fájdalom, magasabb szintű állati jólét.
- Az állatorvos számára: magasabb biológiai hasznosulás a fenntarthatóbb antibiotikum gyógykezeléshez.
- Az állattartók számára: gyors gyógykezelés és a termelés mielőbbi visszaállítása.

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Ketózis az állományban. Mely állatok vannak veszélyben?

Három egyszerű kiválasztási kritérium:

Kövér[1,2]	Beteg[3]	Öreg[4]
Túlkondicionált tehenek (kondíciópontszám ≥ 3,5 a szárazonállási időszakban)	Azok a tehenek, melyeknél az előző laktáció folyamán energiahiánnyal összefüggő betegség (pl. méhgyulladás, magzatburok-visszamaradás, ketózis) fordult elő	A harmadik laktáción túli tehenek

Egy hatásos állatgyógyászati megoldás:

– *A Kexxtone csökkenti a ketózis előfordulási gyakoriságát azokban az ellés körüli tejelő tehenekben/üszőkben, melyeknél várható a ketózis kialakulása*

– *A Kexxtone egy könnyen beadható, egyetlen dózisban szájon át beadandó intraruminális készítmény*

A Kexxtone segít egészségesen tartani a ketózis veszélyének kitett teheneket

1. AZ ÁLLATGYÓGYÁSZATI KÉSZÍTMÉNY NEVE: Kexxtone 32,4 g folyamatos hatóanyag-leadású intraruminális készítmény szarvasmarhák számára. Monenzin. **2. MINŐSÉGI ÉS MENNYISÉGI ÖSSZETÉTEL: Hatóanyag:** Monenzin 32,4 g (35,2 g monenzin-nátrium megfelelője). **Segédanyagok:** A segédanyagok teljes felsorolását lásd: 6.1 szakasz. **Intraruminális készítmény:** Minden intraruminális készítmény tartalma: 12 alegység, amelyek mindegyike (2,9 g monenzin-nátriumnak megfelelő) 2,7 g monenzint tartalmaz. Polipropilénből készült*, adagolónyílással ellátott kupak. Polipropilén* dugattyú. Polipropilén* tartály és oldalszárny. Acélrugó. *A polipropilénből készült komponensek narancssárgával (E110) vannak színezve.**3. GYÓGYSZERFORMA:** Folyamatos hatóanyag-leadású intraruminális készítmény. Szárnyakkal ellátott, henger alakú, narancssárga színű, egyedi azonosítóval ellátott polipropilén intraruminális készítmény, mely 12 alegységből tevődik össze. **4. KLINIKAI JELLEMZŐK: 4.1 Célállat fajok:** Szarvasmarha (tejelő tehenek és üszők). **4.2 Terápiás javallatok célállat fajonként:** A tejelő teheneknél/üszőknél fellépő ketózis előfordulásának csökkentésére olyan állatok esetében, amelyeknél az ellés (után) körüli időben várhatóan fellépne a ketózis. **4.3 Ellenjavallatok:** Nem alkalmazható 300 kg-nál kisebb testtömegű állatok esetében. **4.4 Különleges figyelmeztetések minden célállat fajra vonatkozóan:** Egy adott állat kezelésének szükségességéről állatorvosnak kell döntenie. A kórelőzményben szereplő energiahiánnyal járó betegségek, magas testkondíció pontszám és az ellések száma kockázati tényezőként szerepelnek. Korai regurgitáció esetén azonosítani kell az állatot az állat azonosítószámának az intraruminális készítményen található számmal való megfeleltetésével, és újra be kell adni egy sértetlen intraruminális készítményt. **4.5 Az alkalmazással kapcsolatos különleges óvintézkedések:** A kezelt állatokra vonatkozó különleges óvintézkedések: A kezelt szarvasmarhát a készítmény alkalmazása után 1 óráig elkerített helyen kell tartani, hogy az esetleges félrenyelés vagy regurgitáció felismerésre kerüljön. Ha ez bekövetkezne, az intraruminális készítményt újra be kell adni, ha az nem sérült. Ha sérült, új intraruminális készítményt kell beadni. Az alkalmazás után akár 4 napig is megfigyelés alatt kell tartani a szarvasmarhát, hogy az intraruminális készítmény nyelőcsőben való elakadásának jelei felismerésre kerüljenek. Az elakadás jele lehet a felfúvódás, amelyet köhögés, nyálfolyás, étvágytalanság és a növekedés, illetve súlygyarapodás elmaradása követhet. Az állatok kezelését végző személyre vonatkozó különleges óvintézkedések: A hatóanyaggal való érintkezés allergiás reakciót válthat ki az arra érzékeny személyeknél. A monenzin vagy bármely segédanyag iránti ismert túlérzékenység esetén kerülni kell az állatgyógyászati készítménnyel való érintkezést. Az állatgyógyászati készítmény alkalmazása