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KOŠICE 2009

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Jubilee Middle European Buiatrics Congress

held within the framework of the 60th anniversary of the University of Veterinary Medicine in Košice
3 – 6 June, 2009

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of The Slovak Veterinary Association

in cooperation with:

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P R E F A C E

The Middle European Buiatrics Congress (MEBC) has a long history. Following the initial meeting held in Berlin (Germany) in October 1998 at the Congress of German Buiatrics Association, organization of the Middle European Buiatrics Congress started its history from 1999. From this time onwards the annual Middle European Buiatrics Congresses were held in various countries of the former east European bloc, with the support of European west countries.

It is a great honour for Slovakia to organize the 10th Jubilee Middle European Buiatrics Congress.

Since the beginning of tradition of MEBC, the recent years have been characterized by significant decrease in the practice of bovine medicine, as a result of increasing average milk yield/cow/year due to holsteinisation of herds, decreasing populations of dairy cattle, decreasing price of raw milk for farmers, increasing import of milk and milk products from abroad, and unfavourable effects of milk and its products on health of adult population. Consequently, in the field of bovine medicine, greater emphasis was put on diagnosis, therapy and prevention of infectious diseases, as a result of eastern countries joining the EU (opening of borders – increased market with live animals and their products). This dangerous situation has been supported by important changes in European climate resulting in predisposition to (supporting factors: temperature, humidity, insect distribution, etc.) and dissemination of unusual infectious diseases. On the other hand, we must keep in mind all the time that the health of animals is the basis for adequate reproduction of animals, production of good-quality products for human consumption and securing good health of human population.

Nowadays it is very important for us to continue with the application of some modern methods and knowledge (genomics, proteomics, nutrigenomics, metabolomics) in the field of diagnosis, therapy and prevention of common problems (infectious diseases), organ disorders (metabolic, digestive, respiratory, reproduction, mammary gland, limbs), animal nutrition, animal welfare, farm management, herd health management, food safety and environmental protection. This involves complex management of health status of food animals. We must accept this new situation from the point of view of individual bovine medicine (mostly result of loose housing of cattle without primary contact between stockman and animal) and bovine herd health management (with the complete control of the health status through the preventive diagnosis and herd health control). We must take steps in two directions – to treat the animals individually (disorders of reproduction, locomotory apparatus, mammary gland, metabolic diseases etc.) and to apply herd-level preventive measures (mainly around and through the peripartal period – dry and postpartal period). We must find a sound balance between individual treatments and herd-level preventive measures. We do hope that the 10th Jubilee Middle European Buiatrics Congress will help us to find some answers to our questions in the field of practical veterinary medicine.

The aim of the Middle European Buiatrics Congress is to share scientific and practical knowledge on diagnosis, therapy, and prevention in the ruminant medicine. The congress should provide the basis for scientists from universities and research institutes, practical veterinary surgeons working with ruminants, PhD and graduate students of veterinary medicine as an important form of continuing education.

We are happy that this year 146 papers have been submitted for Middle European Buiatrics Congress held at our university.

Gabriel KOVÁČ
President of the Organising Committee

Herbet SEIDEL
Secretary of the Organising Committee

HISTORY OF MIDDLE EUROPEAN BUIATRICS CONGRESSES

#	MEBC City/Country	Dates	President	Papers	Participants
1st	Balatonfűred, Hungary	May 27-29, 1999	Prof. Dr. Otto Szenci, DrSc.	42/59	228/17
2nd	High Tatras, Slovak Republic	May 11-13, 2000	Prof. Dr. Gabriel Kováč, DrSc.	45/56	232/11
3rd	Milovy near Nové Město na Morave, Czech Republic	May 24-25, 2001	Prof. Dr. Bohumír Hofírek, DrSc.	51/71	263/13
4th	Lovran, Croatia	April 23-27, 2003	Prof. Dr.Sc. Josip Kos, PhD.	61/50	274/11
5th	Hajdúszoboszló, Hungary	June 2-5, 2004	Prof. Dr. Ottó Szenci, DrSc.	68/92	328/20
6th	Cracow, Poland	June 1-4, 2005	Prof. Dr. Edward Malinowski, PhD.	63/80	371/19
7th	Radenci, Slovenia	March 29-April 1, 2006	Mag. Dr. Ožbalt Podpečan	52/ 42/2	217/17
8th	Gura Humorului, Romania	June 5-8, 2007	Dr. Aurelia Ionescu, PhD.	68/48/6	280/19
9th	Budapest, Hungary	July 6-11, 2008	Prof. Dr. Ottó Szenci, DrSc.	*439/823	*2 497/72
10th	Košice, Slovak Republic	June 3-6, 2009	Prof. Dr. Gabriel Kováč, DrSc.	60/86	

*Data obtained from the XXVth World Buiatrics Congress that included also the 9th Middle European Buiatrics Congress

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CURRENT HEALTH, REPRODUCTION, AND PRODUCTION PROBLEMS AND THEIR SOLVING IN CATTLE BREEDING

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ABSTRACT

This study dealt with the current health problems in the herds of food animals (especially the highly productive ruminants). The actual number of different categories of ruminants reared in the Slovak Republic and their productive and reproductive indices, as well as the consumption of some edible animal products by the human population in the Slovak Republic and EU are presented. It is evident that the selection based on the health indices highly fall behind the selection based on the production and reproduction indices. The world economy crisis significantly influenced the animal production in all regions of the Slovak Republic. The diminishing trends in the farming of dairy cows, as well as the decreased use of dietary supplements in their nutrition are the consequence of the low prices for liter of raw milk. The shift could be based on the increased farming of the original beef-milk cattle, as well as the routine using of HACCP program for the preventive diagnosis of health problems (especially the metabolic, reproduction, production and locomotion disorders) on the farms with the highly productive animals.

Key words: production diseases; ruminants; solving of problems

INTRODUCTION

Within the last 200 years, the production of milk per lactation increased 10 times (from 1000 kg to 10 000 kg) on the farms with the highly productive dairy cows (4, 16). There is no doubt that the consequence of this situation is the higher appearance of the postpartum paresis, dislocation of abomasi, steatosis of liver, low fertility, mastitis, laminitis at the period of partum and early lactation which lead to the increased annual culling rates of cows and the economical losses on the farm. (8).

The last decades, the extreme concern was directed to the high development of the production and reproduction indices of food animals, which led to the problems above mentioned. It is evident that the selection based on the health indices highly fall behind the selection based on the production and reproduction indices (10).

The health of animals is influenced by the housing conditions, nutrition, hygiene, infectious situation, breeding, and selection of animals. The high development of the production and reproduction indices leads to the increasing physiological requirements for the dietary nutrients as well. The existence of health problems on the farms with the highly productive animals must be generally accepted (12).

The big feed concerns ruin the farmers because the purchase price of milk is only 15 – 23 cents per liter meanwhile the sale price in the market is often 5 times higher (60.25 – 66.27 cents per liter). The big mistake of the past was to eliminate the original beef-milk cattle from our farms and replace them with the Holstein-Friesian cows. Findings of our farmers have shown the mistaken vision to rear these highly productive dairy cows in the areas higher than 450 m above sea level (14).

Table 1. Survey of numbers and production of ruminants (RIAFIE, 2008)

Number of animals	Amount units	Reality to 31/12/2006	Reality to 31/12/2007
Cattle of them:	thousands	**507.8**	**501.8**
cows	thousands	**218.7**	**215.7**
of them:			
dairy	thousands	**185.0**	**180.2**
rest	thousands	**33.7**	**35.5**
Sheep			
Together	thousands	**332.**	**6 347.2**
of them:			
Ewe	thousands	**229.0**	**231.1**
Goats	thousands	**38.4**	**37.9**
Production			
Slaughter cattle together	t. slaughter weight	**27 994**	**29 499**
Slaughter sheep	t. slaughter weight	**1 162**	**1 021**
Slaughter goats	t. slaughter weight	**322**	**300**
Sheep milk	t.	**9 974**	**10 109**
Sheep wool	t.	**817**	**816**

RIAFIE:
Research Institute of Agricultural and Food Industry Economy

Table 2. Survey of basal herd reproduction of ruminants (RIAFIE, 2008)

Parameter	Amount units	Reality of years 2006	Reality of years 2007
Heifers mating	number/100 cows to 1.1.	29.2	28.6
Cows mating	number/100 cows to 1.1.	69.2	68.6
Transfer of heifers to the category of cows	number/100 cows to 1.1.	30.7	31.7
Culling of cows	number/100 cows to 1.1.	28.0	26.7
Mortality of cows	number/100 cows to 1.1.	6.1	6.2
Confiscation of cow carcasses	number/100 cows to 1.1.	0.5	0.5
Transfer of ewe hoggs to the category of ewes	number/100 ewes to 1.1.	17.1	17.9
Culling of ewes	number/100 ewes to 1.1.	7.4	7.9
Mortality of ewes	number/100 ewes to 1.1.	7.4	7.6

Table 3. Survey of yielding and reproduction properties of ruminants (RIAFIE, 2008)

Parameter	Amount unit	Reality to 31/12/2006	Reality to 31/12/2007
Calves born	number/100 cows	89.2	90.0
Net birth rate	number/100 cows	58.5	58.3
Calves raising	number/100 cows	82.1	83.3
Annual milk yield	kg/dairy cow	5 670.1	5 951.4
Weight gains during fattening period of cattle	kg/feed day	0.779	0.775
Lambs born	number/100 ewes	101.7	97.9
Lamb raising	number/100 ewes	96.9	94.0
Average wool clip	kg/ewe to 1.1.	2.0	1.9
Production of ewe milk	kg/ewe to 1.1.	43.6	43.8

During the recent years, the missing facts about the etiology of morbidity and mortality of food animals are obtained by the studies related to the current health, reproduction, and production problems on our farms. Presented results, which were found by the diploma students during their diploma studies at the Clinic of Ruminants of the University of Veterinary Medicine in Kosice are as follows:

The dominant problem in the category of calves has been the respiratory-diarrhea syndrome. The economical aspect of minimizing of personnel staff in the category of young animals leads to the malpractice in the handling with the newborns (e.g. adspection of the oral cavity, treatment of umbilicus), in the colostral nutrition (e.g. origin of the colostrum, first suckling and its frequency), and in the hygiene conditions in the rearing of young animals. The farmers are not able to supply more money for the nursing of newborns.

The main problems in the category of young cattle have been the metabolic diseases, as a result of non adequate management of grazing, lack of preventive measurements in relation to ruminal dysfunctions, endo, and ectoparasitosis. The traumatic irritation of animals caused by the wrong welfare have had the increasing tendency (e.g. mixed housing of different weight categories of young animals, as well as bad housing conditions).

In the category of dairy cows, metabolic disorders in the peripartal period have been the most frequent cause of the higher occurrence of the *paresis puerperalis*, abomasal displacement, ruminal dysfunction, acidosis or alcalosis, liver steatosis, low fertility, mastitis, laminitis at the period of calving and early lactation. In addition, the infectious agents (e.g. paratuberculosis, IBR, BVDV) significantly influence the morbidity of cattle on our farms.

Table 4. Consumption of selected foodstuffs per inhabitant in SR and EU (kg/year) (RIAFIE, 2008)

Type of foodstuff	During the years										
	2000	2001	2002	2003	2004	2005	2006	2007 (approx.)	Recommended dose		EÚ consumption
Meat on bone	60.9	58.7	59.7	61.5	60.1	61.6	61.1	58.8	57.3		87.4
– beef, veal	9.3	7.0	6.8	6.9	6.4	6.1	5.3	5.4	17.4		18.0
– pork	33.1	31.8	31.3	32.3	31.9	31.1	32.2	32.2	22.2		43.5
– poultry	17.1	18.5	20.14	20.7	20.4	21.1	22.3	19.9	15.0		23.0
– other	1.4	1.4	1.5	1.6	1.4	1.4	1.3	1.4	2.7		2.8
Fish	4.3	4.5	4.4	4.2	4.4	4.4	5.1	4.7	6.0		25.0
Milk and m. products	160.2	161.8	166.2	158.3	153.3	154.6	152.4	155.5	220.0		243.5
– milk	71.5	67.8	67.1	63.9	59.1	55.7	55.9	52.4	91.0		81.2
– cheese, curds	7.9	8.3	9.0	9.3	8.2	9.1	9.5	9.8	10.1		17.3
Eggs (pcs)	210	212	214	219	200	199	207	206	201		246
Fats together	23.9	24.3	25.2	24.6	23.3	23.8	23.3	22.0	22.0		
– butter	2.7	3.0	3.0	2.8	2.2	2.0	2.0	2.2	2.8		4.18
– lard	3.3	2.8	3.2	3.4	3.3	3.3	3.0	3.1	3.0		
– FPFO	17.8	18.4	18.9	18.3	17.7	18.4	18.2	16.6	16.2		19.9
Sugar	31.5	26.6	27.6	27.0	30.2	34.0	31.9	28.7	30.9		33.0
Grain as flour	98.5	95.1	94.8	95.9*	92.8	91.0	84.8	85.0	98.5		89.4
Potatoes	68.1	64.3	74.8	66.3	64.2	60.3	58.6	58.7	80.6		80.5
Legumes	1.9	1.9	1.9	1.6	1.6	1.6	1.6	1.6	2.6		3.9
Vegetables	94.2	80.5	77.3	80.9	89.9	86.7	88.0	89.1	127.9	↓	238.9
Fruit	56.8	51.3	49.7	52.6	49.7	52.6	54.0	58.2	96.7	↑	
Grape wine (litres)	10.8	11.3	11.7	10.8	10.3	10.2	12.2	11.5			33.8

Explanation:
- other meat: mutton, kid, venison, rabbits and other tiny animals
- FPFO: food plant fat and oils

How to solve these problems:

1. The economical crisis should not affect the routine preventive diagnosis and control of health problems on the farms with the highly productive animals on the level of individual and herd health management (8), by means of general and specific indices of organ damage (6, 11).

a) The veterinary surgeons must convince the farmers that the program for the preventive diagnosis of health problems of livestock can result in the production of healthy foodstuffs. Subsequently, these commodities can positively influence the health status of human population.

b) The mind of farmers must be changed from the mistaken path of the strict selection of food animals on the production indices (e.g. milk production, fat and protein content in milk), while the health status of animals has been generally ignored and longevity of animals decreases.

c) Could the highly milk productive Holstein-Friesian cows solve our economical problems? They could not. Only the original beef-milk cattle (Slovak spotted and Pinzgau cattle) on our farms can cover our requirements for the milk and meat products.

2. The health aspects can be performed by:

a) The routine using of the program for the preventive diagnosis of health problems of food animals (8) connected with the well organized external and internal function of management on the farm (2)

b) The application of the HACCP program with the emphasis on the critical and potentially critical control points during the entire production and reproduction cycle (12, 17)

c) Using of current research results and their applications (e.g. genomic, proteomic and nutrigenomic) in the diagnostic, preventive, and therapeutic measures (3, 7, 9,15,18)

d) To balance the selection based on the production and reproduction indices with the selection based on the health indices (e.g. finding and application of the markers, which point out the animals with the predisposition to the diseases) (5).

e) The milk parameters (% of fat, % of protein, somatic cells, urea) must be reconsidered. Liver steatosis (the weight of liver 4 – 7 kg in healthy dairy cow can be increased up to 25 kg in dairy cow with steatosis) found in the highly productive animals results in the significant decrease of longevity. Could the cows with liver steatosis produce the healthy milk when the detoxification ability of liver significantly failed?

Table 5. Survey of slaughterhouse findings at the biggest slaughterhouse in Slovakia during years 2006–2008

Year	No. of animals slaughtered				No. of carcasses confiscated								Kg of meat conf.
	cattle	cows	bulls	heifers	lungs	heart	liver	spleen	kidney	stomach	intestines	udder	together
2006													
I. half-year	5 946	3 700	1 409	837	1 387	322	1 909	1 488	802	645	5 946	1 375	4 343
II. half-year	5 512	3 182	1 821	509	1 655	357	1 456	5 512	887	628	5 512	1 258	3 752
Together	**11 458**	**6 882**	**3 230**	**1 346**	**3 042**	**679**	**3 365**	**7 000**	**1 689**	**1 273**	**11 458**	**2 633**	**8 095**
2007													
I. half-year	5 294	3 389	1 383	522	1 672	414	1 464	5 294	1 132	684	5 294	2 079	4 679
II. half-year	3 743	2 130	1 400	210	1 579	208	1 107	3 743	826	555	3 743	1 387	2 628
Together	**9 037**	**5 519**	**2 786**	**732**	**3 251**	**622**	**2 571**	**9 037**	**1 958**	**1 239**	**9 037**	**3 466**	**7 307**
2008													
I. half-year	3 566	1 912	1 326	328	1 294	231	781	3 564	748	544	3 564	865	2 625
II. half-year	3 105	1 543	1 374	188	1 052	149	674	3 105	577	563	3 105	564	1 427
Together	**6 671**	**3 455**	**2 700**	**516**	**2 346**	**380**	**1 455**	**6 669**	**1 325**	**1 107**	**6 669**	**1 429**	**4 052**

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REFERENCES

1. Agroinštitút Nitra, štátny podnik., 2008: MP SR. Správa o poľnohospodárstve a potravinárstve v Slovenskej republike 2008 (Stav za rok 2007) (Zelená správa). Bratislava, november 2008, 198 p.

2. Brand, A., Peeters, C. A. M., 2008: Farm management: The „invisible cow“ in herd health practices ? *Magyar Allatorvosok Lapja,* 130, (Supplement I.), 20–24.

3. Burvenich, Ch., De Spiegeleer, B., Peelman, L., Paage, M. J., 2008: Innate immunobiology of bovine mammary gland and *E. coli* infections. *Magyar Allatorvosok Lapja,* 130, (Supplement I.), 25–28.

4. De Kruif, A., Opsomer, G., Noordhuizen, J. P., 2007: Dairy herd health management: current state and perspectives. Production Diseases in Farm Animals.13th International Conference. Proceedings, Leipzig, July 29th – August 4th 2007, 337–350.

5. Goff, J. P., 2008:: Immune supression around the time of calving and the impact of metabolic disease. *Magyar Allatorvosok Lapja,* 130, (Supplement I.), 39–41.

6. Ingvartsen, K. L., Friggens, N. C., Larsen, T., Rontved, C. M., 2007: New opportunities in monitoring and early diagnostics and challenges in management and prevention of individual dairy cows. *Production Diseases in Farm Animals. 13th International Conference.* Proceedings, Leipzig, July 29th – August 4th, 2007, 354–361.

7. Hammon, H. M., Stürmer, G., Schneider, F., Engelhard, T. Genzel, A., Staufenbiel, R., Kanitz, W., 2007: Metabolic and endocrine changes and hepatic expression of gluconeogenic enzymes in high-yelding dairy cows with low and high liver fat content during the transitional period. *Production Diseases in Farm Animals. 13th International Conference*. Proceedings, Leipzig, July 29th – August 4th 2007, 196.

8. Kováč, G., Nagy, O., Seidel, H., 2006a: Preventívna diagnostika chorôb a kontrola zdravia v chovoch hovädzieho dobytka. *Infovet,* 13, (1), 45 – 48.

9. Kováč, G., Reichel, P., Nagy, O., Seidel, H., Sirotkin, A., Mikula, I., 2006b: Smerovania k objasneniu porúch látkového metabolizmu dojníc. *Infovet,* 13, 280–282.

10. Kováč, G., Petrovič, V., Tóthová, Cs., 2007a: Súčastnosť a perspektívy zdravia a produkcie dojníc. *Infovet,* 14, 208–214.

11. Kováč, G., Tóthová, Cs., Nagy, O., Seidel, H., Farkašová, Z., Novotný, J., 2007b: Acute phase proteins and variables of energetic profile in dairy cows. *Rev. Rom. Med. Vet.,* 17, 55–64.

12. Kováč, G., Nagy, O., Seidel, H., Petrovič, V., Tothová, Cs., Tomčová, J., 2008[a]: Manažment zdravia v stáde dojníc: súčasný stav a perspektívy. *Infovet,* 15, 26–32.

13. Kováč, G., Tóthová, Cs., Nagy, O., Seidel, H., Novotný, J., Tomčová, J.,2008[b]: Acute phase proteins and variables of enzymatic and hepatic profile in dairy cows during the pre- and postpartal period. Proceedings of the 9th Middle European Buiatrics Congress. Budapest 6 –11 July, 2008, 72–77.

14. Lettrich, R., 2009: Viera v lepšie časy ich neopúšťa – zatiaľ. *Roľnícke noviny,* 79, (11), 1 and 5.

15. Loor, J. J., Dann, H. M., Everts, R. E., Oliveira, R., Green, Ch. A., Guretzky, N. A. J., Rodriguez-Zas, S. L., Lewin, H. A., Drackley, J. K., 2005: Temporal gene expression profiling of liver from periparturient dairy cows reveals complex adaptive mechanisms in hepatic function. *Physiol. Genomics,* 23, 217–226.

16. Martens, H., 2007: The dairy cow: physiological facts and concerns. *Production Diseases in Farm Animals.13th International Conference.* Proceedings, Leipzig, July 29th – August 4th, 2007, 26–42.

17. Noordhuizen, J., Cannas, J., 2008: Veterinary herd health & Production management and quality risk management programmes in dairy practice: what´s new? *Magyar Allatorvosok Lapja,* 130, (Supplement I.), 91–98.

18. Richter, S., Morel, I., Gutzwiller, A., Van Dorland, H. A., Morel, C., Zbinden, Y., Bruckmaier, R. M., 2007: Gene expression of hepatic enzymes related to the carbohydrate and lipid metabolism during the transition period in dairy cows. *Production Diseases in Farm Animals.13th International Conference.* Proceedings, Leipzig, July 29th – August 4th, 2007, 157.

TASKS OF THE STATE VETERINARY AND FOOD ADMINISTRATION OF THE SLOVAK REPUBLIC AFTER JOINING THE EUROPEAN UNION

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Veterinary community owing its presence on farms, communication and cooperation with farmers whether from the position of private or official veterinary service plays a key role in questions of animal breeding under hygiene conditions, rapid diagnostics of diseases, monitoring and control of infections, animal treatment and welfare issues, including public health. At present, the veterinary service is also an extraordinary important information channel for breeders of cattle and other farm animals providing them with information and advice to the questions how to reach, eliminate and control risks of food safety e.g. residues of pesticides in connection with feeding good-quality and safe feed stuff, mycotoxines and environmental contaminants. Veterinary service plays also a central role in achieving a well-advised and responsible usage of veterinary medicinal products, including antimicrobial substances in holdings, what substantially helps to minimize risks of development of antimicrobial resistance and dangerous level of residues of veterinary medicinal products in foodstuffs of animal origin. Owing to animal health measures and a regular veterinary surveillance it is possible to state that in Slovakia there is a very low potential threat of „food pathogens".

In the period prior to the accession of Slovakia to the EU the historical role of the veterinary service was concentrated mainly to control of animal diseases on the farm level. However, this role was significantly extended towards the end of past millennium and at the beginning of this contemporary at the level of slaughterhouses where veterinarians have 2 responsibilities – epidemiological surveillance and monitoring of animal diseases and achieving of safe production of meat and products. The role was considerably strengthened just after the accession to the European Union, where one of the key tasks is a principle of food safety „from Stable to Fork". In all EU countries has to be an operational and introduced system of control and combating very dangerous animal diseases, including control of diseases such as brucellosis, tuberculosis and TSE. Clear rules apply to animal movement, including procedures upon disease eradication and removal and handling animal by-products. The EU legislation, inter alia, clearly defines prevention against the use of infected animals for human nourishment.

The afore-said facts confirm the current main idea of the European Community, presented also during the performed veterinary week in November 2008, which is „One Health: Healthy Animals = Healthy People".

The monitoring of animal health is therefore the basic tool in reaching this aim, and is above all the fundamental component of detection and finding out the diseases and infections, monitoring of trend in respect of disease development and serves also as an important supporting tool for declaration of the country or part of the country as free of certain disease. Slovakia also during the year 2008 continued in retaining the status of the country officially free of:

1. bovine tuberculosis,
2. bovine brucellosis,
3. enzootic bovine leucosis
4 ovine brucellosis (*Brucella melitensis*).
5. Aujezsky´s disease

To retain this status, in Slovakia it was continued in monitoring of tuberculosis by tuberculination of bovine animals, serological examination for bovine brucellosis, examination for enzootic bovine leucosis and Aujezsky´s disease. The determined rate of sheep and goats over 6 months of age and all

breeding rams were examined for ovine brucellosis (*Brucella melitensis)*.

Data obtained from monitoring are used in risk analyses for purposes of animal health and also public health. Even though we know that both domestic animals and wild animals are susceptible to certain agent (we have our own experiences with positive results and presence of classical swine fever virus in wild boar in Slovakia), compliance with strict and effective biological measures for protection of farm animal holdings automatically need not mean detection of the disease in holdings. Thus, the monitoring and surveillance of diseases that includes also results from wildlife population may increase the qualitative level of national reports of monitoring and disease occurrence on its territory and represent additional information source for risk analyses from the view point of international trade. Slovakia just towards the end of April 2009 was sending to the European Commission summary reports on monitoring and occurrence of diseases on its territory for the year 2008 and just reports concerning avian influenza or classical swine fever included also results obtained from wild birdlife or wild boar.

The main idea concerning the animal health at the all-European level, defined as „Prevention is better than Cure", is a part of a new strategy of animal health, adopted for the years 2007-2013. The strategy was also for several times a topic of negotiations of Chief Veterinary Officers of EU Member States and is based on 4 basic pillars:

1. priorities for EU measures – have a decisive importance for the strategy which is based on risk assessment;

2. uniform regulative framework– with regard to an increasing importance of the EU it is efficient an effort for compliance with international standards and to cooperate to the widest extent upon creation of future OIE rules. At the same time the emphasis is put on common responsibility for financing of veterinary policy i.e. veterinary fund and financial responsibility should be maintained. Necessity to notify suspicious cases and also immediate determination of disease outbreak are indicated as extraordinary important and pointed out at flexibility and efficiency in respect of uncertainties concerning reimbursement of direct and indirect costs.

3. Prevention, surveillance and preparedness for crises – inevitability of effective border inspection controls from the side of Member States, general judgement of a concrete risk together with possible consequences and to a great extent also the usage of random controls in border areas with the aim to discourage illegal trade.

4. Science and research – is an assumption to apply research knowledge in production, consultant service and control. From this view it is important to orientate the activities to knowledge transmission.

As we have already mentioned, the mission of veterinary service is not only in detection of animal diseases, adoption of relevant measures, however it plays an important role in increasing of awareness of food produces and operators in relation to required provision of food safety „from Stable to Fork". During our accession process we had to fulfil strict and uncompromising requirements laid down in EU legislation, incorporate (transpose) them into the Slovak legislation and ensure their implementation. As a basis for its brief evaluation, the information indicated in evaluation of this accession process for the whole field of veterinary medicine had been used. Among the most important belongs

1. We transposed the legislation on transmissive spongiform **encephalopathies (TSE) and animal by-products (ABP)**, we created the system of TSE monitoring, including organizing the laboratory diagnostics. The total ban of the feeding of animal meals to farm animals was introduced and we strengthened official controls of specified risk material as well as its destruction. At present, also based on favourable all-European trend in BSE development and also in connection with EFSA opinion it may be predicted that infectivity in bovine animals at the age of 33 months is not detectable or it is still not present and therefore in the year 2008 the age limit for removal of the vertebral column, as a specified risk material has been modified in bovine animals from 24 to 30 months in the Commission Regulation (EC) No 357/2008. As far as ABP is concerned with regard to practical and scientific knowledge and experience obtained from 1.5.2003, when the Regulation (EC) No 1774/2002 started to be applied, the text of a new proposal aimed at update of current valid rules, provision of legal certainty, introduction of endpoint of life-cycle of ABP and modification of the ABP categorization framework with regard to risks connected with them, was finalized in spring 2009.

2. We created the **veterinary control system for internal market** and joined the system of animal movement control within the Community - ANIMO. The legislation for identification and registration of animals was transposed. The process of registration of farms and identification of animals, especially other species than bovine animals continued. The District Veterinary and Food Administrations started with collection of fees for veterinary controls in compliance with Annex IV of Regulation (EC) No 882/2004 of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, and animal health and animal welfare rules. And we laid down rules concerning veterinary controls upon imports from third countries and import rules. 3 border inspection posts at the EU external border were put into operation meeting all the criteria required by EU legislation

3. Regarding **control measures for infectious animal diseases,** inter alia the national contingency plans for foot and mouth disease (FMD), classical swine fever (CSF), Newcastle disease and Avian influenza are elaborated. Slovakia is connected to the system of Animal Disease Notification System (ADNS). Legislation in respect of **placing the animals and animal products on the market** was also transposed**.** In 2008, the State Veterinary and Food Administration of the Slovak Republic worked out within the system of veterinary prevention and protection, guidelines regarding classification of individual holdings and stated measures for consistent introduction of surveillance network as well as health requirements upon movement of live animals and germinal products. In 2008 in the Slovak Republic was realized

- National Control Programme for Salmonella Infections in Flocks of Laying Hens of Domestic Fowl (Gallus gallus) Producing Eggs for Domestic Consumption in the Slovak Republic for the Years 2008 - 2010;
- National Control Programme for Salmonella Infections in Poultry Breeding Flocks of Domestic Fowl (Gallus gallus) in the Slovak Republic for the Period of the Years 2007 - 2009,
- The National Eradication Programme of Poultry Breedings from Salmonella Infections in Slovakia in the Year 2008;
- National Rabies Eradication Programme in Slovakia for the Year 2008;
- Plan of Eradication of Classical Swine Fever in Wild Boar Population in Slovakia in the year 2008;
- Plan of Surveillance of Avian Influenza in Poultry and Wild Birds in Slovakia in the year 2008;
- Plan of Surveillance of Bluetongue in Slovakia for the year 2008;
- National Programme of the Monitoring of American Foul Brood in the Years 2008 – 2009.
- Surveillance of **Salmonella infections** within 2 programmes and one study of Salmonella prevalence. The aim was and is to reduce the occurrence of *Salmonella enteritidis*, *Salmonella hadar*, *Salmonella infantis, Salmonella typhimurium* and *Salmonella virchow* in breeding flocks of *Gallus gallus* so that by 31 December 2009 it remains positive maximum 1% or less from adult breeding flocks involving minimum 250 birds.

4. The legislation for human **health protection** was transposed to a wide extent, gradually so called „Hygiene Packet" of regulations related to food hygiene gradually started to apply. At present, the discussion to modernization of inspections on slaughterhouses as well as process of accreditation of laboratories examining *Trichinella* on slaughterhouses are under way.

5. The legislation on **common measures (including zoonoses)** was transposed and control systems in respect to residues and veterinary medicinal products were introduced. During the year 2008, the legislative proceeding was performed in respect of the proposal of the Ordinance laying down Community procedures in connection with determination of limits of residues of pharmacological active substances in foodstuffs of animal origin with the aim to improve the availability of veterinary medicinal products for food-producing animals, and at the same time it will be also ensured a high level of human health protection, creation of special legal framework for determination of MRL for pharmacological active substances not intended for the use in veterinary medicinal products in EU as well as provision of clear reference values for purposes of the control (i.e. reference values for adoption of measures) in some cases when MRL were not determined.

6. In the field of **animal welfare** administrative structures were developed in compliance with animal welfare legislation for its control and monitoring at central, regional and local level. In the year 2008, negotiations started within the current activities on the proposal of the new legal rule which will govern conditions of animal protection upon slaughter and killing with the aim to support the innovation of stunning of animals in a human manner, to ensure a better integration of good conditions of animals into a production process of slaughterhouses, to increase the knowledge of respecive personnel and to impove the protection of animals in case of massive killing; and these good conditions will have a positive impact on meat quality and also safety at work.

In conclusion, it is possible to state, that regardless of the professional position of each veterinarian in Slovakia, the role of veterinary community in prevention, disease combating and protection of human health is unsubstituable both on national and European level. The veterinarians manage to carry responsibility for presentation of knowledge in various local situations, turn achieved theoretical knowledge to practical solutions and to contribute to prevention and regulation of animal diseases.

ALLERGIC DERMATITIS IN SHEEP

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ABSTRACT

In summer and autumn of 2008, an enzootic dermatitis was observed in a flock of Texel sheep in the western part of Germany. This pruritic dermal condition mainly affected the unprotected skin, as on the head, ears, sternal area and vulva. The animals were free of mange, ticks and *Melophagus* infestations, as well as of fungal diseases. Bacterial examinations of skin scrapings gave only unspecific results. Despite intensive search, no photosensitizing plants could be found on the pastures. The histological examination of skin biopsies taken from three ewes proved severe hyperkeratosis and dermatitis, characterized

by infiltration of inflammatory cells, especially high numbers of eosinophils. Housing of affected sheep led to complete healing within some weeks.

Sera from the three affected and of eight controll sheep were analysed with the use of Allercept® (HESKA, Switzerland), a non-competitive, solid-phase ELISA-test, designed to detect the presence of allergen-specific IgE in sera using the recombinant alpha chain of the high-affinity IgE receptor. Positive reactions against biting insects were found in both groups, with higher ELISA values in affected the sheep especially against *Culicoides*.

These findings suggested that the skin disease resulted of an immediate hypersensitivity reaction to insecte bites, especially *Culicoides* species, resembling the alterations of "sweet itch" or summer dermatitis in horses. This disease can easily be confused with mange, zink deficiency or photosensitization, particularly as latter also improves after housing.

Key words: dermatitis; hypersensitivity; insects; sheep

RETINOL-BOND-PROTEIN4 (RBP4) AND DISLOCATIO ABOMASI (DA)

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OBJECTIVE

DA is regarded to be one of the most common diseases in cattle. The development of a left (lDA) or right sided (rDA) DA is caused by various factors. The lDA results from disturbances in the energy metabolism starting during the dry period and potentiating post partum (p.p.). Its assignment to the fat mobilisation syndrome is likely, however it is not always indicated through body condition scoring (BCS) or back fat thickness.

Aim of the study was therefore to check for signs indicating an irregular distribution of peripheral and visceral fat ante (a.p.) and p.p. in cows with lDA during early lactation.

EXPERIMENTAL DESIGN

In a dairy farm, 969 cows and heifers were examined clinically, including back fat thickness (BFT) and laboratory-diagnostically 4 weeks (w) a.p., 1-2 w a.p., 3 days (d) p.p. as well as 4-5 w p.p.. Out of those cows, 44 healthy and 25 cows with lDA were selected and comprehensively analysed biochemically (Hitachi 912). RBP4 was determined by means of Western-Blot analyses after isolation in 12% SDS-PAGE.

RESULTS

The main results of the energy metabolism are shown in the table (medians).

		4 w a.p.	1–2 w a.p.	3 d p.p.	4–5 w p.p.
BFT	healthy	20	21	22	14
mm	lDA	20	20	19	13
FFA	healthy	82	122	706	319
µmol/l	lDA	76	110	1413	403
Biliru-bin	healthy	1.8	1.6	5.3	3.7
µmol/l	lDA	1.3	1.9	12.4	4.7
BHB	healthy	0.51	0.46	0.84	0.72
mmol/l	lDA	0.51	0.49	1.02	0.69
RBP4	healthy		9.1	7.8	8.1
	lDA		15.7	6.1	9.1

Comparing BFT in both groups, no significant differences were found. In the lDA group, the concentrations of FFA, bilirubin and BHB increase significantly 3 d p.p.. The RBP4 concentration is significantly higher a.p..

Because of studies in oxen in which RBP4 is also reflecting the amount of visceral fat, the results indicate that cows with lDA p.p. have stored significantly higher visceral fat before calving. Because visceral fat also produces cytokine, the motility of the abomasum can be influenced.

CONCLUSIONS

RBP4, being an indicator for visceral fat, shows that cows with lDA p.p. have stored significantly more visceral fat a.p. than healthy cows.

IMMUNOSUPPRESSIVE EFFECTS ON OVINE LYMPHOCYTES DUE TO COMBINED ISOFLURANE ANAESTHESIA

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Key words: immune system; isoflurane; sheep

INTRODUCTION

Isoflurane is frequently used for veterinary inhalational anaesthesia although studies in humans report genotoxic effects and DNA damage in lymphocytes due to halogenated ethers (1, 2). Even though our recent investigation shows that combined isoflurane anaesthesia is associated with impaired immune reactivity in equine lymphocytes (3), little is known about the genotoxicity of isoflurane and its impact in ruminants. Immunosuppression during the perioperative periode might cause postoperative complications and influence the recovery of patients. Hence the biological impact of general anaesthesia with isoflurane on ovine lymphocytes was evaluated by determining potential genotoxic and immunosuppressive effects.

PATIENTS AND METHOD

16 sheep (classified as ASA 1: healthy, no systemic disease) underwent general anaesthesia with isoflurane during laparoscopic-assisted cystotomy. The experimental study protocol was approved by the Institutional Ethics Committee and had governmental approval. Blood samples were collected from the jugular vein before, 1.5 hr and 24 hrs after the induction of anaesthesia. White blood cells were counted in the improved Neubauer haemo-cytometer. Differential blood cell counts were carried out on Diff-Quick® stained blood smears. Lymphocyte proliferation test and comet assay analysis were performed as previously described (3).

RESULTS

Total white blood cell count significantly decreased during anaesthesia. Preliminary data obtained from comet assay analysis revealed only minor changes in levels of DNA damage in lymphocytes due to isoflurane anaesthesia, whereas lymphocyte proliferative reactivity decreased significantly during and after anaesthesia, however, was back to previous levels within 24 hours.

DISCUSSION AND CONCLUSION

Our preliminary results demonstrate - for the first time in sheep - inhibitory effects of isoflurane anaesthesia on lymphocyte proliferation parallel to changes in lymphocyte counts. Our data agree well with human studies showing the influence of volatile anaesthetics on the proliferative activity of peripheral blood lymphocytes. Although anaesthesia affected the total white blood cell count and the lymphocyte proliferative reactivity significantly, only minor lymphocytic DNA damage was detectable.

This study confirms immunosuppression due to inhalation anaesthesia with isoflurane in sheep. Further studies are required to focus on possible postoperative complications due to the anaesthesia induced transient impaired immune reactivity and the influences on the recovery of patients after long-lasting surgery with different general anaesthesia protocols.

REFERENCES

1. Karabiyik, L., Sardas, S., Polat, U., Kocabas, N. A. and Karakaya, A. E., 2001: Comparison of genotoxicity of sevoflurane and isoflurane in human lymphocytes studied in vivo using the comet assay. *Mutat. Res.* 492, 99–107.

2. Sardaş, S., Karabiyik, L., Aygun, N. and Karakaya, A.E., 1998: DNA damage evaluated by the alkaline comet assay in lymphocytes of humans anaesthetized with isoflurane. *Mutat. Res.* 418, 1–6.

3. Strasser, A., Dadak, A., Kühnel, H. and Velde, K., 2008: Immunosuppression during and after castration under inhalation anaesthetic without genotoxic effects on equine lymphocytes. Submitted.

DYNAMICS OF SELECTED ACUTE PHASE PROTEINS IN SURGICAL ABOMASUM REPOSITION IN COWS

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ABSTRACT

The usefulness of the bovine major APPs was examined in surgical reposition of abomasum in cows. Eight cows - patients of Clinic for Ruminants, Veterinary University of Vienna were examined. In 5 cows additionally: bronchitis, postpartal vaginal injury, rumen acidosis, retained placenta, metritis and mastitis was detected. In blood of cows haptoglobin (Hp), fibrinogen (Fb), serum amyloid A (SAA) concentrations as well as total serum protein and their fractions (albumin, α-, β-, γ-globulin) were measured. Blood was taken 3 times: I – at arrival to the Clinic, II – about 3 days later and III – before return to the owner (mostly between 5th and 7th day of hospitalization). The highest mean concentrations of all of the measured APPs was found in I sample. In this moment in one cow no elevated Hp concentration occurred, whereas in one another the concentration was low (0.12 g.l^{-1}). Only in one cow Fb concentration exceeded 7 g.l^{-1}. In all the examined cows serum SAA concentration was over 100 mg/l. In II examination the mean APPs concentrations decreased slightly. In all cows Hp concentration was over 0.1 g.l^{-1}. Fb concentration in one cow was over normal range as formerly. SAA concentration was over 49.6 mg.l^{-1} in all cows. At III sample the concentrations of SAA and Fb decreased, but the increase of Hp concentration was found. In five cows at III sample the Hp concentration exceeded still the maximum of normal range. In no one of the examined cows Fb concentration not exceeded the normal range and the lowest SAA concentration was 42.4 mg.l^{-1}. During surgical treatment of displaced abomasum mean Fb concentrations were normal, only in one cow was higher at I and II sample, in the same time bronchitis was diagnosed in the same cow. The last disease seemed to have deciding influence on the increase of mentioned APP. In last sample the high Hp and SAA concentrations were observed. It may be explained by fact that return of that proteins to normal range occurs normally on 10-14th day of inflammatory response.

Key words: fibrinogen; haptoglobin; serum amyloid A; treatment monitoring

INTRODUCTION

According to rise of dairy farm milk production, the elevated rate of health problems may occur. Displacement of abomasum is typical problem of high yielding cows. In cows with displacement of abomasum about two times more frequently metabolic disturbances are found (e.g. ketosis, milk fever, retained placenta) (4). Economic losses result also from decreased milk production, because it is reduced before diagnosis and treatment (3).

In cattle leukocytosis – classical acute-phase parameter, is not suitable for measuring of disease's intensity. Acute-phase proteins (APPs) are in contrast useful parameters of inflammatory response in cattle. APP concentration correlates with disease severity and with the extent of inflammatory changes (6). Because acute-phase reaction is non-specific, it is possible to use changes in the concentrations of APPs universally to monitor the course of the disease, independently of its nature (9, 10). The aim of this work was the evaluation of usefulness of the three major bovine APPs after surgical reposition of abomasum in dairy cows.

MATERIAL AND METHODS

Eight cows with displaced abomasum, patients of Clinic for Ruminants of Veterinary University Vienna were examined. Blood samples were taken 3 times at occasions of other diagnostic purposes, associated with treatment. First blood sample (I) was taken at arrival to the Clinic, second (II) on third day of hospitalization, and third sample (III) at departure to the owner, mostly on 7th day of stay at the Clinic.

Surgery of abomasum was made at day of arrival by omentopexy with fixation to right abdominal wall (5).

All cows were examined upon arrival at the clinic, and on every day of their stay at the Clinic according to the method of Baumgartner (2005). Each cow was assigned, a clinical number and case history card was created and updated according to Clinic regulations.

Blood was collected from jugular vein using two types of sterile test tubes of the Vacutainer™ system (one for blood serum and the other with the anticoagulant K_2EDTA). Blood collected to obtain serum was left for two hours and then centrifuged at 2000 x *g* for 10 min. at room temperature. The blood serum was collected in test tubes and stored at -20°C

until assay. Concentration of Fb was determined in the whole blood according to the method of Millar *et al.* (1971) in the modification by Brugmans *et al.* (1998) whereas the concentrations of Hp (11), SAA with ELISA (Tridelta Development), and total serum protein (TSP) (Biuret method), fractions of TSP i.e.: albumin, α-, β- and γ-globulins, were determined by paper electrophoresis in the sera of examined patients. At arrival acid-base balance was estimated based on vein blood examination in all patients.

The data underwent unidirectional analysis of variance using the statistical package STATISTICA 7.1. We compare differences during treatment and the significance of differences between subsequent collections was estimated using the Duncan's test.

RESULTS

In six cows displacement of abomasum occurred within first month postpartum, in remaining two cows in later period. Additionally in 5 cows diagnosis of: bronchitis, parturient vaginal injury, rumen acidosis, metritis and mastitis was diagnosed. In four cows left displaced abomasum (LDA) and in remaining 4 right displaced abomasum (RDA) was diagnosed. Only in one cow at arrival to the Clinic urine ketone bodies were found. During hospitalization the presence of ketone bodies was detected in three cows. Body temperature over 38.5 °C was measured in 4 cows, pulse frequency >80 bpm in 6 cows, and breathing frequency >30/min.in 3 cows.

The highest hematocrite level was found at first examination and in next samples decrease of Ht level was observed (30.4; 27.8; 25.3 %, respectively).

Table 1. Selected parameters of acid-base balance

	pH	K^+ (mmol/L)	Na^+ (mmol/L)	Cl^- (mmol/L)	HCO_3^- (mmol/L)	pCO_2 mm Hg
$\bar{x}$ (±)	7.5 (0,1)	2.8 (0,8)	134.5 (3,3)	94.5 (10,0)	36.3 (8.3)	49.4 (5.9)
Min-max	7.4–7.62	1,8–4,2	129–138	81–105	27.3–55	42–56

In Table 2 the mean concentrations of APPs examined during treatment of displaced abomasum are presented.

In first blood sample only one cow showed normal Hp level, and in second examination individual low Hp level was estimated (0.12 $g.l^{-1}$). At the same moment only one cow showed elevated Fb concentration. The highest mean SAA concentration was found in first sample.

At second sample in 4 cows Hp concentration decreased as well as the mean. In all cows Hp was detectable. In cow that formerly presented high Fb concentration, the level of this protein decreased but was still over the normal range (Fb levels of remaining cows were normal). Mean SAA concentration slightly decreased. Higher SAA levels that occurred at first sample were no more observed, but in 3 cows slight SAA increase was found.

Table 2. Concentration of examined APPs in cows with displaced abomasum (P ≤ 005)

APP		Examination number I n=8	II n=8	III n=8
Hp $g.l^{-1}$	$\bar{x}$	**1.21**	**1.10**	**1.14**
	±	**1.08**	**0.63**	**1.28**
	min-max	0.0–2.82	0.24–2.1	0,0–3.75
	Median	0.81	1.17	0.79
Fb $g.l^{-1}$	$\bar{x}$	**6.4**	**5.95**	**5.23**
	±	**2.0**	**1.08**	**1.32**
	min-max	4.26–10.78	4.48–7.69	2.45–6.68
	Median	6.24	5.95	5.48
SAA $Mg.l^{-1}$	$\bar{x}$	**138.59[a]**	**122.86**	**96.77[b]**
	±	**34.72**	**38.57**	**38.88**
	min-max	105.73–214.01	49.59–154.27	42.41–164.94
	Median	130.92	142.08	103.99

In five cows at III sample Hp concentration still significantly exceeded the normal range. Fb was below the normal range only in one cow, in remaining patients it was within physiological range. Mean SAA concentration achieved minimum and differed significantly from first examination (Tab 2). In one animal slight increase, but in remaining animals the decrease of total serum protein occurred. Concentration of serum protein fractions was presented in Table 3.

Table 3. Electrophoretic fractions of total serum protein in cows with displaced abomasum

Examination number		albumin g/L	α-globulin g/L	β-globulin g/L	γ-globulin g/L
I	$\bar{x}$ (±)	**26.51 (6.86)**	**9.3 (1.37)**	**7.52 (2.26)**	**14.33 (4.69)**
	min-max	16.14-39.0	6.68-11.72	5.21-11.1	5.36-21.18
II	$\bar{x}$ (±)	**24.9 (2.6)**	**8.96 (0.91)**	**7.18 (0.85)**	**14.59 (2.9)**
	min-max	21.3-28.8	7.39-10.36	5.68-8.42	8.06-16.84
III	$\bar{x}$ (±)	**25.02 (4.56)**	**9.41 (1.18)**	**7.96 (1.69)**	**15.73 (2.43)**
	min-max	20.93-34.31	7.79-10.88	5.26-10.13	11.44-19.32

Mean albumin concentration at each sapling was below minimum of normal range. The largest differences between individuals were found at first sample. At the last sample normal albumin level occurred only in one cow.

At I examination no cows presented normal α-globulin level (12). In one cow its concentration was below, in remaining cows over the normal range. At second examination mean α-globulin concentration decreased, but in 3 cows this fraction achieved normal range. At third sample slight increase of α-globulin concentration was found.

Determined mean β-globulin concentration was below the normal range. The lowest mean concentration was found at second examination. Normal concentration of this fraction at first examination occurred in three cows, at second in one and in the third in four cows.

In cows with displaced abomasum low γ-globulin concentrations were also found. The lowest levels were determined at first examination, and in following samples an increase appeared. Majority of examined cows showed γ-globulin concentration below the normal range. Normal γ-globulin concentration occurred only in one cow at first and in two individuals at third examination.

DISCUSSION

Displacement of abomasum is multifactorial disease, appearing frequently in periparturient period. Predisposing factors as well as complicating factors may be ketosis and retained placenta (16). Similar conditions occurred also in presented cases. In majority of examined cows the displacement of abomasum occurred within one month postpartum and at arrival to the Clinic ketosis or retained placenta were found. In study of Stengärde and Pehrson (2002) 74% of cows with displaced abomasum showed accompanying disease, which may influence the Hp and SAA concentrations.

Although majority of cases in cows are LDA (17), in our study the same number of LDA and RDA was found. It is similar to results of Hirvonen and Pyörälä (1998).

Only in one cow that exhibited body temperature exceeding 38,5 °C no accompanying disease was found. Slightly elevated temperature may be caused by locomotory activity during transport, because on the next day in was normal. Elevated hearth rate and breathing frequency were probably induced by stress induced by contact with unknown, large group of humans (veterinarians and students) and partly circulatory disturbances associated with hemoconcentration.

In cows metabolic alkalosis was diagnosed based on high blood pH, high HCO_3^-, pCO_2 and low potassium and chloride concentrations (Tab. 1.). Metabolic alkalosis is frequently diagnosed at cases of displaced abomasum (19, 15). Hypokalemia is a result of intracellular movement of potassium or exertion of this cation in kidney to prevent metabolic alkalosis (14, 18).

Low chloride concentration was the consequence that as the component of HCl stayed in displaced abomasum and was not available to absorption in small intestine.

At day of surgery in 6 cows elevated Hp levels occurred. In two cows the increase of Hp from very low levels (0.0 and 0.12 $g.l^{-1}$) at I examination to significantly higher in II examination was induced probably by surgical treatment only, because no additional clinical symptoms were seen. Hirvonen and Pyörälä (1998) determined slight increase of Hp concentration after surgery. In our study the increase was more markedly. Also the increase of SAA in over mentioned two cows was probably induced by surgical treatment.

The highest Hp concentration at all three examinations as well as SAA at first sample were found in one cow that additionally has vaginal wall injury after dystocia, and it probably caused high APPs production. In another cow only the increase of SAA concentration and no reaction of other APP was found. Simultaneously muco-purulent nasal discharge appeared and the type of antibiotic given after reposition was changed. The increase of SAA in this cow was observed because this protein is more sensitive and reacts more rapidly to inflammatory stimuli than Hp (6). Mixing the patients from different farms in the Clinic was probably the factor predisposing to respiratory infection. Adaptation stress may additionally negatively affect the function of immune system.

Mean Fb concentration was normal at I and II examination with exception of one cow which at arrival exhibited also bronchitis. The increase of Fb was in that cow probably caused by this accompanying disease.

At last examination high Hp and SAA levels were estimated. That finding was partly caused by earlier than planned blood taking. It was associated by reducing the hospitalization costs and return the cows to owners at the moment of achieving stable clinical status, at which further stay at Clinic was not necessary. In one cow Fb concentration was below normal range at last examination. In blood sample taken two deays earlier GGT, GLDH activities and bilirubin level were significantly elevated over the normal range. It may indicate on important liver damage and is such case low Fb level agrees with results of McSherry *et al.* (1970).

Low concentration of total serum protein as well as its fractions was found. Stengarde and Pehrson (2002) found that only 1.7 % of cows with displaced abomasum had TSP levels below 59 $g.l^{-1}$, but over 15 % the concentration over 89$g.l^{-1}$. High TSP level was in authors opinion caused by chronic inflammation or with dehydratation. Mean TSP was 81.2 ±10.7 $g.l^{-1}$. In our study the TSP was much lower and the concentration below 59 $g.l^{-1}$ showed 75 % of cows at I and II examination, and the percent decreased to 62.5 % at III sampling. Low TSP concentration may be a consequence of low age of examined cows: four of them were about 2 years old, two 3-years, one 4-years and one 6.5-years. Moreover economic manner of feeding the cows in smaller Austrian farms may also influence the TSP level.

Ht level was normal, although in I examination weak degree of hemoconcentration was found, which may indicate on dehydratation. Similar Ht levels obtained Zadnik *et al.*(2001).

Mean albumin concentration in cows with displaced abomasum was lower in our study than found Stengarde and Pehrson (2002) and Hirvonen and Pyörälä (1998) that obtained respectively 35.1 and 36 $g.l^{-1}$. Low albumin concentration was associated with low TSP and probably with long duration of inflammation. During treatment no increase of this fraction was found, it corresponds with lack of return of Hp and SAA levels to normal range.

Concentrations of α-, β- and T-globulins showed slight changes during treatment. Similarly as in other cow diseases

(10) α-globulin concentration was elevated in majority of cows because of inflammation. Concentration of remaining globulin fraction was below normal range.

CONCLUSIONS

Low Fb concentration in contrast to other APPs indicate on its low availability in monitoring of displaced abomasum surgical reposition. Similarly, in study of Irmak and Turgut (2005) and Hirvonen and Pyörälä (1998) Fb level was normal in cows with displaced abomasum. Relatively high Hp and SAA concentration and their stepwise decrease during treatment indicates that both APPs may be useful in postsurgical monitoring of cows with displaced abomasum.

High percent of cows treated because of DA show simultaneously accompanying diseases that complicate course of basic problem. Because of over mentioned circumstances the evaluation of APPs in monitoring of surgical treatment of displaced abomasums seems helpful in evaluation of cow status and advance of inflammatory response.

REFERENCES

1. **Baumgartner, W., 2005:** *Klinische Propädeutik der Inneren Krankheiten der Haus - und Heimtiere*. Parey, Berlin, 382 pp.

2. **Brugmans, F., Venner, M., Menzel, D., Mischke, R., 1998:** Messung der Fibrinogenkonzentration beim Pferd mit den Hitze-Präzipitationsmethoden nach Schalm und Millar. *Deutsche Tierarztliche Wochenschrift*, 105, 58-61.

3. **Deluyker, H. A., Gay, J. M., Weaver, L. D., Azari, A. S., 1991:** Change of milk yield with clinical diseases for a high producing dairy herd. *J. Dairy Sci.*, 74, 436-445.

4. **Detilleux, J. C., Gröhn, Y. T., Eicker, S. W., Quaas, R. L., 1997:** Effects of left displaced abomasum on test day milk yields of holstein cows. *J. Dairy Sci.*, 80, 121-126.

5. **Dirksen, G., 1967:** Gegenwärtiger Stand der Diagnostik, Therapie und Prophylaxe der Dislocatio abomasi sinistra des Rindes. *Deutsche Tierärztliche Wochenschrift*, 74, 625-633.

6. **Heegaard, P. M. H., Godson, D. L., Toussaintc, M. J. M., Tjùrnehùj K., Larsen, L. E, Viuff, B., Rùnsholt, L., 2000:** The acute phase response of haptoglobin and serum amyloid A (SAA) in cattle undergoing experimental infection with bovine respiratory syncytial virus. *Veterinary Immunology and Immunopathology*, 77, 151-159.

7. **Hirvonen, J., Pyörälä, S., 1998:** Acute-phase response in dairy cows with surgically- treated abdominal disorders. *Vet. J.*, 155, 53-61.

8. **Irmak, K., Turgut, K., 2005:** Disseminated intravascular coagulation in cattle with abomasal displacement. *Vet. Res. Comm.*, 29, 61-68.

9. **Jawor, P., Stefaniak, T., 2006:** Acute phase proteins in treatment of calves with respiratory tract inflammation (in Polish). Universitatis Agriculturae Stetinensis, *Zootechnica*, 48, 51-56.

10. **Jawor, P., Steiner, S., Stefaniak, T., Baumgartner, W., Rząsa, A., 2008:** Determination of selected acute phase proteins during the treatment of limb diseases in dairy cows. *Veterinarni Medicina* 53, 173-183.

11. **Jones, G. E., Mould, D. L., 1984:** Adaptation of Guaiacol (peroxidase) test for haptoglobins to microtitration plate system. *Research in Veterinary Science*, 37, 87-92.

12. **Kaneko, J. J., 1989:** Serum proteins and dysproteinemias and appendix VI w *"Clinical Biochemistry of Domestic Animals"* Ed. Kaneko J.J. Academic Press, San Diego, 142-165.

13. **McSherry, B. J., Horney, F. D., deGroot, J. J., 1970:** Plasma fibrinogen levels in normal and sick cows. *Canadian Journal of Comparative Medicine*, 34, 191-197.

14. **Peek, S. F., Divers, T. J., Guard, C., Rath, A., Rebhun, W. C., 2003:** Hypokalemia, muscule weakness and recumbency in dairy cattle (17 cases 1991-1998). Preconvention Seminar 7: Dairy Herd Problem Investigation Strategies. *Proceedings of the 36th Annual Conference of American Association of Bovine Pracitioners.*

15. **Sahinduran, S., Albay, M. K., 2006:** Haematological and biochemical profiles in right displacement of abomasum in cattle. *Revue de Medicine Veterinaire,* 157, 352-356.

16. **Shaver, R. D., 1997:** Nutritional risk factors in the etiology of left displaced abomasum in dairy cows: a review. *J. Dairy Sci.*, 80, 2449-2453.

17. **Stengärde, L. U., Pehrson, B. G., 2002:** Effects of management, feeding, and treatment on clinical and biochemical variables in cattle with displaced abomasum. *American Journal of Veterinary Research*, 63, 137-142.

18. **Ward, G. M., 1966:** Potassium metabolism of domestic ruminants. A review. *J. Dairy Sci.,* 49 268-276.

19. **Zadnik, T., Mesaric, M., Reichel, P., 2001:** A review of abomasal displacement - clinical and laboratory experiences at the Clinic for Ruminants in Ljubljana. *Slovenian Veterinary Research*, 38, 193-208.

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EXPRESSION OF RBP4-mRNA IN ADIPOSE TISSUE AND RBP4 IN SERUM OF HEALTHY DAIRY COWS

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INTRODUCTION

RBP4 is an adipokine which regulates glucose uptake in adipocytes and seems to be involved in the development of insulin resistance. High serum levels in humans reflect an impaired insulin action and can be used as a predictive marker for type II diabetes. Furthermore it is considered an indicator for visceral obesity. Lipolysis and insulin resistance play a crucial role for predisposition, incidence and outcome of disease in the perparturient period in dairy cows. As proven for mice and human the metabolic activity of adipose tissue, especially in regard to insulin sensivity and lipolytic activity, varies between the different fat depots in the body and there is evidence that similar mechanisms can be assumed for cattle.

AIMS OF THE STUDY

To investigate whether mRNA expression of RBP4 in fat of healthy cows is different according to the body site and whether serum RBP4 levels change during the periparturient period.

MATERIALS AND METHODS

Samples were taken at slaughter immediately after death from 12 cows, slaughtered for non metabolic reasons. Samples were collected from omentum, renal capsule, inguinal region (retroperitoneal), hip (subcutaneous) and heart base. They were immediately deep frozen using liquid nitrogen and stored at -70 °C. mRNA expression for RBP4 was measured using quantitative RT-PCR method. Additionally serum samples from 44 healthy dairy cows were collected 3 wks a.p., 3 d p.p. and 3 wks p.p. and analysed for RBP4 using Western Blot.

RESULTS

Expression of RBP4 mRNA was higher in retroperitoneal, renal and pericardial than in subcutaneous or omental fat. It was significantly upregulated in pericardial fat in comparison to omental fat. Serum RBP4 levels 3 d p.p were 8 (4.05; 13.44) $mg.l^{-1}$ (median and quartiles) and did not change significantly between the three sampling points.

CONCLUSIONS

In healthy cows RBP mRNA expression is highest in pericardial fat what might be due to the high metabolic activity and glucose turnover in the heart muscle. In this study no specifically higher expression of RBP4 in omental fat of cattle could be shown, which was expected for those animals did not show metabolic disorders. However the general mRNA-expression level in subcutaneous fat is lower than in visceral depots surrounding heart or kidney. The constant serum levels of RBP4 show, that in cows with a balanced energy metabolism and no excessive fat mobilization and insulin resistance, RBP4 levels are not alterated during the periparturient period.

NATURAL OCCURRENCE AND ELIMINATION OF 19-NORTESTOSTERONE IN SHEEP BEFORE AND AFTER TREATMENT

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ABSTRACT

The use of hormones in food producing animals for fattening purposes is strictly prohibited throughout the European Union. However, 17α-19-nortestosterone (17α-19-NT), the marker metabolite used for screening of the anabolic steroid 17β-19-nortestosterone (17β-19-NT, nandrolone), is recurrently detected in urine from meat sheep by the Austrian Agency for Health and Food Safety, Competence Centre Veterinary Drugs and Hormones, Vienna.

In order to make an interpretation of 17α-19-NT-positive field samples possible, the spontaneous occurrence and elimination of 17β-19-NT and its metabolites 17α-19-NT and 19-norandrostenedione (19-NAD) were studied under controlled conditions. Six lambs of each sex were treated with a single i.m. dose of 2 mg 17β-19-NT laurate/kg b.w.. Samples of blood, urine and feces were systematically collected over a period starting 6 weeks before and lasting until 32 weeks after the treatment. Samples were analysed by a regulatory HPLC-EIA and/or an LC-MS-technique.

Plasma of untreated animals was free of 17β-19-NT and related metabolites. Following treatment, free parent compound 17β-19-NT (but no unconjugated metabolite) was detectable for about 4 weeks. Maximum mean concentrations of 0.58 $ng.ml^{-1}$ in male and 0.44 ng/ml in female animals were measured on day 3 *post injectionem* (p.i.).

Analyses of *urine* revealed a sporadic excretion of 17α-19-NT in untreated male as well as in female animals (up to 2.95 $ng.ml^{-1}$) but not of 17β-19-NT and 19-NAD. Three days after treatment, 17β-19-NT reached maximum mean concentrations of 17 $ng.ml^{-1}$ in males and 20 $ng.ml^{-1}$ in females. Mean levels of 17β-19-NT fell below the LOD (1.02 $ng.ml^{-1}$) by day 52 p.i. in females and by day 86 in males.

Maximum urinary concentrations were also found for 17α-19-NT at day 3 p.i. with mean values in males of 242 ng/ml and in females of 253 $ng.ml^{-1}$. Concentrations continuously fell thereafter and, by the last sampling date (day 226 p.i.), fluctuated at levels similar to those found before treatment.

Sporadic EIA-signals corresponding to 19-NAD standard were detected in urine for a period of 98 days in females and 114 days in males following treatment. However, this obviously treatment related crossreactive metabolite could not be confirmed by LC-MS and still awaits identification.

The results reveal that 17α-19-NT is endogenously occurring in urine from sheep of both sexes. Its detection alone is, therefore, not necessarily indicative for exogenous administration of 17β-19-NT to sheep. An i.m. dose of 2 mg 17β-19-NT laurate. kg^{-1} b.w., a dose known to be biologically effective following repeated administration to sheep, is definitely assessable for a period of time corresponding to a usual sheep fattening period due to the high levels of urinary 17α-19-NT.

Feces turned out not to be a suitable matrix for residue surveillance purposes since levels of 17β-19-NT related metabolites were found to be low and too irregular for regulatory screening purposes.

BIOCHEMICAL PARAMETERS OF PERITONEAL FLUID ANALYSIS IN DAIRY COWS

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ABSTRACT

Analysis of peritoneal fluid is generally considered to be a useful diagnostic method in gastroenterology because changes in the characteristics of peritoneal fluid generally reflect intraabdominal conditions. In bovine gastroenterology, abdominocentesis and analysis of peritoneal fluid is also an established diagnostic procedure. However in cattle, the peritoneal fluid analysis routinely comprises only a small number of basic parameters to apply the transudate-exudate categorization system. This classic classification system is unable to identify pathogenic pathways or aetiological factors, and in some cases it has only moderate diagnostic accuracy, therefore additional biochemical parameters have been developed for human patients, and partially for horses and dogs. The aim of this study was to examine biochemical parameters of peritoneal fluid in healthy dairy cows and to establish reference ranges to provide more comprehensive information on specific pathological intraabdominal conditions.

The study included 95 clinically healthy Holstein-Friesian cows. Peritoneal fluid and jugular venous blood samples were taken simultaneously. The parameters (albumin, glucose, cholesterol, fibrinogen, L-lactate, D-dimer, LDH, ALP CPK were determined in peritoneal fluid and venous blood. Light's criteria, serum ascites albumin gradient (SAAG), and parameter ratios between peritoneal fluid and venous blood were calculated. In addition the routinely used parameters total protein and WBC have been measured. Data are given as Median [1. Quartile/3. Quartile]. Preliminary reference ranges were calculated using 2.5 and 97.5 percentiles of the parameters.

The parameters of peritoneal fluid and the calculated values obtained in this study could be used for preliminary reference ranges in cattle. Only few RBCs were found in the peritoneal fluid samples (0.03 [0.01/0.13] T/L). The number of peritoneal fluid leukocytes was counted with 2.50 (1.40/3.70) G/L predominantly composed of polymorphnuclear cells (62.0 [53.5/76.5] %) and a lower percentage of lymphocytes (29.5 [16.0/38.5] %). Thus the reference ranges for WBC in peritoneal fluid lay between 0.67 and 4.88 G/L. No bacteria have been found in the peritoneal fluid samples.

Due to species-dependent variations, some biochemical parameters (total protein, albumin, SAAG, D-dimer, LDH) were found to have different reference values compared to monogastric animals and humans. Especially, peritoneal total protein concentration of healthy cows (5.6 - 41.8 $g.l^{-1}$) were found to be higher than in monogastric animals (exudate if > 30 $g.l^{-1}$). However, some values (e.g. peritoneal fluid-blood protein ratio, LDH ratio, glucose concentration, and L-lactate concentration) showed similar reference ranges.

Because the peritoneal fluid measurements obtained in this study increases the diagnostic information from peritoneal fluid analysis in human patients, horses and small animals, it is reasonable to assume the same relationship in cattle. However, clinical studies are necessary to demonstrate the diagnostic value of these measurements.

Key words: D-dimer; glucose; Light's criteria; L-lactate; peritoneal fluid analysis; SAAG

NOVEL MINIMAL INVASIVE TECHNIQUE FOR MEASURING HEPATIC METABOLISM QUANTITATIVELY IN DAIRY COWS EXEMPLIFIED BY STUDYING HEPATIC GLUCOSE-NET PRODUCTION AFTER DEXAMETHASON TREATMENT

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ABSTRACT

Based on the indicator-dilution method using para-aminohippuric acid (PAH) (1, 2), the objective of the study was to establish a minimally invasive technique to quantitatively determine hepatic metabolism and consequently quantify the net glucose production under the influence of dexamethason.

Eight clinically healthy German Holstein cows (age 3.6 ± 0.8 $\bar{x}$ ± s) years, body weigh (BW) 560 ± 59 kg, 382 ± 108 days post partum) were included in the study. Indwelling catheters were implanted in the jugular vein and the abdominal aorta six days prior to the start of the experiment. Furthermore, a transcutaneous ultrasound-guided (B-Mode 2 - 7 MHz convex transducer, 11 - 23 cm image depth range) catheterisation of the portal and hepatic vein as well as the cranial mesenteric vein was performed.

To determine hepatic plasma flow rates, the PAH indicator was infused into the mesenterical vein (5 h after application of 2 g of primer, constant infusion rate of 14.4 $g.h^{-1}$). Blood samples were collected from the hepatic portal and jugular vein as well as the abdominal aorta prior to infusion (base values) and subsequently in 40 minute intervals starting 50 min after delivering the priming dose. Hence, in total, six simultaneous blood samples for analysis of PAH and Glucose were gathered.

The hepatic net production rate of glucose was defined as the concentration difference between afferent and efferent hepatic vessels in relation to hepatic plasma flow (1, 2). To investigate the influence of dexamethason on net hepatic glucose production, this value was determined a day prior to (Day 0) and subsequently on days 1, 2 and 4 after dexamethason treatment (100 $mg.kg^{-1}$ BW; intramuscular application).

In all animals (n=8), treatment with dexamethason led to an increase in hepatic plasma flow as well as in the arterial fraction of the blood flow of the hepatic vein ($P \leq 0.05$). Glucose concentration increased in all vessels under the influence of dexamethason ($P \leq 0.001$). This increase could, however, not be related to an increased net production of glucose (Day 0: 1389 ± 587 $g.day^{-1}$; Day 1: 1229 ± 429 $g.day^{-1}$; Day 2: 1008 ± 322 $g.day^{-1}$; Day 4: 1452 ± 507 $g.day^{-1}$), as in fact a decrease in these levels was observed (Tag 0 vs. 2, $P \leq 0.001$).

Necropsy two weeks post implantation confirmed the correct position of the catheters in all animals. In one cow, a thrombus in the portal vein prevented blood sampling from Day 2 of the study onwards. From the remaining seven animals, all samples were successfully collected according to protocol. In the abdominal cavity a mild localised fibrinous perihepatitis at the passage of the catheter into the liver was observed.

The ultrasound-guided percutaneous multiple centesis of hepatic vessels, which was essential to the study design, proved to be safe, reliable and simple to conduct. The results of the net glucose production imply that the increase in plasma glucose concentration following dexamethason treatment is not due to an increased hepatic gluconeogenesis but rather a reduced peripheral consumption of glucose.

Key words: cow; gluconeogenesis; hepatic plasma flow; implantation; ultrasound-guided catheter

REFERENCES

1. Katz, M. L. and Bergman, E. N., 1969 A: Simultaneous measurements of hepatic and portal venous blood flow in the sheep and dog. *Am. J. Physiol.,* 216, 946-952.

2. Katz, M. L. and Bergman, E. N., 1969 B: Hepatic an portal metabolism of glucose, free fatty acids, and ketone bodies in the sheep. *Am. J. Physiol.*, 216, 953-960.

LAPAROSCOPIC PERFORMED CYSTOTOMY AND CATHETER IMPLANTATION IN MALE SHEEP

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Key words: cystotomy; laparoscopy; sheep; treatment urolithiasis

INTRODUCTION

Urolithiasis is a common disease in small ruminants. It occurs mainly in breeding animals and in early castrated males and is primarily considered to be a nutritional disease. Depending on the mineral distribution of the feed struvite, calcium carbonate and calcium phosphate uroliths can develop. In small ruminants the sigmoid flexure and the urethral process are the most common sites for uroliths to lodge. Clinical signs may be associated with partial or complete urethral occlusion, which causes urine retention and leads to bladder distension, abdominal pain and later on to urethral perforation and rupture of the urinary bladder. The animal then dies from uremia and septicemia. Diagnosis is based on history and physical examination. Treatment can be conservative (administration of antispasmodics, correcting electrolyte imbalances, dietary management) or surgical. Different surgical procedures to remove the uroliths and to re-establish urethral patency are established: amputation of the urethral process, perineal urethrostomy, percutaneous and surgical tube cystostomy. As laparoscopic surgery gains in popularity especially in horses and small animals it was aim of this experimental study to develop a laparoscopic technique in male sheep for cystotomy and following urinary catheter implantation.

PATIENTS AND METHOD

Laparoscopy was performed on 10 male sheep placed under general anaesthesia in dorsal recumbency. A laparoscope was inserted on the right paramedian region between the xiphoid and preputial orifices. After creation of a capnoperitoneum, grasping forceps were inserted at the left paramedian line close to the last pair of teats. The urinary bladder was elevated using grasping forceps and exteriorized through an abdominal incision. The bladder was opened extracorporeally, rinsed, closed, and then repositioned. A pigtail balloon catheter was subsequently inserted percutaneously under laparoscopic control and removed ten days thereafter. A repeat laparoscopy was performed at 14 days after the first procedure for assessing pathological changes.

RESULTS

Laparoscopic-assisted cystotomy with following catheter implantation was successfully performed on all sheep. In one sheep, both the ventral and dorsal bladder walls were inadvertently perforated when placing the urinary catheter. The postoperative course was favourable: all sheep had a good appetite and showed no abdominal symptoms. During the second-look laparoscopy, it was observed that one sheep had developed a focal adhesion of the peritoneum to the bladder catheter portal site.

CONCLUSIONS AND CLINICAL RELEVANCE

Laparoscopic-assisted cystotomy with catheter implantation is feasible in male sheep. This technique may be useful for removal of uroliths from the bladder and to perform urinary diversion for obstructive urolithiasis.

ANESTHETIC CONSIDERATIONS IN SOUTH AMERICAN CAMELIDS

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INTRODUCTION

Familiarity with a species decreases the risk of anesthesia. South American Camelids (SACs) have different behavioral, physiological and anatomical characteristics compared to cattle, sheep or goat. For example, SACs show adaptations for survival at high altitudes where oxygen tension is low: Their oxygen hemoglobin dissociation curve is shifted to the left and their red blood cell configuration and hemoglobin content differ from many other mammalian species (3, 4).

The fleece can be particularly heavy and therefore it can be difficult to judge body weight, however, an accurate body weight is necessary to dose drugs appropriately. Anatomic peculiarities relevant to anesthesia include a small oral cavity, a mouth that does not open wide, and an elongated soft palate which can make intubation difficult.

By understanding the differences and adjusting the protocol accordingly, SACs can be safely sedated or anesthetized.

INSTRUMENTATION AND PREPARATION

Venous access can be a challenge in SACs. The jugular vein lies deep and the skin of the neck is thick, from 1 cm up to 3 cm in male animals. There is no jugular furrow and the veins themselves are well endowed with valves preventing backflow. Therefore, knowledge of the anatomical landmarks is important. The two main venipuncture sites are high and low (at the level of the 6th vertebra) on the neck. Despite difficulty palpating the vessel in the proximal portion of the neck, many prefer this site for catheter placement because of a better separation between the jugular vein and the carotid artery. The vessel is located at the bisection of a line drawn caudally from the lower jaw with a line drawn ventrally from the base of the ear (2). Due to the proximity to the esophagus on the left side the jugular vein is preferentially catheterized on the right side. Alternate sites for catheter placement include the lateral thoracic vein (adults) and the cephalic or saphenous vein (juveniles). Auricular vessels may be used in anesthetized patients.

The main considerations in preparation for anesthesia are similar to those of ruminants: tympany, regurgitation, salivation and aspiration pneumonia in addition to general considerations like hypoventilation, hypoxemia and hypotension. Therefore, it is advisable to fast the animal for up to 24 hours and withhold water for 8 to 12 hours. This will not completely empty the compartment 1 (rumen equivalent), but will substantially decrease gastric volume and gas production. Regurgitation is less likely in right lateral recumbency. Fasting of nursing juveniles is not necessary and may increase their stress and likelyhood of metabolic derangement.

Sedation

Sedation in SACs can be achieved with butorphanol alone or in combination with midazolam/diazepam or xylazine. Keep in mind that the dose for xylazine in SACs is between that reported for cattle (0.03–0.1 $mg.kg^{-1}$) and horses (0.3–1.0 $mg.kg^{-1}$). Ketamin can be added to deepen the level of sedation and provide additional analgesia. All of these drugs can be administered IM or IV and will provide effects ranging from sedation to short-term anesthesia with variable degree and duration. Usually, the qualitiy of induction is good, with animals showing some degree of ataxia before assuming a sternal or lateral position.

Anesthetic techniques

Regional and general anesthetic techniques may be used in SACs. Regional administration of local anesthetics can facilitate surgical intervention and minimize the need for administration of drugs with systemic effects. Most protocols for induction of general anesthesia include either ketamine (2–5 $mg.kg^{-1}$) or propofol (2 $mg.kg^{-1}$, titrated to effect) in combination with a muscle relaxant (midazolam or diazepam). Typically animals become recumbent about 45 seconds following IV administration. As with other ruminants it is recommended to orotracheally intubate SACs. This helps to secure the airway, to prevent aspiration of regurgitated material and to facilitate application of oxygen and anesthetic gases. Llamas and alpacas should be placed in sternal recumbency ("cush") with the head extended after anesthetic induction. A mouth gag, strips of tape or gauze can be used to spread the jaw and facilitate visualization of the larynx. Topical local anesthetic (lidocain spray) is applied to the laryngeal structures and the trachea intubated using a long but narrow laryngoscope blade. When intubation is difficult it is helpful to first place a flexible guide into the trachea to help direct the endotracheal tube into the

trachea. When available, inhalation anesthetic techniques are used to maintain anesthesia in patients undergoing invasive surgical procedures.

Monitoring

As with any other species, good anesthetic protocols require good monitoring techniques to tailor drug administration to the animal's needs. Monitoring anesthetic depth in SACs can be challenging because they maintain ocular reflexes at seemingly all planes of anesthesia. Jaw tone seems to be a useful method as well as rectal tone, drooping of the lower lip and relaxation of the neck. Basic clinical monitoring includes auscultation of the heart and lungs, palpation of the pulse, evaluation of mucous membrane color and capillary refill time. Ideally, monitoring of blood pressure, hemoglobin saturation with oxygen, electrocardiogram and body temperature is performed for the entire duration of general anesthesia.

Perianesthetic support

SACs have prominent eyes that stay open during anesthesia and are prone to damage in lateral recumbency. Eye lube should be used for protecting the cornea. Attention should always be given to patient positioning so as not to cause neural or muscular damage. In lateral recumbency, the lower forelimb should be pulled forward and the upper limbs supported in a natural position. In dorsal recumbency, the limbs should be flexed. For longer procedures padding becomes increasingly important. In cool environments or in young and small patients, external heat should be provided. A balanced electrolyte solution should be administered intravenously in those patients in whom recumbency is expected to last longer than one hour. An administration rate of 5–10 ml per hour is suggested in most patients. In critically ill patients, electrolytes may need to be adjusted and acid-base balance corrected.

Although oxygen is routinely used in patients during inhalation anesthesia, this is less common when using injectable techniques. Because llamas and alpacas have been shown to become hypoxic during injectable anesthetics, oxygen supplementation is highly recommended especially for patients at altitude.

Recovery

SACs are obligate nasal breathers, therefore, nasal congestion needs to be resolved before extubation after anesthesia. When possible, the head should be positioned to allow drainage of regurgitant fluid while still minimizing edema. Additionally, vasoconstrictors like phenylephrine can be diluted and gently placed in the nasal cavity prior to extubation.

In general SACs recover well from anesthesia. When possible it is ideal to place them in sternal posture with the head elevated. Oxygen insufflation during this period is beneficial. If the patient is intubated, the endotracheal tube should be protected so it does not get lacerated by the molars.

Analgesia

Analgesia can be provided with NSAIDS, opioids or local analgesics but unfortunately, there is very little published regarding benefits or side effects of systemically administered analgesics in these animals. Hence, much of the use is based on personal preference and extrapolation from other species. Butorphanol (0.1 $mg.kg^{-1}$) is recommended for somatic pain even though the short half-life following IV administration limits its clinical usefulness (1).

REFERENCES

1. **Carrol, G. L., Boothe, D. M., Harrtsfield, S. M., Martinez, E. A., Spainn, A. C., Hernandez, A., 2001:** Pharmacokinetics and pharmacodynamics of butorphanol in llamas after intravenous and intramuscular administration. *J. Am. Vet. Med. Assoc.*, 219, 1263–67.
2. **Davis, I. A., McGaffin, J. R., Kuchinka, G. D., 1996:** Intravenous catheterization of the external jugular vein in llamas. *Compend. Cont. Educ. Food Anim. Suppl.*,18, 330–35.
3. **Fowler, M. E., Zinkl J. G., 1989:** Reference ranges for hematologic and serum biochemical values in llamas (Lama glama). *Am. J. Vet. Res.*, 50, 2049–53.
4. **Chiodi, H., 1970:** Comparative study of blood gas transport in high altitude and sea level camelidae and goats. *Respir. Physiol.*, 11, 84–93.

LAPAROSCOPIC-ASSISTED CYSTOTOMY AND IMPLANTATION OF A URINARY CATHETER IN RAMS

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ABSTRACT

Urolithiasis is a common disease in rams, which treatment could be laparoscopic-assisted tube cystotomy.

To investigate and compare the influences of Trendelenburg position and capnoperitoneum in dorsal and left lateral recumbent male sheep, 16 healthy rams were used in a prospective study.

Each sheep was anesthetized and prepared for laparoscopy. One group of 8 animals was positioned in dorsal recumbency and the other group in left lateral recumbency, and then both in Trendelenburg position, followed by insufflation, and disufflation. Heart rate, and arterial blood pressures were monitored, and arterial blood samples for blood gas and acid-base status were collected, in 5 time points, before and between changes of position, after insufflation and after disufflation. Afterwards, a laparoscopic-assisted cystotomy, and implantation of a pigtail catheter under laparoscopic guidance, were performed in the right paramedian area. The catheter was removed 10 days after implantation and 14 days after surgery, a second laparoscopic procedure documented eventual pathological findings.

Trendelenburg influenced positively the hemodynamic parameters, more severely in dorsal recumbency. The capnoperitoneum aggravated the hemodynamic changes and influenced positively the blood gas and acid-base status. Laparoscopic-assisted cystotomy and implantation of a urinary catheter were successfully performed in all sheep. There were found no major postoperative complications.

Trendelenburg position and capnoperitoneum have effects in sheep and must be used carefully in patients with hemodynamic and metabolic alterations. Laparoscopic-assisted implantation of a urinary catheter can be a valid choice for the correction of urolithiasis in rams.

ULTRASONOGRAPHIC ASSESSMENT OF LIVER DIMENSIONS AND INTRAOPERATIVE LIVER PALPATION AS TOOLS FOR DIAGNOSIS OF FATTY LIVER IN DAIRY COWS

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ABSTRACT

The lipomobilisation syndrome in dairy cows results in an increasing fatty infiltration of hepatic tissue, which consequentially leads to an increased volume and altered consistency of the liver. In order to determine the degree of fatty liver, the objective was to investigate the informational value provided by transcutaneous ultrasound to detect alterations of liver dimensions and intraoperative liver palpation to detect alterations of liver form as well as liver consistency. Furthermore, it should be investigated to which extent this data could be used to predict overall liver fat content.

One hundred and fifty one lactating German Holstein cows (body weight 571 ± 80 kg; age 4.9 ± 2.0 years (mean ± sd)) with left sided abomasal displacement (LDA) were included in the study. Transcutaneous liver dimensions (distance from dorsal

and ventral liver margin to the ´midline of the back in 12th, 11th and 10th intercostal space (ICS), liver angle at Margo ventralis in 12th, 11th, 10th, and 9th ICS; BRAUN 1990) were measured ultrasonographically and data on withers height, days post partum, body condition score and back fat thickness were collected.

During laparatomy for correction of LDA, palpative liver findings (shape of Margo ventralis, surface, consistency) were noted and a liver biopsy was taken and biochemically analysed to determine Triacylglycerol (TAG). Furthermore, a patho-histological examination was carried out to rule out hepatic diseases other than hepatic steatosis. None of the cows (n=151) suffered from other hepatic disorders than hepatic steatosis.

The hepatic TAG correlated significantly, but regarding to the extent only marginally with the transcutaneous ultrasonographic liver dimensions and the angular dimensions of the Margo ventralis of the liver. The highest correlation between hepatic TAG and liver extent was determined at the 11th ICS ($R = 0.16$, $P < 0.001$) and to the ultrasonographically mesasured liver angle at the 10th ICS ($R = 0.09$, $P < 0.01$).

Considering all dimension parameters in a stepwise multiple linear regression analysis, it was possible to predict hepatic TAG with the formula y = -130 + 4.72*liver distance at 10th ICS + 2.18*liver angle at 10th ICS with a coefficient of determination of $R = 0.29$. Comparably, body weight, age, withers height, days post partum, body condition score and back fat thickness provided statistically sound correlations with the ultrasonographically detected liver dimensions and liver angle (R^2 till 0.25). Above features also correlated as is known with the hepatic TAG.

Intraoperative palpatoric evalutation of the liver margin revealed an increasing blunting of the Margo ventralis of the liver with increasing hepatic TAG, while the liver consistency decreased with increasing TAG content. These subjectively determined palpatory and adspectory findings of the liver provide a superior estimation of the TAG compared to ultrasonographically obtained liver dimesions.

In summary, transcutaneous ultrasonographic measurement of the liver in cows allowed no reliable conclusions to concentraions of hepatic TAG. Therefore, it seems to be unsuitable for diagnostic of the liver fat content.

Apparent reasons for this include individual variances in liver dimensions dependent on body weight and size of the animal. The palpatoric evaluation of the liver margin and the consistency of the parenchyma during laparatomy seems to be more reliable than the ultrasonographic liver measurements to obtain an informative basis on the liver fat content.

Key words: liver angle; liver consistency; transcutaneous ultrasound; triacylglycerol

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GASTROINTESTINAL EMERGENCIES IN CATTLE: CAUSES, DIAGNOSIS AND TREATMENT

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ABSTRACT

Acute diseases of the gastrointestinal tract in calves and adult cows are common and warrant concern.

In adult cattle displacements of the abomasum are most frequently observed, but the number of cases with intestinal obstruction is increasing. In calves during weaning diseases of the rumen are of major importance, but also diseases of the abomasum or intestinal obstructions are diagnosed.

The clinical signs allow in some cases the presumptive diagnosis, but sometimes, depending on duration and location of the disease the presentation of cattle with abdominal diseases presents a diagnostic challenge and may have immediate life threatening consequences. A strong clinical suspicion of a strangulating obstruction of the gastrointestinal tract, such as abomasal volvulus, intussusception, or mesenteric volvulus, warrants prompt laparotomy. Progressive abdominal distension and persistent, severe abdominal pain in calves are also considered indicators for surgical treatment.

In this presentation the clinical signs, diagnostic methods, differential diagnosis, treatment and prognosis of selected gastrointestinal emergencies will be described by means of patients of the Clinic for Ruminants in Vienna submitted from 2005 to 2008. Especially clinical signs and treatment of patients with abomasal volvulus and intestinal obstruction and the tentative diagnosis will be discussed.

The most important clinical findings in both diseases include disturbance of the general behaviour, dehydration, markedly reduced rumen activity, abdominal distention, no or greatly reduced defaecation and metabolic alkalosis.

Laparotomy with the patient standing was performed in adult cattle. In calves surgery was done in left lateral recumbency.

The abomasum was displaced without volvulus to the right of its normal position and with volvulus. The bowel was obstructed by intussusception, volvulus, impaction (by hairballs, clotted blood), compression (diverticulum), strangulation, incarceration and torsion of the root of the mesentery. Correction of the abnormalities was done, if possible.

Usually, there were clear interrelationships between the duration of the disease, the clinical status of the animals and the prognosis. The most important prerequisites for a rapid and complete recovery following abomasal volvulus or intestinal obstructions are early diagnosis and surgical correction.

BOVINE PROGRESSIVE DEGENERATIVE MYELOENCEPHALOPATHY IN TYROLEAN GREY CATTLE

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ABSTRACT

Bovine progressive degenerative myeloencephalopathie (BPDME), or so called Weaver syndrome, is a well known autosomal recessive hereditary condition in Brown Swiss cattle and Brown Swiss crossings. Within the neurodegenerative diseases BPDME is added to primary axonal degenerations, characterized by primary involvement of the axon with either sparing or retrograde degeneration of its soma. Up to now BPDME is distributed in Europe, USA and Canada. First symptoms are regularly recognized in animals from 5 to 8 months of both sex. Clinical signs include "weaving" gait, weakness, ataxia, pelvic limb spastic paresis, dysmetria, abnormalities of proprioception and mild forelimb deficits that precede recumbency. The course of disease is slowly progressive over years. Lesions can be found in the central nervous system, particularly in the thoracic spinal cord white matter as well as in sensory nerves. Pathohistological findings consist of axonal swelling, degeneration and loss of axons with or without liquefaction, secondary demyelination, mobile resorption and reactive astrogliosis. We present three cases of Grey cattle showing the morphology of BPDME. In all cases spinal cord was fixed in 7 % buffered formalin, embedded in paraffin-wax, sectioned and stained with haematoxylin and eosin. Our results indicate a hereditary component in this breed and show that the syndrome described is an important differential diagnosis in Grey cattle showing clincal signs of neurological disease.

PEROSOMUS ELUMBIS IN CALVES

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ABSTRACT

This report presents the most important clinical and morphological aspects of the so-called "Perosomus Elumbis", a congenital vertebral malformation of calves characterized by the segmental lack of vertebrae caudal to the thoracic spine. The etiology has to be defined, but inheritance is suspected.

The report is based on seven calves (five Holstein, one Brown and 1 cross breed) examined at the Veterinary Clinical Department of the University of Bologna (BO66/04; BO107/04; BO46/06; BO107/06; BO290/08) or at the Department of Veterinary Clinical Sciences of Padua (PD34/04; PD66/04). All animals appeared bright and alert at birth, but were completely unable to stand up or to maintain quadrupedal stance if raised

manually. The seven calves, ranging from one to 15 days of age, were euthanized on the first day of hospitalization.

Although having individual clinical presentation, the disorder was morphologically characterized by partial or complete segmental absence of vertebra, mostly of the lumbar and sacral segments. The tail was always missing.

As a consequence of the vertebral malformation, the animals' trunks were shorter than normal, and the posterior part of the body was clearly underdeveloped. More precisely, the girdle showed a reduction in size and width whereas the hind limbs were shorter with evident muscular hypoplasia. In some cases, due to the lack of vertebrae, the hind limbs were attached to the caudal aspect of the thorax by soft tissue only.

On the contrary, the anterior half of the body – from the caudal part of the thorax – was normal. Radiological examination clearly demonstrated the skeletal malformation including the lack of vertebrae. Internal organ malformations, such as intestinal atresia, hydronephrosis, testicular agenesia, uterine and vaginal malformations as well as concentric cardiac hypertrophy were also observed in some animals.

In the context of general scientific interest in genetic diseases of cattle, the Authors suspect Perosomus Elumbis to be a potential inherited disease. However, the reported cases studies did not provide evidence for this hypothesis.

Identification of clinical cases and reports of malformed calves are necessary to improve the knowledge of this defect.

Key words: calf; congenital disease; *Perosomus elumbis*; vertebral column

FIRST EXPERIENCES WITH THE LAPAROSCOPIC MANAGEMENT OF LEFT ABOMASAL DISPLACEMENT IN DAIRY COWS IN THE CZECH REPUBLIC

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ABSTRACT

We have verified a possibility of performing laparoscopic abomasopexy under field conditions. 60 dairy cows with left abomasal displacement were treated, using the Janowitz method with the Dr. Fritz laparoscope. Out of the 60 cows under study, 43 were suffering from a concurrent disease. In 8 cows, peritonitis was diagnosed perioperatively. After the surgery, the cows were monitored for their return to milk production and health status (rectal temperature, rumen auscultation, urine analysis for ketone bodies with diagnostic stripes). The surgery in all the cows under study was successful and without complications. 51 cows were returned back to full performance (85 %). During 30 days post operation, 7 cows died and 2 were culled. Mean daily milk yield in the 51 cured animals was 16.3 litres pre-operation and 32.6 litres 30 days post operation.

The Janowitz method is a safe, little invasive and technically simple way of operating left abomasal displacement. The laparoscopic method appears to be a promising possibility how to manage left abomasal displacement under field conditions.

INTRODUCTION

Left abomasal displacement (LDA) in dairy cows is a common disorder. First cases were described in 1950s and it is assumed that they had occured before. Also due to a high level of expertise of large animal veterinary practitioners, this disease is paid growing attention. In the herds where a well formulated diet is not provided and cows suffer from health disorders post partum, the incidence of LDA achieves 15 %. This implicates the need of efficient and considerate therapeutical approach to the animal, and beneficial for the farmer that would lead to a faster return of the dairy cow to high production.

LDA can be treated either conservatively or surgically. The simpliest conservative method is abomasum repositioning by rolling the cow. In this way, the repositioning can be achieved in most cases. A great disadvantage is a high percentage of recurrent cases – 75 % to 80 % (2, 3, 4). This method has been improved by percutaneous abomasum fixation with toggle (Grymer-Sterner suture, LDA suture) placed through skin and abdominal wall in the lumen of abomasum in dorsal recumbency after the abomasum repositioning, and verified by auscultation and percussion. This method has a high success rate, 80 – 88 % success rate is reported (5, 6). A disadvantage

is that complications if they should occur, are very serious. The complications include inadequate abomasum fixation, fixation of another organ, abomasum fistulation, suture tear and recurrence, organ lacerations and subsequent peritonitis, abomasum stabilization in an abnormal position or damage to the pyloric region of abomasum with subsequent partial obstruction, trombophlebitis of milk veins and formation of local abscesses and fistulae along the surgery wound (7).