során tilos az evés, az ivás és a dohányzás. Az intraruminális készítménnyel való érintkezés közben, illetve a regurgitált intraruminális készítmény visszanyerésekor védőkesztyűt kell viselni. Az intraruminális készítménnyel való érintkezést követően le kell venni a védőkesztyűt, és meg kell mosni a kezet, valamint az érintkezésnek kitett bőrterületeket. Egyéb óvintézkedések: A monenzin lenyelése vagy szájba jutása kutyáknál, lovaknál, egyéb lóféléknél vagy gyöngytyúkoknál halálos lehet. Nem szabad engedni, hogy kutyák, lovak, más lófélék vagy gyöngytyúkok érintkezzenek monenzin-tartalmú készítményekkel. A készítmény regurgitálásának kockázata miatt ezen fajokat nem szabad arra a területre engedni, ahol a kezelt szarvasmarhát tartják. **4.6 Mellékhatások (gyakorisága és súlyossága):** Ritkán emésztőszervi tüneteket (úgymint hasmenés, kérődző gyomorrendellenesség) figyeltek meg. Igen ritkán nyelőcső elzáródás volt megfigyelhető. A mellékhatások gyakoriságát az alábbi útmutatás szerint kell meghatározni: nagyon gyakori (10 állatból több mint 1-nél jelentkezik egy kezelés során), gyakori (100 állatból több mint 1-nél, de kevesebb mint 10-nél jelentkezik), nem gyakori (1000 állatból több mint 1-nél, de kevesebb mint 10-nél jelentkezik), ritka (10 000 állatból több mint 1-nél, de kevesebb mint 10-nél jelentkezik), nagyon ritka (10 000 állatból kevesebb mint 1-nél jelentkezik, beleértve az izolált eseteket is). **4.7 Vemhesség, laktáció vagy tojásrakás idején történő alkalmazás:** A vemhesség és laktáció ideje alatt alkalmazható. **4.8 Gyógyszerkölcsönhatások és egyéb interakciók:** Nem ismert. **4.9 Adagolás és alkalmazási mód:** Intraruminális alkalmazás. A megfelelő beadóeszközt használva, 3–4 héttel a tejelő tehén/üsző ellésének várható időpontja előtt egy intraruminális készítményt kell alkalmazni. A Kexxtone átlagosan naponta 335 mg-os monenzin adagot biztosít, körülbelül 95 napon keresztül. Az utasításokat gondosan kell követni. Az intraruminális készítmény beadásához szükség van az állat megfelelő megfékezésére. Korlátozni kell az állat előre/hátra való mozgását, illetve biztosítani kell, hogy az állat fejét előrenyújtott helyzetben lehessen tartani, nyaktájéki nyomás nélkül, a fulladozás elkerülése érdekében. 1. Minden intraruminális készítményen egy egyedi szám található. Ezt a számot az állat azonosítószámával együtt fel kell jegyezni, hogy egy esetleges regurgitáció esetén az állat azonosítható legyen. 2. Hajtsuk vissza az intraruminális készítményen található szárnyakat, és helyezzük be a készítményt az erre a célra kifejlesztett eszközbe, a nyílással ellátott végével előre. 3. Tartsuk az állat nyakát és fejét előre nyújtott pozícióban. Ragadjuk meg az állatot egy kézzel a szájszegletnél fogva. Vezessük be az adagolóeszközt az állat szájába, az elülső fogakat kikerülve. A garat és nyelőcső traumás sérülésének elkerülése érdekében, ne fejtsünk ki túlzottan nagy erőt. 4. Helyezzük be az adagolóeszközt a nyelvtő mögé, az őrlőfogakat kikerülve. Miközben az állat nyel, az adagoló eszköz könnyedén túlcsúszik a nyelvtőn. NE FEJTSÜNK KI TÚL NAGY ERŐT. Ha ellenállásba ütközünk, kissé húzzuk vissza az eszközt, és ismételjük meg az eljárást. 5. Győződjünk meg róla, hogy az adagolóeszköz fejrésze túlhaladt-e a nyelvtőn. Miközben az állat éppen nyel, lövelljük ki az intraruminális készítményt az adagolóeszközből. **4.10 Túladagolás (tünetek, sürgősségi intézkedések, antidotumok), ha szükséges:** Több mint egy intraruminális készítmény véletlen alkalmazása a monenzin túladagolásra jellemző mellékhatások kialakulásához vezethet, úgymint étvágycsökkenés, hasmenés és levertség. Ezen mellékhatások általában átmeneti jellegűek. A legmagasabb tolerált monenzin adag naponta 1 mg és 2 mg között van testtömeg-kilogrammonként. 