That is why surgical methods of LDA treatment are preferred, especially in valuable animals. All the surgical approaches are based on laparotomy, decompression and abomasum repositioning (reduction) and subsequent abomasum fixation. The most popular method is right laparotomy with ometopexy (Dirksen method). The advantage of this technique is an easy assessment of the abomasum position and a relatively simple technique of the omentum fixation. A possible drawback is a more difficult palpation and decopression of the abomasum, and the abomasal reduction in case the rumen is very full and a possibel postoperative damage of the omentopexy (particularly in animals with high body condition whose omentum is infiltrated with fat). Another method is left laparotomy with abomasopexy (the Utrecht method). The advantage of this method is that it enables to peform abomasal wall adspection and rumenotomy (if indicated). A disadvantage is a more difficult placing of the stay suture in the less distended abomasum, and it is more difficult to achieve the bottom of the abdominal cavity in large patients, and intestinal loops might be injured when the Gerlach needle moves in the abdominal cavity.

A relatively new method of left abomasal displacement management is ventral laparoscopic abomasopexy (according to Janowitz), described in 1998. This method enables laparoscopic control of the abomasal decompression, reduction and fixation. The laparoscopic method shows a minimum invasiveness and therefore it is less painful for the animal that may resume a high performance sooner. The success rate of this method is about 98 %, it is fast and safe (mainly due to a visual guidence of the tools). Unlike the laparotomy techniques, there is no need to use a systemic antibiotic therapy. Thus, this method is more cost-effective because there is no need to discard milk and medical costs are lower. Laparoscopy can be used for any left abomasum displacements, i.e. for different degrees of filling the abomasum with gas or fluid, or with different rumen sizes. The visual guidance enables to reveal adhesions between abomasum and rumen or abdominal wall or other pathologies in the abdomen. In the studies comparing the laparotomic technique based on omentopexy with laparoscopic abomasopexy, high success rates were found (about 90 %). The laparoscopic method, however, is faster, has less postoperative complications and cows recover faster and achieve higher performance. A higher feed intake has been reported after a laparoscopic surgery and an earlier assumption of normal serum levels of glutamate dehydrogenase and total bilirubin (4, 5, 8, 9). The most common posoperative complication with the Janowitz method is the wound infection that, however, can be easily managed, and a possible recurrence when the fixation ligature is torn apart (8).

The aim of this study was to verify the method of ventral laparoscopic abomasopexy according to Janowitz under field conditions and investigate its effect on postoperative production performance in the dairy cow.

MATERIAL AND METHODS

The study included 60 Holstein cows from 3 herds in the Czech Republic with a diagnosed left abomasal displacement. The cows were on 1st to 7th parity, 5 to 90 postpartum. Before the surgery, in most animals ketoneuria was found, decresed feed intake, retarded ruminal function and decreased in milk yield.

In 43 patients, a concurrent disease was diagnosed at the time of surgery. 17 cows suffered from endommeritis, in 11 cows had retained secundina, 4 cows suffered from digital disorders, 2 from mastitis, 1 had had a cesaerian section, and 8 were diagnosed severe peritonitis perioperatively. Of the latter 8 cows with peritonitis, 4 had had an unsuccessful omentopexy previously, in 3 cows, the cause of peritonitis was a severe puerperal endometritis, and in 1 cow (3 months postpartum) we did not manage to find the cause of peritonitis.

The abomasal displacement was managed by the Janowitz method in all the cows, using the Dr. Fritz laparoscope.

The operative procedure was as follows: Sites for the insertion of trocar and scope were shaved, washed and desinfected and local infiltration with 2 % lidocain was performed. The site for laparoscope insertion was located in the left flank a palm width ventrally from costal processes and a palm width caudally from the last rib. The site for the trocar insertion was located in the last intercostal space, a palm width ventrally from costal processes. In the middle, between the two sites, a short skin incision was made. Through the incision at the laparoscope site the Veress needle was inserted in the abdominal cavity through which the air was insufflated in the abdomen. When the abdomen was sufficiently distended, the Veress needle was removed and the laparoscope end was inserted in the abdomen. Then the left side of the abdominal cavity was scoped and the displaced abomasum was identified and the prensence of adheions was excluded. Then a long trocar was inserted in the abdominal cavity through the incision in the last intercostal space that was subsequently under the laparoscopic control inserted in the abomasal lumen. Subsequently, the abomasum was decompressed and a toggle with a double fibre was inserted in it in such a way that the fibre remained sticking outside the abomasum in the abdominal cavity. Then the cow was placed in right lateral recumbency and a rolled to dorsal recumbency. Then the sites on the bottom of the abdomen were prepared and insensitized, and laparoscope and forceps were inserted. These sites were located a palm width laterally from the midline, cranially from the naval. After the insertion of laparoscope and forceps in the abdominal cavity, a fibre protruding from the abomasum was grasped by the forceps and pulled through the laparoscope channel out of the abdominal cavity. The cow was turned to the right lateral recumbency and by pulling the fibre the abomasum was repositioned and pulled tightly to the abdominal wall. The abomasum was fixed in this position by pulling the ends of double fibre through the roll of bandage and the ends were knotted. All the insertion

sites were treated with antibiotic spray.

The postoperative care included oral infusion with probiotics and glycerol, fluid thrapy with hypertonic solution of sodium chloride, and one cow received the vitamin-mineral infusion. The cows with retained secundina, peritonitis and mastitis were given systemic antibiotics. After 3 or 4 weeks the stay sutures fixing the abomasum were cut off.

After the surgery, the health status was monitored (rectal temperature, rumen auscultation, analysis of ketone bodies in urine with diagnostic stripes).

The return to milk production was monitored by watching the values received from the milk parlour software. Losses in patients in 30 days after the surgery were assessed.

RESULTS

In all the cows under study, a successful abomasal reduction and subsequent abomasopexy were performed. Mean length of the surgery was 45 minutes. The ruminal function was normal in most cows already the next day after the surgery. During five days after the surgery, the urine analysis values became normal.

During the 30 days of monitoring, 9 animals were lost (15 %). 6 of them were cows affected with perioperatively diagnosed peritonitis, and in spite of a massive use of antibiotics we did not manage to save them and they died soon after the operation. Another cow developed clinical paratuberculosis in the postoperative period and she was culled. One cow was accidentaly trapped between the bars and got strangled, 5 days after the operation. The cow that had undergone the caesarian section and then ventral laparoscopic abomasopexy was culled due to a low performance.

Out of total 60 cows under study, 51 cows (85 %) were returned to full production. Mean performance of these cows before the surgery was 16.3 liters (from 0 to 28 liters). 1 month after the surgery, the milk yield increased to 32.6 liters (from 15 to 45 liters).

DISCUSSION

The success rate we achieved (85 %) is lower than that reported in the reference studies. It can be explained by the fact that 43 out of 60 animals under monitoring were suffering from a concurrent disease during the surgery. The success rate was negatively influenced by the fact that the monitored group included 8 cows with peritonitis, in 4 of which an unsuccessful laparotomy had been performed before the laparoscopy. From 17 cows that did not suffer from a concurrent disease, none were lost.

The benefits of the laparoscopic method for the management of left abomasal displacement can be seen in the fact that is is possible to shorten the duration of surgery to about 30 minutes. This approaches the duration of percutanneous abomasum fixation. As compared with the latter method, the laparoscopy enables visual control in the abdomen. This technique can be easily performed under field conditions. A disadvantage is a higher price of the device. This fact, with respect to the size of Czech herds, is not necesarrily a decisive factor. When using the laparoscopic technique, were consider necessary to treat concurrent diseases such as endometritis, mastitis, hoof diseases. General treatment should be aimed at the optimizing of hydratation and acid-base balance, curing of ketosis and protective treatment of the liver.

REFERENCES

1. Ames, N. K., 1987: Left displacement in dairy cows. *Agri-Practice*, 8, 11–16.

2. Dirksen, G., 1967: Gegenwärtiger Stand Diagnostik, Therapie und Prophylaxe der Dislocacio abomasi sinistra des Rindes. *Dtsch Tierärztl Wochenschrift*, 74, 626–633.

3. Begg, H., Whiteford, W. A., 1956: Displacement of the abomasum in the cow. *Vet. Rec.* 68, 122–125.

4. Grymer, J., Sterner, K. E., 1982: Perkutaneous fixation of left displacement abomasum, using a bar suture. *J. Am. Vet. Med. Assoc.*, 180, 1458–1461.

5. Harvey, D., Bouchard, E., Cecyre, A., 1984: Abomasopexie chez la vache par la technique „Navette" Résultants cliniques *Méd. Vét. Québec*, 14, 95–99.

6. Janowitz, H., 1998: Laparoskopische Reposition und Fixation des nach links verlagerten Labmagens. *Tierärztl. Prax,* 26 (G), 308–313.

7. Markusfeld, O., 1986: The Association of displaced abomasum with various periparturient factors in dairy cows. A retrospective study. *Prev. Vet. Med. A.*, 172–183.

8. Seeger, T., Kumper, H., Failing, K., Doll, K., 2006: Comparison of laparoscopic-guided abomasopexy versus omentopexy via right flank laparotomy for the treatment of left abomasal displacement in dairy cows. *Am. J. Vet. Res.,* 67, 472–478.

9. Tithof, P. K., Rebhun, W. C., 1986: Complications of blind-stitch abomasopexy: 20 cases (1980 - 1985). *JAVMA*; 189, 1489–1492.

MONITORING CERTAIN METABOLIC PARAMETERS FOR PREDICTION OF STILLBIRTH IN DAIRY COW

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ABSTRACT

During the last decades all over the world there is a declining trend in conception and calving rates in high yielding dairy Holstein cows. At the same time the gradual increase of stillbirth rates especially in Holstein heifers has until recently received relatively little attention. Birth weight and sex of calf, parity and age of dam and season of calving are all factors associated with calving difficulties and stillbirths. Other causes of stillbirths not related to calving difficulties are, for example, infections (e.g. Bovine Virus Diarrhoea), insufficient placenta development, metabolic disorders of the cow, and congenital malformations of the calf. There is also a management part of the problem. This is further supported by a study in which 67 stillborn calves from Holstein heifers in 41 farms were examined post mortem in order to find possible reasons for stillbirths. Malformations were found in only approximately 5 % of the calves while 46 % had signs of calving difficulties (trauma). One third (32 %) of stillborn calves were clinically normal and full-term and without any signs of calving difficulties. It was concluded that only half of the stillborn calves could be explained as caused by calving difficulties.

The objective of the study was to investigate the possible effect of subclinical metabolic disorders on the incidence of stillbirth in dairy cattle on a large scale dairy farm in Hungary.

MATERIALS AND METHODS

In the first experiment 94 dairy cattle were sampled three times in the periparturient period (at dry-off, 3 weeks prior to expected calving, and < 1 h after calving). In the second experiment 86 dairy cattle were sampled two times in the periparturient period (at 3 weeks prior to expected calving and < 1 h after calving). Blood parameters measured were as follows: glucose, -hydroxy-butyrate (BHB)-, non-esterified fatty acid (NEFA)-, urea /UN/-, total protein (TP)-, albumin (ALB)-concentration, AST-activity, and -carotene (-C)-level. Urine parameters were as follows: pH, and net acid base excretion (NABE). The following physiological values were used for the comparisons: glucose: 3.0–3.9 mmol.l^{-1} (it should be > 2.3 mmol.l^{-1}), BHB: < 0.8 mmol.l^{-1}, NEFA: < 0.2 mmol.l^{-1}, UN: 3.3–5.0 mmol.l^{-1}, TP: 65 – 85 g.l^{-1}, ALB: 39–43 g.l^{-1}, AST: < 80 U.l mmol.l^{-1-1}, β-C: > 5.6 μmol.l^{-1}, urine pH: 7.8 – 8.4, NABE: >100 mmol.l^{-1}. Differences from the normal values were evaluated by percentage changes and analysis of variance.

RESULTS

Comparing the data of the cows with normal calving (Group 1, n = 159) and stillbirth (Group 2, n = 21) only NEFA-concentration 1 h after calving showed 12.8 % difference between the two groups which reached a significant level ($P < 0.05$). While > 5 % difference could be detected between the normal and the stillbirth groups regarding BHB (5.1 %), UN (5.5 %), ALB (5.5 %) and β-C (5.2 %) values at 1 h after calving. In contrast, higher TP values could be detected in the normal cows (10.8 %) than those in the stillbirth group ($P > 0.05$).

CONCLUSIONS

Changes in the NEFA concentration in the prepartum period may be one of the reasons for stillbirth in dairy cows however more frequent samplings are needed to confirm its effects. Other metabolic parameters measured in our studies showed no effect on the stillbirth rate.

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ENERGY PROTEIN METABOLISM AND FERTILITY IN THREE CATTLE BREEDS IN TIROL

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ABSTRACT

One major cause for poor fertility is the disturbed energy metabolism around parturition characterised by increased FFA (free fatty acids) and reduced insulin as well as IGF-1 concentrations. IGF1 is an indicator of energy supply and plays a key role in follicle maturation and ovulation. Several cattle breeds in Tyrol have also reached high milk yields. However, their fertility is better than the fertility in HF cows (1). Therefore our investigations aimed at characterising the peripartal energy and protein metabolism in three cattle breeds in Tyrol.

MATERIALS AND METHODS

For the investigations, 254 cows of the breeds „Fleckvieh“ (FV), „Braunvieh“ (BV) and „Grauvieh“ (GV) were examined 1–2 weeks before plus 1 and 4 weeks after parturition during autumn with the start of stable housing as well as during spring at the end of stable housing. The food intake was mainly grass, grass silage and also pellets. In blood serum the following was tested: FFA, ß-0H-Butyrate (BHB), cholesterol, glucose, bilirubin, protein, albumin, urea, creatinine, AST, GLDH, GGT, AP as well as CK. Additionally, insulin, IGF-1 and haptoglobin was tested in 67 non (nms; physiological FFA and BHB-concentrations) and metabolic stressed (ms; highest FFA and BHB concentrations) FV-, BV- and GV-cows.

RESULTS

The FFA-, insulin- as well as IGF-1- concentrations in nms and ms cows are shown in the table (medians). The table also contains the milk yield per year, period of inter parturition and insemination index.

Although, ms FV and BV cows have with ca. 8000 $kg.year^{-1}$ a high milk yield, the IGF-1-concentrations increase 1 week p.p. ($P < 0.05$) and remain significantly higher 4 weeks p.p.

In contrast to that, in HF cows fed a diet based on corn silage we found IGF1- concentrations around 30–40 $pg.ml^{-1}$ before parturition whilst after parturition the level was below detection limits (10 $pg.ml^{-1}$) (1). Parameters of the protein metabolism show no significant differences between nms and ms cows. Enhanced IGF-1-concentrations clearly explain the better fertility state in FV- and BV-cows.

State	parameter	Fleckvieh weeks after parturition			Braunvieh weeks after parturition			Grauvieh weeks after parturition		
		- 1-2	1	4	- 1-2	1	4	- 1-2	1	4
nms	FFA	170	**195**	**180**	**111**	**198**	155	220	**330**	290
ms	µmol.l^{-1}	395	**920**	**390**	**260**	**610**	230	290	**575**	198
nms	insulin	0.14	0.10	0.16	0.14	**0.08**	0.12	0.16	0.13	0.14
ms	nmol.l^{-1}	0.13	0.07	0.05	0.08	**0,06**	0.07	0.14	0.08	0.10
nms	IGF-1	**40**	**52**	**58**	58	94	48	41	45	38
ms	pg.ml^{-1}	78	118	98	92	118	60	58	83	56
		MY/a	DO	FSC	MY/a	DO	FSC	MY/a	DO	FSC
nms		7081	**58**	1.93	**6308**	**83**	**1.78**	5024	65	1.14
ms		7994	**154**	2.02	**8130**	**138**	**2.25**	5293	77	1.40

MY/a: milk yield/a; DO: days open; FSC: first service conception rate; bold = contemporary samples nms:ms $P < 0.05$

CONCLUSION

The cattle breeds FV, BV and GV which mainly received green fodder plants and silages, respectively, in the stable are more fertile than HF-cows with diet based on corn silage. The main difference occurs in IGF-1; its concentration does not decrease peripartal, but increases under the circumstances mentioned earlier.

REFERENCES

1. Fürll, M., Hädrich, G., Heckel, F. et al., 2006: Beziehungen zwischen peripartalem Stoffwechsel sowie fruchtbarkeitsrelevanten Funktionen. *Slov. Vet. Res.,* 43, Suppl 10, 154–157.

PUERPERAL CHANGES IN INTRAUTERINE PRESSURE IN DAIRY COWS AFTER STILLBIRTH

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ABSTRACT

Decreased mechanical activity of the uterus during delivery may play an important role in the pathogenesis of stillbirth in dairy cows. A decline in intensity and frequency of uterine contractions prior to parturition may lead to a prolonged delivery. This delay may increase the risk of perinatal mortality of the offspring. It might be hypothesized that the mechanical function of such uterus also differs from optimal after calving. Therefore, we examined the possible relationship between foetal death and the mechanical activity of the uterus in dairy cows, based on alterations in intrauterine pressure (IUP) early postpartum.

At a large-scale dairy cattle farm, eight puerperal Holstein-Friesian cows after stillbirth of their singletons were included into the recordings of IUP (Group 1). Although three of these cows had shed their foetal membranes within 12 hours after parturition (NRFM, no retention of foetal membranes), and the remaining five cows suffered with retention even 36 hours postpartum (RFM, retained foetal membranes), we considered them as members of the same group. Twelve healthy NRFM dams with vital offsprings (Group 2) were used as controls. We started the non-invasive digital IUP-recordings (with 4 Hz sampling frequency) 14 to 17 hours after calving, for which we used an open tip catheter system. We continuously recorded 4 hours, and using 12-hour breaks, further 1 hour periods twice (Labview 5.0; National Instruments). During analysis, we gained various IUP parameters (contraction frequency, FREQ; amplitude or intensity, AMP; duration, DUR; mean area under the curve, AUC; total area under the curve, TAUC) using an individual curve analysis procedure. By using a portable acid-base and electrolyte analyzer (ABL77, Radiometer), we measured blood Ca^{2+} concentrations from the samples taken before the first and at the end of all recordings. For the statistical analysis we used Student t-test and repeated measures analysis of variance.

We found that although FREQ and AMP from stillbirth cows 14 to 17 hours after calving did not differ from those of the controls, and they were significantly higher at 26 and 38 hours (FREQ: $P < 0.05$ and AMP: $P < 0.01$). We could not find similar significant differences in AUC or DUR. Based on the time elapsed from parturition, the means of all parameters, except those of DUR, resulted in various significant differences. Based on the concentrations of Ca^{2+} before the first recordings, two RFM cows and one NRFM cow showed hypocalcaemic levels.

During the early postpartum period of cows with stillbirth, mechanical activity of the uterus was more intensive, despite the concomitant decline in blood Ca^{2+} concentrations in three cows. Therefore, our hypothesis that stillbirth occurred due to a prolonged parturition as a result of a decreased prepartal uterine motility is very unlikely, however further investigations are needed to clarify more details.

Key words: dairy cow; intrauterine pressure; puerperium; stillbirth

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CHRONOLOGICAL APPEARANCE OF APOPTOSIS DURING PREIMPLANTATION DEVELOPMENT OF BOVINE, RABBIT AND MOUSE EMBRYOS

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ABSTRACT

Apoptosis (programmed cell death) is self-directed process based on a genetic mechanism and characterized by complex cascade of biochemical events. This study was undertaken to obtain information about chronological appearance and incidence of spontaneous and induced apoptosis during preimplantation development of bovine, rabbit and mouse embryos.

After reaching appropriate developmental stages (2-cell to blastocyst), preimplantation embryos were cultured *in vitro* with either no supplementation or with apoptotic inductor (bovine embryos with staurosporin for 24 h, rabbit and mouse embryos with actinomycin D for 10 h) at standard conditions. Then they were subjected to morphological staining and evaluated by fluorescence microscopy. The identification of apoptotic cells was based on the morphological assessment of nuclei, the detection of specific DNA degradation in the nucleoplasm (TUNEL assay) and the immunochemical detection of active caspase-3 in the cytoplasm.

The occurrence of spontaneous apoptosis during early development of embryos (from the 2-cell stage to the morula stage) was sporadic and its presence was observed only at stages following the embryonic genome activation (in 4-cell and later embryos in mouse, in 8-cell and later embryos in cow and in morulas in rabbit). The susceptibility of embryos at early stages to apoptotic inductor was also relatively low. Nevertheless, the presence of staurosporin or actinomycin D caused negative effect on embryo growth and triggered earlier appearance of some apoptotic features (at 4-cell stage in bovine and rabbit embryos).

On the opposite, majority of untreated bovine, rabbit and mouse embryos at blastocyst stage contained at least one apoptotic cell. The incidence of spontaneous apoptosis reached 8.8 %, 1.4 % and 6.6 % of apoptotic cells per embryo, respectively for bovine, rabbit and mouse blastocysts. The presence of apoptotic inductors significantly decreased the average number of blastomeres ($P < 0.001$) and increased the incidence of apoptotic cells in blastocysts of all evaluated species ($P < 0.001$).

The results show that apoptotic processes in preimplantation embryos are species- and stage-specific.

Key words: apoptosis; cow; mouse; preimplantation embryo; rabbit

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NEW ENDOCRINE REGULATORS OF BOVINE OVARIAN FUNCTIONS

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ABSTRACT

Progress in reproductive biology, biotechnology, veterinary and human medicine and animal production depends of the new knowledge concerning regulation of reproductive functions. Most efficient regulators of long-term physiological events including reproduction are hormones, growth factors and related substances. This is the short review of the results of our and our partners studies of „classical“ and „alternative“ regulators of bovine ovarian functions. The aim of our study was to identify biological active substances produced by reproductive system, involvement of these substances in control of basic ovarian functions (proliferation, apoptosis, secretory activity, follicle-, oo- a embryogenesis) and intracellular mediators of these effects.

Our experiments demonstrated expression, accumulation and secretion of steroid and peptide hormones, prostaglandins, growth factors and their binding proteins, receptors for hormones, cyclic nucleotides, proliferation-, apoptosis-related intracellular substances, protein kinases and transcription factors by bovine ovarian cells. Experinments with hormonal treatments showed, that basic ovarian functions (proliferation, apoptosis, secretory activity, follicle-, oo- a embryogenesis) are controlled not only by classical gonadotropins, steroid hormones and prostaglandins, but also by less known nonapeptide hormones oxytocin and its analogues, by growth hormone, prolactin, indoleamines serotonin and melatonin, growth factors IGF-I, EGF and their binding proteins. Hormonal treatments resulted changes in accumulation of cyclic nucleotides and some protein kinases. Pharmacological blockade or activation of production or action of these intracellular substances substantially changed basic ovarian functions and modified effects of peptide hormones and growth factors. These observations demonstrates, that hormones regulate ovarian functions through protein kinases.

These new molecules and pharmacological regulators of their intracellular mediators could be useful for characterisation, prediction and regulation of bovine ovarian functions, as well as for treatment of reproductive disorders in reproductive biology, biotechnology, veterinary medicine and animal production.

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RISK ASSESSMENT OF METRITIS AND CONSEQUENCES OF PUERPERAL METRITIS FOR SUBSEQUENT METABOLIC STATUS, REPRODUCTION AND MILK YIELD IN DAIRY COWS

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ABSTRACT

The objective of this study was to determine some metabolic and other factors predicting the risk of metritis, and the effect of puerperal metritis (PM) on metabolic status, reproduction and milk yield were analysed. A total of 105 Holstein-Friesian cows were included, and sampled on day < -14 prepartum and 4, 10-14, 28-35 and 56-63 postpartum for metabolic tests. From day 4, the development of metritis, and from days 28-35 the ovarian activity was monitored. When grade ≥1+ ketonuria was present on day 4 postpartum, this indicated a higher probability of metritis [odds ratio (OR) 2.64; P < 0.05] including PM occurring on days 10-14 (OR: 2.65; P < 0.05). Plasma non-esterified fatty acid (NEFA) concentrations > 0.200 mmol/l in days < -14 prepartum indicated a higher risk of metritis (OR: 3.44; P < 0.05). The odds of metritis increased, depending on whether a body condition score (BCS) loss of ≥ 1.0 occurred between days < -14 and 28-35 (OR: 2.82; P < 0.05], between days < -14 and 10-14 (OR: 4.79; P < 0.01) or between days 10-14 and 28-35 (OR: 10.81; P < 0.01). PM was more probable (OR: 27.3; P < 0.001) in cows with retained placenta. The risk of metritis was lower in multiparous than in primiparous cows (OR: 0.29; P < 0.01). PM increased the risk of ovarian inactivity between days 28-35 (OR: 2.83; P < 0.05). Cows affected with PM (PM+ cows) showed lower milk production on day 4 (kg; P < 0.05) and lower milk production (P < 0.05), milk fat and milk protein production (kg; P < 0.01; P < 0.01) in the first 100 days of lactation than did PM- cows.

Key words: dairy cow; metabolic status; metritis; milk production; reproduction; risk indicators

UTERINE CONTAMINATION IN COWS WITH PUERPERAL METRITIS, CLINICAL ENDOMETRITIS AND IN COWS WITHOUT SYMPTOMS OF THESE DISEASES

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ABSTRACT

Comparison of uterine bacterial spectra in cows with puerperal metritis and clinical endometritis diagnosed by standard clinical examination as well as in cows without symptoms of these diseases represents the object of this trial. In addition, resistance to usual intrauterine antibiotics was tested. Evaluation of the secret manually obtained from vagina and transrectal palpation of uterus were performed in 37 cows on day 10±3 post partum within the framework of puerperal metritis diagnosis on the one hand and, on the other hand, in 78 cows on day 25 ± 3 post partum as part of clinical endometritis diagnosis on a dairy farm. The cows were divided into groups CPM (cows without symptoms of puerperal metritis, n=13), MPM (cows with mild puerperal metritis, n=16), SPM (cows with severe puerperal metritis, n=8), CCE (cows without symptoms of clinical endometritis, n=10), MCE (cows with mild clinical endometritis, n=28) and SCE (cows with severe clinical endometritis, n=40). Bacteriological samples obtained by Uterine Culture Swab (EQUI-VET) were inserted to transport media (Amies) and they underwent aerobic as well as anaerobic culture in laboratory. A wider bacterial spectrum was found in the cows on day 10 ± 3 compared to day 25 ± 3 (6 vs. 3). Occurrence of E. coli was higher in Group SPM compared to Group SCE (2/8 vs. 0/40, P < 0.05). A. pyogenes was not demonstrated in cows not suffering from uterine inflammation, therefore occurrence of A. pyogenes was expressively higher in Groups MPM and SPM compared to Group CPM (7/16 and 6/8 vs. 0/13, P < 0.05 and P < 0.01) as well as in Groups MCE and SCE compared to Group CCE (14/28 and 18/40 vs. 0/10, P < 0.05 and P < 0.05). Generally, though insignificantly, a higher occurrence of A. pyogenes resistance to tetracycline and ampicilin was found. Higher occurrence of A. pyogenes resistance to rifampicin was found on day 25 ± 3 compared to day 10 ± 3 (10/32 vs. 0/13, P < 0.05).

Key words: bacteriology; cow; endometritis; metritis; resistance

INTRODUCTION

Puerperal metritis as well as clinical endometritis represents a major cause of sub/infertility in cows and their negative effect on subsequent reproductive performance has been evidenced (16, 10, 17, 13, 12, 14, 9, 28). Depending on diagnostic methods and various internal and external factors, the incidence of this type of postpartal disorders can be any value between 10 % and 80 % (20, 15, 25, 19, 8, 9, 7, 24). Data concerning uterine bacteria are necessary for effective treatment and prevention of these diseases. The spectrum of uterine bacteria changes in relation with postpartum interval as well as the condition of uterus (3, 10, 4, 22, 23, 7). The object of this trial was to compare uterine bacterial spectra both in cows with puerperal metritis and clinical endometritis, as well as in cows without clinical symptoms of these diseases..

MATERIALS AND METHODS

A total of 115 postpartum dairy cows (Holstein) were used in the study. Thirty seven cows were examined on day 10 ± 3 with the aim to diagnose puerperal metritis and 78 cows were examined on day 25 ± 3 post partum with the aim to diagnose clinical endometritis. The examination included manual withdrawal of secret from vagina and transrectal palpation of uterus. The cows were divided into experimental groups on the basis of clinical findings. Thus groups were established as follows: CPM (cows without symptoms of puerperal metritis - normal lochia, n=13), MPM (cows with mild puerperal metritis - purulent lochia, n=16), SPM (cows with severe puerperal metritis – putrid lochia, n=8), CCE (cows without symptoms of clinical endometritis - clean mucus, complete involution of uterus, n=10), MCE (cows with mild clinical endometritis – muco-purulent secret, complete or incomplete involution of uterus, n=28) and SCE (cows with severe clinical endometritis - purulent secretion, incomplete involution of uterus, n=40). Uterine swabs for bacteriological examination (Uterine Culture Swab, EQUI-VET) were aseptically collected from each cow, the samples were immediately inserted in the transport media (Amies) and they underwent aerobic as well as anaerobic culture in laboratory. Bacterial resistance to tetracycline, oxytetracycline, rifampicin, cephalotin, ampicilin, ceftiofur and florfenicol was assessed.

The differences among the groups were evaluated using Chi-square test.

RESULTS

Wider bacterial spectrum was found in the cows on day 10 ± 3 compared to day 25 ± 3 (6 vs. 3). Occurrence of E. coli was higher in Group SPM compared to Group SCE (2/8 vs. 0/40, P < 0.05). A. pyogenes was not demonstrated in cows not suffering from clinical symptoms of uterine inflammation, therefore occurrence of A. pyogenes was expressively higher in Groups MPM and SPM compared to Group CPM (7/16 and 6/8 vs. 0/13, P < 0.05 and P < 0.01) as well as in Groups MCE and SCE compared to Group CCE (14/28 and 18/40 vs. 0/10, P < 0.05 and P < 0.05) (Tables 1 and 2).

Table 1. Occurrence of uterine bacteria in experimental groups CPM (cows without symptoms of puerperal metritis), MPM (cows with mild puerperal metritis) and SPM (cows with severe puerperal metritis) on day 10 ± 3 post partum

	CPM$^{n=13}$ (%)	MPM$^{n=16}$ (%)	SPM $^{n=8}$ (%)
A.pyogenes	0^{ab}	43.75^{a}	75*
Bacillus spp.	46.15	12.5	25
E.coli	23.08	0	25
Proteus mirab.	15.38	6.25	12.5
E.coli hem.	15.38	12.5	0
Staph. CN	0	12.5	12.5

^{a} – P < 0.05; ^{b} – P < 0.01

Table 2. Occurrence of uterine bacteria in experimental groups CCE (cows without symptoms of clinical endometritis), MCE (cows with mild clinical endometritis) and SCE (cows with severe clinical endometritis) on day 25 ± 3 post partum

	CCE$^{n=10}$ (%)	MCE $^{n=28}$ (%)	SCE $^{n=40}$ (%)
A.pyogenes	0^{ab}	50^{a}	45^{b}
Bacillus spp.	20	7.14	7.5
E.coli	0	0	0
Proteus mirab.	0	0	0
E.coli hem.	0	0	0
Staph. CN	10	7.14	5

^{a} – P < 0.05; ^{b} – P < 0.05

Generally, though insignificantly, a higher occurrence of A. pyogenes resistance to tetracycline and ampicilin was found. A higher occurrence of A. pyogenes resistance to rifampicin was found on day 25 ± 3 compared to day 10 ± 3 (10/32 vs. 0/13, P < 0.05) (Table 3).

Table 3. Percentage of cows with Arcanobacter pyogenes resistance to defined antibiotics on days 10 ± 3 and 25 ± 3 post partum

	Day 10 ± 3 pp $^{n=13}$	Day 25 ± 3 pp $^{n=32}$
Rifampicin	0^{a}	31.3^{a}
Tetracykline	53.8	28.1
Oxytetracykline	7.7	37.5
Cephalotin	7.7	25
Ceftiofur	7.7	0
Florfenikol	7.7	0
Ampicilin	38.5	31.3

^{a} – P < 0.05

DISCUSSION AND CONCLUSION

Puerperal metritis and clinical endometritis present an important issue in bovine reproduction. Although many methods of diagnosis of uterine inflammation have been described (vaginal inspection or palpation, transrectal palpation, ultrasonography, bacteriology, cytology, histology, measurement of body temperature, determination of certain parameters in peripheral blood such as the number and activity of polymorphonuclear leucocytes, the occurrence of specific proteins as haptoglobin or α_1 acid-glycoproteid, concentration of 13,14-dihydro,15-keto-$PGF_{2\alpha}$ or the level of interleukin-6), very few of them are applicable in practice. Thus vaginal and rectal examinations have remained as the most important diagnostic methods. Transrectal palpation is not an accurate method for diagnosis of puerperal metritis because it completely lacks clear distinction between a normal and an affected uterus during 14 days post partum. Therefore, vaginal inspection or palpation and evaluation of lochia samples taken manually represent methods which are most often applied in diagnosing puerperal metritis A purulent or fetid character of lochia clearly demonstrates the disease (6, 27, 7). Non-specific symptoms such as anorexia, drop in milk yield, dehydration and pyrexia can help to diagnose puerperal metritis. However, these symptoms are not sufficiently accurate because they are present only in some cases (24). The signs of systemic illness we found only sporadically. These were the reasons why we considered vaginal examination and evaluation of lochia on the day 10 ± 3 pp to be a sufficiently accurate diagnostic method for puerperal metritis in this field trial. Later the progress of uterus involution can be evaluated more accurately by transrectal palpation. Nevertheless this method is not sufficiently accurate because uterine involution varies among cows due to an existing relation with various factors, except uterine inflammations. Moreover, evaluation of uterine involution based on transrectal palpation is subjective (24). But examination of vaginal content for presence of pus is considered as an accurate and a practical method for diagnosis of clinical endometritis (2, 21, 13, 24). Therefore transrectal palpation as well as vaginal palpation aimed at manual withdrawal of exudates was also used for diagnosis of clinical endometritis on day 25 ± 3 post partum in our trial.

The main causative microorganisms associated with uterine inflammation in cows seem to be *A. pyogenes* and *E. coli*, also

including various bacteria such as Bacillus spp. and G- anaerobes (4, 29, 11, 18, 7). The results of several trials confirmed the coexistence and interaction among causative pathogens such as *A. pyogenes, E.coli*, G-anaerobes especially *F. necrophorum* and *Bacteroides melaninogenicus* (26, 3, 10, 1, 29, 4, 18). In our trial *A. pyogenes* was not demonstrated in cows not suffering from clinical symptoms of uterine inflammation and we found a higher occurrence of *E. coli* in cows with severe puerperal metritis on day 10 ± 3 compared to cows with severe clinical endometritis on day 25 ± 3 post partum. There is lack of data on resistance to usual intrauterine antibiotics. We found generally, though insignificantly, a higher occurrence of *A. pyogenes* resistance to tetracycline and ampicilin. A higher occurrence of *A. pyogenes* resistance from rifampicin was found on day 25 ± 3 compared to day 10 ± 3. Interpretation of these results must be carried out with causion because resistance depends, among other factors, on local epidemiological situation as well as on the kind of antibiotics used in observed herds.

In conclusion, bacterial spectrum in bovine uterus seems to be an important factor influencing the genesis of inflammation. The bacterial spectrum is being reduced simultaneously with the development of uterine inflammation and it is larger in the acute, compared to the chronic, stage of the disease. *Arcanobacterium pyogenes* represents the most important pathogen in puerperal metritis as well as clinical endometritis in cows. More data on bacteria resistance to usual antibiotics in practice are desirable.

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REFERENCES

1. Bekana, M., Jonsson, P., Ekman, T., Kindahl, H., 1994: Intrauterine bacterial findings in postpartum cows with retained fetal membranes. *Zentralblatt fur Veterinarmedizin*, 41, 663–670.

2. Bretzlaff, K., 1987: Rationale for treatment of endometritis in the dairy cow. *Veterinary Clinics of North America.* Food Animal Practice, 3, 593–607.

3. Dohmen, M. J. W., Lohuis, J. A. C. M., Huszenicza G., Nagy, P., Gacs, M., 1995: The relationship between bacteriological and clinical findings in cows with subacute/chronic endometritis. *Theriogenology,* 43, 1379–1388.

4. Dohmen, M. J. W., Joop, K., Sturk, A., Bols, P. E., Lohuis, J. A. C. M., 2000: Relationship between intra-uterine bacterial contamination, endotoxin levels and the development of endometritis in postpartum cows with dystocia or retained placenta. *Theriogenology*, 54, 1019–1032.

5. Drillich, M., 2006: An update on uterine infections in dairy cattle. *Slovenian Veterinary Research*, 43, 11–15.

6. Drillich, M., Schroder, A., Tenhagen, B. A., Heuwieser, W., 2005: Efficacy of a treatment of retained placenta in dairy cows with prostaglandin F2 alpha in addition to a local antibiotic treatment. *Deutsche* Tierärztliche *Wochenschrift,* 112, 174–179.

7. Földi, J., Kulcsár, M., Pécsi, A., Huyghe, B., de Sa, C., Lohuis, J. A. C. M., Cox, P., Huszenicza G., 2006: Bacterial complications of postpartum uterine involution in cattle. *Animal Reproduction Science*, 96, 265–281.

8. Garcia, M. E., Quintela, L. A., Taboada, M. J., Alonso, G., Varela-Portas, B., Diaz, C., Barrio, M., Becerra, J. J., Pena, A. I., Herradon, P. G., 2003: The effect of metritis in reproductive performance in dairy cows. *Archivos de Zootecnia*, 52, 409–412.

9. Gilbert, R. O., Shin, S. T., Guard, CH. L., Erb, H. N., Frajblat, M., 2005: Prevalence of endometritis and its effects on reproductive performance of dairy cows. *Theriogenology*, 64, 1879–1888.

10. Huszenicza, G., Fodor, M., Gacs, M., Kulcsar, M., Dohmen, M. J. W., Vamos, M., Porkolab, L., Kegl, T., Bartyrik, J., Lohuis, J. A. C. M., Janosi, S., Szita, G., 1999: Uterine Bacteriology, Resumption of Cyclic Ovarian activity and Fertility in Postpartum Cows kept in Large-Scale Dairy Herds. *Reproduction in Domestic Animals*, 34, 237–245.

11. Kaczmarowski, M., Malinowski, E., Markiewicz, H., 2004: Influence of various treatment methods on bacteriological findings in cows with puerperal endometritis. *Pol. J. Vet. Sci.*, 7, 171–174.

12. Kim, I. H., Kang, H. G., 2003: Risk factors for postpartum endometritis and the effect of endometritis on reproductive performance in dairy cows in Korea. *J. Reprod. Dev.*, 49, 485–491.

13. LeBlanc, S. J., Duffield, T. F., Leslie, K. E., Bateman, K. G., Keefe, G. P., Walton, J. S., Johnson, W. H., 2002: Defining and diagnosis postpartum clinical endometritis and its impact on reproductive performance in dairy cows. *Journal of Dairy Science*, 85, 2223–2236.

14. Maizon, D. O., Oltenacu, P. A., Grohn, Y. T., Strawderman, R. L., Emanuelson, U., 2004: Effect of diseases on reproductive performance in Swedish Red and White dairy cattle. *Preventative Veterinary Medicine*, 66, 113–126.

15. Markusfeld, O., 1987: Periparturient traits in seven high dairy herds. Incidence rates, association with parity, and interrelationship among traits. *Journal of Dairy Science*, 70, 158–168.

16. Nakao, T., Moriyoshi, M., Kawata, K., 1992: The effect of postpartum ovarian dysfunction and endometritis on subsequent reproductive performance in high and medium producing dairy cows. *Theriogenology*, 37, 341–349.

17. Noakes, D. E., Parkinson, T. J., England, G. C. W., 2001: *Arthur´s Veterinary Reproduction and Obstetrics* (8th ed). W. B. Saunders, London, 868 s.

18. Olson, J. D., Ball, L., Oetzel, G. R. (1987): Metritis and Pyometra. *J. Soc. Therio.*, 14, 65–65.

19. Peeler, E. J., Otte, M. J., Esslemont, R. J., 1994: Recurrence odds ratios for periparturient diseases and reproductive traits of dairy cows. *Br. Vet. J.*, 150, 481–488.

20. Sagartz, J. W., Hardenbrook, H. J., 1971: A clinical, bacteriologic and histologic survey of infertile cows. *J. Am. Vet. Med. Assoc.*, 158, 619–622.

21. Sheldon, I. M., Noakes, D. E., 1998: Comparison of

three treatments for bovine endometritis. *Veterinary Record, 142,* 575-579.

22. Sheldon, I. M., Dobson, H., 2004: Postpartum uterine health in cattle. *Anim. Reprod. Sci.*, 82/83, 295-306.

23. Sheldon, I. M., Rycroft, A. N., Zhou, C., 2004: Association between postpartum pyrexia and uterine bacterial infection in dairy cattle. *Vet Rec.*, 154, 289-293.

24. Sheldon, I. M., Lewis, G. S., LeBlanc, S., Gilbert, R. O., 2006: Defining postpartum uterine disease in cattle. *Theriogenology*, 65, 1516-1530.

25. Stevenson, J. S., Call, E. P., 1988: Reproductive disorders in the periparturient dairy cow. *J. Dairy Sci.*, 71, 2572-2583.

26. Watson, E. D., 1989: In vitro function of bovine neutrophils against Actinomyces pyogenes. *Am. J. Vet. Res.*, 50, 455-458.

27. Williams, E. J., Fischer, D. P., Pfeiffer, D. U., England, G. C. W., Noakes, D. E., Dobson, H., Sheldon, M., 2005: Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. *Theriogenology,* 63, 102- 117.

28. Youngquist, R. S., Threlfall, W. R., 1997: *Current Therapy in Large Animal Theriogenology.* W.B. Saunders Company, Philadelphia, 898 s.

29. Zerbe, H., Ossadnik, C., Leibold, W., Schuberth, H. J., 2001: Influence of Escherichia coli and Arcanobacterium pyogenes isolated from bovine puerperal uteri on phenotypic and functional properties of neutrophils. *Vet. Microbiol.*, 79, 351-365.

EVALUATION OF CHANGES IN METABOLIC HORMONES AND METABOLIC INDICES DURING PHYSIOLOGICAL AND PATHOLOGICAL COURSE OF PUERPERIUM IN LACTATING COWS

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ABSTRACT

The metabolic hormones and metabolites are important mediators of reproduction and nutrition status of the cows. A total of 20 Slovak Holstein spotted breed multiparous cows were used for the study. Animals were allocated in two groups; first dairy cows with physiological course of puerperium (G_1) (n=11) and second group dairy cows with pathological course of puerperium (G_2) (n=9). Milk production in cows was 8568 ± 428 kg.year^{-1}. Blood samples for analysis of IGF-I, insulin, non-esterified fatty acid (NEFA), aspartat-aminotransferase (AST), alanin-aminotransferase (ALT),glucose and total cholestrerol (TCH) were collected twice weekly from 21 days antepartum. to 56 days postpartum. Significantly lower concentrations of IGF I in G_2 were found only during first and second weeks in postpartum period period (89.675 ± 11.5112 ng.ml^{-1} vs. 105-58 ± 10.76 ng.ml^{-1} and 77.433 ± 7.226 ng.ml^{-1} vs. 91.5516 ± 10.0875 ng.ml^{-1}) ($P < 0.05$) Within five weeks in postpartal period were recorded significantly higher concentrations of insulin in G_1 (average 3.676 ± 0.08 ng.ml^{-1} vs. 3.12 ± 0.18 ng.ml^{-1}) ($P < 0.01$). Were found higher concentrations of NEFA in G_2 during eight weeks postpartum opposite G_1 within eight weeks postpartum (average 0.51± 0034 mmol.l^{-1} versus 0.34 ± 0.029 mmol.l^{-1}) ($P < 0.01$). Were recorded significantly higher concentrations of AST during four weeks postpartum in G_2 (average 1.86 ± 0.0315 µkat.l^{-1} vs. 1.67 ± 0.024 µkat.l^{-1}) ($P < 001$). During late pregnancy and transition period have recorded significantly changes of metabolites concentrations between cows with different course of puerperium.

Key words: cow; energy balance; metabolic indices; puerperium

INTRODUCTION

Many studies have described that reproductive disorders are associated with postpartum metabolic diseases (2, 1, 5) These data suggest that postpartum diseases which are correlated with nutrition or imunne function during late pregnancy have effect on occurence of another metabolic and reproduction diseases (3). The metabolic hormones and metabolic parameters are

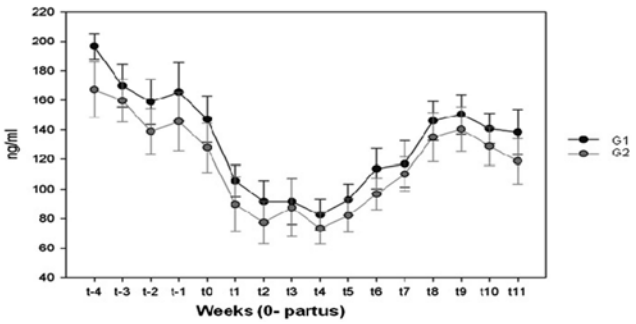

Fig. 1. Dynamic concentrations of IGF in cows with physiological (G1) and pathological (G2) course of puerperium

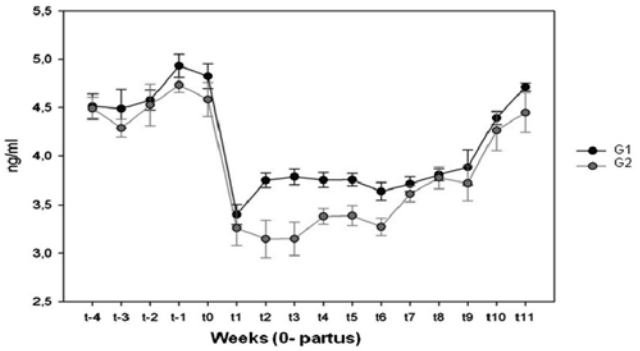

Fig. 2. Dynamic concentrations of INSULINE in cows with physiological (G1) and pathological (G2) course of puerperium

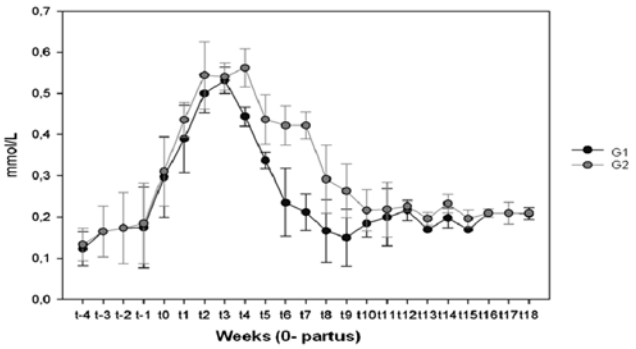

Fig. 3. Dynamic concentrations of NEFA in cows with physiological (G1) and pathological (G2) course of puerperium

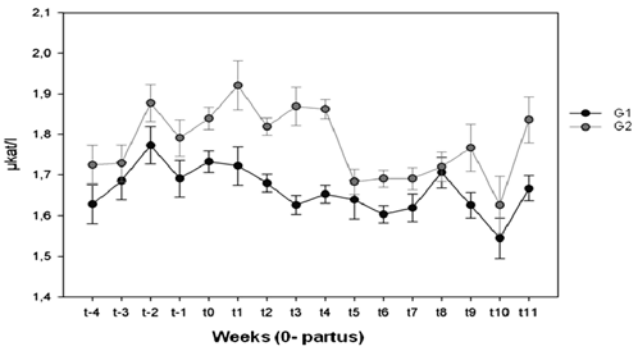

Fig. 4. Dynamic concentrations of AST in cows with physiological (G1) and pathological (G2) course of puerperium

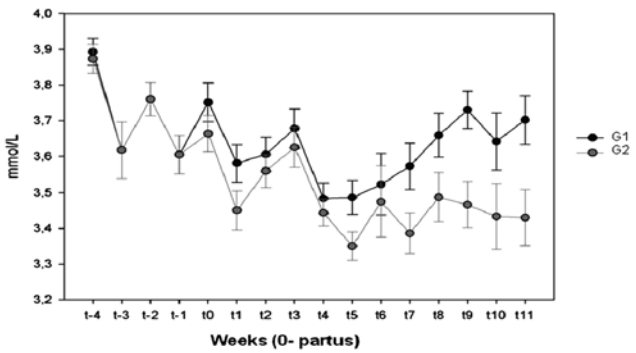

Fig. 5. Dynamic concentrations of GLUCOSE in cows with physiological (G1) and pathological (G2) course of puerperium

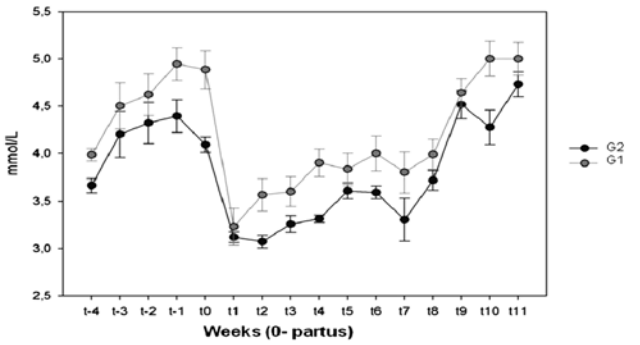

Fig. 5. Dynamic concentrations of CHOLESTEROL in cows with physiological (G1) and pathological (G2) course of puerperium

important mediators of reproduction and nutrition status of the cows..In high milk production cows and also in cows with negative energy balance the concentrations of insulin, IGF-I, NEFA and glucose have been significant signals of quality of nutrition and reproduction efficiency in the cows (6). IGF-I, cholestrerol, phospholipid, AST have evaluated in cows with retained placenta and endometritis versus physiologically course of perperium (7, 4) The aim of this study was determine metabolic hormons and metabolic indices during physiologically and pathologically course of puerperium in lactating cows.

MATERIALS AND METHODS

A total of 20 Slovak Holstein spotted breed multiparous cows were used for the study.Animals were allocated in two groups; dairy cows with physiologically course of puerperium (G_1) (n=11) and second group dairy cows with pathologically course of puerperium (G_2) (n=9) (retained of placenta, endometritis). Milk production in cows was 8568 ± 428 kg.year^{-1}. Body condition score (BCS) was assesed in same operator in 5 scale (0-poor and 5-obesity) of BCS. Cows had from 3.5–4 scale of BCS in opening of experiment. Blood samples

for analysis of IGF-I (insulin-like growth factor I), insulin, NEFA (non-esterified fatty acids), TG (triglycerides) aspartat-aminotransferase (AST), alanin-aminotransferase (ALT), total cholestrerol (TCH), and glucose were collected twice weekly, from 21 days prepartum to 56 days postpartum. IGF-I was determined by radioimmunoassay (RIA) using human kits S-2143 (Linco ltd.) validated for bovine analysis. Insulin was analysed by ELISA (set EIA 2340, Roner) and metabolites by automatic biochemical analysis (ALIZE,Lisabio) with diagnostic test firms Mérieux and Randox. NEFA was determined in blood serum by spectrophotometry (SPECOL 211, Carl Zeiss Jena). All data were compared by statistical Student *t* test.

RESULTS AND DISCUSSION

There are few reports showed mechanisms of relationship of postpartal reproductive diseases based on metabolic status in late pregnancy and transition period in lactating cows (9, 8, 4). Were recorded higher IGF I concentrations in postpartum in cows with physiological course of puerperium. Significantly lower concentrations of IGF I in cows with pathological course of puerperium were found only during first and second weeks in postpartum period (89.675 ± 11.5112 ng.ml^{-1} vs. 105.58 ± 10.76 ng.ml^{-1} and 77.433 ± 7.226 ng.ml^{-1} vs. 91.5516 ± 10.0875 ng.ml^{-1}) ($P < 0.05$) (Fig. 1). The concentrations of IGF-I in cows with retained placenta and endometritis tended to be lower than in cows without postpartum diseases (5). Within five weeks in postpartal period were recorded significantly higher concentrations of insulin in cows with physiological course of puerperium (average, 3.676 ± 0.08 ng.ml^{-1} vs. 3.12 ± 0.18 ng.ml^{-1}) ($P< 0.01$) (Fig. 2). Subclinical imbalance of metabolic during late pregnancy is risk factor of postpartum diseases (5; 2). During period of negative energy balance increase of NEFA concentrations in first week post partum (10) Were found higher concentrations of NEFA in G_2 opposite G_1 within eight weeks postpartum (average 0.51± 0.034 mmol.l^{-1} vs. 0.34 ± 0.029 mmol.l^{-1}) ($P < 0.01$) (Fig. 3). Were recorded significantly higher concentrations of AST during four weeks postpartum in G_2 opposite G_1 (average 1.86 ± 0.0315 µkat.l^{-1} vs. 1.67 ± 0.024 µkat.l^{-1}) ($P < 0.01$) (Fig. 4). Concentrations of glucose have tended non significantly higher in G_1 versus G_2 during four weeks postpartum (average, 3.52 ± 0.054 mmol.l^{-1} vs. 3. 41± 0.43 mmol.l^{-1}) but next four weeks were significantly higher in G_1 versus G_2 (average, 3.61 ± 0.07 mmol.l^{-1} vs. 3.32 ± 0.045 mmol/l) ($P < 0.01$) (Fig. 5). Were found non significantly differences between goups in concentrations of total cholesterol but have tended higher levels within postpartum in G_1 opposite G_2 (Fig. 6). The concentrations of total cholesterol were lower in cows with postpartum diseases (8, 3). During late pregnancy and transition period have recorded significantly changes of metabolites concentrations between cows with different course of puerperium.

CONCLUSIONS

Significantly lower concentrations of IGF I in G_2 were found only during first and second weeks in postpartum period period (89.675 ± 11.5112 ng.ml^{-1} vs. 105.58 ± 10.76 ng.ml^{-1} and 77.433 ± 7.226 ng.ml^{-1} vs. 91.5516 ± 10.0875 ng.ml^{-1}) ($P < 005$) Within five weeks in postpartal period were recorded significantly higher concentrations of insulin in G_1 (average 3.676 ± 0.08 ng.ml^{-1} vs. 3.12 ± 0.18 ng.ml^{-1}) ($P < 0.01$). We found higher concentrations of NEFA in G_2 during eight weeks postpartum opposite G_1 within eight weeks postpartum (average 0.51 ± 0.034 mmol.l^{-1} versus 0.34 ± 0.029 mmol.l^{-1}) ($P < 0.01$). We recorded significantly higher concentrations of AST during four weeks postpartum in G_2 (average 1.86 ± 0.0315 µkat.l^{-1} vs. 1.67 ± 0.024 µkat.l^{-1}) ($P < 0.01$).

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REFERENCES

1. Grohn, Y. T., Rajala-Schultz, P. J., Allore, H. G., DeLorenzo, M. A., Hertl, J. A., Galligan, D. T., 2003: Optimizing replacement of dairy cows: Modeling the effects of diseases. *Prev. Vet. Med.,* 61, 27–43.

2. Houe, H., Ostergaard, S., Thilsing-Hansen, T., Jorgensen, R. J., Larsen, T., Sorensen, J. T., Agger, J. F., Blom, J. Y., 2001: Milk fever and subclinical hypocalcaemiaan evaluation of parameters on incidence risk, diagnosis, risk factors and biological effects as input for a decision support system for disease control. *Acta Vet. Scand.,* 42, 1–29.

3. Ishikawa, Y., Nakada, K., Hagiwara, K., Kirisawa, R., Iwai, H., Moriyoshi, M., Sawamukai, Y., 2004: Changes in interleukin-6 concentration in peripheral blood of pre- and post-partum dairy cattle and its relationship to postpartum reproductive disease. *J Vet. Med. Sci.,*; 66, 1403–1408.

4. Kikukawa, S., Asaga, M., Ishikawa, Y., Nakada, K., Moriyoshi, M., Sawamukai, Y., 2002: Relationships between changes in peripheral IGF-1 concentration in prepartum and the crisis of ovarian and uterine disease in postpartum dairy cattle. *J Reprod. Dev.*, Suppl. 48, 151.

5. LeBlanc, S. J., Herdt, T. H., Seymour, W. M., Duffield, T. F, Leslie, K. E., 2004: Peripartum serum vitamin E, retinol, and beta-carotene in dairy cattle and their associations with disease. *J Dairy Sci.,* 87, 609–619.

6. Lucy, M. C., 2003: Mechanismus linking nutrition and reproduction in postpartum cows. *Reprod. in Domestic Ruminants,* 61, 415–417.

7. Nakada, K., Ishikawa, Y., Matsumoto, Y., Yamamoto, T., Moriyoshi, M., Sawamukai, Y., 2004: Effects of feeding and environmental change during dry period on homeostasis maintenance mechanism and its relationships to postpartum reproductive disease in dairy cattle. *J Reprod. Dev.,* Suppl. 50, 81.

8. Nakada, K., Ishikawa, Y., Sato, T., Moriyoshi, M., Sawamukai, Y., 2005: Relationship between peripartum peripheral immunoglobulin concentrations and occurence of reproductive disorders in cows. *J Rakuno Gakuen Univ.,* 30, 5–14.

9. Rajala, P. J, Grohn, Y. T., 1999: Effects of dystocia, retained placenta, and metritis on milk yield in dairy cows. *J Dairy Sci,* 81, 3172–3181.

10. Rizos, D., Kenny, D. A, Griffin, W., Quinn, K. M., Duffy, P., Mulligan, F. J, Roche, J. F, Boland, M. P., Lonergan, P., 2008: The effect of feeding propylene glycol to dairy cows during the early postpartum period on follicular dynamics and on metabolic parameters related to fertility. *Theriogenology,* 69, 688–699.

CONSEQUENCES OF UTERINE DISEASE ON REPRODUCTIVE PERFORMANCE OF DAIRY COWS

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ABSTRACT

Retained placenta, metritis and endometritis are diseases of immune function in the transition period, which begin at least 2 weeks prepartum. Clinical and subclinical uterine diseases in cows are associated with sub-fertility and infertility. It is characterised by longer intervals from calving to first insemination, reduce conception of affected animals, and more cows culled for failure to conceive. These effects on fertility and costs of treatment mean that uterine disease in one of the most expensive conditions challenging the dairy industry. The infertility associated will uterine disease is caused by damage to the endometrium and disruption of ovarian cyclic activity. The effects of uterine disease on ovarian function are likely mediated at multiple levels: ovary, hypothalamus and pituitary.

Key words: dairy cows; reproductive performance; uterine disease

INTRODUCTION

Parturition is a period of high risk for mother and offspring in all species, and cattle are no exception. As well as the risk of physical damage during the birth process or failure to release the placenta after parturition, there is often an upsurge of microbial injection in the cow. Before parturition the uterine lumen is sterile. During parturition, the physical barriers of the cervix, vagina and vulva are compromised providing there opportunity for bacteria to ascend the genital tract from the environment as well as the animals skin and feces. Indeed bacterial contamination of uterine lumen is almost ubiquitos in cows and notably greather than in other mammals (31, 35, 16). The greatest impact on health and productivity is associated with microbial contamination of the uterine lumen after parturition. Amongst the mammals *Bos Taurus*, and particularly dairy cattle farmed in intensive systems, commonly acquire microbial contamination of the uterus. Indeed 80-100 % of animals have bacteria in their uterine lumen within the first 2 weeks after calving. Although immune responses progressively eliminate the microbes up to 40 % of animals still have a bacterial infection 3 weeks after calving (24, 38).

Reproduction is one of the key pillars of dairy production. Many dairy herds do not achieve their targets for reproductive performance and incur substantial economic opportunity cost. Despite the fact that postpartum uterine disease is only one component of reproductive performance, and that it generally has secondary importance to insemination efficiency (19), it has traditionally occupied a substantial amount of veterinarians attention. Reproductive performance is linked the health in the weeks immediately and after calving, and timely achievement of subsequent pregnancy in turn has a substantial impact on profitability (4).

This paper reviews current information on consequences of uterine disease in dairy cows.

Incidence of uterine disease

The placenta is normally expelled within 6 h of expulsion of the calf but if still present by 24 h, it is defined as a retained placenta. The incidence of retained placenta in a herds is up to 10% (median 8.6%) but can be increased in cows with twins, after dystocia and where infectious agents are endemic. The expression of clinical uterine infection depends on the balance between factors such as the animal, immunity, the number and pathogenicity of the microbes, and the uterine environment the metritis and endometritis are associated with reduced feed intake, a more negative energy balance, and reduced immune function, and these differences are measurable beginning 2

weeks before calving, i.e., 3–7 weeks before the conditions are diagnosed (23, 41, 11, 15). Typically, 25–40 % of animals have clinical metritis in the first 2 weeks after calving, and disease persists in up to 20 % of animals as clinical endometritis.

Although the clinical signs of uterine disease such as purulent material discharging from the uterus into the vagina are readily detected, the role of subclinical uterine disease is less well characterised but is an emerging issue. Up to 50 % of cows 40–60 days after calving had neutrophils in the uterine lumen or endometrium, concomitant with inflammation of the tissues, and subclinical endometritis reduced conception rates (17, 7).

Definitions of uterine diseases

The term metritis has been used across a broad range of intervals postpartum to descicibe a variety of heterogenous conditions including puerperal metritis, endometritis, pyometra, and various subjective clinical findings that were assumed to correlate with bacterial infection of the uterus or slow uterine involution. Recently, case definitions have been proposed (37). **Puerperal metritis** is defined as an animal with an abnormally enlarged uterus and a fetid watery red-brown uterine discharge, associated with signs of systemic illness (decreased milk yield, dullness or other signs of toxaemia) and fever > 39.5 C, within 21 days after parturition. Animals that are not systemically ill, but have an abnormally enlarged uterus and a purulent uterine discharge detectable in the vagina, within 21 days after calving, may be classified as having **clinical metritis**. **Clinical endometritis** is characterised by the presence of purulent (> 50 % pus) uterine discharge detectable in the vagina 21 days or more after parturition, or mucopurulent (approximately 50 % pus, 50 % mucus) discharge detectable in the vagina after 26 days. In the absence of clinical endometritis, a cow with **subclinical endometritis** is defined by > 18% neutrophils in uterine cytology samples collected 21–33 days after calving, or > 10 % neutrophils at 34–47 days. **Pyometra** is defined as the accumulation of purulent material within the uterine lumen in the presence of a persistent corpus luteum and a closed cervix.

In particular it is important to differentiate animals with metritis from those with endometritis. Metritis is infection of the cavity, lining and deeper layers of the uterus. On the other hand, endometritis is a localised infection of the lining of the uterus, which is inflamed with white pus mixed with mucus discharging from the uterus into the vagina. The deeper layers of the uterus are not affected by endometritis, so the uterus is not much bigger than that of a normal animal. Clearly, metritis is a much more severe disease than endometritis, requiring a different therapeutic approach. Firstly, it is much more urgent to identify cows with metritis promptly and, secondly, these animals need systemic treatments to counter the uterine infection and alleviate the generalized ill-health.

The use of the term pyometra should also be differentiated from clinical endometritis. Pyometra implies accumulation of pus within the uterine lumen associated with a closed cervix and a corpus luteum. There is often a corpus luteum present in animals with endometritis but the cervix is patent, often with pus discharging from the uterus into the vagina.

Consequences of uterine disease

Clinical and subclinical uterine diseases are associated with sub-fertility and infertility. At the herd level this is characterised by longer intervals from calving to first insemination or conception for affected animals, and more cows culled for failure to conceive in a timely manner (18, 5). These effects on fertility and the costs of treatment mean that uterine disease is one of the most expensive conditions challenging the dairy industry. Furthermore, the high incidence of uterine disease in cattle compared with other domestic species suggests that there may be critical flaws in dairy cow husbandry or a fundamental problem with some breeds of cow. Thus, considerable effort needs to be made to understand the risk factors for uterine disease and the biological mechanisms underlying how the uterus is able to detect infection, respond to the microbes and how infection modulates normal uterine function.

In a typical study the first service conception rate was lower for cows with endometritis (29.8 % vs. 37.9 %), the median calving to conception interval was longer (151 vs. 119 days) and there were more animals culled for failure to conceive (6.7 % vs. 3.8 %), than unaffected animals (20). Similarly, cows with a purulent cervical discharge have lower submission rates, lower pregnancy rates and more culls for failure to conceive (25). The financial losses associated with uterine infection are dependent on the cost of treatment, reduced milk yield, and infertility.

Uterine disease such as retained placenta and uterine infection are key risk factors for the occurrence of abnormal progesterone profiles indicating delayed ovulation, cystic ovarian disease or long luteal phases (27, 33, 9, 10). The effects of uterine disease on ovarian function are likely mediated at multiple levels: ovary, hypothalamus and pituitary. Similarly, the low conception rates in cattle with subclinical endometritis or after resolution of other uterine disease are probably the consequence of disruption of endocrine pathways and physiology as well as associated with uterine inflammation (38).

It is assumed that a healthy endometrium is necessary for the nutrition of the blastocyst and embryo, and the successful establishment of pregnancy. Certainly infection with pathogenic bacteria appears to preclude conception. Furthermore, there is embryo mortality if uterine infection occurs with these bacteria after conception (34). Viruses such as bovine virus diarrhoea have a similar effect (26).

As the uterus is usually sterile, the presence of microbes or pathogen-associated molecules appears to provoke a substantial immune response. The uterine immune response is generated by immune cells within the endometrium and by the endometrial stromal and epithelial cells. Indeed, it is the epithelial cells that are the first line of defence against microbes in the uterine lumen.

Innate immunity in the genital tract is highly dependent on the expression of pattern recognition receptors (PRRs) to detect pathogen-associated molecular patterns (PAMPs). Binding of PAMPs to PRRs activates signal transduction pathways for mitogen-activated protein kinase (MAPK) and the nuclear factor-kappa B (NFkB) transcription factors, leading to secretion of prostaglandins, cytokines and chemokines (6, 22, 1). Epithelial and stromal cells express toll-like receptor 4 (TLR4),

the innate immune receptor for lipopolysaccharide (endotoxin, LPS), which is the key PAMP of the common uterine pathogen *E. coli* (12). This concept of endometrial cell expression of PRRs is supported in other species by expression of other toll-like receptors for bacteria and viruses (39, 14, 40). However, bovine cell cultures have the advantage over other species because they are free of contamination with professional immune cells (13). The bovine TLR4 signalling pathways are also emerging (3). This makes the bovine system a useful model for exploring the mechanisms of uterine disease in other mammals as well as cattle (12).

The effect of pathogen-associated molecules on uterine cells is not limited to inflammation, but also affects endocrine function. The principal hormones secreted by the endometrium are PGF2α and PGE2, respectively, and the secretion of these hormones is modulated by *E. coli* or LPS (13). The prediction from the in vitro work is that uterine disease would extend the luteal phase, which is what is observed clinically. Furthermore, exogenous PGF2α is an effective treatment for uterine disease and eicosanoids may modulate uterine immunity directly (21). In vitro, LPS stimulates progesterone secretion from mixed populations of luteal cells (including steroidogenic, endothelial and immune cell types) to a level similar to that seen with luteinising hormone (LH), but at higher concentrations LPS kills the cells (8).

A large healthy oestrogenic follicle at the time of ovulation is important for establishment of a successful pregnancy (28). However, as well as effects on luteal function, uterine infection also perturbs ovarian follicle growth and function (35). Cows with uterine disease have smaller ovarian follicles and lower peripheral plasma oestradiol concentrations. Furthermore, this might be a localised effect of uterine infection on ovarian function because when uterine bacterial growth scores were high fewer first or second postpartum dominant follicles were selected in the ovary ipsilateral to the previously gravid uterine horn than the contralateral ovary (35).

At the level of the hypothalamus and pituitary, the oestradiol-induced preovulatory LH surge is blunted when bacterial endotoxin is infused into the uterus or administered intravenously (29, 30, 2). Indeed, LPS or various intermediary cytokines such as interleukin (IL)-1 or tumour necrosis factor (TNF)- block gonadotrophin releasing hormone (GnRH) secretion and the pituitary responsiveness to GnRH pulses (32, 2, 42). However, uterine infection does not appear to affect peripheral plasma FSH concentration profiles, or ovarian follicle wave emergence.

REFERENCES

1. **Akira, S., Takeda, K., 2004:** Toll-like receptor signalling. *Nature Rev. Immunol.*,4, 499–511.

2. **Battaglia, D.F., Krasa, H. B., Padmanabhan, V., Viguie, C., Karsch, F.J., 2000:** Endocrine alterations that underlie endotoxin-induced disruption of the follicular phase in ewes. *Biol. Reprod.*, 62, 45–53.

3. **Connor,E. E., Cates, E. A., Williams, J. L., Bannerman, D. D., 2006:** Cloning and radiation hybrid mapping of bovine toll-like receptor-4 (TLR-4) signalling molecules. *Veterinary Immunology and Immunopathology*, 112, 302–308.

4. **De Vries, A., 2006:** Economic value of pregnancy in dairy cattle. *J. Dairy Sci.*, 89, 3876–3885.

5. **Esslemont, D., Kossaibati, M. A., 2002:** *The Cost of Poor Fertility and Disease in UK Dairy Herds*. Intervet UK Ltd., City, 146.

6. **Ghosh, S., May, M. J., Kopp, E. B., 1998:** NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. *Annual Rev. Immunol.,* 16, 225–260.

7. **Gilbert, R. O., Shin, S. T., Guard, C. L., Erb, H. N., Frajblat, M., 2005:** Prevalence of endometritis and its effects on reproductive performance of dairy cows. *Theriogenology*, 64,1879–1888.

8. **Grant, E. J., Lilly, S. T., Herath, S., Sheldon, I. M., 2007:** *E. coli* lipopolysaccharide modulates bovine luteal cell function. *Vet. Rec.*, 161, 695–696.

9. **Hajurka, J., 2006:** Bovine ovary-luteal phase during pregnancy and period of reproductive rest. *Veterinarstv*i, 56, 692–695.

10. **Hajurka, J., 2008:** Ovaries of cows-luteal phase after insemination. *Veterinarstvi*, 58, 52–57.

11. **Hammon, D. S., Evjen, I. M., Dhiman, T. R., Goff, J. P., Walters, J.L., 2006:** Neutrophil function and energy status in Holstein cows with uterine health disorders.*Vet. Immunol. Immunophatol.*, 113, 21–29.

12. **Herath, S., Dobson, H., Bryant, C. E., Sheldon, I. M., 2006a:** Use of cow as a large animal model of uterine infection and immunity. *J. Reprod. Immunol.,* 69, 13–22.

13. **Herath, S., Fischer, D. P., Werling, D., Williams, E. J., Lilly, S. T., Dobson, H., Bryant, C. E., Sheldon, I. M., 2006b:** Expression and function of toll-like receptor 4 in the endometrial cells of the uterus. *Endocrinology*, 147, 562–570.

14. **Hirata, T., Osuga, Y., Hirota, Y., Koga, K., Yoshino, O., Harada, M., Morimoto,C., Yano, T., Nishii, O., Tsutsumi, O., Taketani, Y., 2005:** Evidence for presence of tool-like receptor 4 system in the human endometrium. *J. Clinical Endocrinology and Metabolism*, 90, 548–556.

15. **Huzzey, J. M., Veira, D. M., Weary, D. M., von Keyserlingk, M. A. G., 2007:** Prepartum behaviour and dry matter intake identify dairy cows at risk for metritis. *J. Dairy Sci.*, 90,3220–3233.

16. **Kaczmarowski, M., Malinowski, E., Markiewicz, H., 2004:** Bacteria isolated from uterus of cows with foetal membrane retained. *Bulletin of the Veterinary Institute in Pulawy*, 48, 33–36.

17. **Kasimanickam, R., Duffield, T. F., Foster, R. A., Gartley, C. J., Leslie, K. E., Walton, J. S. et al., 2004:** Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. *Theriogenology*, 62, 9–23.

18. **Kossaibati, M. A., Esslemont, R. J., 1997:** The costs of production diseases in dairy herds in England.*Vet. J.*, 154, 41–51.

19. **LeBlanc S. J., 2005:** Overall reproductive performance of Canadian dairy cows: challenges we are facing. *Advances in Dairy Technology*, 17, 137–157.

20. **LeBlanc, S. J., Duffield, T. F., Leslie, K. E., Bate-**

man, K. G., TenHag, J., Walton, J. W. 2002: The effect of prepartum injection of vitamin E on health in transition dairy cows. *J. Dairy Sci.*, 85, 1416–1426.

21. Lewis, G. S., Wulster-Radcliffe, M. C., 2006: Prostaglandin F2alpha up regulates uterine immune defences in the presence of the immunosuppressive steroid progesterone. *Am. J. Reprod. Immunol.*, 56, 102–111.

22. Li, Q., Verma, I. M., 2002: NF-kappa B regulation in the immune system. *Nature Rev. Immunol.*, 2, 725–734.

23. Markiewicz, H., Kuzma, K., Malinowski, E., 2001: Predisposing factors for puerperal metritis in cows. Bulletin of the Veterinary Institute in Pulawy, 45, 281–288.

24. Markiewicz, H., Kuzma, K., Malinowski, E., 2004: Relation between particular hormones, cytokines and metabolites during the periparturient period in cows. *Medycyna Weterinaryjna*, 60, 356–359.

25. McDougall, S., 2001: Effect of intrauterine antibiotic treatment on reproductive performance of dairy cows following periparturient disease. *New Zealand Vet. J.*,49, 150–158.

26. McGowan, M. R., Kirkland, P. D., Rodwell, B. J., Kerr, D. R., Carroll,C. L., 1993: A field investigation of the effects of bovine viral diarrhea virus infection around the time of insemination on the reproductive performance of cattle. *Theriogenology*, 39,443–449.

27. Opsomer, G., Grohn, Y. T., Hertl, J., Coryn, M., Deluyker, H., De Kruif, A., 2000: Risk factors for post partum ovarian dysfunction in high producing dairy cows in Belgium: a field study. *Theriogenology*, 53, 841–857.

28. Perry, G. A., Smith, M. F., Lucy, M. C., Green, J. A., Parks, T. E., MacNeil, M. D., Roberts, A. J., Geary,T. W., 2005: Relationship between follicle size at insemination and pregnancy success. *Proceedings of the National Academy of Science USA*, 102, 5268–5273.

29. Peter, A. T., Bosu, W. T. K., DeDecker, R. J., 1989: Suppression of preovulatory luteinizing hormone surges in heifers after intrauterine infusions of *E. coli* endotoxin. *Am. J. Vet. Res.*, 50, 368–373.

30. Peter, A. T., Bosu, W. T. K., Gilbert, R. O., 1990: Absorption of *E. coli* endotoxin (Lipopolysaccharide) from the uteri of postpartum dairy cows. *Theriogenology*, 33, 1011–1014.

31. Regassa F., Noakes D. E., 1999: Acute phase protein response of ewes and the release of PGFM in relation to uterine involution and the presence of intrauterine bacteria. *Vet. Rec.*, 144,: 502–506.

32. Rivest, S., Lee, S., Attardi, B., Rivier, C., 1993: The chronic intracerebroventricular infusion of interleukin-1 alters the activity of the hypothalamic-pituitary-gonadal axis of cycling rats. I. Effect on LHRH and gonadotropin biosynthesis and secretion. *Endocrinology*, 133, 2424–2430.

33. Royal, M. D., Darwash, A. O., Flint, A. P. F., Webb, R., Wooliams, J. A., Lamming, G.E., 2000: Declining fertility in dairy cattle: changes in traditional and endocrine parameters of fertility. *Anim. Sci.*, 70, 487–501.

34. Semambo, D. K., Ayliffe, T. R., Boyd, J. S., Taylor, D. J., 1991: Early abortion in cattle induced by experimental intrauterine infection with pure cultures of actinomyces pyogenes. *Vet. Rec.*, 129, 12–16.

35. Sheldon, I. M., Noakes, D. E., Rycroft, A. N., Pfeiffer, D. U., Dobson, H., 2002: Influence of uterine bacterial contamination after parturition on ovarian dominant follicle selection and follicle growth and function in cattle. *Reproduction*, 123, 837–845.

36. Sheldon, I. M., Noakes, D. E., Rycroft, A. N., Pfeiffer, D. U., Dobson, H., 2003: The effect of Intrauterine administration of estradiol on postpartum uterine involution cattle. *Theriogenology*, 59, 1357–1371.

37. Sheldon, I. M., Lewis, G. S., LeBlanc, S., Gilbert, R.O., 2006: Defining postpartum uterine disease in cattle. *Theriogenology*, 65, 1516–1530.

38. Sheldon, I. M., Williams, E. J., Miller, A. N. A., Nash, D. M., Herath, S., 2008: Uterine diseases in cattle after parturition. *Veterinary Journal*, 176, 115–121.

39. Schaefer, T. M., Desouza, K., Fahey, J. V., Beagley, K. W., Wira, C. R., 2004: Toll-like receptor (TLR) expression and TLR-mediated cytokine/chemokine production by human uterine epithelial cells. *Immunology*, 112, 428–436.

40. Soboll, G., Schaefer, T. M., Wira,C. R., 2006: Effect of toll-like receptor (TLR) agonists on TLR and microbicide expression in uterine and vaginal tissues of the mouse. *Am. J. Reprod. Immunol.*, 55, 434–446.

41. Urton, G., von Keyserlingk, M. A. G., Weary, D. M., 2005: Feeding begaviour identifies dairy cows at risk for metritis. *J. Dairy Sci.*, 88, 2843–2849.

42. Williams, C. Y., Harris, T. G., Battaglia, D. F., Viguie, C., Karsch, F. J., 2001: Endotoxin inhibits pituitary responsiveness to gonadotropin – releasing hormone. *Endocrinology*, 142, 1915–1922.

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SONOMICROMETRY - A METHOD FOR OBJECTIVE MEASUREMENT OF UTERINE INVOLUTION IN CATTLE

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ABSTRACT

The pivotal factor for good fertility is an uncomplicated puerperal period. Until now it is not possible to evaluate the progress of uterine involution accurately, because there is a lack of suitable methods. Sonomicrometry is a technique utilizing piezoelectric crystals to measure distances. Each crystal is able to transmit and receive waves of ultrasound. Distances between crystals are then calculated based on the time taken for a wave to travel between a transmitting crystal and its corresponding receiver and its velocity of 1540 m.sec^{-1} in soft tissue. The dynamic resolution of these distance measurements can be as small as 0.0016 mm. Sonomicrometry has been widely used in experimental cardiovascular physiology studies, but there are also studies on gastrointestinal motility, muscle function and cervical diameter in larger animals. In a first step, we tested the sonomicrometry system *in vitro*, within a water bath. By implanting the piezoelectric crystals into the myometrium of an isolated uterus within the water bath, the maximal measurable distance was approximately 20 cm. In a second step, four crystals were implanted into the myometrium of the pregnant uterine horn via laparotomy of a heifer two weeks prior to calving. The crystals were positioned in a row on the curvature major with a distance between two crystals of approximately 10cm. Recordings started on the day of operation. They were performed daily for four hours until two weeks after parturition. In the third and fourth week after birth recordings were performed on every other day. Parallel to sonomicrometric measurements, the behaviour of the animals has been documented and saved on video. During the first week after surgery there were just moderate uterine contractions. Amplitude and frequency increased towards calving and contractions demonstrated a regular pattern and coordinated propagation, with a few short periods of uterine quiescence. Directly after expulsion of the calf, a distinct decrease by 40% of uterine length was observed. During the first week after calving uterine involution was most prominent. The uterine contractions showed a regular pattern with high frequencies and amplitudes, which decreased in the course of time. While involution continued during the second and third week after parturition, there were just irregular uterine contractions. Approximately at the beginning of the fourth week p.p., the involution was completed and uterine contractions showed a regular pattern with high frequencies and low amplitudes. In conclusion, sonomicrometry is a precise and objective method to measure uterine contractions and uterine involution during the peripartal period in cows. Using this method in the future it may be possible to evaluate the effects of different treatments on the uterus in cows during pregnancy and the puerperal period in cows.

OCCURRENCE AND SOME CONSEQUENCES OF TWIN CALVING ON HUNGARIAN DAIRY FARMS

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ABSTRACT

Four Hungarian dairy farms were examined in order to screen the occurrence and consequence of twin pregnancies in dairy cattle. Between 2002 and 2007, data were collected from twinning cows and their calves. In the 4 farms, 446 twin-calvings out of 12 995 parturitions were evaluated. During the evaluation the farms' own annual mean productions were used as control data. Data collection was based on a computer program (Riska) and local documentations.

The mean occurrence of twinning in the 4 farms was 3.4 %. The highest incidence rate was found on Farm 1 (8.2 %), while the smallest on Farm 4 (1.8 %), where the incidence was constantly the smallest during the 6-year study period. The incidence of twinnings showed a clear seasonality with higher rates between April and July. There was a close correlation between twin-calvings and parity. From the third or higher pregnancies twin-calving was more likely to occur than in younger dams. The gestation length of twin-carrying cows was shorter (4 to 8 days, depending on farm). Milk production of the cows with twins and the mean annual milk production of the farms were also compared. Our results showed that milk production of the cows with twins was higher than the farms' average. on Farm 2, this production difference was very low, but in the other 3 farms, it was more than 400 kg, so that in one of them it reached 2200 kg. Twinning also had consequences on stock replacement, as heifers originated from twin-calvings could be bred approximately 0.5–2.5 months later than those from single parturitions.

Postpartum reproductive performance showed that 33 to 54 % of the cows carrying twins developed retained fetal membranes, while it varied between 8 and 20 % among singleton dams. Cows that delivered twins needed in average more than 10 days (5 to 23 days) from calving to first AI, more than 41 days for the service period and more than 19 days for calving interval.

It can be concluded that twin-calvings have a harmful effect on the reproductive performance despite the higher milk production level of such dams. The higher frequency of calving difficulties, the need of multiple uterine treatments, the longer service period and the higher AI index are all additional cost factors.

CHANGES IN UDDERS OF COWS BELONGED TO DIFFERENT FARMS IN POLAND

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ABSTRACT

The aim of the study was to characterize the health status and occurrence of pathological changes in mammary glands as well as isolation of etiological agents of mastitis in different herds. A single clinical examinations of 2457 cows udders and bacteriological examination of 6930 aseptically taken quarter milk samples were performed in 20 farms that milked from 9 to 542 cows. At the moment of examination clinical forms of mastitis were stated in 0 % to 32.5 % of cows (7.31 % on average). Acute forms of inflammations were noted in 1.1% and chronic mastitis were noted in 2.62 % of quarters. Different degree of

damages of teat end and teat canal ranged from 0 % to 14 % of examined cows (3.54 % on average). Subclinical forms of mastitis were noted in 35.72 % quarters (range from 12.35 % to 57.7 % in particular farms) of 63.51 % of examined cows (from 36.75 % to 78.95 % in particular farms). *Staphylococcus aureus* was isolated on 80 % of farms, coagulase-negative staphylococci on 100 %, CAMP-negative streptococci on 100 %, *Str. agalactiae* on 15 %, Gram negative rods on 35 %, *Arcanobacterium pyogenes* on 10 %, yeasts on 5 % and *Prototheca spp.* on 5 % of examined farms. Predipping was applied regularly on 10% of farms and sporadically on 5% of farms and postdipping was regularly performed on 90% of farms. Full program of dry cow therapy was applied in all farms. Infections and inflammations were connected mainly with poor hygiene of udders and milking equipment and faults in milking procedures.

Key words: cows; health status; mammary glands

DYNAMIC OF HEALTH STATUS OF MAMMARY GLAND IN 154 DAIRY COWS DURING TWO MONTHS

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ABSTRACT

The study was carried out on herd of 154 dairy cows in free stabling conditions with elevated boxes technologies. The standard housing conditions were observed, and were used a milking shed. At the beginning of the experiment health status in herd was following: 21 healthy dairy cows, 68 cows with abacterial form of mastitis, 42 with potential (latent) mastitis, 10 cows with subclinical, 2 with subacute, and 11 dairy cows with the acute form of mastitis. At the end of experiment, from the group of cows with healthy mammary gland, all cows had a different form of mastitis: 9 cows abacterial, 5 potential (latent), 3 subclinical, one dairy cow subacute mastitis, and 3 cows were in dry period. Marked changes in dairy cows group with abacterial mastitis were determined: in 15 cows we found out free restoring to health, in 22 cows initial status was live, 6 cows had a latent mastitis, 9 subclinical, 1 subacute, 1 cow with acute form of mastitis, and 11 dairy cows were in drying off, if they were healed. In the work were studied the dynamic and distribution of udder health status in the dairy cows in free stabling conditions, the standard farms conditions, applying hygienic programs of milk store, and without therapy of mastitis in first moment.

Key words: bacterial pathogens; dairy cow; intramammary infection; mastitis

INTRODUCTION

Mastitis is the most common and most costly disease of dairy cattle, shared the losses about 27 % from herd. More factors, as for example changing housing conditions, are complicate in understanding of aetiology of intramammary infection (4). The several antimastitis measures, for purpose of the prevention of mastitis and the reduction of udder infection, were working-out.

All preventative measures have an identical signs centred on: standardization dairy and housing technology, correct function of the milking machine, supporting of specific and nonspecific defence of the organism, therapy of the dairy cows with other forms of mastitis, culling the chronically affected cows (1, 5). The cows herd in term of genesis of the udder infection is continuous varied complex. Unfavourable effects from the backgrounds and the continual negative effects of pathogenic bacteria influence the udder health status. These factors accelerated changes in the udder health status.

Aim of our study was, in cow herd with constant housing conditions, udder health status in cows was followed during the two months.

MATERIAL AND METHODS

The characterisation of herd: The herd was consisted from 180 (Holstein breed, Slovak spotted breed) dairy cows. Dairy cows were kept in cowshed K-167 using free stabling with the elevated boxes. In the farm they used seasonal feeding with grazing-land in the summer. Milking took place in the tandem milking shed 2 x 5 MIELE, while in the maternal ward (stall K-98 with tie stall) was used pipe line or movable can milking. The study was preformed in 154 dairy cows, kept in separated spaces of stall. Measures for the prevention of udder health status in this group of cows during the 60 days of examination not established. From this group were cull 24 chronically affected cows.

Methods of examination: Complex examination of the 154 cows was done three times during 60 days: on 0 day, 30th day, and 60th day. Individually examination consisted of:

- clinical examination of mammary gland according to IDF (Bulletin, No 211, 1987)
- examination the first portion of milk, NK test reaction
- bacteriology examination of collected milk samples (quarter samples)

Microbiology analyses were carried out according to IDF Bulletin, No. 132, 1981; STN 56 0080; STN 56 0081; STN 56 0082. Bacteriology analyses were centred on the isolation of *Staphylococcus sp., Streptococcus sp., Arcanobacterium sp., E. coli.*

Cultivation and identification of bacteria was performed on the 5 % blood agar, Medium No. 110, Baird Parker agar, Edwards, Endo agar. Colouring by Gram, catalase activity, coagulation of the rabbit's plasma, haemolysis, and the pigments production were carried out, too.

Isolated bacteria were examined by the commercial set STAPHYtest 16, STREPTOtest, ENTEROtest (PLIVA-Lachema, Brno, Czech republic), and results were evaluated using the identifying programme TNW, version 6.0 (PLIVA-Lachema, Brno, Czech republic).

Table 1. Classification of udder infection according to IDF (International Dairy Federation)

Mastitis		Examination		
		Clinical	NK test reaction	Bacteriology
Clinical	acute	+	±	+
	subacute	-/flock of casein		
	chronic	+		
Subclinical		-	+	+
Latent		-	-	+
Abacterial		+/ flock of casein	+	-
		±/ flock of casein	-	
		-	+	

RESULTS AND DISCUSSION

Abundance of the bacterial agens of mastitis, and the number of infected cows in single examination in 154 cows are presented in Table 2. Any measures were not applied on the farm (therapy of the dairy cows with mastitis, standardization dairy program, the stable disinfection), have had the complex negative effect, which consisted in increasing the incidence of mastitis from 42.2 % to 49.2 %. The number of dairy cows infected by *Streptococcus uberis* and coagulase-negative staphylococci increased. In 3 of the dairy cows was found a new infection due to *Streptococcus dysgalactiae.* On the other side, free restoring 10 dairy cows with *Arcanobacterium sp.* infection, and 5 cows infected by *Prototheca sp.* we determinated.

Table 2. Abundance of the bacterial agens of mastitis, and the number of infected dairy cows in single examination in 154 cows

Examinations day	Day 0		Day 30		Day 60	
Number of samples	154		137		130	
Diagnosis	n	%	n	%	N	%
Infected cows	65	42.2	57	41.6	64	49.2
Non infected cows	89	57.8	80	58.4	66	50.8
Streptococcus uberis	23	14.9	23	16.8	29	22.3
Streptococcus dysgalactiae	0	0.0	2	1.5	3	2.3
Proteus sp.	1	0.6	0	0.0	0	0.0
Bacillus sp.	1	0.6	4	2.9	1	0.8
Coagulase-negative stapfylococci	25	16.2	21	15.3	31	23.8
Arcanobacterium sp.	10	6.5	7	5.1	0	0.0
Prototheca sp.	5	3.2	0	0.0	0	0.0

The dynamics of udder health status in 154 cows on 0, 30th and 60th day of following are presented in table 3. On the 60th day of experiment, from the group of cows with healthy mammary gland, all cows had a different form of mastitis: 9 cows abacterial, 5 potential (latent), 3 subclinical, one dairy cow subacute mastitis, and 3 cows were in drying off.

In the group of 68 dairy cows with abacterial mastitis at the start of the experiment, after 30 day health status was following: 29 cows with abacterial, new infection was found in 24 cows, from these 8 cows had acute form of mastitis and 9 cows were rejected. On the 60th day of experiment was following status: number of healthy cows increased, but also the cows with subclinical mastitis increased. Culling the chronically affected cows increased, too.

In the cows with latent form of mastitis on 0 day, on 30th day were udder health status followed: 7 cows with healthy mammary gland, 16 dairy cows with abacterial mastitis, 8 with latent form, and in one by one cow with subclinical and subacute form of mastitis, 4 cows with acute the mammary infection. At the end of experiment 11 cows had healthy mammary gland, number of latent, subclinical and restored cows increased.

In the groups with subclinical, subacute and acute forms of mastitis at the beginning of experiment, were found improvement of udder health status, during the following. From the unfavourable initial status, only 1 cow with the acute and 3 cows with the subclinical infection was detected, at the end of following.

From 133 affected cows on 0 day of experiment, on 30th days were 117, and on 60th day were only 100 affected cows. Abacterial form of mastitis was determinate, most frequently. Higher increase in the incidence of subclinical form of mastitis in the herd, was recorded from 30th to 60th day. Prevalence of other forms of mastitis decreased during the two months of the experiment.

Table 3. Dynamics and distribution of udder health status in 154 cows on days 0, 30 and 60

	The time of examination and udder health status														
	Day 0	Day 30							Day 60						
Udder health status	Number	Healthy	Abacterial	Latent	Subclinical	Subacute	Acute	Rejected	Healthy	Abacterial	Latent	Subclinical	Subacute	Acute	Rejected
Healthy	21	3	7	7	1	1	-	2	-	9	5	3	1	-	3
Abacterial	68	6	29	11	3	2	8	9	15	22	6	9	1	4	11
Latent	42	7	16	8	1	1	4	5	11	2	13	8	-	1	7
Subclinical	10	2	4	3	-	-	1	-	3	2	1	2	-	1	1
Subacute	2	-	1	1	-	-	-	-	-	-	2	-	-	-	-
Acute	11	2	3	3	2	-	-	1	1	1	2	2	1	2	2
summary	154	20	60	33	7	4	13	17	30	36	29	24	3	8	24

Table 4. Dynamics of the incidence of abacterial mastitis in 154 cows herd on days 0, 30 and 60 of investigation

Day 0		Day 30							Day 60						
		negative			positive				negative			positive			
Udder health status	Number	Healthy	Abacterial	Latent	Subclinical	Subacute	Acute	Rejected	Healthy	Abacterial	Latent	Subclinical	Subacute	Acute	Rejected
Abacterial	**68**	**6**	**29**	**11**	**3**	**2**	**8**	**9**	**15**	**22**	**6**	**9**	**1**	**4**	**11**
Acute	9	-	4	3	0	-	2	-	3	1	-	-	1	2	1
Subacute	7	1	2	1	1	1	1	-	-	-	-	1	-	1	-
Subclinical	52	5	23	7	2	1	5	9	12	21	6	8	-	1	10

Dynamics of the incidence of abacterial mastitis in 154 cows herd on days 0, 30 and 60 of examination are presented in Table 4. Most favourable changes in the incidence of abacterial acute mastitis we recorded after 60 days of experiment. Little decrease in the incidence of the subclinical form of abacterial mastitis was determined.