4.11 **Élelmezés-egészségügyi várakozási idők:** Hús és egyéb ehető szövetek: nulla nap. Tej: nulla nap. **5. FARMAKOLÓGIAI TULAJDONSÁGOK:** Farmakoterápiás csoport: Acetonémia kezelésére szolgáló gyógyszerek. Állatgyógyászati ATC kód: QA16QA06. A monenzin a poliéter-ionoforok farmakoterápiás csoportjának, ezen belül a karboxil-alcsoportnak a tagja. Ezek a *Streptomyces cinnamonensis* faj természetes fermentációs termékei. **5.1 Farmakodinámiás tulajdonságok:** A monenzin a baktérium sejthártyájához kapcsolódva megakadályozza egyes fontos iongradiensek fenntartását a sejten belül, melyek a tápanyagok szállításához és a protonmozgató erő létrehozásához feltétlenül szükségesek. A monenzin legfőképpen a Gram-pozitív baktériumokkal szemben hatékony. A Gram-negatív baktériumok összetett szerkezetű külső membránjuknak köszönhetően eredendően ellenállóak az ionoforok hatásával szemben. Eszerint a monenzin végső hatása a bendő belsejében nem más, mint a mikrobapopuláció arányainak megváltoztatása, mely az acetát- és butiráttermelő baktériumok számának csökkenését, illetve a propionáttermelő (a glükoneogenézis prekurzora) baktériumok számának növekedését jelenti. A bendő belsejében élő baktériumpopulációban bekövetkező változások az energiametabolizmus hatékonyságának növekedését eredményezik. Az ellés körüli időszakban lévő tejelő tehén esetében a monenzin pozitív hatásaként megemlíthető a vér csökkent ketontartalma, a megnövekedett szérum glükózszint, illetve a ketózis alacsonyabb előfordulási gyakorisága. **5.2 Farmakokinetikai sajátosságok:** Az intraruminálisan alkalmazott monenzin a gyomor-bél traktuson belül fejti ki hatását. A monenzin intraruminális alkalmazását jelentős first pass metabolizáció követi, ebből kifolyólag a szisztémás keringésben a monenzin koncentrációja alacsony marad. A metabolitok, illetve az anyavegyület az epével választódnak ki. Az intraruminális készítményben lévő tabletta-alegységek bendőtartalommal való érintkezésekor a készítmény nyílásánál gél képződik, amely lassan szabadul fel az intraruminális készítményből. A monenzin körülbelül 335 mg/nap átlagos dózisban szabadul fel az intraruminális készítményből. **6. GYÓGYSZERÉSZETI JELLEMZŐK: 6.1 Segédanyagok felsorolása:** Szacharóz zsírsav-észter, Karbomer, Laktóz-monohidrát, Magnézium-sztearát, Vízmentes kolloid szilícium-dioxid, Polipropilénből készült*, adagolónyílással ellátott kupak. Polipropilén* dugattyú. Polipropilén* tartály és oldalszárny. Acélrugó. *A polipropilénből készült komponensek narancssárgával (E110) vannak színezve. **6.2 Inkompatibilitások:** Nem ismert. **6.3 Felhasználhatósági időtartam:** A kereskedelmi csomagolású állatgyógyászati készítmény felhasználható: 3 évig. A közvetlen csomagolás első felbontása után felhasználható: 6 hónapig. **6.4 Különleges tárolási előírások:** A fóliatasak jól lezárva tartandó. **6.5 A közvetlen csomagolás jellege és elemei:** Alumínium fóliatasak, amely 1, 3 vagy 5 intraruminális készítményt tartalmaz. Előfordulhat, hogy nem minden kiszerelés kerül kereskedelmi forgalomba. **6.6 A fel nem használt állatgyógyászati készítmény vagy a termék felhasználásából származó hulladékok megsemmisítésére vonatkozó különleges utasítások:** A fel nem használt állatgyógyászati készítményt, valamint a keletkező hulladékokat és a regurgitált intraruminális készítményeket a helyi követelményeknek megfelelően kell megsemmisíteni. **7. A FORGALOMBA HOZATALI ENGEDÉLY JOGOSULTJA:** Eli Lilly and Company Limited, Elanco Animal Health, Priestley Road, Basingstoke, Hampshire RG24 9NL, Egyesült Királyság. **8. A FORGALOMBA HOZATALI ENGEDÉLY SZÁMA(I):**EU/2/12/145/001-003. **9. A FORGALOMBA HOZATALI ENGEDÉLY ELSŐ KIADÁSÁNAK/MEGÚJÍTÁSÁNAK DÁTUMA:** 2013/01/28. **10. A SZÖVEG FELÜLVIZSGÁLATÁNAK DÁTUMA:** 2016/04/15. Erről az állatgyógyászati készítményről részletes információ található az Európai Gyógyszerügynökség honlapján (http://www.ema.europa.eu). **A FORGALMAZÁSRA, KIADÁSRA ÉS/VAGY FELHASZNÁLÁSRA VONATKOZÓ TILALMAK:** Nem értelmezhető.

Kérjük, hogy további információkért keresse helyi Elanco képviselőjét: Lilly Hungária Kft.
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