From our following of dairy cows during two months resulted:

- the number of affected dairy cows increased from 42.2 % to 49.2 %
- the number of dairy cows infected by *Streptococcus uberis* increased from 14.9 % to 22.3 %
- coagulase-negative infection increased from 16.2 % to 23.8 %
- in 2.3 % of the dairy cows was found a new infection due to *Streptococcus dysgalactiae*
- *Arcanobacterium* sp. infection, and cows infected *Prototheca* sp. were not found at the end of experiment
- after 60 days in 21 healthy cows, inflammatory process was determinate
- from 68 cows with abacterial form of mastitis, 15 were free restored, in 22 inflammatory process continued, 20 cows were infected
- from 42 cows with latent mastitis – 15 were free restored, in 2 cows was abacterial form determinate, in 13 inflammatory process continued, in 9 cows the inflammatory process progressed
- in the cows with subclinical mastitis at beginning improvement of health status in 6 cows were determinate (3 cows restored, 2 with abacterial and one of these had a latent form of mastitis), persist infection were found in 2 cows and acute mastitis had one cow
- two cows came over from subacute to latent mastitis
- in the group (n=11) with acute form of mastitis was terminal status following: in 2 cows was found acute mastitis, in other cows improvement of health was recorded
- from all 154 cows, number of healthy cows increased from 21 to 30 cows

On the base of our results, can be observe, that the udder health status changed (from healthy mammary gland to acute mastitis and rejecting from herd) without changes in housing conditions during the production period.

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REFERENCES

1. Durel, L., Guiidarini, C., Moroni, P., Locateli, C., Scaccabarozzi, L., Smith-Van de Lemput, E., 2008: Prevalence Intramammary Infections in Heifers around calving in 40 Dairy Herds in the West of France. *Magyar Allatorvosor Lapja,* 130, Supplementum I., 46.

2. *International Dairy Federation Bulletin.* 1981: Laboratory Methods for use in Mastitis Work. Document No. 132, IDF Brussels 27.

3. *International Dairy Federation.* 1987: Bovine Mastitis-definition and Guidelines for diagnosis. Document No. 211, IDF Brussels, 22.

4. Malinovski E., Lassa, H., Klosowska A., Markiewicz, H., Smulski, S., 2006: Relationship between mastitis agents and somatic cell in foremilk samples, *Bull. Veterinary Institute Pulawy*, 50, 349–352.

5. Pyörälä, S., 2003: Indicators of inflammation in diagnosis mastitis. *Veterinary Research*, 24, 565–578.

6. STN 56 0080, 1983: Potravinárske výrobky. Spôsob odberu vzoriek pre mikrobiologické skúšanie.

7. STN 56 0081, 1983: Potravinárske výrobky. Príprava vzoriek pre mikrobiologické skúšanie.

8. STN 56 0082, 1983: Potravinárske výrobky. Zásady kultivácie mikroorganizmov a spôsob spracovania výsledkov pri mikrobiologickom skúšaní.

THE ROLE OF COAGULASE-NEGATIVE STAPHYLOCOCCI ISOLATED FROM BOVINE AND OVINE MAMMARY GLANDS IN SLOVENIAN DAIRY HERDS AND FLOCKS

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ABSTRACT

321 dairy cows with 912 udder quarters from nine dairy herds and 143 ewes with 278 halves from three flocks were included in the study. In bovine dairy herds 29 % of cows and 9.6 % of udder quarters were infected with coagulase-negative staphylococci (CNS). Somatic cell count in bovine milk did not significantly differ between infected and uninfected udder quarters, irrespective of the lactation number, on the contrary to ovine where the difference was significant. Bovine mammary glands, infected with CNS did not show any signs of inflammation, while in cases of infected ovine mammary glands clinical signs were considerable. Two of the most frequently isolated CNS species from bovine mammary glands were *S. haemolyticus* and *S. chromogenes*. In ovine were *S. epidermidis* and *S. caprae*. Considerable resistance rates to betalactams were observed in *S. haemolyticus*.

Key words: coagulase negative staphylococci; dairy cows; dairy sheep; intrammary infection

INTRODUCTION

Coagulase-negative staphylococci (CNS) are considered to be minor mastitis pathogens. CNS can be isolated from subclinical and clinical mastitis cases and also from other part of the udder e.g. teat skin, teat canals and ducts (5, 15, 16). CNS intramammary infections (IMI) in bovine usually cause no or mild clinical signs, however, Taponen *et al.* (15) reported 14.3 % of CNS IMI caused considerable clinical signs and Coolcs *et al.* (3) showed 29.1 % prevalence of clinical IMI.

Mastitis is also an important disease in sheep. Clinical cases are often severe; systemic signs are present and the condition is obviously painful. Clinically affected halves frequently suffer partial or complete damage and do not resume normal function. Reduced milk yield leads to decreased growth of the lambs (7, 9). Although a wide range of microorganisms may cause ovine mastitis, most cases are reported to be due to staphylococci (2). Several reports indicate that CNS are the most common cause of subclinical mastitis in dairy ewes (1, 11).

MATERIALS AND METHODS

Animals

321 dairy cows with 912 udder quarters from nine dairy herds and 143 ewes with 278 halves from three flocks were included in the study.

Milk samples

Milk samples were taken from individual udder quarter/half of cows/sheep and collected into autoclaved glass tubes following teat desinfection with 70% alcohol after discarding first streams of milk. Collected samples were kept cool and sent to a lab the same day for bacteriological determination and somatic cell counting.

Bacterial determination

Milk samples were streaked with a sterile swab within 24 h on quarter plates of washed blood agar with sheep/bovine blood and incubated at 37°C. After 48 h plates were examined for aerobic bacterial growth. Gram positive cocci were considered as *Staphylococcus* or *Micrococcus* species if they were catalase positive. Differences in hemolysin production were classified visually by an experienced observer as either -hemolysin positive or negative. The slide coagulase test was performed as described by the manufacturer of the rabbit plasma (Biokar diagnostics, France). Later on the API-Staph test (Biomerieux, Macy I´Etolle, France) was used for the final determination of CNS strains.

Somatic cell counts

Milk samples collected for the bacteriological analysis were used also for SCC. The samples were heated to 40°C in a water bath for 15 min. Later on samples were double processed in a Fossomatic 360 (A/S N. Foss Electric, Hillerod, Denmark)

Agar disk diffusion method

In bovine CNS isolates the tops of 4-5 colonies were picked up from pure culture with a sterile loop. The colonies were suspended in 5 ml of sterile physiologic saline. The inoculum turbidity was standardized to equivalent of a 0.5 McFarland standard. The entire surface of a Mueller-Hinton agar plate was inoculated using a sterile swab. Disks containing ampicillin, amoxicillin, kanamycin, linkomycin, neomycin, oxytetracycline, penicillin, cephoperazone, oxacillin, cloxacillin and cefquinom were placed using a sterile forceps onto agar surface and gently pressed down to ensure contact. Plates were incubated at 35°C for 20 h. Subsequently the diameter of the inhibition zone around each disk was measured. This procedure is conforming to the National Committee for Clinical Laboratory Standards, documents M31-A2 and M2-A7.

RESULTS

Table 1. Prevalence of CNS IMI in nine dairy herds

Herd	A	B	C	D	E	F	G	H	I	Total
Prevalence (%)	14.8	4.5	13.5	6.8	3.9	13.2	5.8	10.7	8.3	9.6

Table 1 shows prevalence of CNS IMI on a herd level. Prevalence differs from 3.9 % (Herd E) to 14.8 % (Herd A). The mean lactation of infected animals was 1.52.

Table 2. Mean somatic cell counts associated with CNS intramammary infections in cows

CNS	n	%	Mean SCC(10^3)*	Min./max. SCC (10^3)*
S. chromogenes	24	30	105.7	6/224
S. haemolyticus	23	28.8	123.7	18/302
S. simulans	9	11.2	105.4	12/415
S. xylosus	9	11.2	267.9	5/605
S. epidermidis	6	7.5	134.2	23/315
S. hyicus	5	6.3	167	4/275
S. sciuri	4	5	79.8	28/212
Total	**80**	**100**	**140.4**	**5/605**

*cells.ml^{-1} milk

Table 2 shows average number of SCC according to different CNS. SCC differs between isolates of the same CNS species as well between different CNS. Border line of 400.000 somatic cells/ml milk is exceeded in few cases. Two of the most frequently isolated CNS species from bovine mammary glands were *S. haemolyticus* and *S. chromogenes*.

Table 3. Mean somatic cell counts associated with CNS intramammary infections in ewes

CNS	n	Mean SCC (10)
S. epidermidis	22	2487
S. caprae	19	2083
S. hyicus	14	1253
S. simulans	9	930
Total	64	1878
Bacteriol. negative halves	194	92

Table 3 shows average number of SCC according to different CNS isolated from ovine mammary glands.

Table 4. Antimicrobial susceptibility of CNS isolated from bovine mammary glands

CNS	Penicillin resistance rate	Ampicillin resistance rate
S. haemolyticus	83.3 %	94.4 %
S. chromogenes	39.1 %	39.1 %

Results of the agar disk diffusion method show high resistance rate of *S. haemolyticus* and *S. chromogenes* strains against penicillin and ampicillin.

DISCUSSION

Prevalence of CNS in dairy herds in a present study was lower (Table 1) comparing to some studies (13, 14) and comparable to the others. High percentage of *S. haemolyticus* strains (Table

2) isolated from bovine mammary glands is not very common. We found only one study that reported high percentage of *S. haemolyticus* strains among CNS (8). Our results comfirm conclusions of other authors that CNS are found in younger animals, mostly heifers (5, 6). Antimicrobial resistance of CNS against betalactams is high (Table 4) and comparable to others (3). Sampimon *et al.* reported 14.6 % prevalence of CNS in mammary glands associated with high SCC. They found out that quarters infected with *S. epidermidis* and *S. simulans* had significantly higher SCC. In a present study mean SCC in affected quarters was 140 400 cells/ml in milk (Table 2) which was far below border line. Only in two isolates SCC exceeded 400 000 cells/ml. No clinical signs were observed in affected quarters nor animals. That leads to conclusion that CNS isolated from bovine mammary glands in present study did not play an important role in epidemiology of IMI.

The prevalence of CNS in ovine udder on a half level was 23 % and comparable to some studies (4, 11), but much higher then in others (10). The mean SCC in infected ovine mammary glands was similar to mean SCC caused by major mastitis pathogens in ewes and goats (4, 11) as well as in cows (12). On the contrary to bovine CNS in ewes provoked a significant immune response which could be shown in higher SCC (Table 3).

REFERENCES

1. Ariznabarreta, A., Gonzalo, C., San Primitivo, F., 2002: Microbiological quality and somatic cell count of ewe milk with special reference to staphylococci. *J. Dairy Sci.,* 85, 1370–1375.

2. Bergonier, D., Berthelot, X., 2003: New advances in epizootiology and control of ewe mastitis. *Livest. Prod. Sci.,* 79, 1–16.

3. Cools, S., Catry, B., Vanrobaeys, M., de Kruif, A., Opsomer, G., 2007: Antimicrobial resistance of staphylococci isolated out of suclinical and clinical cases of mastitis. In: *Proc. Heifer Mastitis Conference*, Ghent, Belgium, 38–39.

4. Deinhofer, M., Pernthaner, A., 1993: Differentiation of staphylococci from sheep and goat milk samples. *Dtsch Tierarztl Wochensch*, 100, 234–236.

5. De Vliegher, S., 2004: *Udder Health in Dairy Heife rs: Some Epidemiological and Microbiological Aspects.* Ghent: Faculteit Diergeneeskunde. PhD thesis.

6. Fox, L. K., 2007: Prevalence and incidence of clinical and subclinical heifer mastitis. In: *Proc. Heifer Mastitis Conference*, Ghent, Belgium, 62–63.

7. Fthenakis, G. C., Jones, J. E. T., 1990: The effect of experimentally induced subclinical mastitis on milk yield of ewes and on the growth of lambs. *Br. Vet. J.*, 146, 43–49.

8. Jemeljanovs, A., Konosonoka, I. H., Bluzmanis, J., 2007: Coagulase negative staphylococcal mastitis in dairy farms of Latvia. In: *Proc. Heifer Mastitis Conference*, Ghent, Belgium, 44–45.

9. Larsgard, A. G., Vaabenoe, A., 1993: Genetic and environmental causes of variation in mastitis in sheep. *Small Rumin. Res.*, 12, 339–347.

10. M rk, T., Waage, S., Tollersrud, T., Kvitle, B., Sviland, S., 2007: Clinical mastitis in ewes; bacteriology, epidemiology and clinical features. Acta Vet. Scand., 49 (1), 23.

11. Pengov, A., 2001: The role of coagulase-negative Staphylococcus spp. and associated somatic cell counts in the ovine mammary gland. J. Dairy Sci., 84, 572–574.

12. Podpečan, O., Zrimšek, P., Pate, M., Ocepek, M., Zdovc, I., Pengov, A., 2008: Epidemiology of *Staphyloccoccus aureus* intramammary infection in dairy cows in Slovenia. In *Proceedings of the 9th Middleeuropean buiatrics congress*, Budapest, Hungary, 157–160.

13. Sampimon, O. C., Barkema, H. W., Berends, I. M. G. A., Sol, J., Lam, T. J.G. M., 2007: Prevalence of coagulase negative staphylococci on dairy farms in The Netherlands. In: *Proc. Heifer Mastitis Conference*, Ghent, Belgium, 32–33.

14. Schukken, Y.H., 2007: CNS mastitis: nothing to worry about. In: *Proc. Heifer Mastitis Conference*, Ghent, Belgium, 21–22.

15. Taponen, S., Simojoki, H., Harveri, M., Larsen, D., Pyörälä, S., 2006: Clinical characteristics and persistence of bovine mastitis caused by different species of coagulase-negative staphylococci identified with API or AFLP. *Vet. Microbiol.*, 115, 199–207.

16. Waage, S., M rk, T., R ros, A., Aasland, D., Hunshamar, A., degaard, A., 1999: Bacteria associated with clinical mastitis in dairy heifers. *J. Dairy Sci.*, 82, 712–719.

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FRACTURE DIAGNOSIS AND TREATMENT IN SMALL RUMINANTS - RETROSPECTIVE CLINICAL STUDY OF 15 CASES (2005-2008)

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ABSTRACT

In this retrospective study medical reports (2005–2008) of 15 small ruminant patients suffering from fracture were reviewed. Species, breed, age, sex, results from clinical and orthopedic examination, radiographic findings, therapeutic measures, time until recovery and outcome were recorded. 2/15 cases were caused by blunt trauma. In 13/15 cases no clear aetiology was emerged. In the frontlimb (n=6) the following types of fractures were diagnosed: three cases of epiphyseal fracture – two in the distal radius and one in the distal metacarpal bone. Furthermore one case of radius-ulna fracture and two cases of metacarpal bone fractures were found. In the hindlimb (n=9) six cases of femur fractures, two cases of metatarsal fractures and one case of medial and lateral first phalanges fracture were reported.

Two patients were euthanized without therapy because of poor prognosis. The remaining thirteen patients were treated either by splint bandage application (n=7), osteosynthesis (n=5) or amputation (n=1). Additional measures included anti-inflammatory medication, stabling in a well-padded box with a skid-proof floor and a controlled exercise programme. Systemic antibiosis was administered in all patients with surgical therapy. 12/13 patients showed complete recovery, 1/13 was euthanized after surgery because of failure of osteosynthesis.

SURGICAL TREATMENT OF A COMMINUTED METACARPAL FRACTURE IN AN 800 KG BULL

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ABSTRACT

To describe the clinical and radiographic findings and the surgical treatment of a comminuted fracture of the metacarpus 3+4, and its long-term outcome in an 800 kg Simmental test bull from a breeding station.

MATERIALS AND METHODS

After clinical and orthopaedic examination of the symptomatic right forelimb, radiographs were taken in three different planes (dorso-palmar, latero-medial, oblique).

The fracture was treated in general anesthesia applying the

technique of percutaneous transfixation. Two 6 mm Steinmann pins were drilled into the distal radius, and the axis of the fractured metacarpus was adjusted in a physiological alignment under closed reduction. The Steinmann pins were incorporated in a full limb cast covering the limb completely from the level of the elbow to the claws (positioned in a straight axis of 180° including the radius, metacarpus and phalanges).

RESULTS

The 19-month-old bull showed a non-weightbearing right forelimb lameness, despite a "first-aid transport bandage using PVC splints". After positioning the bull on the surgical table in left lateral recumbency, the transport bandage was removed under sedation. The metacarpus showed an abnormal movement within the bone shaft, an abnormal axis alignment, crepitus and a large soft tissue swelling without skin injuries. After radiography, a closed comminuted mid-metacarpal fracture was diagnosed.

First change of cast was done 5 weeks after the surgery. The Steinmann pins were removed as they had become loose due to a slight superficial bone infection around the pins. Radiographs already showed a moderate callous formation. From this day on, only a full limb cast was applied for fracture fixation. The cast was supported by an iron splint incorporated in its lateral wall.

Twelve weeks after the surgery, only the cranial half of the cast was retained. An exercise program was introduced where the bull was made to walk twice a day for 10 minutes each. After 14 weeks, a Robert Jones bandage was applied and the exercise program was continued. After 17 weeks, the fracture site showed a good and strong callus formation, and therefore only a supporting bandage was applied from the claws to the carpus.

The bull was hospitalized for a total of 131 days. At the breeding station, first mounting of the bull was 33 weeks after the surgery. It yielded 900 portions of best quality semen with two services. Now, two and a half years after the treatment, the bull still gives good breeding performance.

CONCLUSIONS

Due to economic reasons, comminuted metacarpal fractures in adult and heavy dairy cows are rarely an indication for surgical intervention. However, for highly valuable patients like ours, a surgical treatment of such a fracture with a primarily bad prognosis should be attempted. In valuable breeding bulls, the costs of the treatment and a 33-week recovery period are of secondary importance to the owner because if the surgery is successful then the high treatment and recovery costs will be soon amortized through semen production.

SEPTIC ARTHRITIS OF THE FETLOCK JOINT IN CATTLE - DIAGNOSIS AND TREATMENT USING ARTHROTOMY AND JOINT LAVAGE

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ABSTRACT

To describe the clinical, radiographic and ultrasonographic findings, treatment protocol and outcome of septic arthritis of the fetlock joint in 32 cattle (1998–2008).

MATERIAL AND METHODS

The case records of 32 cattle (1 week to 7.5 years old) suffering from septic arthritis of the metacarpophalangeal and metatarsophalangeal joint were reviewed retrospectively (1998–2008). We evaluated the age and breed of the animals, the cause and duration of the joint infection, the clinical, radiographic and ultrasonographic findings, involvement of other synovial cavities of the affected digit, the results of arthrocentesis and cytology of the aspirated fluid. Additionally, the intraoperative findings, the number of joint lavages applied in each case, the peri- and postoperative medical treatment, the time of recovery, the outcome and the reasons for euthanasia after stating the diagnosis were assessed.

RESULTS

Arthrotomy and joint lavage was carried out in 12/32 cattle under intravenous regional anesthesia applying a rubber tourniquet at the proximal metacarpus/metatarsus. The

patients were fixed in lateral recumbency on the surgical table. Arthrotomy, using one or two surgical approaches, was performed in all the cases showing a sero-fibrinous, fibrinous or fibrino-purulent arthritis. This classification of joint inflammation was carried out by the macroscopic evaluation of the synovial fluid aspirated by arthocentesis. Repeated joint lavages (3 to 10 times) were also performed under intravenous regional anesthesia. Treatment was successful in 10 of these 12 cattle. Two cattle had to be euthanized 12 and 15 days after arthrotomy due to persistent bone infection. Peri- and postoperative systemic antibiotic treatment was given for 7 to 18 days. NSAIDs were administered for a maximum of 5 days. The mean time of postsurgical recovery was 18.8 days (± 4.6). Twenty cattle showing a purulent arthritis of the fetlock with osteolysis/osteomyelitis and concurrent septic inflammations of other digital joints and/or the digital flexor tendon sheath were euthanised without treatment.

In 27 cases, the fetlock joint infection was caused by penetrating infected wounds. The mean age of the wounds at the time of first examination was 7.6 days (1 to 35 days; ± 6.3). Only in 4 cattle, the wounds were 1 to 3 days old. In 3 cattle, the fetlock joint infection developed from septic claw diseases and in 2 cattle a haematogeneous spread was responsible.

CONCLUSIONS

Treatment of septic sero-fibrinous, fibrinous or fibrino-purulent arthritis of the fetlock joint in cattle is a challenging procedure. Besides the primary surgical approach, there is need for repeated joint lavages, long-term administration of antibiotics, and special bandage techniques for immobilisation of the limb, especially for dorsally located large wounds. The cost of such a treatment is high, requiring the owners consent and readiness to take the risk. The prognosis of septic fetlock arthritis caused by penetrating wounds strongly depends on the age of the wound, and extent of involvement of the joint structures at the time of surgical intervention.

IS THERE ANY POSSIBILITY TO ASSESS LAMENESS IN TIED COWS?

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ABSTRACT

There are many publications about diagnosing lameness in cattle, but it is currently only one published article about how to assess lameness in tied cows. Although welfare standards in the future may lead to the phasing out of the tied systems especially in Middle Europe majority of cows are still in tied systems. The aim of the project was to determinate indicators for lameness in tied cows. Among the indicators was rotation of the feet, way of resting a foot, standing of the edge of a step, repeated stepping, and uneven weight bearing feet when moving sideways in the stall. Additional to direct signs we try to assess also some secondary signs as decubitus signs on skin on level of carpal, tarsal and hip joint.

One observer firstly recorded presence or absence of these indicators in 100 cows in different herds and afterwards every cow was put on the table and all eight claws were pared and controlled for pathological changes. The optimum combination of sensitivity and specificity of lameness detection in the stall was achieved by requiring at least two positive indicators to confirm lameness.

With such combination of any proposed primary and secondary indicators it was proved that lameness can be scored reliably also in tied stalls.

INTRODUCTION

There are only few publications about diagnosing and assessing lameness in tied cows. In central European countries as Slovenia and Austria still most animals are tied. Although pasture was one of very common and popular part of the system, in last years because less and less people are involved in breeding and production procedure, pasture is time and labour consuming, especially in tied cows system. Therefore it is difficult for owner and also for veterinarian to assess lameness in such conditions.

Our team try to follow protocol of some Austrian authors who try to assess lameness in tied cows with the difference that we trimmed all cows after assessment on their standing place. In housing systems where cows are generally kept tied assessing of lameness is often very difficult, due to lack of space, unsuitable layout of buildings or the fact that animals are unfamiliar or owner is not able to manage to move the cows and observe them during the movement.

During the research time we try to determine indicators for lameness in tied cows and prove all the diagnosis with correction in the booth.

MATERIAL AND METHODS

In our practice we follow process of diagnosis and afterwards the results of correction process in 100 tied cows in 37 different stalls. After the owners was alert and ask for help in their herds because of some problems they have with their animals, firstly four veterinarians made assessment of posture of animals and according to certain criteria assess potential reason for lameness of the animals.

Among the indicators was rotation of the feet, way of resting a foot, standing of the edge of a step, repeated stepping, and uneven weight bearing feet when moving sideways in the stall. Additional to direct signs we try to assess also some secondary signs as decubitus signs on skin on level of carpal, tarsal and hip joint.

Afterwards one skilled veterinarian performs a correction of all assessed animals and photographically documented the lesions found.

An assessment procedure was performed as follows:

The cow was observed standing undisturbed in the stall and presence of any indicators were noted. If the animal was lying at the time of arrival of assessors we try to raise the cow and afterwards left her for few minutes to make their normal, standard position.

The cow was also moved on the tied place left and right as far the system allows. Afterwards assessor made an assessment and put most possible diagnosis as the reason for lameness.

Every animal in the project was afterwards put in to the booth and only one veterinarian made correction with documentation of the findings.

Within all animals assessed by other three assessors an additional assessment was made just before the correction procedure and both assessment were compared. We also take data about regular claw correction in the herd and opinion of the owner about assumed percentage of lame cows in the herd.

RESULTS

Out of 100 cows in the project 86 were assessed to be lame because of some changes on a claw or at the very near of the claw. 14 animals were not diagnosed as lame.

In the 86 lame animals after correction we found in 77 cows pathological changes, which were definitively reason for the lameness. In 9 cows we did not find any pathological changes, only small deformations, which obviously lead to show some difficulties in standing and rising of the animal.

In 14 negative animals during assessment in 3 cases we found during correction procedure pathological signs, what means that those animals were false negative during the assessment procedure and no of the indicators were present at the time of assessment.

The optimum combination of sensitivity and specificity of lameness assessment in tied cows systems were presence of at least two indicators. The best indicators were definitively way of resting a foot and repeated stepping. Also finding of decubiti on skin at the level of tarsal and metatarsal joint on the outer side of affected leg was very good indicator.

In 86 % of all animals assessment was made correctly in 9 % of cases we have to deal with false positive detection and in 3 % of all cases the result was false negative. Sensitivity of the procedure is 0.9663 what actually show high liability of the procedure. Specifitiy is 0.8235 what is actualy folow up of specific population in the research.

	Diseased animals	
Animal population	**Positive**	**Negative**
Diseased animals	86	9
Healthy animals	3	14

DISCUSSION

In the research the majority of the indicators we decided proved to be specific for lameness and also in tied systems there is quite high probability to diagnose lameness when at least two indicators are positive. Secondly there is necessity to have an experienced person for assessor. The best indicator in our cases was way of resting a foot and repeated stepping. Also rotation of the foot for more than 15 degree was relatively reliable indicator of lameness. The fact is that rotation may occur as consequence of conformation or even claw overgrowth without to have any pathological changes on claw. In some false positive assessment we have to deal with this problem.

Second problem of research population was, that we did not look for lameness in healthy herds and pick out diseased animals, but we used this assessment in cases where the owner already notice some problems and we only try to prove if with only observation it is possible to diagnose what kind of changes we would find on feet. With decision for such population we actually minimise the possibility to make mistakes in assessment.

In other experiment made by group of Welfare Quality Project they suggested that weight shifting with a foot could be also result due to nervousness or other irritation. In our cases we can not agree with such statement. We agree that degree of movement in tied stalls is very limited and it can reveal a reluctance to bear weight on a foot as the cow moves sideways. Movement of the front feet cannot often be achieved, so the method is likely to underestimate or not to detect front foot lameness more than hind foot lameness.

In the case of sole ulcer on the outer claw the most reliable indicator was always standing on the edge of the step, because cows try to unload pressure on diseased point on the sole.

The final decision of the method of assessment was that all mentioned indicators are very reliable in the case we have to do with suspicion of pathological changes already at the time before visit. If all those indicators are reliable also in healthy herd where we must pick out animals suspected to be lame among haelthy population is matter of further investigation. Any method where reliability of detection is under 70 % could be questionable. Also it is necessary to produce such a method which could be performed also from relatively inexperienced veterinarian or even lay person.

In conclusion, using a combination of proposed indicators with an experienced professional, lameness can be detected reliably also in tied systems. The sensitivity of the method depends on experience of the professional and on step of development of pathological changes on foot and is in optimum constellation not worse than in the case of using methods requiring the cows to walk.

REFERENCES

1. Leach, K., Huber, J.,Whay, H., Winckler, Ch.,March, S., Dippel, S, 2008: Assessing Lameness in Tied Cows. *Proc. of 15th Symp. on Lameness in Ruminants*, June 2008, Kuopio, Finland, 126–128.

2. Winckler, Ch., Willen, S.,: The reliability and repeatability of a lameness scoring system for use as indicator of welfare in dairy cows. *Acta Ag Scand A* Supplement 30, 103–107.

CASE REPORT: TIBIAL NERVE PALSY IN 2 CALVES AND COMMUNE PERONEAL NERVE PALSY IN 1 CALF

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ABSTRACT

We report the case of 3 Holstein calves which were sent to **our clinic from a farm that has many problems with bronchopneumonia and diarrhoea in newborn calves. Despite a lot of drugs there was not even a positive effect observable. When visiting the farm we also noticed 3 calves (one male, 2 females) age ranged from 4 to 6 weeks with foot drop due to unilateral weakness of active dorsiflexion of the fetlock. The clinical examination offers that 2 calves (one male, one female) are passive but not active able to extend and to flex both the tarsal articulation and the fetlock of the right respectively the left hind limb. These clinical findings lead to the diagnosis: tibial nerve palsy. We decide to treat this calves conservatively by a cruropedious cast over 2 weeks. The additional therapy consists of using non steroidal anti-inflammatory drugs and antibiotics. After removing the cast the tibial nerve palsy seems to be nearly healed and the 2 calves have achieved active extension and flexion of the tarsal articulation and the fetlock. In the following weeks the calves get physiotherapy by leading them over rough ground a few times per day. 12 weeks after sending to our clinic the calves were released.**

The clinical examination of the third calf (female) offers an inability to extend the tarsal articulation and the fetlock and a reduced flexion of the fetlock of the left hind limb. Additionally this calf has a temperature and suffers from a phlegmone the whole hind limb. We decide to euthanize this calf due to the complications and order a diagnostic autopsy. The pathological and histopathological examination shows a commune peroneal nerve palsy. Because of knowing the effort to treat bronchopneumonia and diarrhoea by lots of drugs we suppose that the nerves palsies are probably associated with a wrong placed injection.

Furthermore all 3 calves suffered from bronchopneumonia caused by Mannheimia haemolytica and diarrhoea caused by Cryptosporidia.

Key words: calf; tibial nerve palsy; commune peroneal nerve palsy; cast

DEVELOPMENT OF THE ORTHOPAEDICS DISEASES IN COWS AT SLOVAKIA AND ACTUAL ASSESMENT

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ABSTRACT

We are presenting overview of hoof diseases of high-productive dairy cows in Slovakia and assessment of contemporary situation in relationship with farming conditions associated with intensive farming technologies. Full-grate floor (unsuitable materials and slimy surface, very abrasive surface, thin walkway surface of grates) caused intensive traumatization of horny claw and consequently corium of dairy cows. Insufficient hygiene, inadequate correction of foot increased incidence of different diseases in region of digital skin (necrobacilosis dermatitis interdigitalis) and specific traumatic inflamation of the corium.

In the case of high increase of yield of milk in different farms in connection with unkeeping of optimal technologic parameters we found changes of incidence and disease types of claws and skin of limbs (laminitis, dermatitis digitalis) of dairy cows.

We determined increased food demand. Inadequate diet of the high-productive dairy cows resulted in decreased occurrence of hoof diseases, especially diseases of claws, and reduced age of dairy cows.

We are presenting an overview of prevention principles of hoof diseases from the point of view of necessary and carefully analysis of high claw morbidity of dairy cows bred in 3 individual farms. Hoof diseases in some dairy cows breeding are today the most common causes of their early elimination from reproduction. Incidence of lameness like clinical sign of pain of dairy cows claws in monitored farms was about 25-60 %.

Functional correction of claws, desinfecting baths, routine lameness diagnosis, adequate food and sufficient hygiene of environment are the main points for reduction of economical looses because of claw diseases of dairy cows.

COMPARISON OF THE EFFECT OF FEEDING OF CRIMPED AND DRY CORN GRAIN IN HIGH PRODUCING DAIRY COWS

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ABSTRACT

The aim of the study was to measure the effect of feeding of crimped corn grain with high moisture (DM ≈ 70 %) vs. dry one on the rumen fermentation, energy, protein and acid-base metabolism, health status, reproductive performance, daily milk production and milk composition of high producing dairy cows. Kemisile 2 S was used as preservative at crimping with the dosage of 4.2 kg.t^{-1}. The experiment was carried out at a large scale dairy farm with 1000 head of Holstein Friesian cows. The milk production of the cows was 99593 litres yearly. Two hundred in calf second and third parity Holstein Friesian cows were selected randomly to *control* and *experimental* groups, 3 weeks prior to expected parturition. Rumen fluid, blood and urine samples were taken for laboratory examinations once a week in the first month and once month onwards the experiment four times. The corn silage based diet was identical for both groups except for the control animals received dry and the experimental cows crimped corn with 30 % moisture content during the trial. The *total concentration of volatile fatty acids* in rumen fluid of

the experimental cows was superior to the controls by 12.1 % on average. The *acetate ratio* of the experimental cows was superior to the controls by 13.4 % on average (P ≤ 0.0003). The *glucose concentration* in the blood samples was higher in the experimental cows than in the controls by 5.7 % on average. The *aceto-acetic acid concentration* in blood samples of the experimental cows was inferior to the controls by 46.8 % on average (P < 0.0496). The *NEFA concentration* of blood samples in the experimental cows was inferior to the controls by 22.4 % on average. The BCS loss was less in the experimental cows than in the controls. The period of calving to first heat was shorter by 1.6, calving to first AI by 6.6 and calving to conception by 4.4 days in the experimental cows. The daily milk yield of the experimental cows exceeded the control's milk production by 4.33 kg on average (P ≤ 0.001). Conclusions can be drawn as following: more favourable rumen fermentation, more balanced energy and protein metabolism, better health status, higher daily milk yield, higher milk protein concentration, better reproductive performance can be achieved by feeding of crimped corn instead of dry one. The favourable biological effect of feeding of crimped corn could be in relation with may be better digestibility and consequently better utilisation of that.

Key words: crimped corn; dairy cow; rumen fermentation; health; milk production

INTRODUCTION

In several parts of the world increasing size of the dairy farms and the demand for high production of the cows as well as the health and good reproduction performance of the herds are conditions to survive the competition is found. The expertise within the field of dairy nutrition is of importance to be able to optimize the production, health and reproduction of the animals and their welfare in the future. Due to the high incidence of metabolic diseases in connection with the modern milk production technology is it important to take nutrition and animal welfare into considerations.

MATERIAL AND METHODS

Aim of the study

The aim of the study to measure the effect of feeding of crimped corn grain with high moisture (DM ≈ 70 %) on the

- energy metabolism and rumen fermentation,
- protein metabolism
- acid-base balance,
- health status,
- daily milk production,
- milk composition,
- cost of the milk production of high producing dairy cows under field condition.

Crimping

In the late 1960's a Finnish farmer and engineer Aimo Korte developed a method of processing and storing grain with this moisture content: crimping. Crimping means rolling grain by using a roller mill, applying acid or some other preservatives to it and than putting it into storage.

For the crimping process the grain is harvested when its moisture is between 30 % and 45 % and it is at its nutritional peak. Crops will normally be at this level three to four weeks before conventional harvest date. It is also possible to harvest the grain a little bit dryer, but then some water needs to be added during preservation. As added water is absorbed slowly it is recommend combining while the moisture rate is suitable. Than the grain is rolled and the seed coat is cracked exposing the starch by passing through a hopper fed crimping machine. During this process the crimping machine adds a special preservative to the grain[1] which maximises nutrient retention in the clamp and at feed out. All common crops are suitable for crimping such as corn, barley, wheat, oat, maize, peas, beans, soy, linseed and lupines. In Scandinavia and UK they feed cattle, pigs, poultry and horses successfully with crimped grain.

Design of the experiment

Two hundred in calf second and third parity Holstein Friesian cows were selected randomly continuously to control and experimental groups with 94 head of cows in the control group and 102 animals in the experimental one, 3 weeks prior to expected parturition. The grouping was carried out just some days before starting the experiment, according to the stage of gestation of the cows. The age of the cows, previous lactation and actual BCS of cows was taken into consideration at the selection.

The duration of the experiment was 140 days.

Within the groups "group nucleus" of 13 clinically healthy cows was assigned in each group for taking rumen fluid, blood and urine samples for laboratory examinations.

The health and the nutritional status of the cows were monitored by metabolic profile test (MPT). Only clinically healthy cows were sampled. Clinically healthy cows did not show any symptom of any disease (e.g. *mastitis*, lameness, *diarrhoea* etc).

Feeding regime

The feeding regime of the control animals was the same as usual at the dairy farm during the duration of the trial. The corn grain receiving the controls was dry. The daily ration of the experimental cows was the same one as the controls except of corn grain. The experimental cows received crimped corn grain with high moisture content (DM ≈ 70 %) during the trial.

The ration composition is summarised below:

[1]In the present experiment Kemisile 2 S was used with the dosage of 4,2 kg/t

Stage of lactation Þ	Close-up		Early and peak lactation	
Feeds	Control	Experi-mental	Control	Experi-mental
Corn silage (kg)	15	15	22	22
Alfa-Alfa haylage (kg)	3	3	7	7
Grass hay (kg)	2	2		
Pea straw (kg)			1	1
Wheat straw (kg)	1	1	1	1
Wheat bran (kg)	1	1	0.9	0.9
CGF (kg)	1	1	1.5	1.5
Sunflower meal, expeller (kg)	1.6	1.6	1.3	1.3
Premix (kg)	0.2	0.2		
Dairy sprinter (Cargill conc[1], kg)			0.9	0.9
Lactoplus energy (Cargill conc, kg)			2.7	2.7
Corn grain (dry; kg)	1.4		5.7	
Crimped corn		2.0		9

Rule of sampling

Milk composition was measured according to the rule of Hungarian Milk Recording (Gödöllő, Hungary). Rumen fluid, blood and urine samples were always taken 3 hours after the morning feeding. Rumen fluid was taken by using of Dirksen tube into glass bottles and they were corked hermetically in order to avoid the slipping away of VFAs. Blood and urine samples (20 ml each) were taken from the subcutaneous abdominal vein and via metal catheter from the urinary bladder into test tubes, respectively. Samples were cooled to 4°C and transported to laboratory for further analysis. Clotting of the blood was prevented by adding of 50 µl of heparin in each test tube.

Timing of samplings

The rumen fluid, blood and urine samples were taken from the cows of "group nucleus" according to the time[2] scheduled here:

Sampling/ Groups	I	II	III	IV	V	VI	VII	VIII
Control	-2 wks	+2-5 d	+14 d	+3 wks	+30 d	+60 d	+90 d	+120 d
Experi-mental	-2 wks	+2-5 d	+14 d	+3 wks	+30 d	+60 d	+90 d	+120 d

[2]The times of sampling related to calving date. E.g. -2wks, two weeks prior to expected parturition, +14 d, 14 days after calving.

RESULTS

Rumen fermentation

The rumen fermentation was monitored by the determination of VFAs and lactic acid concentration of rumen fluid, calculation of VFAs ratio as well as the rumen fluid pH was measured.

The **rumen fluid pH** was ranging within the physiological range apart from the controls at sampling 5 and the experimental animals at sampling 6 and 8 it exceeded the upper limit of the physiological range by 2.71, 0.71 and 0.43 %, respectively Although in these cases the pH exceeded the upper limit of the physiological range but it was no remarkable and no significant. So it can be stated that the pH did not indicate ruminal acidosis or alkalosis during the trial.

The **acetate concentration** in rumen fluid of the experimental cows was superior to the controls by 26 % on average ($P \leq 0.0333$). At the 1st, 2nd, 3rd, 4th, 5th, 6th, 7th and 8th sampling the acetic acid concentration was superior to the controls by 27.8, 58.9, 27.5, 34.4, 11.6, 14.5, 31.5, 4.9 and 26.0 %, respectively, indicating more favourable rumen fermentation. The difference between these values was significant at sapling 1, 2 and 3, at $P \leq 0.04$, $P \leq 0.00001$ and $P \leq 0.01$, respectively. The acetate is one of the most important energy sources because 1 mol acetate produces 10 mol ATP in the intermediary metabolism.

The **propionate concentration** in rumen fluid of the experimental cows was inferior to the controls by 5.8 % on average. The difference in the average concentration of propionate was 1.08 mmol/l during the trial, which is not so considerable and not significant. At sampling 1, 6, 7 and 8 the propionate concentration of control animals was superior to the experimental' one by 6.6, 30.5, 27.7, and 20.8 %, respectively, and at the 2nd, 3rd, 4th and 5th sampling the propionate concentration of experimental cows was superior to the controls by 46.8, 0.8, 6.3 and 0.2 %, respectively. The difference between the ruminal propionate concentration of the two groups was significant at sapling 2 and 6, at $P \leq 0.02$ in both cases.

The **n-butyrate concentration** in rumen fluid of the experimental cows was superior to the controls by 17.0 % on average. At sampling 1, 2, 3, 4, 6, 7 and 8 the n-butyrate concentration of experimental animals was superior to the controls by 5.7, 52.9, 2.7, 57.5, 8.3, 0.8, and 23.8 %, respectively and at the 5th sampling it was inferior to the controls by 4.4 %. The difference between the n-butyrate concentration of the two groups was significant at sapling 2 and 4, at $P \leq 0.004$ and $P \leq 0.014$, respectively.

There was considerable and significant difference in the **i-butyrate concentrations** of both groups.

The **total concentration of volatile fatty acids** (TVFAs) in rumen fluid of the experimental cows was superior to the controls by 12.1 % on average. At the 1st, 2nd, 3rd, 4th, 5th, 7th and 8th sampling it was superior to the controls by 12.6, 49.2, 10.9, 25.7, 2.2, 8.1 and 3.3 %, respectively, indicating more favourable rumen fermentation, consequently more balanced energy metabolism. The difference between the TVFAs concentration of the two groups was significant at the 2nd sampling at $P \leq 0.0004$ level. At sampling 6 the TVFA con-

centration in the experimental animals was inferior to the controls by 3.6 %.

There was no remarkable and significant difference in the **concentration of valeric, i-valeric and capronic acid** of both groups.

The **acetate: propionate ratio** in rumen fluid of the experimental cows was 2.83 : 1 and in the controls 2.09 : 1 on average indicating more favourable rumen fermentation in the experimental ones because in this case the ratio is nearer to the optimal 3 : 1. The difference between the values of the two groups was significant at $P \leq 0.01$ on average.

The **acetate ratio** in rumen fluid of the experimental cows was superior to the controls by 13.4 % on average ($P \leq 0.0003$). At the 1st, 2nd, 3rd, 4th, 5th, 6th, 7th and 8th sampling it was superior to the controls by 16.4, 6.1, 14.9, 8.0, 10.8, 19.6, 23.1 and 9.5 %, respectively indicating more favourable rumen fermentation. The difference between the acetate ratio of the two groups was significant at sampling 3, 4, 5, 6, and 8 at the level of $P \leq 0.0007$, $P \leq 0.044$, $P \leq 0.01$, $P \leq 0.0002$, $P \leq 0.015$, respectively.

The **propionate ratio** in rumen fluid of the experimental cows was inferior to the controls by 17.6 % on average ($P \leq 0.0127$). At the 1st, 2nd, 3rd, 4th, 5th, 6th, 7th and 8th sampling it was inferior to the controls by 18.0, 0.6, 10.6, 18.5, 6.3, 29.5, 34.0 and 17.2 %, respectively. The difference between the propionate ratio of the two groups was significant at sampling 1, 4, 6, 7 and 8 at the level of $P \leq 0.0017$, $P \leq 0.0096$, $P \leq 0.0009$, $P \leq 0.0009$ and $P \leq 0.049$, respectively.

The **n-butyrate ratio** in rumen fluid of the experimental cows was inferior to the controls by 3.0 % on average. At the 2nd, 4th, 6th and 8th sampling it was superior and the 1st, 3rd, 5th and 7th sampling it was inferior to the controls by 1.0, 27.0, 12.2, 19.3 and 12.7, 6.2, 3.5, 6.7 %, respectively. The difference between the values of the two groups was significant at sapling 1, 4, and 8 at the level of $P \leq 0.028$, $P \leq 0.011$ and $P \leq 0.026$, respectively.

There was no remarkable and significant difference in the **concentration of valeric, i-valeric acid ratio** of both groups.

There was no detectable **lactic acid** concentration in the rumen fluid samples of both groups at all the samplings during the experiment.

Health status of the cows

The **health status** of the cows in connection with the feeding was tested by performing metabolic profile test (MPT) and data were collected concerning the reproduction performance of the cows. The MPT involved the monitoring of energy balance, revealing the subclinical fat mobilisation syndrome (fatty liver) and hyperketonaemia (subclinical ketosis) as well as monitoring the acid-base metabolism.

The **energy balance** (carbohydrate and lipid metabolism) was monitored by determination of glucose, aceto-acetic acid and NEFA concentration in blood samples, as well as the data concerning to the acid-base metabolism in urine samples were also taken into consideration because these values give indirect information on the energy metabolism. Performing the MPT subclinical hyperketonaemia was monitored by determination of aceto-acetic acid concentration in blood samples, subclinical fat mobilization syndrome was detected by measuring NEFA and AST values in blood samples.

The **glucose concentration** in the blood samples was ranging within the physiological range apart from the control cows at last sampling and it was higher in the experimental cows than in the controls by 5.7 % on average during the experiment. At the 1st, 4th, 5th, 6th, 7th and 8th sampling the glucose concentration was superior to the controls by 4.1, 15.9, 11.4, 2.8, 6.9 and 39.0 %, respectively. At the last sampling the low glucose concentration indicated *hypoglycaemia* in the control animals. The difference between the glucose concentration of the two groups was significant at the 5th ($P < 0.05$) and the 8th samplings ($P < 0.001$).

The **aceto-acetic acid concentration** of blood samples in the experimental cows was inferior to the controls by 46.8% on average during the trial ($P < 0.0496$). It remained within the physiological range at all the samplings except for the controls at sampling 2 to 5, it was remarkable high indicating *hyperketonaemia* but in spite of this fact no significant difference was found at these sampling times. The aceto-acetic acid concentration in the controls was superior to the experimental cows by 44.9, 56.4, 79.4, 70.8 % at the 2nd, 3rd, 4th and 5th sampling, respectively (Table 17 and Figure 17-18). The *hyperketonaemia* (subclinical ketosis) was diagnosed by the ≥ 0.1 mmol/l aceto-acetic acid concentration in blood plasma.

The **occurrence rate of *hyperketonaemia*** was 76.9, 42.9, 28.6, 7.1, 21.4 and 15.4 % in the control cows at the 2nd, 3rd, 4th, 5th, 7th and 8th sampling as well as 7.7, 16.7, 16.7, 7,7 and 15.4 % in the experimental animals at sampling 1, 2, 3, 7 and 8, respectively.

The **NEFA concentration** of blood samples in the experimental cows was inferior to the controls by 22.4 % on average and no significant difference was found during the trial. It was remarkable elevated in the experimental cows at the 2nd sampling and in the controls at sampling 2 to 5 indicating increased lipid mobilization. The NEFA concentration in the controls was superior to the experimental cows by 23.4, 42.9, 45.9, 37.0, 29.1 % at the 2nd, 3rd, 4th, 5th and 6th sampling, respectively. At the 1st sampling the NEFA concentration of the experimental cows was higher than in the controls.

The **AST activity value** in blood plasma exceeded the upper limit of the physiological range during the experiment, which may be caused by the relatively high concentration of copper (≈ 50 ppm) in the ration offered to all the cows. It was remarkable elevated in the experimental cows at the 6th, 7th and 8th sampling and it was superior to the controls by 73.7, 79.9 and 67.8 % at the level of $P \leq 0.0015$, $P \leq 0.0025$, $P \leq 0.0017$, respectively.

The **subclinical fat mobilisation syndrome** (subclinical fatty liver) was detected by the ≥ 0.2 mmol/ NEFA concentration and the ≥ 80 AST activity value in blood plasma.

The **occurrence rate of subclinical fat mobilisation syndrome** was 100.0, 78.6, 50.0 and 35.7 % in the control cows at the 2nd, 3rd, 4th and 5th sampling as well as 46.2, 84.6, 46.2, 23.1 and 27.3 % in the experimental animals at sampling 1, 2, 3, 4 and 5, respectively.

The acid base metabolism

The **pH value** of the blood was ranging within the physiological range during the experiment and did not show any disturbances of acid-base metabolism.

The **pCO_2, TCO_2, ABE, SBE, HCO_3** and **SBC values** were higher by ≤ 10 % than the upper limit of the physiological range, which may be caused by the inorganic buffer (sodium bicarbonate) offered ad libitum to all the cows traditionally, in order to prevent the rumen acidosis. In spite of this fact the rumen fluid pH was ranging within the physiological range apart from the 5th, 6th and 8th samplings.

The **urinary pH** was ranging within the physiological range at the 1st sampling and from the 2nd sampling onwards the experiment it exceeded the upper limit of the physiological range by 11.3 - 11.7 % on average and it ranged between 8.7 - 14.9 %.

The ≤ 100 mmol/l **NABE values** indicated moderate *acid load* in both groups of cows 2 weeks prior to expected parturition at the 1st sampling but from the 3rd sampling onwards the experiment the NABE value exceeded the 200 $mmol.l^{-1}$ in both groups, which may be caused by inorganic buffer offered to the cows.

PRACTICAL RESULTS (Farm data)

Body condition

The BCS of control cows was superior to the experimental animals by 0.1 score on average and exceeded the experimental at sampling 1-5 by 9.3, 2.6, 4.9, 6.1 and 15.2 % respectively. From the 6th sampling (two months after calving) onwards the experiment the BCS of experimental cows exceeded the control's by 3.9, 4.6 and 3.9 %. It means the BCS loss was less in the experimental cows than in the controls indicated more balanced energy metabolism.

Reproductive performance

The days from calving to first heat, days from calving to first artificial insemination (AI) and days from calving to conception were evaluated. The period of calving to first heat was by 1.6, calving to first AI by 6.6 and calving to conception by 4.4 days shorter in the experimental cows, than in the controls.

Milk production and milk composition

The **daily milk yield** of the experimental cows exceeded the control's milk production by 4.33 kg on average. The difference between the daily milk production was significant at $P \leq 0.001$.

The **butter fat** concentration in milk of the control cows was superior to the experimental animals by 0.2 % on average and the difference between them was significant at $P \leq 0.001$.

The **milk protein** concentration of the experimental cows was higher by 3.65 % than in the controls on average and the difference between them was significant at $P \leq 0.001$.

CONCLUSIONS

Evaluating and summarising the results of the study the conclusions can be drawn as following:

- &Better rumen fermentation,
- More favourable molar ratio of VFAs,
- More balanced energy and protein metabolism,
- Better health status,
- Higher daily milk yield,
- Higher milk protein concentration,
- Better reproductive performance can be achieved by feeding of crimped corn instead of dry one.

The favourable biological effect of feeding of crimped corn could be in relation with may be better digestibility and consequently better utilisation of that. The scientific verification of the better digestibility and better utilisation of the crimped corn needs further research work

The references are available at the authors if they are requested.

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METABOLIC STATUS OF HEALTHY DAIRY COWS IN LATE PREGNANCY AND EARLY LACTATION

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ABSTRACT

Aim of this work was to determine following blood serum parameters in healthy dairy cows in late pregnancy and early lactation: glucose concentration (G), non-esterified fatty acids (NEFA), triglycerides (TG) and total bilirubin concentration (Bil). Total number of 17 dairy cows, with average 3 lactations and 7625 L of milk per 305 days of lactation, were investigated during late pregnancy (7 cows, 1-4 days before parturition) and early lactation period (10 cows, 1-4 days after parturition). Blood samples were obtained from the v. jugularis, and blood serum was separated and stored at -18oC until analyzed. The statistical significance of differences between mean values and the correlation between serum metabolic parameters was determined using ANOVA, LSD test, and correlation coefficient.

Blood serum glucose, NEFA, and TG concentration decreased, while total bilirubin concentration increased significantly in healthy dairy cows during early lactation, compared to late pregnancy period. High negative correlation coefficient was determined for blood serum glucose and NEFA (r = -0.73), and glucose and TG (r = -0.56) concentrations in healthy dairy cows during late pregnancy period. During the early lactation period high positive correlation coefficient was determined between blood serum glucose and NEFA (r=0.64), and between NEFA and TG (r=0.70) concentration. Results of this investigation indicate significant metabolic changes during peripartal period in healthy dairy cows. These changes are the result of physiological adaptation to the increased mammary gland metabolic demands during early lactation period.

Key words: dairy cows; lactation; metabolic status; pregnancy

INTRODUCTION

At the onset of lactation the nutrient demand increases dramatically and faster than the increase in feed intake. Thus most dairy cows face negative energy balance (NEB) in early lactation. Most diseases in dairy cows occur during the first two weeks pp (9). Metabolic disorders are highly multi-factorial and a wide range of animal, management and feed factors may lead to such problems. Fatty liver may occur around calving when the cow is in NEB and blood levels of non-esterified fatty acids (NEFA) increase as the cow mobilizes adipose tissue. Blood profiles have frequently been used to assess nutrient status of cows in the transition period (15, 5). More recently, metabolites such as NEFA and t-hydroxybutyrate (BHB) have been added to the profiles to monitor energy balance (30). Blood profiles are considered useful to identify nutritional shortcomings even before the productivity is impaired. Such profiles have also been used to monitor herd health and to find subclinical disease, to predict risk of ketosis or abomasal displacement as well as investigate herd problems with metabolic disorders (20, 16, 18). The aim of this study was to examine some parameters of metabolic profile reflecting both energy and liver status around calving. Establishing reference values for dairy cows in this period (transition period) is a challenge. Due to great variation between methods, laboratories and cow material, reference values in literature vary.

MATERIAL AND METHOD

Total number of 17 dairy cows, with average 3 lactations and 7625 litres of milk per 305 days of lactation, were investigated during late pregnancy (7 cows, 1–4 days before parturition) and early lactation period (10 cows, 1–4 days after parturition). Blood samples were obtained from the v.jugularis 4 hours after the morning feeding and blood serum was separated and stored at -18oC until analyzed. Blood parameters studied were glucose, non-esterified fatty acids, triglicerides and total bilirubin concentration. Glucose concentration in blood determined with enzymic method specific for glucose (Dialab, Vienna, Austria). Concentration of NEFA in blood serum was determined with colorimetric method (test N0 001 INEP-ZEMUN). Triglyceride concentration in blood serum was determined using an enzymatic test kit (bio Merieux Ref.6.123.6). Total bilirubin concentration in blood serum was determined with method by Jendrossik-Grofu, using test kit Alfapanon-Novi Sad. Blood profile has been used to monitor management and herd health in dairy cows. The statistical significance of differences between mean values and the correlation between serum metabolic parameters was determined using ANOVA, LSD test, and correlation coefficient.

RESULTS AND DISCUSSION

Blood serum parameters in healthy dairy cows in late pregnancy and early lactation: glucose concentration (G), non-esterified fatty acids (NEFA), triglycerides (TG) and total bilirubin concentration (Bil) are presented in Table 1.

Table 1. Blood serum metabolic parameters (Mean±SD) in ante and peripartal dairy cows

Time of investigation	n	Glucose (mmol.l^{-1})	NEFA (mmol.l^{-1})	TG (mmol.l^{-1})	Bil mmol.l^{-1})
Late pregnancy	7	3.12 ± 042	0.54 ± 0.26	0.41 ± 0.03	4.83 ± 1.70
Early lactation	10	2.71 ± 0.35**	0.46 ± 0.10*	0.35 ± 0.04**	5.80 ± 1.05*

Legend: * P < 0.05; ** P < 0.01.

Table 1 shows that, in the last days of pregnancy in cows, the average glucose concentration in blood was x = 3.12 ± 0.42 mmol.l^{-1}. The concentration of glucose in ruminants is lower compared to monogastric animals, and according to most authors, its value ranges from 2.2 to 3.2 mmol.l^{-1}. Our results show that the concentration of glucose in the last days of pregnancy is within physiological levels and is a result of balanced metabolism which can provide normal regulation of metabolic processes in early lactation (22, 17, 11). After parturition, the average concentration of glucose in blood sera of healthy cows was significantly lower x = 2.71 ± 0.35 mmol.l^{-1}. It is thought that requirements for glucose in early lactation are higher than the levels that organism can provide in the conditions of high milk production (27, 28, 10). In the conditions of negative energy balance in the early lactation, the process of the mobilization of fatty acids from tissue depots is intensive, which leads to their depositing in liver, consequently decreasing the process of gluconeogenesis in hepatocytes. A statistically significant difference in mean values of glucose concentration in blood in the examined groups was established (P < 0.05).

Free fatty acids in blood in cows represent the most dynamic derivates of fats and their concentration in blood depends directly on the degree of lipomobilization in fat tissue. NEFA are the best indicator of the degree of energy balance and the intensity of lipomobilization from tissue depots. The average values of free fatty acids in blood in healthy cows ante partum were x = 0.54 ± 0.26 mmol.l^{-1} and were statistically different in comparison to the average values of free fatty acids (x = 0.46 ± 0.410 mmol.l^{-1}) in the blood of healthy post partum cows (P < 0.05). This is in accordance with reference data stating that concentration of NEFA in blood of dairy cows significantly increase between 17 and 2 days ante partum, only to reach the highest value on the day of parturition (3, 27). It is undoubted that the process of lipomobilization starts in late pregnancy (1).

In this period, it represents a physiological process of adaptation of organism to the new conditions of hormonal regulation and preparation for the lactation to follow. The results of investigation in our experiment show that cows in late pregnancy have a significantly higher level of NEFA in blood, compared to physiological values typical for cattle (19). In healthy cows in the first days of lactation the concentration of NEFA decreases compared to their concentration in the last days of pregnancy and, regardless of body energy demands, their concentration remains within physiological levels. Part of the increase in plasma NEFA was hormonally induced. Cows undergo tremendous endocrine changes during and immediately after calving (6). Most of these changes are also involved in adipose mobilization, such as that mediated by cytokines, catecholamines, estradiol, insulin, and somatotropin (2). Significant individual variations in NEFA concentrations in blood sera indicate that in some animals, the process of lipomobilization is very intensive in the condition of higher body energy demands.

According to numerous authors, the best indicator of negative energy balance and the degree of mobilization of fats from tissue depots in peripartal cows is the elevated concentration of NEFA in blood (7, 29, 25, 22, 26). The degree of lipomobilization is much higher in high-yielding dairy cows than it is the case in those which have no such predisposition for high milk production. In some individuals in early lactation there can be cases of diseases (fatty liver, ketosis) due to very intensive lipomobilisation, because regulatory mechanisms can no longer provide a balanced ratio between metabolic processes and the demands of mammary glands. In cows with ketosis, NEFA concentration is significantly higher than in healthy cows during pregnancy and in early lactation. NEFA values ≥ 0.5mmol/L during the first week of lactation were considered as the most suitable criterion for identifying limited adaptive performance (12). Whitaker (30) suggests a pp reference value for NEFA of < 0.7 mmol.l^{-1} day 10–20 pp.

The average concentration of triglycerides in blood sera in ante partum cows was X = 0.41 ± 0.03 mmol.l$^{-1,}$ and was

significantly statistically different ($P < 0.01$), compared to the average value of triglycerides in blood of healthy post partum cows of $X = 0.35 \pm 0.04$ mmol.l^{-1}. Numerous authors have also confirmed that in post partum period there is a decrease in the concentration of triglicerides in blood (21, 8). Positive corelation ($r = 0.70$) (Table 3) between concentrations of free fatty acids and triglyceride in blood serum was revealed in those cows with no fatty infiltration and degeneration of hepatocyte was found, which means that functional ability of liver cells in healthy cows was preserved for the turnover and metabolism of fats, hence triglycerides are not accumulating in these cells but are transported by blood up to the tissues to meet their needs. In fatty liver cows the concentration of triglycerides is very low immediately after parurition (13). The intensity of lipomobilization and the percentage of decreasing the level of triglycerides in blood can be taken as most reliable indicators of the degree of pathological changes in liver.

The concentration of overall bilirubin is a parameter in determining the metabolic profile of dairy cows in peripartal period. Every increase in bilirubin concentration in the blood of cows indicates a damage to the liver function and/or a disorder in bilirubin metabolism in the body. The average concentration of overall bilirubin in blood serum of ante partum cows was $X = 4.83 \pm 1.70$ µmol.l-1, and was significantly statistically different ($P < 0.05$) compared to the average values of overall bilirubin in healthy post partum cows, which was $X = 5.80 \pm 1.05$ µmol.l-1. In all the examined cows, the values of bilirubin concentration were within physiological levels (4, 14). Hyperbilirubinemia can indicate pathological states of the liver and is one of the reliable indicators of functional liver status in dairy cows (21, 24).

Blood serum glucose, NEFA, and TG concentration decreased, while total bilirubin concentration increased significantly in healthy dairy cows during early lactation, compared to late pregnancy period. High negative correlation coefficient was determined for blood serum glucose and NEFA ($r = -0.73$), and glucose and TG ($r = -0.56$) concentrations in healthy dairy cows during late pregnancy period (Table 2). During the early lactation period high positive correlation coefficient was determined between blood serum glucose and NEFA ($r = 0.64$), and between NEFA and TG ($r = 0.70$) concentration (Table 3).

Table 2. Correlation coefficient between metabolic parameters in late pregnancy dairy cows

	NEFA	TG	Bil
Glucose	-0.73	-0.56	-0.41
NEFA	-	0.47	0.32
TG	-	-	-0.21

Table 3. Correlation coefficient between metabolic parameters in early lactation dairy cows

	NEFA	TG	Bil
Glucose	0.64	0.28	0.49
NEFA	-	0.70	0.49
TG	-	-	0.33

Results of this investigation indicate profound metabolic changes during peripartal period in healthy dairy cows. This changes are the result of the process of adaptation to the increased mammary gland metabolic demands during early lactation period and they don't indicate a disorder in liver function.

REFERENCES

1. Adewuyi, A. A., Gruys, E., van Eerdenburg, F. J., 2005: Non esterified fatty acids (NEFA) in dairy cattle. A review. Vet Q., 27, 117–126.

2. Bell, A. W., 1995: Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J. Anim. Sci., 73, 2804–2819.

3. Betrics, S., Grummer, R. R., Valiano, C., Stodderd, E., 1992: Effect of prepartium dry matter intake on liver triglicerydе concentration and early lactation, J. Dairy Sci., 75, 1914–1922.

4. Damnjanović, Z., 1990: Koncentracija lipida i lipoproteina u krvnom serumu zdravih i ketoznih krava. Magistarska teza, Beograd.

5. Đokovic, R., Šamanc, H., Jovanović, M., Nikolic, Z., 2007: Blood concentrations of thyroid hormones and lipids and content of lipids in the liver in dairy cows in transitional period. Acta Vet. Brno, 76, 525–532.

6. Drackley, J. K., Overton, T. R., Douglas, G. N., 2001: Adaptations of glucose and long-chain fatty acid metabolism in liver of dairy cows during the periparturient period. J. Dairy Sc., 84, E Supplementum, E100–E112.

7. Gaal, T., Roberts, J., Reid, I. M., Dew, M., Coop, M., 1983: Blood composition and liver fat in post parturient dairy cows. Veterinary Record, 113, 53–54.

8. Gerloff, B., Herdt, T., Emery, R., 1986: Relationship of hepatic lipidiosis to health and performance in dairy cattle. JAVMA, 8, 845–849.

9. Goff, J. P., Horst, R. L., 1997: Physiological changes at parturition and their relationship to metabolic disorders 1, 2. J. Dairy Sci., 80, 1260–1268.

10. Greenfield, R. B., Cecava, M. J., Johnson, T. R., Donkin, S. S., 2000: Impact of dietary protein amount and rumen undegradability on intake, prepartum liver triglyceride, plasma metabolites and milk production in transition dairy cattle. J. Dairy Sci., 83, 703–710.

11. Guo, R., Peters, R., Kohn, R. A., 2007: Effect of a Transition Diet on Production Performance and Metabolism in Periparturient Dairy Cows. J. Dairy Sci., 90, 5247–5258.

12. Hachenberg, S., Weinkauf, C., Hiss, S., Sauerwein, H., 2007: Evaluation of classification modes potentially suitable to identify metabolic stress in healthy dairy cows during the peripartal period. J. Anim Sci., 85, 1923–1932.

13. Husvet, F., Karsai, F., Gaal, T., 1982: Peripartal fluctation of plasma and hepatic lipid components in dairy cows. Acta veterinaria Academie Scientarium Hungaricae, 30, 97–112.

14. Jovanović, M. J., Šamanc, H., Damjanović, Z., Marković, S., Djoković, R., 1993: Funkcionalno stanje jetre krava u visokom graviditetu i ranoj laktaciji. Vet. Glasnik, 4–5, 295–310.

15. Kida, K., 2002: Use of every ten-day criteria for metabolic profile test after calving and dry off in dairy herds. J. Vet. Med. Sci., 64, 1003–1010.

16. LeBlanc, S. J., Leslie, K. E., Duffield, T. F., 2005: Metabolic predictors of displaced abomasum in dairy cattle. J. Dairy Sci., 88, 159–170.

17. Lotthamer, K. H., 1991: Uticaj i posledice neuzbalansirane ishrane na zdravlje i plodnost mlečnih krava, Zbornik predavanja 20. seminara za inovaciju znanja veterinara, Beograd, 71–103.

18. Macrae, A. I, Whitaker, D. A., Burrough, E., Dowell, A., Kelly, J. M., 2006: Use of metabolic profiles for the assessment of dietary adequacy in UK dairy herds. Vet. Rec., 159, 655–661.

19. Mayes, P., 1989: Harperov pregled biohemije, Beograd.

20. Oetzel, G. R., 2004: Monitoring and testing dairy herds for metabolic disease, Vet. Clin. North Am. Food Anim. Pract., 20, 651–674.

21. Reid, I. M., 1983: Reproductive performance and fatty liver in Guernsey cows. Anim. Prod., 5, 273–279.

22. Šamanc, H., Ana-Nikolić, J., Damnjanović, Z., Stojić, V., Begocvić Jevrosima, Djoković, R., 1994: The influence of sodiumpropionate on blood glucose and serum cortisol concentrations in healthy and spontaneosly ketotic lactating cows. Acta Veterinaria Belgrade, 4, 203–214.

23. Šamanc, H., Damjanović, Z., 1987: Stepeni prepartalne glikemije na nastanak ketoze posle teljenja kod krava. Vet. Glasnik, 41, 983–984.

24. Staufenbiel, R., Meier, R., Hachbart, K. H., Staufenbiel, B., 1992: Untersuchungen zum optimacen fettansaltz bei der Milchkuh. Mh. Vet. Med., 47, 125–136.

25. Staufenbiel, R., Staufenbiel, B., Rossow, Weidemann, F., 1993: Energie-und fettstoff wechsel des Rindes. Vergleich der Aussage der Rucken fett dicke mit anderen Vuntersuchungsqzoben. Mh.Vet. Med., 48, 167–174.

26. Stengärde, L., Tr vén, M., Emanuelson, U., Holtenius, K., Hultgren, J., Niskanen, R., 2008: Metabolic profiles in five high-producing Swedish dairy herds with a history of abomasal displacement and ketosis. Acta Veterinaria Scandinavica, 50:31doi:10.1186/1751-0147-50-31.

27. Studer, V. A., Grummer, R. R., Betrics, S. J., 1992: Effect of prepartum propylen glycol administration on preparturient fatty liver in dairy cows. J. Dairy Sci., 75, Supplementum 1, 184–186.

28. Vazquez-Anon, M., Bertics, S., Luck, M., Grummer, R. R., 1994: Peripartum liver triglyceride and plasma metabolites in dairy cows. J. Dairy Sci., 77, 1521–1528.

29. Veenhuizen, J. J., Drackley, J. K., Richard, M. J., Sanderson, T. P., Miller, L. D., Joung, J. W., 1991: Metabolic changes in blood and liver during development and earlytreetment of Experimental fatty liver and ketosis in cows. J. Dairy Sci., 74, 4238–4253.

30. Whitaker, D. A., 2004: Metabolic profiles. In Bovine Medicine: Diseases and Husbandry of Cattle. Blackwell Science, Oxford, 804–817.

METABOLIC PROFILE IN HIGH-PRODUCING DAIRY COWS IN DIFFERENT PHASES OF THE CALVING-TO-CALVING INTERVAL

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ABSTRACT

In the Holstein dairy herd with mean milk yield of 9.671 kg per 305 day lactation, the metabolic profile was monitored in some cows. The monitoring included 12 cows on 2nd to 4th parity. Metabolic profile was monitored in dry period (group A), in close-up period (group B), 3–1 days prepartum (group C), 1–6 hours postpartum (group D), 3–5 days postpartum (group E), in early lactation (group F), in peak lactation (group G) and 180–220 DIM (group H).

Blood samples were collected from the coccygeal vein, using the HEMOS set. Serum was analysed with the automatic analyser HITACHI 902. Hormones T3, T4 and insulin were analysed by the chemiluminiscence method, IMULITE analyser.

Total protein and albumine levels were within the reference range throughout the period monitored. TP levels were decreasing towards the parturition and the lowest values were observed 1–3 days prepartum.

Urea levels were decreasing towards the parturition, in accordance with crude protein intake. The lowest urea levels were found on the calving day, and in the post-calving period they were increasing rapidly, reaching the maximum in the peak lactation.

Glucose levels were gradually decreasing in the postpartum period and were the lowest in the early lactation. Hypoglycemia was observed in the cows from 3 days postpartum to peak lactation and was associated with lipomobilization and subclinical ketosis. The first symptoms of lipomobilization occurred in the close-up period and were the most pronounced on the calving day and in the postpartum period. With the progressing lactation, NEFA

and BHB levels were decreasing. Thy dynamics of NEFA and BHB indicates the development of lipomobilization and ketosis in the periparturient and peak lactation cows.

AST, GMT and CK enzymes showed a correlation with parturition and early lactation period. AST values were the lowest in the dry cows and were gradually increasing with the oncoming calving. On the calving day and in the postpartum to peak lactation period, the AST values exceeded the physiological limit. The highest values were found 3–5 days postpartum. CK activity reached the peak in the postpartum period. The dynamics of AST and, above all, CK indicates the muscle degradation.

Calcium levels were reduced in the peripartum period. A slight drop was observed in the prepartum period, the greatest decrease on the calving day, and the low levels persisted till 5 days postpartum. Afterwards, hypocalcaemia was not diagnosed. Magnesium levels were leveled throughout the period under study. In some cows, decreased phosphorus levels were observed in the dry period in and the first few days postpartum. Copper levels were suboptimal in the dry cows, zinc levels were suboptimal in the peripartum period.

T3 levels were leveled throughout the period under study. Greater changes were found in T4 levels, with the most pronounced decrease in the early and peak lactation. Insulin levels started to decrease in the prepartum period, on the calving day and early lactation they were the lowest and gradually increased with the progressing lactation.

Key words: hypocalcaemia; insulin; ketosis; lipomobilization; metabolic profile; T3; T4

VACCINATION OF CATTLE AGAINST BLUETONGUE WITH BLUEVAC 8TM BY MEANS OF A NEEDLE-FREE INJECTION TECHNIQUE (ACUSHOTTM) IN CATTLE

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ABSTRACT

Bluetongue virus (BTV), an orbivirus of the Reoviridae family encompassing 24 known serotypes, is transmitted to ruminants via Culicoides biting midges. Symptoms of bluetongue disease include fever, lesions of nasal mucous membrane, conjunctivitis, salivation, dysphagia, serous nasal discharge, apathy, lesions of teats, and lameness.

In order to contain viral spread and reduce economical losses after an outbreak of blue tongue disease following BTV serotype 8 (BTV-8) infection of cattle and sheep, a compulsory national vaccination campaign was started in Germany in 2008.

Commonly, vaccinations are delivered by injection via needle and syringe into the subcutaneous (SC) space. Although vaccination delivered in this way has led to tremendous advances in the control of many infectious diseases in livestock, this technique holds the risk of transmitting blood-borne diseases, especially in large animal practice, where, to save time, a single needle is often used repeatedly. Therefore, in order to search for easier and safer ways to administer vaccines, while at the same time increasing the speed of vaccine delivery, needle-free injection systems have been developed as an alternative to conventional subcutaneous medication delivery devices. In that respect, the ACUSHOTTM Needle-Free Injection System (ACUSHOTTM, Winnipeg, Kanada; www.acushot.ca) represents a novel device for the transdermal delivery of any liquid diagnostic or therapeutic agent directly into the SC space. Thus the aim of the study was to investigate the practicability and efficacy of needle-free vaccination of cattle against bluetongue virus.

Twenty nine clinically healthy, non-lactating, non-pregnant, 2- to 8-year-old female Holstein cattle were randomly assigned to one of three groups (A/B: n=10, C: n=9). All cattle from groups A–C were vaccinated first on Day 0 (D0), and subsequently boostered on Day 28 (D28). Cows from Group A were vaccinated using 4.0 ml of commercial bluetongue vaccine (Bluevac 8TM; CZ Veterinaria, Spain) administered via a needle-free delivery device (ACUSHOTTM, Winnipeg, Kanada). Cows from Group B received a SC injection of 4.0 ml of bluetongue vaccine using a 1.2 mm (18 gauge) needle, while cows in Group C served as negative controls and received a SC injection of 4.0 ml of sterile, isotonic saline. All vaccinations were performed with the same batch of vaccine. The first injection was applied on the left, the booster on the right hand side of the animals' neck. To mark the injection site and to ease the follow up of possible local adverse reactions, hair around, but not at the actual injection site was

clipped and the skin thickness (in mm) was measured using a spring loaded cutimeter. Before inoculation, blood samples of all cattle were collected and analyzed for BTV-Antibodies and BTV-Virus to ensure the cows had no prior vaccination or contact to the field virus. Furthermore, to prevent seroconversion due to field virus, all animals were treated with cyfluthrin (BayoFly®, Bayer Vital GmbH, Germany) prior to and throughout the study, in two-week intervals.

All studied cows remained free of BTV-8 throughout the study period. As expected, no seroconversion was observed in the control cows (Group C) after injection of saline. All vaccinated cows seroconverted within eight weeks of the first vaccination. Four weeks after the first vaccination (D0), as well as four weeks after boostering (D28), administration with the needle-free ACUSHOTTM injection device (Group A) was associated with significantly higher titers compared to immunisation with needle and syringe (Group B).

In none of the cows fever or general depression was observed after vaccination. Local reactions at the injection site (the majority of which were mild) occurred in all vaccinated cows and disappeared completely within two to three weeks post injection. However, local reactions in the cows from Group A were more prominent compared to Group B and C. There were distinct differences regarding the tolerance to vaccination technique: While SC vaccinated cows showed the typical pain and stress reactions on injection, little reaction was seen when using the needle-free ACUSHOTTM device.

In conclusion, vaccination with Bluevac 8TM (CZ Veterinaria, Spain) yields high serum titres against BTV-8. Needle-free delivery of the vaccine by means of the ACUSHOTTM device elicits a faster immune response while at the same time yielding higher antibody titres in cattle compared to SC injection with a syringe and needle.

Local reactions at the injection site were overall mild but slightly more prominent in cows vaccinated by means of the needle-free vaccination device. The injections were very well tolerated; nearly no pain and defence reaction occurred when cows are vaccinated with the ACUSHOTTM device. Thus, the needle-free delivery of vaccines by means of the ACUSHOTTM device appears to be a promising alternative to SC injections with needle and syringe, the reference standard mode of injection. Efficacy of preventing transmission of infectious agents by needle-free delivery of vaccines still needs to be tested.

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DEXAMETHASONE-INDUCED INSULIN RESISTANCE IN DAIRY CATTLE IN EARLY LACTATION

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ABSTRACT

Glucocorticoids are widely used as therapeutical tools in ketotic dairy cows to increase effectively blood glucose concentration. This well-known effect has been explained until now predominantly by an increase of hepatic gluconeogenesis. The aim of this study was to investigate whether dexamethasone affects furthermore insulin resistance in early lactating cows.

Eighteen clinically healthy Holstein cows (2–4 weeks post partum, 5 d after right flank omentopexie performed due to left displacement of abomasum) were assigned to one of three treatment groups: dexamethasone group I (DG-I; 40 µg/kg BW dexamethasone-21-dinatriumphosphate i.v.; N = 6), dexamethasone group II (DG-II; 40 µg/kg BW dexamethasone-21-isonicotinate i.m.; N = 6), control group (CG, 15 ml saline i.v., N = 6). To characterise the peripheral insulin resistance, hyperinsulinemic euglycamic clamps (HIEC) were performed 18 hours after administration of the drugs. Thus, each cow received five consecutive infusion periods (two hours each) with increasing doses of bovine insulin (0.1, 0.5, 2, 5, 10 mU.kg-1 BW.min-1). Blood glucose concentration was clamped at the basal level by adjusting the intravenous infusion rate of glucose according to blood glucose level assessed every 10 min.

After dexamethasone-treatment, serum glucose concentrations were significantly higher in DG-I and DG-II compared to CG ($P < 0.001$; $P < 0.01$ respectively). The increase was significantly higher in DG-I than in DG-II ($P < 0.001$).

During HIEC, steady-state glucose infusion rates (SSGIR) were significantly lower in DG-I ($p < 0.01$) and DG-II ($P < 0.05$) compared to CG during the second infusion period (0.5 mU.kg-1 BW.min-1), and in DG-II compared to CG during the fourth infusion period (5 mU.kg-1 BW.min-1; $P < 0.05$). The

maximal insulin response, defined as SSGIR in the last infusion period (10 mU.kg-1 BW.min-1) was not different between dexamethasone-treated and not-treated cows.

In conclusion, dexamethasone-21-dinatriumphosphate and dexamethasone-21-isonicotinate exhibited almost the same effects on glucose homeostasis. The administration of both formulae increased blood glucose level. One involved mechanism was found to be an increase of insulin resistance due to reduced peripheral insulin sensitivity. Peripheral insulin response was not affected.

THE RELATION OF BACKFAT THICKNESS WITH METABOLIC DISORDERS, FERTILITY PROBLEMS AND CERTAIN DISEASES POST PARTUM - A LONG TERM STUDY WITH DAIRY COWS

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ABSTRACT

Cows within 42 days p.p. and a BFT loss over 35 % have a significant higher risk to develop problems with cycling or mastitis and to suffer from abomasal displacement. In the lactation period until 100 days p.p. and a BFT loss over 35 %, cows have a significant higher risk to develop mastitis or ketosis. Finally a significant higher risk for orthopedic problems was found for cows within 42 days p.p. and a BFT loss over 40 % (see results in Table 1). There is no significant higher risk for problems after parturition related to uterus due loss of BFT.

Key words: back fat thickness; cattle; herd health; ultrasound

INTRODUCTION

In this retrospective study the influence of back fat thickness (BFT) of cattle on fertility, metabolic problems, mastitis and milk yield was investigated. It is hypothesised that a higher decrease of back fat thickness between parturition and 42 and 100 days post partum (p.p.) has a negative impact on fertility, ketosis and certain diseases.

MATERIALS AND METHODS

Five dairy farms in Germany with 292 Holstein Friesian cows and two farms in Austria with 111 Simmental cows participated in this trial. All cows together had 1045 lactation

Table 1. The risk of certain diseases related to the loss of back fat thickness

Decrease of back fat thickness %	Disease	OR	95 % CI	95 % CI	P
			LL	UL	
< 35 % within 42 days post partum	Problems with cycling	1.750	0.990	3.184	0.052
	Displaced abomasum	14.192	1.265	159.278	0.044
	Mastitis	2.100	1.138	3.876	0.016
< 35 % within 100 days post partum	Mastitis	2.064	1.234	3.452	0.005
	Ketosis	2.738	1.066	7.032	0.030
< 40 % within 42 days post partum	Orthopedic problems	3.410	1.070	10.877	0.028

periods, which were considered in this project. The back fat thickness was measured with an ultrasound (3.5 MHz) between the tuber coxae and the tuber ischia at the following days: end of lactation period (8 weeks before parturition), 42 days before the calculated parturition day, day of parturition, 42 days and 100 days after parturition. The programme „Inter-Herd"(Interagri, Earley Gate, UK) was used to record the data. Statistic evaluation was done using odds ratio (OR > 1 increased risk OR < 1 decreased risk) as risk parameter.

RESULTS

The accounted value indicates the relative chance to become sick with the respective disease, if the decrease of back fat thickness is higher than the denoted percentage. Level of significance $P < 0.05$, OR Odds Ratio, LL lower and UL upper limit of 95 % confidence interval (CI) respectively.

THE CONCENTRATION OF ESSENTIAL AMINOACIDS IN CALVES BLOOD SERA DURING THE NEONATAL PERIOD

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ABSTRACT

The aim of this paper was to study the concentration of essential aminoacids (histidine, threonine, arginine, valine, methionine, isoleucine, leucine, phenylalanine) in blood sera of newborn calves and the possible influence of the mineral adsorbent on a change in their concentration.

The experiment was performed on 30 calves, divided into two groups: the control calves group (15 animals) was given the first colostrum (first-collection colostrum after partus) 2 and 12 hours after birth, and the second colostrum (second collection after partus) 24 and 36 hours after birth; the treated calves group (n=15) was fed in the same way, but with the addition of 5 g zeolite.l-1 of colostrums. The blood samples for analysis were taken 6, 16, 30 and 40 hours after birth, and the serum was isolated and kept at -18 oC before analysing.

The concentration of most essential aminoacids in blood sera rises in the period between 6 and 40 hours after birth. The rising tendency is less noticed in histidine and leucine. The addition of zeolite mineral adsorbent affects an earlier increase in the concentration of essential aminoacids in calves blood sera.

Key words: amino-acids; calves; proteins; zeolite

INTRODUCTION

Newborn development is characterized by high protein synthesis and nitrogen (N) turnover rates in many tissues, especially in the gastrointestinal tract (4, 10). As a consequence, nutritient requirement for nitrogen and for amino-acids, as well as for protein are relatively high. Colostrum provides particularly large amounts of nutritients (especially proteins) and immunoglobulins as well as non-nutritient growth-promoting factors, and it therefore plays a major role in the development of neonatal gut, especially protein synthesis, and other organs, as shown in several species including cattle. Protein synthesis and turnover in newborn are reduced following food withdrawal and are enchanced by colostrum feeding, as shown in lambs (13). Rates of body protein synthesis and N turnover were greater during enteral than during parenteral feeding indicating considerable involvement of the gastrointestinal tract in protein methabolism (5, 3). The rate of absorption may have been greater in calves during the first 24–36h of life not only for various proteins and peptides, but also for amino-acids (19). Post absorptive clearance and metabolism of amino-acids was possibly smaller in newborn calves than in older calves. Clearance from the plasma pool is enhanced by stimulation of tissue uptake by insulin, whose concentrations increased more markedly in 28-day old calves than immediately after birth (19) in accordance with studies in humans (2).

Protein components in colostrum provided about 35 % of amino-acids (AA) in newborn lambs, suggesting that amounts of ingested protein with colostrum greatly influence the AA status of the neonate (18). Protein syntheses are higher during the early post-natal period than during any other period of life (8, 17, 3). There are marked post-natal changes in the concentration of plasma amino-acids in calves (16).

Calves are born with a mostly inadequate essential amino-acid (EAA) status. The sum of EAA and NEAA decreased from the first to the fifth milking of colostrum to levels in mature milk (19). The EAA and non-essential amino-acid (NEAA) status were rapidly normalized after intake of the first colostrum, but normal plasma levels of some amino-acids were also reached during the first 24 h of life even before the first meal was provided. Delay colostrum intake had only transienteffect

on EAA and NEAA concentrations in contrast to plasma IgG and total protein levels (1, 14, 6, 7, 9).

MATERIALS AND METHODS

The experiment was performed on 30 cattle, divided into two groups: the control cattle group (15 animals) was given the first colostrum (first-collection colostrum after partus) 2 and 12 hours after birth, and the second colostrum (second collection after partus) 24 and 36 hours after birth; the treated cattle group (n=15) was fed in the same way, but with the addition of 5g zeolite.l-1 of colostrums. Colostrum was fed in amounts of 1.5 l per meal. The blood samples for analysis were taken 6, 16, 30 and 40 hours after birth, and the serum was isolated and kept at -18 oC before analysing. Measurement of amino-acids in serum samples pool were performed by high-performance liquid chromatography (HPLC, GBC Australia).

RESULTS AND DISCUSSION

Concentration of the sum of essential amino-acids (EAA) (His, Thr, Arg, Val, Met, Ile, Leu, Phe) is shown in Table 1.

The sum of serum EAA concentrations increased after colostrum intake in both groups. At 6 h after birth concentrations of EAA in blood serum of treated group was three times higher than in control group. The addition of zeolite affects an earlier increase in the concentration of essential amino-acids in calves blood sera. Hammon and Blum (10) showed that the sum of free EAA increased on day 1 after colostrum intake, but not after milk replacer intake, although milk replacer contained higher amounts of free EAA than first colostrum. It appears that the increase of EAA concentrations depends more on the intake of high amounts of protein-bound EAA than on the intake of free EAA with colostrum or milk.

Blood serum EAA concentrations remained high on day 2 in both groups. Our results were in accordance with the findings of the other investigators (4, 10, 19). The importance of colostrum intake for plasma free EAA concentrations was still seen on day 2 when plasma EAA decreased in group fed with milk replacer (group was fed colostrum at the first meal, followed by twice-daily feeding of milk replacer in the same volumes). In the study of Yvon et al, (18) plasma free AA originated mainly from ingested casein, -lactoglobulin and IgG1. Thus high protein intake provided by colostrum is very important for total EAA status in the newborn calves. Some of the ingested amino-acids are well known to be metabolized in the intestinal cells, other amino-acids appearing in the portal vein are directly taken up by the liver and do not enter the general circulation. Previous studies with milk fed calves had shown that plasma concentrations of amino-acids administered as free amino acids (3 methylhistidine) increased within 2 h of ingestion, whereas for protein-bound amino-acids (arg) it took considerably longer for them to appear in blood plasma (15). Thus, increases in plasma amino-acids 2 h after meal ingestion probably primarily mirrored absorption of free amino- acids more than that of protein-bound amino acids in colostrum or milk.

Protein synthesis in the neonate is maximally stimulated after food intake in virtually all tissues of the body (12) and the dietary amino-acids (AA) are efficiently utilized as direct precursors for protein deposition (4). The AA derived from intestinal absorption can stimulate hepatic protein synthesis and are a primary stimulus for liver protein synthesis in neonates (4, 12). The enhanced stimulation of liver protein synthesis by feeding in the neonate appears to be dependent primarily on amino-acids supply.

An increase of EAA during the first 40 h was noted in control group (except histidine and leucine). Great differences of acute post-prandial responses (within 2 h) between individual amino-acids were primarily seen after the intake of first colostrum. The rise of the most individual EAA in both or at least one of the groups and hence the sum of individual EAA suggested that colostrum intake helped to normalize the potentially the relatively deficient EAA status of newborn calves. Concentration of EAA in treated group increased within 30h (except valine, isoleucine, phenylalanine, methionine), and than slightly decreased. Rapid Phe liver metabolizm is probably responsible for prevention of its rise in blood plasma (11). Concentrations of EAA in treated group were much higher than those of EAA in control group. At 40 h after birth there were no group differences in sum of essential amino-acids in blood sera of calves.

Table 1. Serum concentrations (μmol.l^{-1}) of essential aminoacids in neonatal calves

EAA	Control group				Treated group			
	6 h	16 h	30 h	40 h	6 h	16 h	30 h	40 h
his	49.2	149.7	87.8	145.2	186.1	179.1	190. 1	244.4
Thr	54.9	170.3	197.4	218.8	134.1	225.6	215.6	175.5
Arg	34	91.4	162.9	208.4	147.8	144.5	202.9	189.1
Val	67.3	256.8	279.6	340.7	220.5	365.1	357.7	356.3
Met	17.8	28.3	29.2	29.4	14.3	56.5	38.1	21.3
Ile	20.9	72.8	79	87.1	87.2	94.3	110.1	76.3
leu	34.4	149.7	128.7	157.2	140.7	192.2	148.3	140.3
Phe	40.3	100.1	105.5	85.7	102.5	126.1	80.6	74.6
Sum	318.8	1019.1	1070.1	1272.5	1033.2	1383.4	1343.4	1277.8

The differences between individual EAA within 40 h after birth in control and treated calves are shown in Table 2 and Figure 1.

Table 2. Concentration of EAA (µmol.l-1) within 40 h after birth in control and treated calves

EAA	Control group			Treated group		
	Mean	SE	CV	Mean	SE	CV
His	107.98	24.13	44.70	199.93*	15.00	15.00
Thr	160.35	36.52	45.56	187.70	20.89	22.26
Arg	124.18	38.51	62.03	171.08	14.68	17.16
Val	236.10	58.99	49.97	324.90	34.85	21.45
Met	26.18	2.80	21.41	32.55	9.42	57.86
Ile	64.95	14.97	46.10	91.98	7.09	15.41
Leu	117.50	28.35	48.25	155.38	12.41	15.98
Phe	82.90	14.80	35.71	95.95	11.70	24.39

*($P \leq 0.05$)

The average concentration of investigated EAA was higher in treated group but only concentration of histidine was statistically significant ($P \leq 0.05$).

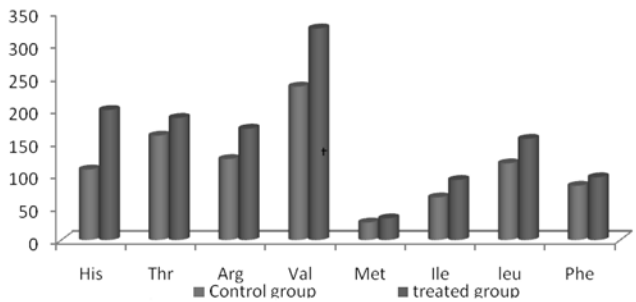

Fig. 1. Concentration of EAA (µmol.l-1) within 40 h after birth in control and treated calves

Following intake of free amino-acids or release of amino-acids from ingested proteins and after absorption from the gastrointestinal (GI) tract amino acids follow one of the three major metabolic fates: firstly, they act as substrates for the maintenance or net protein synthesis; secondly they provide nitrogen and carbon skeletons for the formation of NEAA and serve as a precursors for various metabolically non-protein nitrogen containing compounds; or thirdly, they enter catabolic pathways leading to the elimination of the nitrogen (ammonia and urea). Postprandial rise of EAA suggested that these amino-acids, by definition, were probably absorbed, whereas a postprandial fall in any amino-acids possibly suggested enhanced clearance due to tissue uptake, metabolizm and excretion.

REFERENCES

1. Arthington, J. D., Cattell, M. B., Quigley, J. D., McCoy, G. C., Hurley, W. L., 2000: Passive Immunoglobin Transfer in Newborn Calves Fed Colostrum or Spray-Dried Serum Protein Alone or as a Supplement to Colostrum of Varying Quality. J. Dairy Sci., 83, 2834–2838.

2. Aynsley-Green, A., 1988: The management of islet cell dysregulation syndromes in infancy and childhood. Z. Kinderchir., 43, 267–72.

3. Davis, T. A., Burrin, D. G., Fiorotto, M. L., Nguyen, H. V., 1996: Protein synthesis in skeletal muscle and jejunum is more responsive to feeding in 7-than in 26-day-old pigs. Am. J. Physiol., 270, 802–809.

4. Davis, T. A., Burrin, D. G., Fiorotto, M. L., Reeds, P. J., Jahoor, F., 1998: Roles of insulin and amino-acids in the regulation of protein synthesis in the neonate. J. Nutr., 128, Supplementum 2, 347S–350S.

5. Duffy, B., Pencharz, P., 1986: The effect of feeding route (i.v. or oral) on the protein metabolism of the neonate. Am. J. Clin. Nutr., 43, 108–111.

6. Fratric, N., Stojić, V., Janković, D., Šamanc, Gvozdić, D., 2005: The effect of a clinoptilolite based muneral adsorber on concentrations of immunoglobulin g in the serum of newborn calves fed different amounts of colostrum. Acta Veterinaria, 55, 11–21.

7. Fratric, N., Stojic, V., Rajčić, S., Radojičić, B., 2007: The effect of mineral adsorbent in calf diet colostrum on levels of serum immunoglobulin G, protein and glucose. Acta Veterinaria, 57, 2–3, 169–180.

8. Goldspink, D. F., Kelly, F. J., 1984: Protein turnover and growth in the whole body, liver and kidney of the rat from the foetus to senility. Biochem. J., 15, 507–16.

9. Gvozdić, D., Stojić, Šamanc, H., Fratrić, N., Daković A., 2008: Apparent efficiency of immunoglobulin absorption in newborn calves orally treated with zeolite. Acta Veterinaria, 58, 345–355.

10. Hammon, H. M., Blum, W. J., 1999: Free amino-acids in plasma of neonatal calves are influenced by feeding colostrum for different durations or by feeding only milk replacer. J. Anim. Physiol. a. Anim. Nutr., 82, 193–204.

11. Newsholme, E. A., Leech, A. R., 1983: Biochemistry for the medical science. J. Wiley & Son, Chichester.

12. O'Connor, P. M., Kimball, S. R., Suryawan, A., Bush, J. A., Nguyen, H. V., Jefferson L. S., Davis, T. A., 2004: Regulation of neonatal liver protein synthesis by insulin and amino acids in pigs. Am. J. Physiol. Endocrinol. Metab., 286, E994–E1003.

13. Patureau-Mirand, P., Mosoni, L., Levieux, D., Attaix, D., Bayle, G., Bonnet, Y., 1990: Effect of colostrum feeding on protein metabolism in the small intestine of newborn lambs. Biol. Neonate, 57, 30–36.

14. Stojić, V., Šamanc, H., Fratrić, N., 1995: The effect of clinoptilolite based mineral adsorber on colostral immunoglobulin G absorption in newborn calves. Acta Veterinaria Beograd, 45, 67–74.

15. Vacher, P. Y., Schmitz, M., Hirni, H., Blum, W. J., 1990: Postprandial plasma variations of 3-methylhistidine compared to those of lysine, homoarginine and xylose on the

normal conditions and in malabsorption in the suckling calf. Reprod. Nutrit. Develop., 30, 471–482.

16. Vermorel, M., Vernet, J., Dardillat, C., Saido e Demigne, C., e Davicco, M. J., 1989: Energy metabolism and thermo-regulation in the new-born calf: effect of calving conditions. Canadian Journal of Animal Science, 69, 113–122.

17. Young, V. R., Fukagawa, N. K., Storch, K. J., Hoerr, R., Jasik, T., Bier, D. M., 1988: Stable isotope probes:potential for application in studies of amino-acid utilization in the neonate. In: Lindblad, H. ed. Perinatal Nutrition, Academic Press, San Diego, 221–241.

18. Yvon, M., Levieux, D., Valluy, M. C., Pélissier, J. P., Mirand, P. P., 1993: Colostrum protein digestion in newborn lambs. J. Nutr., 23,586–96.

19. Zanker, I. A., Hammon, H. M., Blum, J. W., 2000: Plasma amino acid pattern during the first month of life in calves fed the first colostrum at 0–2 h or at 24–25 h after birth. J. Vet. Med. A Physiol. Pathol. Clin. Med., 47, 107–21.

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METABOLIC STATUS OF PERIPARTAL DAIRY COWS IN HEALTH AND KETOSIS

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ABSTRACT

The peripartal period is a critical time for the occurrence of metabolic diseases in dairy cows. Total number of 19 dairy cows, with average 2.7 lactations and 7175 litres of milk per 305 days of lactation were investigated two weeks before and after parturition. There were no clinical cases of ketosis before parturition, and after parturition 8 cows were diagnosed with clinical ketosis (loss of appetite, ruminal atonia, urine ketone bodies > 17.20 mmol.l^{-1}). Blood samples were obtained from the *v. jugularis*, and blood serum was separated and stored at -18 °C until analyzed. The following blood serum metabolic parameters were evaluated: 1) glucose, 2) NEFA, 3) triacylglycerol (TG), and 4) total bilirubin concentration (tBIL). The statistical significance of differences between mean values and the correlation between serum metabolic parameters was determined using ANOVA, LSD test, and correlation coefficient.

Blood serum glucose concentration decreased significantly in dairy cows during early lactation, with a more pronounced decrease in ketotic cows, compared to the late pregnancy period. Blood serum NEFA concentration decreased in healthy and increased significantly ($P < 0.01$) in ketotic postpartum cows. Dairy cows diagnosed as clinical ketosis did have a significantly lower ($P < 0.05$) blood serum TG level and higher ($P < 0.05$) BIL concentrations. High negative correlation coefficient was determined for blood serum glucose and NEFA concentrations (r=-0.82) in ketotic cows during early lactation. Results of this investigation indicate a close relationship between some metabolic parameters in dairy cows with ketosis and liver function disorders.

Key words: ketosis; metabolic status; peripartal dairy cows

INTRODUCTION

Peripartal period in high-milking dairy cows represents "transition period" (17), with inevitable imbalance between available energy from feed intake and energy requirements for milk production during early lactation (15). This net shortage of energy resulting from less energy input than output is called negative energy balance (NEB) (22). Metabolic diseases in dairy cows most frequently occur during the first two weeks postpartum (15). Ketosis is a metabolic disorder closely associated with fatty liver, and those two pathophysiological conditions are discussed together (15).

Ketosis respresents incomplete oxidation of fatty acids (FA) during the time of impared carbohydrate metabolism. Fatty liver develops when FA hepatic uptake overcomes their oxidation and secretion (10). Many blood serum parameters have been used to evaluate the degree of hepatic fatt accumulation, from blood glucose concentration (7, 25), total bilirubin concentration (tBIL) (32; 9), to the activity of serum enzimes such as aspartate aminotransferaze (AST), glutamate dehydrogenase (GDH), γ-glutamyltransferaze (γ-GT), alkaline phosphatase (AP), or alanine aminotransferaze (ALT) (16, 1, 42, 43, 12, 32).

Metabolic profile testing usually have been used to assess nutrient status of cows in the transition period (32, 24, 20, 4). In the begining metabolic profiles included packed cell volume and haemoglobin along with glucose, proteins and minerals (32). Nowdays metabolites such as NEFA and β-hydroxybutyrate (BHB) have been added to the profiles to monitor energy balance. Metabolic profile is considered useful to identify nutritional shortcomings even before the produc-

tivity is impaired (45). Such profiles have also been used to monitor herd health and to find subclinical disease, to predict risk of ketosis or abomasal displacement as well as investigate herd problems with metabolic disorders (30, 26, 13). Blood parameters that may reflect nutrient status of the cow, such as glucose, insulin, NEFA, BHB and cholesterol, also enzymes and proteins that reveal liver status are of interest to include in transition period profiles.

Aim of this work was to investigate metabolic status of dairy cows in health and ketosis during peripartal period.

MATERIAL AND METHODS

Total number of 19 dairy cows, with average 2.7 lactations and 7175 litres of milk per 305 days of lactation were investigated two weeks before and after parturition. There were no clinical cases of ketosis before parturition, and after parturition 8 cows were diagnosed with clinical ketosis (loss of appetite, ruminal atonia, urine ketone bodies > 17.20 mmol.l^{-1}). Blood samples were obtained from the v. jugularis, and blood serum was separated and stored at -18°C until analyzed. The following blood serum metabolic parameters were evaluated: 1) glucose, 2) NEFA, 3) triacylglycerol (TG), and 4) total bilirubin concentration (tBIL). The statistical significance of differences between mean values and the correlation between serum metabolic parameters was determined using ANOVA, LSD test, and correlation coefficient.

RESULTS AND DISSCUSSION

Blood serum metabolic parameters in healthy and ketotic peripartal dairy cows are presented in Table 1.

Blood serum glucose concentration was significantly lower in healthy and ketotic dairy cows in early lactation, compared to the late pregnancy period. Ketotic dairy cows in early lactation have had significantly increased blood serum NEFA and total bilirubin, and decreased blood serum TG concentration.

Clinical cases of ketosis in dairy cows have been characterized by metabolic conditions such as hypoglicaemia, hyperketonemia, hypoinsulinemia, increased blood plasma NEFA, decreased hepatic glycogen and increased hepatic triglycerides (2, 8). Ketosis can be usefully categorized into tree general types: 1) type I or primary ketosis (spontaneous, underfeeding), 2) type II or secondary ketosis (fatty liver-ketosis complex) and 3) butyric acid silage ketosis (19, 18, 29, 31). Ketosis in dairy cows can be classified as: 1) primary underfeeding ketosis (cow is not offered enough "acceptable" feed, 2) secondary underfeeding ketosis (reduced feed intake due to another disease), 3) alimentary ketosis (feed containing high ketogenic precursors), and 4) spontaneus ketosis (nutritionaly adequate diet), wich is the least understood condition. Primary ketosis can be manifested in clinical or subclinical form (2). Our experimental design and results indicate primary spontaneous clinical ketosis (type I) in the dairy cows at group E.

Blood serum glucose concentration in dairy cows during the early lactation is completely dependant on hepatic gluconeogenesis (46), and nutrition-inuced ketosis results in downregulation of glucogenic genes such as phosphoenolpyruvate carboxykinase 1 (PCK1). Inabilty of early lactation dairy cows to sutisfy an increase mamary gland glucose demands induces a decrease of blood serum glucose concentration, since mammary gland glucose utilization is almost completely independent on blood glucose concentration (39). Our results indicate that blood serum glucose concentration in early lactation dairy cows is significantly lower comparing to the late pregnancy period (Table 1), with very significant decrease in ketotic cows relative to the other experimental groups (1.51 ± 0.64 mmol.l^{-1}, P < 0.01).

Part of the homeorhetic adaptation of dairy cows metabolism during peripartal period to support lactation is mobilization of fatty acids from adipose tissue with increase of nonesterified fatty acids blood serum concentration (35, 3). Metabolic fate of NEFA in the liver is ether to be oxidized or reesterified and exported as very low density lipoproteins (VLDL). The level od fatty acid mobilization during early lactation, however, could be so high that liver is overwhelmed with NEFA, which may accumulate within hepatocytes as tryacilgliceride (TG) droplets. Development of fatty liver is a common metabolic change associated with ketosis during lactation (40, 38, 8). Certain degree of hepatic accumulation of TG in early lactation dairy cows is normal (34, 36), but 90 % of ketotic cows could have moderate (20–40 % fat; 60 % of ketotic cows) to high (> 40 % fat; 30 % of ketotic cows) liver fatt accumulation, with significant increase of blood serum NEFA concentration (9).

Table 1. Blood serum metabolic parameters (Mean ± SD) in healthy and ketotic peripartal dairy cows

Time of investigation	group	n	Glucose (mmol.l^{-1})	NEFA (mmol.l^{-1})	TG (mmol.l^{-1})	tBil (mmol.l^{-1})
Late pregnancy	A	19	$2.98 \pm 0.63_{a}$	$0.33 \pm 0.13_{a}$	$0.56 \pm 0.13_{a}$	$5.10 \pm 2.51_{a}$
	B	11	$3.08 \pm 0.71_{a}$	$0.32 \pm 0.17_{a}$	$0.55 \pm 0.11_{a}$	$4.91 \pm 2.51_{a}$
	C	8	$2.83 \pm 0.48_{a}$	$0.33 \pm 0.06_{a}$	$0.56 \pm 0.16_{a}$	$5.37 \pm 2.67_{a}$
Early lactation	D	11	$2.11 \pm 0.58_{ab}^{*}$	$0.21 \pm 0.14_{a}$	$0.47 \pm 0.09_{ab}$	$4.18 \pm 2.18_{a}$
	E	8	$1.51 \pm 0.64_{b}^{**}$	$0.65 \pm 0.12_{b}^{**}$	$0.43 \pm 0.15_{b}^{*}$	$7.87 \pm 6.15_{b}^{*}$

Legend: A – dairy cows before parturition, B – healthy cows before parturition, C – ketotic cows before parturition, D – healthy cows after parturition, E – ketotic cows after parturition; * P < 0.05; ** P < 0.01; values with different subscript are significantly different

Feed restriction in nutritional ketosis results in nearly twofold increase in serum NEFA concentration, decrease of serum glucose level and treefold increase in serum beta-hydroxybutirate concentration.

Fatty liver diagnosis utilizes many laboratory methods, and most commonly used are blood serum total bilirubin concentration (tBIL) (32, 9), NEFA (33, 14), glucose (7) and ketone concentrations (21, 43, 44, 28), activity of enzymes aspartate aminotransferaze (AST) (12, 32), glutamate dehydrogenase (GDH) (42), sorbitol dehydrogenase (SDH) (7), g-glutamyltransferaze (g-GT) (16, 12, 32), alkaline phosphatase (AP) (1), alanine aminotransferaze (ALT) (1, 43) and ornitine carbamoyl transferaze (OCT) (23). Among the laboratory tests OCT and AST activity and tBIL concentration correlate well with the degree of fatty liver accumulation (23). Total BIL blood serum concentration increses with progesssion of ketotis in dairy cows (6). Our results of tBIL concentration in ketotic cows (7.87 ± 6.15 $\mu mol.l^{-1}$) indicate fatty changes level in liver from GdL 2 to GdL 3 ("Grades der Leberverfetting", GdL), based on the data presented by K a l a i t z a k i s et al. (23). However, blood serum tBIL level could vary between dairy cattle breeds, and Š a m a n c et al., (37) reported significantly different tBIL in fresh East-Fresian and Holstein heifers (4.85 ± 1.7 and 6.99 ± 1.9$\mu mol.l^{-1}$, respectively). It obviously important to establish reference range intervals for metabolic parameters values in local dairy cow heards, in order to make good evaluation of their nutrition, health and management. In dairy cows heards with high incidence ketosis, dislocation of abomasum, purulent endometritis and decreased milk production tBIL concentration in fresh cows (10–20 DIM) is relatively high (7.77 ± 1.96$\mu mol.l^{-1}$) (5). However, significant increse of tBIL concentration in ketotic cows (group E) compared to the healthy cows after calving (group D) indicate fatty liver-ketosis complex problem in those animals.

The degree of correlation between metabolic parameters in ketotic dairy cows after parturition (group E, n=8) is presented in the Table 2.

Table 2. Correlation coefficient between metabolic parameters in ketotic dairy cows (group E, n=8)

	NEFA	TG	tBil
Glucose	-0.82	0.34	-0.40
NEFA	-	-0.31	0.19
TG	-	-	-0.02

Results presented in the Table 2. indicate high negative correlation between glucose: NEFA (r = -0.82), glucose: tBIL (r = -0.40), and NEFA : TG (r = -0.31), with positive correlation between glucose: TG (r = 0.34). Metabolic parameters important in fatty liver diagnostics research reported by D j o k o v i ć *et al.* (9) also indicate negative correlation between glucose: FFA and FFA : TG, with somewhat different correlation coefficient values.

Negative energy balance in dairy cows during early lactation period is closely linked to carbohydrate and lipid metabolism. Seemingly paradoxal situation exists in transition dairy cows: more positive energy balance during dry period producing increase of body condition could be the risk factor for patological negative energy balance followed with metabolic diseases. This hypothesis was tested by S m i t h *et al.* (38) and it was suggested that obese cows cows are in a delicate metabolic balance during early lactation and are highly susceptible to ketosis. Animals that have high fatt reserves in adipose tissue mobilize them faster than they can be metabolized in the liver, producing increased hepatic TG accumulation. Interestingly, research conducted by D a n n *et al.* (8) did not establish clear conection between prepartum intake, periparturient disorders and metabolic status of dairy cows. However, cows fed ad libitum generally had greater body fat mobilization and had greater lipid accumulation in liver immediately after calving then restricted diet cows (8). Our results indicate that as long as there is positive correlation between blood NEFA and TG concentration fresh dairy cow can be considered in "normal" or "physiological" NEB, meaning that liver cells can reesterified and export FFA. If and when blood serum TG level starts do decrease, and blood NEFA concentration stay high or continue to rise (reaching levels above 1.0 $mmol.l^{-1}$) it indicates abnormal NEB.

REFERENCES

1. Abdelkader, S. V, Hauge, J. G., 1986: Serum bile acids and enzymes in the study of liver disease in dogs and cattle. Is. J. Vet. Med., 42, 385–392.

2. Baird, G. D., 1982: Primary ketosis in the high-producing dairy cow: Clinical and subclinical disorders, treatment, prevention and outlook. J. Dairy Sci,. 65, l.

3. Bertics, S., Grummer, R., Valiono, C., Stodderd, E., 1992: Effect of prepartium dry matter intake on liver triglicerylide concentration and early lactation. J. Dairy Sci., 75, 1914–1922.

4. Blowey, R. W., 1975: A practical application of metabolic profiles. Vet Rec., 97, 324–27.

5. Bugarski, D., Kovačević, M., Milovanović, A., 2007: Metabolic profile in diagnosing health status of dairy cows herds. In Feeding, reproduction and health protection of cattle, p.157–163.

6. Bugarski, D., 2002: Koncentracja insulina, insulinu sličnog faktora rasta-I i parametara pokazatelja funkcionalnog stanja jetre u krvi zdravih i ketoznih krava. Master thesis, University of Belgrade.

7. Cebra, C. K, Garry, F. B, Getzy, D. M, Fettman, M. J., 1997: Hepatic lipidosis in anorectic, lactating Holstein cattle: A retrospective study of serum biochemical abnormalities. J. Vet. Intern. Med., 11, 231–237.

8. Dann, H. M., Morin, D. E., Bollero, G. A., Murphy, M. R., Drackley J. K., 2005: Prepartum intake, postpartum induction of ketosis, and periparturient disorders affect the metabolic status of dairy cows. J Dairy Sci., 88, 3249–3264.

9. Đoković, R., Šamanc, H., Jovanović, M., 2005: Koncentracija hormona tireoideje i lipida u krvnom serumu krava u peripartalnom periodu. Etiopatogeneza i dijagnostika poremecaja

metabolizma i reprodukcije goveda, 4-ti simpozijum Ishrana, repodukcija i zastita zdravlja goveda, Subotica, 147-161.

10. Drackley, J. K, Overton, T. R, Douglas, G. N., 2001: Adaptations of glucose and long-chain Fatty acid metabolism in liver of dairy cows during the periparturient period. J. Dairy Sci., 84, E Suppl: E100-E112.

11. Dann, H. M, Morin, D. E, Bollero, G. A, Murphy, M. R, Drackley, J. K., 2005: Prepartum intake, postpartum induction of ketosis and periparturient disorders affect the metabolic status of dairy cows. J. Dairy Sci., 88, 3249-3264.

12. Garry, F. B, Fettman, M. J, Curtis, C. R, Smith, J. A., 1994: Serum bile acid concentrations in dairy cattle with hepatic lipidosis. J. Vet. Intern. Med., 8, 432-438.

13. Geishauser, T., Leslie, K. E., Duffield, T. F., Edge, V.,1997: Evaluation of aspartate transaminase activity and beta-hydroxybutyrate concentration in blood as tests for prediction of left displaced abomasum in dairy cows. Am. J. Vet. Res., 58, 1216-1220.

14. Gerloff, J. B, Herdt, T. H, Emery, R. S., 1986: Relationship of hepatic lipidosis to health and performance in dairy cattle. J. Am. Vet. Med. Assoc., 188, 845-849.

15. Goff, J. P, Horst, R. L., 1997: Physiological changes at parturition and their relationship to metabolic diseases. J. Dairy Sci., 80, 1260-1268.

16. Gröhn, Y., Lindberg, L. A., Bruss, M. L., 1983: Fatty Infiltration of liver in spontaneously ketotic dairy cows. J Dairy Sci., 66, 2320-2328.

17. Grummer, R. R., 1995: Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. J. Anim Sci., 73, 2820-33.

18. Herdt, T. H., 2000: Ruminant adaptation to negative energy balance: Influence on the etiology of ketosis and fatty liver. Vet. Clin. North Am. (Food Anim Pract), 16, 215-230.

19. Holtenius, P., Holtenius, K., 1996: New aspects of ketone bodies in energy metabolism of dairy cows. A review. J. Vet. Med. A, 43, 579-587.

20. Ingraham, R. H, Kappel, L. C., 1988: Metabolic profile testing. Vet. Clin. North Am. Food Anim. Pract., 4, 391-411.

21. Johannsen, U., Menger, S., Staufenbiel, R., Klukas, H., 1993: The morphology and function of the liver in high performance cows 2 weeks post partum. Dtsch. Tierarzt.l Wochenschr., 100, 177-181.

22. Jorritsma, R., 2003: Negative Energy Balance in Dairy Cows as Related to Fertility. Dissertation Utrecht University, Faculty of Veterinary Medicine, ISBN 90-393-3286-X.

23. Kalaitzakis, E., Roubies, N., Panousis, N., Pourliotis, K., Kaldrymidou, E., Karatzias, H., 2007: Clinicopathological evaluation of hepatic lipidosis in periparturient dairy cattle. J. Vet. Intern. Med., 21, 835-845.

24. Kida, K., 2002: Use of every ten-day criteria for metabolic profile test after calving and dry off in dairy herds. J. Vet. Med. Sci., 64, 1003-1010.

25. Kureljušić, B., Radovanov, M., 2007: The influence of fatty liver on blood glucose concentration in cows. In Feeding, reproduction and health protection in cattle, 165-171.

26. LeBlanc SJ, Leslie KE, Duffield TF. 2005: Metabolic predictors of displaced abomasum in dairy cattle. J. Dairy Sci., 88,159-170.

27. Macrae, A. I., Whitaker, D. A., Burrough, E., Dowell, A., Kelly, J. M. 2006: Use of metabolic profiles for the assessment of dietary adequacy in UK dairy herds. Vet. Rec., 159, 655-661.

28. Morrow, D. A. 1976: Fat cow syndrome. J. Dairy Sci., 59, 1625-1629.

Oetzel, G. R. 2004: Monitoring and testing dairy herds for metabolic disease. Vet. Clin. North Am. Food Anim. Pract., 20, 651-674.

29. Oetzel, G. R., 2007. Herd-level ketosis - diagnosis and risk factors. In: Pre-Conference seminar 7C: Dairy herd problem investigation strategies: transition cow troubleshooting, 40th Annual Conference of the American Association of Bovine Practitioners, Vancouver (BC), Canada, pp.67-91.

30. Panousis, N., 2008: Types, diagnosis and treatment of ketosis in dairy cows. Veterinary Journal of Republic of Srpska, VIII, 107-115.

31. Payne, J. M, Dew, A. M, Manston, R., Faulks, M., 1970: The use of a metabolic profile test in dairy herds. Vet. Rec., 87, 150-158.

32. Rehage, J., Mertens, M., Stuckhofe-Zurwieden, N., 1996: Post surgical convalescence of dairy cows with left abomasal displacement in relation to fatty liver. J. Schweizer Archiv Tierheilbunde., 138, 361-368.

33. Reid, I. M, Roberts, C. J., 1983: Subclinical fatty liver in dairy cows: Current research and future prospects. Ir. Vet. J., 37, 104-110.

34. Reid, I. M and Collins, R. A., 1991: The pathology of post parturient fatty livers in high-yielding dairy cows. Invest. Cell Pathol., 237.

35. Reid, I. M, Collins, R. A, Baird, G. D, Roberts, C. J and Symonds H. W., 1979: Lipid production rates and the pathogenesis of fatty liver in fasted cows. J. Agric. Sci. (Camb.), 93:253.

36. Reid, I. M, Harrison, R. D, and Collins R. A., 1977: Fasting and refeeding in the lactating dairy cow. 2. The recovery of the liver cell structure and function following a six-day fast. J. Comp. Pathol., 87,253.

37. Šamanc, H., Jovanović, M., Damnjanović, Z., Ivanov, I., 1992: Koncentracija aminokiselinskog azota i ukupnog bilirubina u krvnom serumu visoko-gravidnih i tek oteljenih junica istočno-frizijske i holstajn rase. Vet. Glasnik, 46, 377.

38. Smith, T. R, Hippen, A. R., Beitz, D. C., Young, J. W., 1997: Metabolic characteristics of induced ketosis in normal and obese dairy cows, J. Dairy Sci., 80, 1569-1581.

39. Stamatović, S., Šamanc, H., Jovanović, M. 1983: Uporedo ispitivanje koncentracije glikoze u krvi v. auricularis magna i v. subcutanea abdominis mlečnih krava. Vet. glasnik, 37, 273-256.34.

40. Vazquez-Anon, M., Bertics, S., Luck, M., Grummer, R. R., 1994: Peripartum liver trglyceride and plasma metabolites in dairy cows. J. Dairy Sci., 77, 1521-1528.

41. West, H. J., 1989: Liver function of dairy cows in late pregnancy and early lactation. Res. Vet. Sci., 46, 231-237.

42. West, H. J., 1990: Effect on liver function of acetonaemia and the fat cow syndrome in cattle. Res. Vet. Sci., 48, 221-227.

43. West, H. J., 1991: Evaluation of total serum bile acid concentrations for the diagnosis of hepatobiliary disease in cattle. Res. Vet. Sci., 51, 133-140.

44. West, H. J., 1997: Clinical and pathological studies in cattle with hepatic disease. Vet. Res. Com., 21, 169–185.

45. Whitaker, D. A., 2004: Metabolic profiles In Bovine Medicine: Diseases and Husbandry of Cattle 2nd edition. Edited by: Andrews AH, Blowey RW, Boyd H, Eddy RG. Oxford, Blackwell Science, 804–817.

46. Young, J. W., 1977: Gluconeogenesis in cattle: significance and methodology. J. Dairy Sci., 60, 1–15.

METABOLIC DISORDERS IN COWS WITH DISPLACEMENT OF ABOMASUM

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ABSTRACT

The objective of the study was to compare some blood biochemical indicators in cows diseased with displacement of abomasum (DA) that recovered or died after treatment. Examinations were performed on 60 multiparous cows (with left (L) or right (R) displacement. Diagnosis was made by an experienced practitioner on the basis of clinical examination. Surgical treatment was undertaken during the first 24 hours after diagnosis. Almost all animals (55 = 91.5 %) became sick in post parturient period (21 days p.p. in average) with the exception of 5 (8.3 %) that were 5 – 8 months pregnant. Blood samples were taken from each cow immediately before surgical procedure. Serum nonesterified fatty acid (NEFE), glucose, cholesterol, aspartate aminotransferase (AST), total bilirubin and BUN were measured. Seven cows (11.67 %) died after surgical correction and all remaining recovered.

No significant differences in NEFE, cholesterol, aspartate aminotransferase, total bilirubin and urea levels were observed dependently on the efficacy of treatment (survival, deaths).

All animals were characterized by low mean values of cholesterol (≤ 2 mmol.l^{-1}), higher levels of NEFA (> 600 µmol.l^{-1}) and bilirubin (> 1.3 mg.dl^{-1}), higher activity of AST (> 100 U.l^{-1}) and level of BUN > 12 mg.dl^{-1}. It was stated that cows which died after surgical treatment were characterized by significant higher levels of glucose (5.05 mmol.l^{-1}) comparing to survive cows (2.93 mmol.l^{-1}).

Key words: cows; displacement abomasum; metabolic disorders

ENVIRONMENTAL FAECAL SAMPLES FOR DETECTION OF PARATUBERCULOSIS IN SMALL CATTLE FARMS

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ABSTRACT

Paratuberculosis (Johne´s disease) is known as one of the most important diseases in ruminants today and can cause high economic losses in the cattle industry. Diagnosis of the disease is difficult, cost intensive and shows, due to the chronic disease devolution, limited validity. Detection of Mycobacterium avium subsp. paratuberculosis (MAP) in environmental faecal samples has recently been described to determine the paratuberculosis herd status of large dairy herds. The lack of information about usability of this cheap method for investigation of the MAP-herd level in small middle European herds is one of the major work fields in paratuberculosis research at the Clinic for Ruminants at the University of Veterinary Medicine in Vienna.

Austrian Dairy herds were sampled for MAP three times

within a period of one and a half years by collecting of environmental and pooled faecal samples. The participating farms were divided in herds with known MAP-infection, documented by results of individual cow testing and farms with unknown herd infection status.

Environmental samples were collected from different sites within the stable and manure storage. Additionally pooled faecal samples from 5 randomly selected cows were collected. All samples were tested for presence of MAP by bacterial culture and PCR (Polymerase Chain Reaction).

Single faecal culture of the environmental samples from the stable identified in almost 70 % of the herds a correct MAP infection status. Environmental samples from the manure storage also identified 70 % of the herds correctly respectively. When 3 consecutive environmental samples were tested for MAP, 80 % of the farms could be assigned the correct herd infection status. All farms with a high within herd prevalence of MAP or an increasing frequency of clinical cases of Paratuberculosis could be detected by environmental faecal sampling. Culture for MAP showed better results than PCR, with most of the positive samples from manure storage sites.

These results indicate that environmental faecal samples could also be used to detect the paratuberculosis herd status of small farms as a cheap alternative to other testing schemes. Repeated testing for MAP by bacterial culture seems to be the most effective way to determine paratuberculosis herd status according to these results and might be more accurate and economic than other screening methods.

Environmental sampling for MAP could be used to establish a basically program for classification of cattle herds, national and international livestock trading and to identify and protect possible MAP-free herds from paratuberculosis.

Key words: environmental faecal samples; Mycobacterium avium subsp. paratuberculosis

MUTATION DETECTION IN THE TOLL LIKE RECEPTORS (TLR1, TLR2 AND TLR4) IN AUTOCHTHONOUS CENTRAL EUROPEAN CATTLE

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ABSTRACT

Toll like receptors (TLR1, TLR2 and TLR4) are known to recognize the bacterial PAMPs (Pathogen associated membrane proteins), and initiate the innate immune signalling. Recently, close linkage between mutations/SNPs in the protein coding region of TLRs and altered responsiveness to bacterial PAMPs has been described. Here we attempted to map SNPs in the coding regions of TLR1, TLR2 and TLR4 in autochthonous breeds from central European cattle. Population of 559 cattle consisting of Slovak spotted cattle (n=10), Polish red (n=64), Pinzgauer (n=247), Slovakian Simmental (n=206) and Dark brown Carpathian (n=32) breeds. TLR gene segments were amplified by PCR using genomic DNA as template. PCR product was then subjected for single strand conformation polymorphism analysis to group the samples according to similar SSCP profiles (Genotypes). Representative sample from each genotype was sequenced and SNPs were validated by SeqScape 2.1 software. Only two TLR4 genotypes occurred in the population, and interestingly both was in homozygous state. This indicates highly conserved nature of TLR4 gene. Although, only three TLR2 genotypes were found, two of them were heterozygous. Novel missense SNPs found in bovine TLR2 gene were Ile680 (Ile, Leu), Glu738 (Asn, Ser) and Lys754 (Lys, Arg) with 0.09, 0.04 and 0.04 frequencies, respectively. TLR1 was the most heterogeneous in the population with 14 SNPs, three silent and six missense distributed in six genotypes. The TLR1 missense SNPs were Ser216 (Ser, Phe), Leu226 (Leu, Phe), Ser228 (Ser, Phe), Ser234 (ser, Phe), Thr236 (Thr, Ile) and His244 (Gln, His). Till to date, SNPs like Pro681His and Arg677Trp in TLR2 gene, and Asp299Gly in TLR4 gene are reported as SNPs that cause increased susceptibility (risk SNPs) of host to mycobacterial and streptococcal infections. Many novel mutations and SNPs are being investigated in vitro and in vivo to assess their effect on disease resistance (linkage studies). SNP mapping presented by us in TLR genes, the key genes of innate immunity, make benchmarking for the linkage studies. Culling of the animals having risk SNPs can be the effective tool in animal breeding to increase the natural resistance of heard against selected bacterial infections.

Key words: cattle; mutation; resistance; toll like receptors

BORDER DISEASE, A POSSIBLE RISK FACTOR FOR THE REINTRODUCTION OF PESTIVIRUSES IN THE AUSTRIAN BVDV FREE CATTLE POPULATION?

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ABSTRACT

Infections with BDV are widespread in most sheep breeding countries throughout the world and BVDV infections are endemic in cattle worldwide; they are the cause of the substantial economic losses to the sheep and cattle industries due to their impact on reproduction and health. In Austria a national BVD eradication program in the cattle population has been implemented in 2004. The goal of this program is to identify and cull all persistently infected cattle, which happen to be the main source for the spreading of this virus infection. In case of success, the eradication programme will result in a zero seroprevalence and full susceptibility in cattle in several years.

Described is a summarisation of studies regarding the pestivirus epidemiology in sheep and goats in Austria. The objective of these investigations was, to find out, whether small ruminants are a risk factor for reintroduction of pestiviruses in BVDV free cattle herds in Austria.

Serological studies carried out in sheep and goats between 2001 and 2003 showed that the overall individual prevalence in sheep was 29.4 percent and the herd prevalence 62.9 percent. Marked regional differences in the seroprevalence among sheep and goats were seen. Moreover, the estimated herd and individual prevalence was significantly higher in flocks in comparison to housed cattle on the farm. Comparative neutralisation studies on ELISA positive samples carried out on blood samples collected in sheep and goats between 2001 and 2003 showed that the main source of BVDV infections were cattle. However, these studies also showed that a pestivirus epidemiology independent from cattle is present in Austrian.

During an epidemiology study in 2003 in the west region of Austria, sheep persistently infected with BDV were found. These animals were completely healthy and were pastured during summertime on an alpine meadow with other sheep. The prevalence of BDV antibody positive sheep was 67.6 % at the beginning of the pasturing and 83 % at the end of pasturing. We described the importance of communal alpine pasturing in the spread of Pestiviruses.

In 2005 a clinical healthy goat, persistently infected with BVDV in a flock housing cattle and goats was detected. In the study described the goat was potentially the source of BVDV infection in the cattle herd.

In a further study we documented the spread of Border disease virus from sheep to calves.

Border disease of sheep characterised by abortion and fetal death was described for the first time in Austria 2008 on a mixed farm (sheep and cattle). The BVDV status of the cattle herd was certified "free of Bovine viral diarrhea" since 2005. However, in correlation with the Border Disease outbreak BDV antibodies were diagnosed in cattle on this farm.

However, the results of our studies showed that BDV and BVDV infected sheep and goats are a high risk factor for pestivirus introduction in susceptible cattle herds and therefore a significant revision of the Austrian BVDV-program is necessary.

Key words: border disease; BDV; BVDV; pestivirus; cattle

NEOSPORA CANINUM - POSSIBLE CAUSATIVE AGENT OF ABORTIONS IN DAIRY FARM IN SLOVAKIA

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ABSTRACT

The worldwide distributed protozoan intracellular parasite, Neospora caninum, is considered a major cause of abortions and reproductive failure of dairy cattle. The transplacental transmission from infected dams to their offspring appears to be a major natural route of the infection, but neonatal calves may become infected by lactogenic transmission of the parasite. Dogs are definitive host for N. caninum, which play special role in horizontal transmission of the parasite by shedding oocyst into the environment. In three north-eastern Slovak dairy farms (A, B, C) reproduction analysis was performed. By reason that a specific diagnosis is achieved only in 23–46 % of abortions additive methods for diagnosis are recommended (precise anamnesis, blood and impression smears, serology, microbiology, electron microscopy, toxicology and genetic testing). Sera of 128 post aborting dairy cows (Bos taurus) of "Slovak spotted breed" were examined to anti-Neospora antibodies. Up to 39.8 % mean seroprevalence of the infection was recorded (Farm A 45.6 %; Farm B 20.4 %; Farm C 68.2 %). DNA of N. caninum was at first time confirmed in brain of aborted foetus from seropositive dam in field condition of Slovakia. The mother has aborted in her second lactation in 5th month of the gravidity. In two heifers from seropositive cows specific antibodies were found. In dogs from two farms anti-Neospora antibodies were also detected. These findings strongly indicate both, the vertical and horizontal transmission of N. caninum in herds, respectively. The high occurrence of anti-Neospora antibodies warrants the attention and a wider surveillance in Slovak herds. The examination of heifers prior their serving would help to discover and interrupt the transmission and persistence of N. caninum in stock-rising.

Key words: Neospora caninum; dog; dairy cattle; heifer; abortion; sylvatic circulation; Slovakia

INTRODUCTION

In the past decade, the protozoan parasite Neospora caninum has become increasingly recognized as an important cause of reproductive failure in dairy and beef cattle. N. caninum is closely resembles Toxoplasma gondii. Wild or domestic canids are the natural definitive host for N. caninum. Infected animals may shed large numbers of oocysts in their feces, which may then be ingested by intermediate hosts such as cattle. It may results in foetal death or birth of live congenitally infected offspring. The seroprevalence of neosporosis in Europe varied between 16–76 % for dairy cattle and 41–61 % for beef cattle (1). Cattle infected with N. caninum are three to seven times more likely to abort compared to uninfected cattle (2, 3). There is no direct transmission between cows; however, the parasite can be transmitted by the vertical rout from mother to foetus. Vertical transmission, which may occur over several generations, is a major factor contributing to the persistence of Neospora in herd (4). Congenitally infected calves may have neurologic signs, be underweight, unable to rise, or have no clinical signs. Chronically infected heifers have a higher rate of abortion, particularly during their first pregnancy, and a high rate of vertical transmission to their offspring. Therefore, N. caninum presents a major economic problem to cattle producers' worldwide (5). By comparing the Neospora status of aborting versus non-aborting cows, the seropositivity correlates with the tendency to abort (6).

To understand the epidemiology of N. caninum associated abortion in cattle it is important to discriminate between those animals that acquire infection exogenously (via ingestion of oocyst) or endogenously (transplacentally). Therefore, our aim was to analyse in addition to the occurrence of neosporosis key factors of breeding (structure and condition of breeding, reproduction analysis and the occurrence of the infection in definitive hosts) in selected farms in north-eastern Slovakia, where according to our former screening the high seropositivity against Neospora was recorded.

MATERIAL AND METHODS

Serum samples of cows

On the basis of former pilot screening of neosporosis in the north-eastern Prešov region from 305 randomly chosen dairy cattle with reproductive failures till 38.7 % mean seropositivity to Neopsora caninum was estimated. Therefore, in the selected farms with the highest seropositivity of neosporosis

a field investigation has been carried out. Totally 128 serum samples of dairy cows (Bos taurus), 17 sera of heifers originated from seropositive dams, four sera of farm dogs were examined to the presence of anti-Neospora antibodies. Seven dairies with reproductive failures were serologically followed up for the presence of specific antibodies during one year in three months intervals.

In all farms "Slovak spotted cattle" were bred with stanchion housing of dairies and free stabling of heifers. These farms, located on the north-east are neighbouring the National Park of Poloniny, characterized by broad-leaved forests and a high population density of red foxes. Farms keep from 300 to 900 animals (Table 1), with mainly stabling stock-raising (usually with stanchion housing of dairies and free housing of heifers). In all three farms, pasture breeding is used from spring to autumn, and their pastures are fenceless.

Reproduction analysis

For the reproduction analysis in first dairy farm (A) 220, in second farm (B) 90 and in third farm (C) 136 Slovak spotted breed and its crossbred multiparous cows were used. Milking in the farms was performed three times per day in separate parklour. Dosage and quality of nitrition were adjusted precise according to milk production calculated by computers schedule used in the farms. Milk production of dairies in year 2007 was 4000 $kg.year^{-1}$ in average.

Serological methods used

Anti-Neospora antibodies from serum samples were detected using an indirect ELISA (ID-VET, Innovative diagnostics, France). The test procedure was performed according to the manufacturer's instructions. The absorbance – optical density (OD) was measured at 450 nm by spectrophotometer (ThermoLabsystems, Opsys MR, USA). For each sample, the ratio of optical densities of examined serum to mean OD of positive control was calculated as S/P % according to the formula: S/P % = OD sample/ OD positive control x 100

Samples with the S/P % < 50 % were classified as negative and the samples with S/P % ≥ 50 % were considered as positive.

Specific antibodies from fat-free skimmed milk (supernatant) of 6 monitored Neospora-positive cows were analysed using a competitive ELISA (VMRD, Inc., USA). The optical density was measured at 630 nm by the same spectrophotometer. The test results were expressed as percentage of inhibition (% I) according to the formula: % I = 100 - [(OD sample x 100) / (Mean OD negative control)]

When a tested samples produced ≥ 30 % inhibition were considered to be positive and < 30 % were considered to be negative.

Isolation of DNA

Genomic DNA from the milk sediment, foetal and dog's tissues was prepared from the reference strain and from the milk sediment using the Nucleospin^® Tissue DNA extraction kit (Machery-Nagel, Germany). DNA samples were collected at 4 °C and were used in PCR.

PCR analyses

Primers Np6 and Np21 for PCR were prepared with minor modifications according to (7). The PCR reagents (MBI Fermentas, USA) were applied according to instruction of the manufacturer. Each PCR reaction was performed in a final volume 50 µl consisting of 10 pM of each primer, 1 U Tag polymerase and sample. The amplification was carried out under the next condition: 1 cycle 94 °C for 4 min as initial denaturation of templates, followed by 30 cycles with denaturation at 94 °C for 20 sec, annealing at 55 °C for 30 sec and primer extension at 72 °C for30 sec and finally 1 cycle of 72 °C for 10 min. After amplification, 15 µl of PCR products was visualised on a 1.5 % agarose gel containing 1 $\mu g.ml^{-1}$ ethidium bromide and visualised under UV light.

RESULTS AND DISCUSSION

Reproduction parameters in three north-eastern Slovak farms are showed in the Table 2. Abortion in cattle is defined as foetal death and expulsion between day 45 and day 265 of the pregnancy. If death occurs before day 40 of gestation, it is usually termed as early embryonic death, when the embryo is resorbed by the uterus with no external signs evident that the pregnancy has terminated. After 2 months of gestation, there is the expulsion of the foetus and placental tissues. Abortion in cattle is a significant cause of reproductive wastage. There are two main cases of bovine abortion. Non-infectious cases are due to genetic, nutritional, environmental, insemination, twinning trauma, drugs motives. Infectious cases may occure due to bovine virus diarrhea (BVD), brucellosis, campylobacter (Vibrio), chlamydiosis, infectious bovine rhinotracheitis (IBR), leptospirosis, trichomoniasis, sarcocystis and last but not least neosporosis. Clinical symptoms are spontaneous or induced premature cessation with expulsion of a foetus too immature for survival (< 260 days), abortion with resorption of placental fluids, foetal dehydration and uterine involution, subsequent abortion or expulsion during parturition may occur (dried, shrunken, brown/black – mummification). Other additional parameters are maceration of foetus due to bacterial infection, the bones are intact in the liquefactive, foul-smelling remains of soft tissues and accompanied by pyometra, uterine abscesses and foetal emphysema. A specific diagnosis is achieved only in 23–46 % of abortions. Requirement methods for diagnosis of abortion are precise anamnesis, blood and impression smears, serology, microbiology, electron microscopy, toxicology and genetic testing.

From 128 post-aborting dairy cattle in 51 anti-Neospora antibodies were detected with 39.8 % mean seroprevalence (Farm A 45.6 %; Farm B 20.4 %; Farm C 68.2 %) (Table 3).

From Farm C, in seven aborting dairies a persisted high level of specific antibodies was detected, during one year every three months of the monitoring (Fig. 1). Therefore, this constant pattern of increase of N. caninum antibody titres in several pregnancies suggests reactivation of a latent infection in the cows, rather than suggesting re-infection. Reactivation has also been suggested by other researchers as an explanation for transmission of the parasite during several successive

pregnancies, and for repeated abortions to occur in seropositive animals (8, 9). Moreover, seropositivity was detected in two 6 months old heifers bird from seropositive mothers. In regard of their age results indicate possible vertical transmission of N. caninum. From March 2008 seventeen new cases of abortions were recorded in Farm A and C with 47 % seropositivity of dams.

Two heifers, more than 6 months old, originated from seropositive mothers were also seropositive. This strongly indicates that N. caninum in this herd spread via vertical transmission. The vertical infection as the main form of the transmission in dairy herds was confirmed by other authors (10, 11, 12).

Totally five abortions were analysed by molecular approaches. Just from the Farm A in the brain of aborted foetus, originated from seropositive mother DNA of Neospora caninum was confirmed by PCR (Fig. 2). This is the first molecular confirmation of Neospora caninum as causative agent of abortion in Slovakia. It was abortion of dairy in her second lactation in 5th months of gravidity, without any other clinical signs. The kinetics of specific antibodies in first month of pregnancy was lower in comparison with antibody level after abortion.

In two from four examined dogs specific antibodies with relatively high S/P % (90 and 92 %) were detected. These dogs had access to the potential infected tissues of aborted foetus in pasture and in the housing zone. Thus, the presence of farm dogs may be linked with horizontal transmission of the parasite by shedding oocyst in a feed manger or into the environment. N. caninum associated abortions are not always linked with horizontal transmission, but also occur in chronically infected dams by vertical transmission. Moreover, it cannot be expected that there is always a positive association between the presence or number of farm dogs and bovine abortion (13).

These studied large breeding farms with the high Neospora-seroprevalence of 39.8 % are characterised by the pasture style of breeding. Free pastures are surrounded by temperate forests with scrubs and greenwood with a high population density of wild animals. The fauna is characterised by high biological diversity, with majority of deer. There are survive here all Slovak carnivores, mainly red foxes and sporadically from neighbouring areas of Poland penetrate bison and elk. There have been all conditions for a circulation of N. caninum by domestic and sylvatic interface and a formation of the new focus of neosporosis is expected. This hypothesis is confirmed by 11.3 % seroprevalence of N. caninum in 53 hunted wild boars from some localities in eastern and central Slovakia (14). Many aspects of the life cycle of N. caninum are unknown, but hunted wild animals represent a potential risk in N. caninum transmission to hunting dogs and ultimately to domestic livestock (15).

Since dogs are the natural definitive host for Neospora caninum, control programs should start with preventing access by dogs to infectious material such as dead calves, aborted fetuses, stillborn calves, and fetal membranes. Furthermore, cattle feed and water should be protected from contamination by dog feces.

Table 1. Structure of the farms according to the number of kept animals

Farms	Total no. of animals	Dairy cattle	Beef cattle	< 1 year	> 1 year	< 6 months
A	900	300	150	150	100	200
B	300	250	-	-	-	50
C	300	90	50	90	60	10

Table 2. Reproduction parameters in farms performed in selected animals

Farm/ no. of examined cattle	Milk production kg.year^{-1}	Days to first insemination	Days open	No. of inseminations/ cow	Conception rate at first insemination (%)
A/220	4000	75	105	1.8	50
B/90	3800	78	110	1.9	49
C/136	3900	74	106	1.9	52

Table 3. The occurrence of neosporosis in dairy cattle with the reproduction failures in farms from north-eastern Slovakia

Farms	No. examined dairies	*Neospora*-positive	%
A	57	26	45.6
B	49	10	20.4
C	22	15	68.2
Total	128	51	39.8

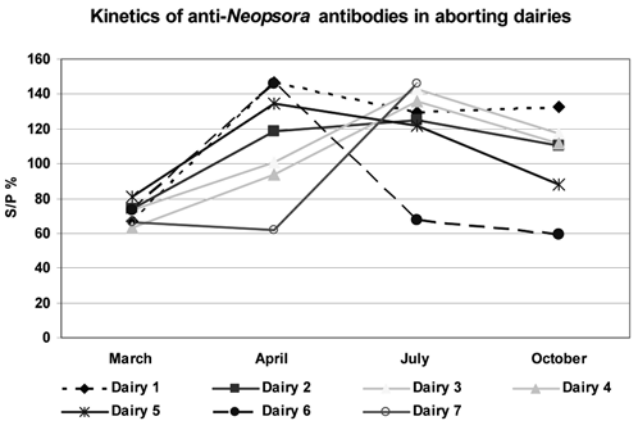

Fig. 1. Konetics of anti-Neopsora antibodies in aborting dairies

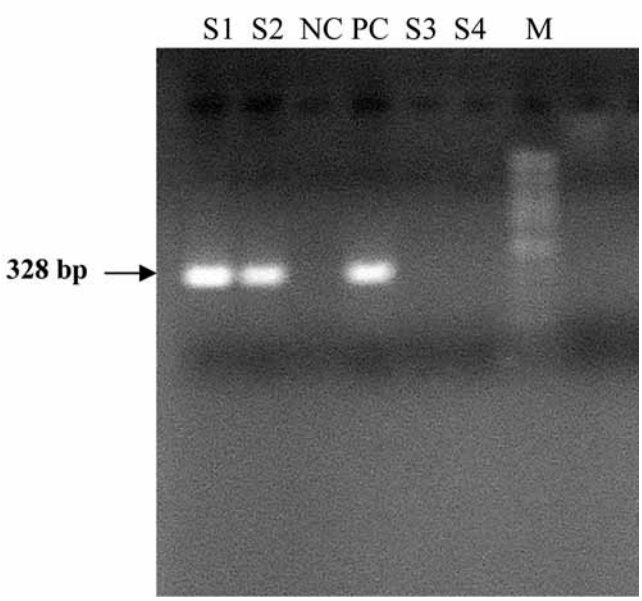

Fig. 2. DNA of Neospora caninum obtained in PCR with Np6 and Np21 primers: M –molecular mass marker; lines 1, 2 – samples of brain of aborted foetus from seropositive dam; line 3 – negative control; line 4 – positive control; lines 5, 6 – samples of brain of aborted foetus from seronegative dam

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REFERENCES

1. Bartels, C. J., Arnaiz-Seco, J. I., Ruiz-Santa-Quitera, A., Bjorkman, C., Frossling, J., von Blumroder, D., 2006: Supranational comparison of Neospora caninum seroprevalences in cattle in Germany, The Netherlands, Spain and Sweeden. Vet. Parasitol., 137, 17–27.

2. Thurmond, M. C., Hietala, S. K., 1997: Effect of congenitally acquired Neospora caninum infection on risk of abortion and subsequent abortions in dairy cattle. Am. J. Vet. Res., 58, 1381–5.

3. Wouda W., Moen, A. R., Schukken, Y. H., 1998: Abortion risk in progeny of cows after a Neospora caninum epidemic. Theriogenology, 49, 1311–1316.

4. Dubey, J. P., Buxton, D., Wouda, W., 2006: Pathogenesis of bovine neosporosis. J. Comp. Pathol., 134, 267–89.

5. Trees, A. J., Davison, H. C., Innes, E. A., Wastling, J. M., 1999: Towards evaluation the economic impact of bovine neosporosis. Int. J. Parasitol., 29, 1195–200.

6. Reiterová, K., Špilovská, S., Antolová, D., Dubinský P., 2009: Neospora caninum, potential cause of abortions in dairy cows: The current serological follow-up in Slovakia. Vet. Parasitol., 159, 1–6.

7. Yamage Yamage, M., Flechtner, O., Gottstein, B., 1996: Neospora caninum: specific oligonucleotide primers for the detection of brain "cyst" DNA of experimentally infected nude mice by the polymerase chain reaction (PCR). J. Parasitol., 82, 272–279.

8. Conrad, P. A., Sverlow, K., Anderson, M., Rowe, J., BonDurant, R., Tuter, G., Breitmeyer, R., Plamer, C., Thurmond, M., Ardans, A., Dubey, J. P., 1993: Detection of serum antibody response in cattle with natural or experimental Neospora infections. J. Vet. Diagn. Invest., 5, 572–578.

9. Pare, J., Thurmond, M. C., Hietala, S. K., 1996: Congenital Neospora caninum infection in dairy cattle and associated calfhood mortality. Can. J. Vet. Res., 60, 133–139.

10. Schares, G., Peters, M., Wurm, R., Barwald, A., Conraths, F. J., 1998: The efficiency of vertical transmission of Neospora caninum in dairy cattle analysed by serological techniques. Vet. Parasitol., 80, 87–98.

11. Davison, H. C., Otter, A., Trees, A. J., 1999: Estimation of vertical and horizontal transmission parameters of Neospora caninum infections in dairy cattle. Int. J. Parasitol., 29, 1683–1689.

12. Wouda, W., Dijkstra, T., Kramer, A. M. H., van Maanen, C., Brinkhof, J. M. A., 1999: Seroepidemiological evidence for a relationship between Neospora caninum infections in dogs and cattle. Int. J. Parasitol., 29, 1677–1682.

13. Dubey, J. P., Schares, G., Ortega-Mora, L. M., 2007: Epidemiology and control of Neosporosis and Neospora caninum. Clin. Microb. Rew., 20, 323–367.

14. Špilovská, S., Reiterová, K., Hisira, V., 2008: Seroprevalence of Neospora caninum in wild boars from selected localities in Slovakia. Infovet, 5, 222–223.

15. Gondim, L. F. P., McAllister, M. M., Mateus-Pinilla, N. E., Pitt, W. C., Mech, L. D., Nelson, M. E., 2004: Transmission of Neospora caninum between wild and domestic animals. J. Parasitol., 90, 1361–1365.

IMPORTANT DISEASES IN NEW WORLD CAMELIDS UNDER MIDDLE EUROPEAN CONDITIONS

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ABSTRACT

Llamas and alpacas as the domesticated representatives of New World Camelids became more and more popular in Europe and consequently also in Austria in the last years. Hence veterinarians are increasingly confronted with these "exotic" animals, either with routine work like vaccination, deworming and castration or when health problems occur.

Therefore the Clinic for Ruminants at the University of Veterinary Medicine in Vienna founded a working group dealing with camelids starting with a questionnaire of New World Camelid owners in German-speaking countries at the end of 2002, followed by further studies (mainly concerning endoparasites). Furthermore the number of cases at the Clinic for Ruminants increased.

In Austria approximately 3,000 llamas and alpacas are registered. They are predominantly kept as companion animals, some for breeding and trekking. The production of wool and meat however does not play a role in Europe.

I would like to present an overview of the main health problems of New World Camelids kept under Middle European conditions. Results are based on questionnaires, investigative studies and experiences at the Clinic of Ruminants.

The main health problems in these species are endoparasite associated diseases, disturbances of the (fore-) stomach (atony, acidosis, ulcers), skin lesions (mainly caused by parasites or zinc deficiency) and injuries.

Anatomy and physiology of camelids differ in some ways from our domesticated ruminants. Consequently diagnostic methods (clinical examination, sampling) as well as therapy varies between these species.

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BOVINE BESNOITIOSIS - A NEW EMERGING DISEASE?

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ABSTRACT

In August 2008 bovine besnoitiosis was diagnosed for the first time in Germany. Previously, this disease was reported from Africa, Asia, Israel, Venezula and the European countries France, Spain, Portugal, and Italy. The causative agent is the cyst-forming coccidian *Besnoitia besnoiti*, which is predominantly localized in tissue-cysts in the skin, mucous membranes and scleral conjunctiva. Clinical signs in early stages of the disease include fever, lymphadenitis, subcutaneous edema, loss of body condition and orchitis. During the chronic stage typical signs are cutaneous lesions such as thickening and folding of the skin and hypotrichia or alopecia. Severe necrotising orchitis may result in permanent infertility. Apparent cases of besnoitiosis are easily diagnosed by the combination of clinical signs and the identification of tissue cysts by histological and cytological investigations. However, most infected cattle remain asymptomatic. Besnoitiosis may have a significant economic impact on affected herds due to adverse effects on male fertility, reduction of slaughter weight, rejection of hide for leather production, and a mortality rate of 10 %. No effective treatment is known. The complete life cycle of *B. besnoiti* remains yet to be elucidated. Cattle are believed to be the intermediate host while the definite host is still unknown. Mechanical transmission by biting flies, such as tabanids, and various medical devices is possible and a direct animal-to-animal transmission seems likely. It is suspected that the infectious agent was introduced to Germany by an infected animal possibly from France.

In addition to a detailed overview on bovine besnoitiosis we will present results of investigations on the first known affected herd case in Germany.

THE EFFECT OF YEAST SUPPLEMENTATION ON RUMEN FERMENTATION DEPENDS ON THE FEEDING RATION AND YEAST STRAIN

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INTRODUCTION

Viable Saccharomyces cerevisiae (Sc) yeast strains as zootechnical additives in ruminant feedstuffs are widely used. Some of the benefits associated with Sc include increased DM and NDF digestion (10, 1) and milk production (10, 5). Yeast cultures also have been shown to stimulate utilization of hydrogen by ruminal acetogenic bacteria (2) and to decrease methane production of rumen bacteria (3). The effect of Sc strains differs depending on in vitro or in vivo circumstances, and the results of recent experiments are often contradicting. W a l l a c e and N e w b o l d (9) reported that the responses of yeast culture are highly variable and apparently in uenced by the composition of the diet. The various effects of different Sc strains are also proven (7, 4). As we do not clearly know the interactions between the rumen and the yeast we can only suppose that the reason behind it is that different yeast strains differently adapt to the conditions in the rumen.

In a previous in vitro study we have concluded that a higher trehalose content in the cell wall of yeast cells improve their rate of survival in the rumen fluid (6).

The aim of our study was to investigate the effects of trehalose producing yeast (Live Sacc Dairy, LSDairy) supplementation on the rumen fermentation of sheep.

MATERIALS AND METHODS

Three rumen cannulated merino wethers were used in a self controlled experimental design. This study served as a precedent for a study on high-yielding dairy cows, therefore the sheep were fed two types of rations similar to the rations high-yielding Holstein-friesian cows are fed with (Table 1 and 2). The high energy diet (HE) contained more concentrate (mainly barley and corn), the high fibre diet (HF) contained more forage (corn silage and alfalfa hay). The daily amount of TMR was 2 kg per animal. Licking blocks and drinking water were offered ad libitum.

Table 1. Ingredients of the two diets

Ingredients	HE diet	HF diet
Corn silage (%)	48.0	54.5
Meadow hay (%)	16.0	6.0
Barley grain (%)	6.0	12.0
Corn grain (%)	16.0	15.0
Extr. sunflower hull (%)	8.0	6.0
Extr. soybean meal (%)	6.0	6.5

Table 2. Nutrient content of the two diets

Nutrients	HE diet	HF diet
Dry matter (%)	59.7	62.4
Crude protein (% in DM)	16.5	16.4
Crude fibre (% in DM)	13.0	16.5
Net Energy L (MJ in DM)	7.2	6.8
Crude fat (% in DM)	2.8	2.8
NDF (% in DM)	30.2	34.8
Concentrate ratio (%)	60.2	51.3

In the control phase of the experiment (CO) rations were not supplemented. The control phase was followed by two experimental phases using 2.5 g trehalose producing (LSDairy) or 2.5 g trehalose non-producing (LS) yeast in the daily TMR, respectively. Rumen fluid samples were taken 3 hours after the morning feeding. Ruminal pH, total volatile fatty acid concentration and molar proportion of acetate, propionate and butyrate

Table 3. Rumen fermentation parameters during the experiment (Mean ± SD)

Items	Treatments					
	HE diet			HF diet		
	CO	LS	LSDairy	CO	LS	LSDairy
pH	^{a1}5.5 ± 0.3	^{a}5.5 ± 0.3	^{b}5.7 ± 0.2	^{a2}5.8 ± 0.2	^{b}5.5 ± 0.3	^{a}5.6 ± 0.2
Acetate, mol%	^{a}91.7 ± 11.3	^{a1}94.3 ± 14.1	^{b}101.2 ± 19.9	^{a}87.4 ± 17.8	^{b2}112.6 ± 17.8	^{b}109.7 ± 22.1
Propionate, mol%	59.3 ± 8.7	59.4 ± 3.9	60.2 ± 2.3	62.7 ± 3.9	61.0 ± 4.8	61.9 ± 4.7
Butyrate, mol%	^{a}18.9 ± 5.9	^{a}20.5 ± 2.3	^{b}22.2 ± 2.1	19.1 ± 4.1	18.7 ± 2.3	20.2 ± 2.8
Total VFA, mmol/l	14.9 ± 4.1	15.8 ± 1.8	16.1 ± 1.7	15.2 ± 2.1	15.8 ± 1.8	15.4 ± 2.6

^{a,b}Means with different superscripts within a diet are significantly different ($P < 0.05$).
^{1,2}Means with different superscripts mark significant difference between the items at HE and HF diets ($P < 0.05$).

Table 4. In sacco degradability (Mean ± SD)

Items	Treatments					
	HE diet			HF diet		
	CO	LS	LSDairy	CO	LS	LSDairy
Pelleted alfa NDF (%)	^{a1}19.7 ± 1.3	^{a}17.8 ± 2.8	^{b}21.7 ± 0.6	^{a2}23.7 ± 1.1	^{b}20.5 ± 2.9	^{b}21.4 ± 1.4
Corn grain starch (%)	53.5 ± 15.0	49.5 ± 8.6	50.7 ± 9.0	54.1 ± 7.9	50.6 ± 8.5	50.7 ± 9
Barley grain starch (%)	^{a}84.9 ± 8.7	^{a}84.2 ± 3.9	^{b1}93.7 ± 2.8	^{a}89.1 ± 2.7	^{b}85.9 ± 5.0	^{a2}89.4 ± 3.4

^{a,b}Means with different superscripts within a diet are significantly different ($P < 0.05$).
^{1,2}Means with different superscripts mark significant difference between the items at HE and HF diets ($P < 0.05$).

were measured. The degradability of corn and barley starch and NDF of alfalfa were also measured with in sacco method.

For hypothesis testing, a general linear mixed model was fit to the studied parameters. Estimation of treatment differences were calculated using contrast matrices. P-values were adjusted by Tukey-Kramer correction at multiple testings. The statistical analysis was carried out in R 2.6 statistical software (8).

RESULTS

The main results of the experiment are summarised in Table 3 and 4.

As it was supposed, rumen fluid pH was lower in HE diet in the control animals. Using LSDairy supplementation in HE, a higher rumen fluid pH was found compared to CO.

Compared to CO, TVFA concentrations were significantly higher when yeast supplementations were used in HF however LS supplementation did not changed TVFA in HE. Molar proportion of acetate in CO tended to be lower in HE. Molar proportion of propionate was higher using LSDairy in HE compared to CO ans LS in HE.

Degradability of barley starch was significantly better in HE but did not change in HF when LSDairy had been used. There was no difference in the degradability of corn starch. The degradability of NDF was significantly better in LSDairy compared to CO and LS in HE but, unsupposedly, lower in HF.

CONCLUSIONS

It was shown that rumen fermentation can be modified by yeast strains and significant differences can occur depending on the substrate (TMR) or yeast strain used.

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REFERENCES

1. Carro, M. D., Lebzien, P., Rohr, K., 1992: Effects of yeast culture on rumen fermentation, digestibility and duodenal flow in dairy cows fed a silage based diet. Livestock Production Science, 32, 219–229.

2. Chaucheyras, F., Fonty, G., Bertin, G., Gouet, P., 1995: In vitro H2 utilization by a ruminal acetogenic bacterium cultivated alone or in association with an archea methanogen is stimulated by a probiotic strain of Saccharomyces cerevisiae. Applied Environmental Microbiology, 61, 3466–3469.

3. Lila, Z. A., Mohammed, M., Yasui, T., Kurokawa, Y., Kanda, S., Itabashi, H., 2004: Effects of a twin strain of Saccharomyces cerevisiae live cells on mixed ruminal microorganism fermentation in vitro. Journal of Animal Science, 82, 1847–1854.

4. Könyves, L., Jurkovich, V., Tirián, A., Tegzes, L., Fébel ,H., Kutasi, J., Brydl, E. 2007: Comparative examination of the biological effects of Saccharomyces cerevisiae yeast cultures in dairy cows (In Hungarian). Magyar Állatorvosok Lapja (Hungarian Veterinary Journal), 129. 400–409.

5. Kung, Jr., L. E., Kreck, M., Tung, RS., 1997: Effects of a live yeast culture and enzymes on in vitro ruminal fermentation and milk production of dairy cows. Journal of Dairy Science, 80, 2045–2051.

6. Kutasi, J., Both, G., Bata, Á., Jurkovich, V., 2005: Induction of trechalose synthesis in Saccharomyces cerevisiae yeast strains (In Hungarian). Proceedings of 16th Hungarian Buiatrics Congress, 5-8 October, 2005, Balatonfüred, Hungary.

7. Kutasi, J., Jurkovich, V., Brydl, E., Könyves, L., Tirián, A.E., Bata, Á., 2004: Influence of different Saccharomyces cerevisiae strains on the oxygen concentration in the rumen fluid. Journal of Animal and Feed Sciences, 13, Suppl. 1, 131–134.

8. R Development Core Team 2007: R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL: http://www.R-project.org

9. Wallace, R. J., Newbold, C. J., 1992: Probiotics for Ruminants. The Scienti c Basis. R. Fuller, ed. Chapman and Hall, London, U.K. 317

10. Williams, P. E. V., Tait., C. A. G., Innes, G. M., Newbold, C. J. 1991: Effects of the inclusion of yeast culture (Saccharomyces cerevisiae plus growth medium) in the diet of cows on milk yield and forage degradation and fermentation patterns in the rumen of sheep and steers. Journal of Animal Science, 69, 3016–3026.

CURRENT HEALTH SITUATION IN SHEEP IN SLOVAKIA

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ABSTRACT

The work analyses health situation on farm sheep in Slovakia. Poops at morbidity and fatality were acquired following clinical and laboratory analysis and registration farmers. 80% loses of lambs into weaning time is bound on perinatal period, it means deadly born and loses till 3.day age. Main reason loses of lambs in perinatal period are deadly born lambs, dystocia, syndrome of hypotermy and starvation, congenital defects and various wounds. Observations showed that vitality, good health lambs and optimally immunized functions are straight depending on health state mother, lonely course pregnancy and own delivery. The most commonly diseases at all ranks sheep are endo and ecto parasitosis (lungworm infestation, intestinal nematodes, liver fluke disease, tapeworm infestation, sheep ked, tick infestation, psoroptic, sarcoptic, chorioptic mange). From infection diseases most frequently to diagnose at lambs listeriosis and mature sheep mastitis on various aetiology, chelodermatits infections and sporadic scrapie. Noninfectious diseases most often to wear in form metabolic or production disorders (nutritional muscular dystrophy, energy-protein malnutrition (ketosis), calcium and phosphorus deficiency, parakeratosis, enzootic ataxia, goiter, alopecia, alotriophagia and photosenzitization). Bed nutritious stage of sheep look like breeding and health phenomenon and be connected with deficiency at nutrition (quantity and quality feed), disorders of health stage and inconvenient animal hygiene and breeding conditions. From poisonings most frequently to diagnose mycotoxicosis (aflatoxicosis, stachybotryotoxicosis, fusariotoxicosis) and chronic intoxication with heavy metals (arsenic, lead, mercury) and acute copper poisoning.

Key words: health; morbidity; sheep

INTRODUCTION

Sheep farming in Slovakia has a long-standing tradition. Actually in Slovakia circa 370 thousand all categories sheep

have been raised. The breeding programe is in small ruminants specialized for production of milk (commodities of sheep and goat milk) and meat (Christmas and Easter lambs). Production of wool is marginal. Key component in small ruminants farming is landcreating. Small ruminats farming has been the economically not so attractive as another livestock farming for a long period of time but it has been one of the most stabile animal-raising branch nowadays. Diagnostic, preventive and therapeutic measures in sheep are carried out less regularly compared to other livestock. On the other hand risk of morbidity in small ruminants with impact on public health is high (scrapie, zoonoses).

RESULTS AND DISCUSSION

Diseases of young

Successful raising of lamb is the basic assumption of healthy population of adult animals. Lamb morbidity in perinatal and postnatal period essentially affects growth ability, economy of lamb raising and in a manner designates future performance (2). Total losses of lambs and kids in our sheep farms vary between 6.2–10.9 % until weaning time. Main cause of lamb losses in perinatal period are stillborn lambs, dystokia (complicated parturition), syndrome of hypothermia and starvation, congenital defects and diferrent injuries. Syndrome of hypothermia and starvation contributes to perinatal losses with 35–50 % and its contribution to the losses until weaning time is 70–80 % (1). Vitality and good health state of lambs including optimal immune functions is directly dependent on the health state of mother, course of gravidity and parturition itself. Lower vital weight at birth and viability can be noticed in lambs, whose mothers were fed with insufficient and imbalanced feed ration, were stabled in inconvenient zoohygienic conditions or they suffered by different diseases (at ewes in our farming conditions these are chronical endo and ecto parasitosis, chronical bronchopneumony, infectious limping, etc.) during gravidity. Fetus during intrauterine growth is fully dependent on the nutrients that are accepted from mother. Most critical period for mother and young is in first 24 to 48 and in some cases till 78 hours post partum (perinatal period). In general lambs at birth are relatively sensitive to the syndrome of hypothermia and starvation. It is associated with insufficient developed thermoregulation system until the third day of life, low reserves of body fat at birth (low source of emergency energy), big surface of body compared to weight (assumption of considerable heat loss in cold and wet enviroment evaporation through the wet body surface at birth). Limiting factor for good health state and optimal youngs is early intake of sufficient amount of good-quality colostrum. Similar as it is in other ruminants colostrum is the basic source of essencial antibodies, vitamins, minerals, sacharides, fat, protein and other biologically active matter. Colostrum must verify quality requirements (designates health state a condition of ewes, numbers of parturitions, nutrition), quantity (optimal colostrum uptake 50 $ml.kg^{-1}$ of body weight/feeding or 210 $ml.kg^{-1}$ of body weight/first 24 hours, in bad climatic conditions – cold and wet environment requirement for colostrum is increased with 15 %) and the speed of intake (lamb is able to accept sufficient amount of colostrum during first 30 to 60 minutes at birth). In breeding practice it is important to respect the rule that young must get colostrum birth as soon as possible immediately after birth and suckle 6 times during first 24 hours of life. This argument corresponds with the fact that ewe produces colostrum approximately to 18 hours post partum and permeability of youngs´ intestines for antibodies is highest directly at birth, after 7 hours begins to decrease and after 24 hours it becomes very low. In case of colostrum immunoglobulines transport to lamb blood, they are protected against infections or systemic diseases. Another diseases, which influence lamb and kid losses until weaning time negatively are diarrhoic and respiratory syndrome (ratio on global losses until weaning time 10–15 %), omphalophlebitis and arthritis (ratio on global losses until weaning time 3 %). Another diseases, which play role in lambs a kids losses until weaning time or after weaning are infectious diseases (e.g. listeriosis, pasteurelosis, ecthyma, tetanus, ratio on global losses 5–10 %), parasitosis (coccidiosis, ratio on global losses 12 %, moniesiosis, ratio on global losses 10 %, other endoparasitosis, ratio on global losses 5 %, ectoparasitosis, ratio on global losses 1 %), nutritional muscle dystrophy (ratio on global losses 5 %), hoof and bone diseases (ratio on global losses 2 %), cerebrocortical necrosis (ratio on global losses 0.5 %), and other metabolic diseases (ratio on global losses 5 %) (Figure 1).

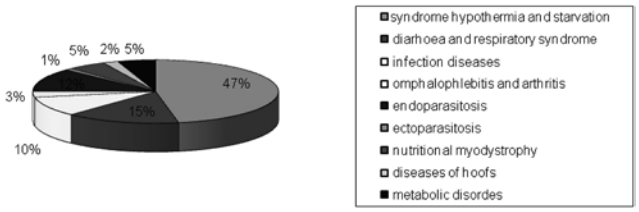

Figure 1.

Diseases of adults

The highest morbidity at mature sheep is diagnosed during breeding time. Diseases originate on multifactorial base as disorders energetic, protein, and mineral metabolism, functional disorders of liver, disorders of reproduction and mastitis. Predisposition on their genesis participate effect of nutrition, metabolic, parasitic, infection, toxic, immunologic, stressful, and technological factors.

Keeping of health of population sheep, their productive and reproductive efficiency begins by selection health and production criteria in selective breeding or goal-directed top cross e.g. on higher milk and meat production, resistance against some diseases (genetics of health) (3). Nutrition of ewes during first 8 weeks pregnancy must be organized so as during hereof terms avoid to slump weighted. Losses of weight ewes in excess of 2.5 kg during first 60 days of pregnancy (approximately 40g/day) results in an retardation development of placenta and growth of fetus. During interim 9 to 16 weeks of pregnancy optimal nutrition of ewes is warranty them good nutrition state. During these terms ewes showing low condition state look like 2.5 are feeding under separate cover. Goal of

optimal nutrition of ewes is achievement at the end pregnancy condition state in all animals from 2.5 into 3.5. The most important terms for development of fetus and health state of ewes is their nutrition 6 weeks before parturition. Almost of 70% fetal growth overshoots in these terms. Shortcomings in nutrition of ewes at the end of pregnancy induce low weight lambs at birth, low natality, advanced perinatal and postnatal morbidity and mortality of lambs, lower production of milk in ewes and occurrence ketosis. Till 50 % nutrients multi they need ewes at the end pregnancy look like on its start alternatively 1.5 multi in comparison with terms without production of milk. In lactation ewes are claim to feed 2.5 till 3.0 times advanced in comparison with animals without production milk. Following presentation is with reason take upon one to feeding with energetic food in minimally 6 weeks before assuming parturition (0.30 till 0.50 kg concentrate/piece/day).

Total losses mature sheep in breeding conditions move from 4 in 11%. The highest share in morbidity mature sheep have they endoparasitosis and ectoparasitosis (10–50%) , diseases of hoofs (3–30%), ketosis (7–13%), disorders of fertility (abortion, infertility prolapsus, dystocia, postpartal complication, metritis 1–12%), infection diseases (e.g. listeriosis, pasteurelosis, Maedi-Visna, mycoplasmosis, scrapie 8–13%), disorders of mineral metabolism (Zn, I, Se, Cu, Co, Ca, P, Mg 5–10%), mastitis (3-8%), deficiency of vitamins (E, A, C, B, D-1-5%), intoxication with organic and inorganic polutants, contaminated feeds, mycotoxicosis, fotosensitization (1–4%) Figure 2. Difference in prevalence diseases of small ruminants at study farms be affected levels of nutrition, sanitation rearing, welfare , nutrition state, age, lactation curve, stage of reproduction, general health state, and genetic lineage several strain.

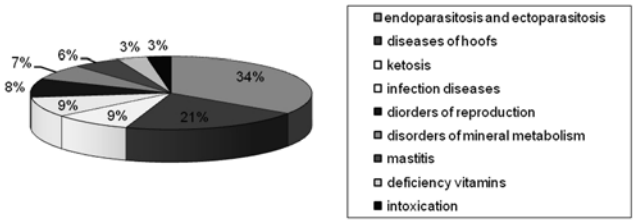

Figure 2

Interim markers that are mention violation interaction of small ruminants with living condition on farm belong e. g. slump milk production, changes of physical, chemical and biological in composition of milk, distinction in required body condition of animals (upwards of 10% in heard), infertility (quiet estrus, low fertility), dystocia, prolapsus, complication after parturition, higher morbidity of lambs and kids in period peripartal and pospartal, occurrence noninfection (organs and metabolic disorders), infection, parasitic and rest diseases in herd upwards. Look like subsidiary markers for arbitration health state of sheep possible in herd watch e. g. intake dry matter (detached evaluate up to the mark flock than individuals), quantity and quality of feeds (analysis of all feeds is optimally have to disposition before their integrate into nutrition dosage), quantity and appearance faeces (primer parameter for arbitration health state and quality of feeding) and behaviour of animals.

Following analyses diseases of sheep in Slovak republic is with reason inspection health state of small ruminants look like integral component of economics health and effectiveness of rearing. Application program of health on levels of farm is bottom on working-out diagnostic, preventive and therapeutic measures and practices. Therefore so as programs of health on farm sheep were effectively to must out from analyses existent rearing (morbidity, mortality, epizootic situation, body condition, behaviour of animals, intake of feeds, succeed parameters of production and reproduction, etc.), condition of outer environs (soil and climatic conditions) and system of technology of stabling, feeding, nursing and milking.

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REFERENCES

1. Bíreš, J., 2007: Starostlivosť o zdravie jahniat pri narodení. Chov oviec a kôz. 27, 27–28.

2. Nash, M. L., Hungerford, L. L., Nash, T. G., Zinn, G. M. 1996: Risk factors for perinatal and postnatal mortality in lambs. Vet. Rec., 139, 64–67.

3. Dwyer, C. M., 2000: Genetic and physiological effects on maternal behaviour and lamb survival. J. Anim. Sci., 85, Suppl. 1, 458.

METHODS TO IMPROVING PERIPARTURIENT HEALTH IN DAIRY COWS – A NEW CONCEPT OF PERIPARTURIENT MANAGEMENT

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ABSTRACT

An efficient and sustainable dairy production requires healthy cows with a relative long span in fair wellness. The periparturient (transition) period of dairy cows is withal the most important phase of their re/production cycle. Nutritional and welfare mismanagement, especially during the transition period, leads to metabolic disorders and subsequently to impaired production – as a syndrome of periparturient crisis in the dairy cow. We tested, as a new components of dairy herd and periparturient management of cow, new methods of their nutrition and care, including: permanent monitoring and data analysis of relevant health, production and economic parameters; checking of the udder and claw (limb) health and BCS before drying; use of uniform high fibre, low energy dry cow diet from drying to calving; guarantee of high comfortable stabling; determination of clinical, milk and biochemical score as a part of controlling of fresh (postparturient) cow.

Key words: dairy cows; periparturient period; syndrome of periparturient crisis; nutrition; The Goldilock´s Diet; welfare; monitoring of relevant health, production and economic parameters; clinical, milk and biochemical score

ABBREVIATIONS

BHB – ß-hydroxybutyrate, BCS – body condition score, DCAB – dietary cation-anion balance, NEB – negative energy balance, NEFA – non-esterified fatty acids, NEL – net energy lactation, ROS – reactive oxygen species, SARA – subacute ruminal acidosis, TMR – total mixed ration.

INTRODUCTION

Dairy farmers, industries, politicans and consumers put heavy demands on dairy production. Such demands challenge dairy cows to produce high quantities of high-quality and healthy milk. Nutrient and welfare management play a crucial role in supporting the dairy cow to cope with these challenges. The quality of care and efforts to achieve financial effects often do not provide enough chance to sustainably secure optimum condition for the animals (welfare) and health. The requirement for the economic prosperity and need to ensure optimum health can become contradictory. Occurrence of metabolic disorders, mastitis, metritis, laminitis, fertility disorders, calf diseases, high mortality and culling after the calving give an evidence of validity and consequences of this approach. But an opposite situation is desirable – good health is a basic prerequisite of high quality dairy production and cost-efficiency of dairy farming (4).

Production diseases

In past decades, the intensive genetic improvement has lead to dairy cows having to spend extremely high amounts of nutrients to provide for high milk production. It happens quite often that herd management level is not adequate to the genetic potential of cows which brings the cow organism to the patophysiological limits. This situation often results in the development of clinical or subclinical production diseases. Production diseases are the most significant health, welfare and economy problems in dairy industry_(5).

Physiological prerequisites of milk production

The basic etiological factor of production diseases is insufficient securing of physiological prerequisites of milk production, i.e.: 1) continuous comfort (high level of welfare) of the animals; 2) as high as possible levels of dry matter intake, high quality diets; 3) proper processes of rumen fermentation and proteosynthesis, digestion and absorption in the digestive tract; 4) efficient utilization of metabolites and substrates (from the digestive tract and from muscle, adipose and bone tissue) in metabolic processes that take place in the body; 5) intensive milk secretion in tubuloalveolar mammary cells that does not harm the body (7).

Stress

If the above prerequisites are met, even cows predisposed to high yield show the adaptability to stress at a required extent. Stress can be characterized as a complex of different burden factors (stressors) and their effects on the living organism and a complex of the organism's responses. According to current stress conceptions the general response of the body consisting in the activation of stress axes (Selye´s concept) is a little more complex. The response reflects complicated interactions at the level inputs (different nature of stressors and possible interactions), outcomes (division of consequences in the form of

reactions and effects) and feedback. The mechanisms of stress development are executed by the central nervous system with the involvement of cerebral cortex, limbic system and reticular formation, sympaticus-adrenal system, classic structures of the endocrine system, diffuse neuroendocrine system and others. The roles of different mediators are also important such as cytokines, ROS, acute phase reactants, etc.

Syndrome of periparturient crisis in the dairy cow

In summary, disorders and diseases related to the periparturient period are called – based on chronology and interlinks between causes and effects of the cow's body – a syndrome of periparturient crisis in the dairy cow (7, 9). The development of this syndrome is caused mainly by the following forms of stress situations: 1) Low level of care of cows and insufficient welfare may cause the environmental stress and subsequent adaptation mechanisms to the stress, including the neurohumoral production of cortisol and catecholamines, accompanied by a decreased feed intake and negative energy balance. 2) Excessive energy intake in the end of lactation and during the dry period, causing the overconditioning. The body of the cow predisposes to the development of insulin resistance and intensive periparturient lipolysis, associated with loss of appetite. In fresh cows this leads to the elevation of plasma concentrations of NEFA, which cannot be fully oxidized by the liver, and BHB. As a consequence, liver steatosis develops, and ketone bodies formation increases and liver insufficiency develops. The effect of excessive energy intake can be regarded as energy stress with the development of hyperglycemic and hyperinsulinemic ketosis. 3) Low dietary calcium intake, disorders such as impaired absorption in the gut, limited release from the bone tissue, impact of metabolic disorders (alkaloses) and high calcium requirements for the production of colostrum and milk cause the impairment of calcium homeostasis in the cow. The induced hypocalcaemia and related stress reactions (so called calcium stress) are accompanied by a retardation of digesta passage, resulting in a further decrease in feed intake and deepening of NEB. A decrease in the abomasum muscle tone due to hypocalcaemia may contribute to abomasum displacement. The inhibition of the uterine muscle contraction may worsen the course of parturition and along with immunosupression it may lead to retained placenta and metritis development. Also contractions of teat sphincter muscles are inhibited, which increases the risk of mastitis. 4) A sudden increase in the dietary intake on non-structural, rapidly fermentable saccharides, causing an incorrect ratio to the content of slowly fermentable fiber and crude protein, or their different nitrogen fractions. This is a stress situation, so called rumen stress, characterized by the development of rumen fermentation disorders (most frequently SARA), accompanied with indigestion and associated with the effects of many toxic substances leading to deterioration of rumen mucosa and hoof corium (laminitis). 5) A drop in the levels of immune response (immune suppression) as a result of physiological hormonal (neurohumoral) processes. In the case of stress induced by the above-mentioned processes (an increase in cortisol levels due to the environmental stress, the impact of acute deficiency of readily available energy, intensive lipolysis, formation and action of adipokines, the development of insulin resistance, development of hepatopathies, compromising normocalcaemia, development of rumenopathies, disorders of acid-base balance) and combined effects, this drop is further potentiated (so called immune stress).

Prerequisites of full value dairy production

During the whole reproduction cycle, but particularly in the periparturient period, the interactions between production diseases and insufficient level of meeting known prerequisites of full value dairy production are typical, in the following areas: 1) management; 2) stockmanship; 3) welfare (technological and hygiene conditions in the herd); 4) herd improvement, genetic control; 5) herd replacement (rearing young animals); 6) production and preparation of feeds, techniques of feeding, nutrition and dietetics, nutrient conversion; 7) preventative care of animal health, metabolism control, immunity and reproduction functions, ensuring optimum body condition, good health and longevity (8).

Cascade effect

Original conception of production diseases included mainly patogenetic aspects such as ketosis, hypocalcaemia and ruminal dysfunctions (i.e. metabolic and nutritional diseases). This conception was later complemented to include many clinical diseases, often influencing each other: difficult calving, prolapses of vagina and uterus, retained placenta, downer cow syndrome, fat liver syndrome, SARA, metritis, mastitis, displaced abomasum, laminitis, ovarian cysts, infertility and others. Animals suffering of these problems show decreased feed intake. This further decreases their intake of energy, Ca, P, Mg, trace elements and vitamins (antioxidants) and further worsens the above-mentioned processes and diseases. Because of this type of development, we talk about „cascade effect" in production diseases, or circulus vitiosus (9).

METHODS, RESULTS AND DISCUSSION

A new concept of periparturient management (NC PPM) – prevention of problems

For several decades it has been recommended to feed dry cows a diet with a high fiber content and in the last 3 weeks before parturition provide several kilos of concentrate (2). However, this approach has shown not to be able to prevent the occurrence of health disorders in fresh cows. There is no clear evidence in the literature to support the theory that the ruminal microflora requires several weeks before the calving to adapt to the diet with a high starch content. On the contrary, it seems that the inclusion of maize silage in the dry period diet is sufficient for the adaptation to a higher starch intake after the calving, particularly if a high quality of TMR is used that prevents the cows from ingesting large amounts of concentrate. Moreover, feed intake after the calving is limited and increases slowly (3). The traditional cow nutrition in the prepartum period provides amounts of nutrients exceeding requirements. The animals receive up to 40 % more energy than they need. This leads to overconditioning and increased

internal fat deposition, which is the main patogenetic triggering factor of the cascade of interlinked periparturient diseases resulting in intensive postparturient (periparturient) lipolysis (1). High losses in lactating cows and performance after the calving (with regard to the above-mentioned processes) require a preventative medical solution.

This should include (within the new complex conception of periparturient management) these elements:

Element A) Permanent monitoring programme of the health, production and economic parameters

The basis of this approach (not only in the periparturient period but over the reproduction cycle or lifetime of a cow) includes:

1) daily recording of relevant information on individual cow and all the herd by people responsible (lifetime records of the cow);

2) continuous health, production and economy analysis of data-set from farm and central databases;

3) systematic use of the data at pre-defined intervals, including the comparison with defined production and economy goals, previous periods, other cattle herds and populations;

4) planning of preventive medicine interventions according to the health-production programme of the herd;

5) software modifications based on the results of summarized monitoring (6).

Element B) Checking of the udder and claw (limb) health and BCS before drying

An important part of periparturient management is the monitoring of udder health, limbs and body condition score. Each cow should be examined 4–6 weeks before drying, focusing on these areas. If health disorders are detected, the cow must be subjected to therapy and the disorders corrected. A BCS check is relatively easy if a cause of a pathologic development is incorrect nutrition in the end of lactation. There are greater problems with keeping physiological BCS values (2.75–3.25) in animals with long calving to calving interval, mainly in herds with transferring cross-breeding (from one breed to another) or several breeds.

Element C) Changes in dry period and pre-fresh nutrition

Recently, one dry cow diet (from drying to calving) with low energy and high fiber has been used with good effects on the health of cows and newborn calves (1). The authors called it „The Goldilocks Diet". It leads to a decrease in NEFA and BHB in the serum and to less difficult calvings, stillborn calves and health disorders. The diet is based on maize silage (32 % DM of the diet), grass, clover or alfalfa silage (20 %), soybean extracted meal and mineral feeds (up to 18 %) and wheat straw (30 %). TMR dry matter must be lower than 50 % and separation of components by the animal should not take place. The length of straw particles should be 3–5 cm. Daily dose of straw is about 3.5 kg which prevents even a cow with good appetite to consume too much energy. Intake of Ca^{2+} and K^{+} is decreased and maintaining a required level of DCAB and normocalcaemia are facilitated. A few days before the calving it is beneficial to provide – in heifers, cows with twins and other cows at risk – dietary additives (propylenglycol, glycerol, yeasts, vitamins, aminoacids etc.). It is also recommended to include 0.5 kg cut straw a day in the fresh cow diet.

Element D) Reduction of environmental stress in the periparturient period (securing welfare).

As mentioned above, the development of periparturient indigestion, lipolysis, ketosis, liver steatosis, hypocalcaemia and immunosuppression are influenced by the levels of cortisol released in the body during the environmental stress. An increased cortisol level is associated with decreased feed intake and (mainly in high BCS cows, or those with higher internal fat) worsens the above-mentioned pathological processes. This is why the periparturient management should include the efforts to achieve well-being and welfare_(5). Heifers and older cows should be in different pens, groups of animals at risk should be made: cows with twins, low BCS, diseased cows, cows affected by the syndrome of periparturient crisis during the previous calving, etc. The move to the pens for the pre-fresh cows can be done 8 to 14 days before calving in non-problem cows; cows with problems should be moved 5 to 7 days earlier. It is necessary to provide enough fresh water, air and space for comfortable lying, optimum temperature and humidity and free access to feed bunk (there should be enough bunk space to enable all the cows in the pen to eat at the same time). An important recommendation ensuing from the new concept (if a design of the barns enables it) is gradual calving of the groups of cows (housing 4 3 2 1 cows), with no other cows coming in the calving pen until it is empty. Then it must be thoroughly disinfected, bedded and kept very clean, and cleaned after every calving. It is also recommended to reduce moves (mixing) of cows in the whole transition period. It is recommended to make one group of cows at dry-off and the other groups 10–14 days before the calving. There should also be a post-fresh cow pen from 1 to 14 days after the calving (in smaller herds to 30 days), with stocking density to occupy up to 80 % of freestalls and 75–80 cm bunk space per cow.

Element E) Systematic physical examination of fresh cows (postparturient protocol)

In many herds, a regular physical examination of fresh cows is performed. In the new concept, clinical index of the cow is determined. The index is calculated by adding negative points (minus 3 when the deviation from the physiological value is small, minus 5 when great) assigned to different pathological processes. The evaluation includes the status before the calving, appetite, course of calving, placenta retention, hypocalcaemia, body temperature, locomotive activity, rumen fill and motorics, diarrhea, ketone bodies in urine, urinary pH, abomasum displacement, udder diseases, reproductive tract and limb diseases, pneumonia development, changes in milk yield, BCS dynamics and other factors. The traditional postparturient examination takes place 7 to 10 days after the calving, the new concept includes the whole periparturient period, with an emphasis put to first 14 days of lactation. The index value is used for the determination of dairy cow clinical score (score 1–5). Score 1 is physiological condition, score 5 stands for a deeply impaired health status. Based on the results of milk analysis

Table 1. Use of Elements NC PPM on Farm 1

	Element A	Element B	Element C	Element D	Element F	Element G
February 2008	Yes	No	No	No	Yes	No
February 2009	Yes	No	Yes	No	Yes	No

Table 2. Health and production parameters of herd on Farm 1

	Daily milk yield to 50th day of lactation	Culling to 50th day of lactation	Metritis	Calcium1. Day of lactation	NEFA 1. day of lactation	BHB 10. day of lactation	Clinical score	Milk score	Biochemical score
	l	% of animals	% of animals	mmol/l	mmol/l	mmol/l	Points	Points	Points
February 2008	42.1 ± 4.3	4	11	1.97 ± 0.14	0.42 ± 0.18	0.84 ± 0.23	2.17 ± 0.75	1.73 ± 0.45	2.15 ± 0.32
February 2009	32.2 ± 5.7	9	23	1.42 ± 0.25	1.03 ± 0.28	1.72 ± 0.37	3.86 ± 1.15	2.59 ± 0.75	3.81 ± 0.51
Significance	*			*	**	**	**	*	**

P ≤ 0.05 = *, P ≤ 0.01 = **

Table 3. Use of Elements NC PPM on Farm 2

	Element A	Element B	Element C	Element D	Element F	Element G
February 2008	Yes	No	No	No	Yes	Yes
February 2009	Yes	Yes	Yes	Yes	Yes	Yes

(fat and protein content, fat/protein ratio, lactose content, fat-free dry matter, ev. urea), and SCC values, milk score of the cow (score 1–5) is determined at the first test (or second). A new feature of periparturient control, enabling the evaluation of nutrient conversion and accurate diagnosis of pathological processes is the biochemistry score of the cow (score 1–5). It is based on results of serum analyses on the calving day and days 10 and 30 postpartum. It is determined in 5–10 % cows. The following parameters are analyzed: total protein, albumin, urea, AST, GMT, creatinkinase, C, anP, Mg, total billirubin, NEFA, BHB, and other parameters.

Element F) Efficient modern therapy

The efficient therapy should follow each detection of a pathological process in a cow that has just fallen ill.

Element G) Biosecurity programs

Modern management should observe all the principles of disinfection and rodent and insect control (particularly in calving pens and fresh pens) and infectious diseases control (elimination of causative agents, vaccinations).

Farm 1

February 2008 – classical nutrition and management (87 parturitions, blood samples – 20 cows):

1040 H cows, drying 57 ± 11 days before calving, dry period 42 ± 6 days (one group, outside shed plus yard), close-up period 15 ± 3 days (14 outside parturition pens, straw bedding), fresh I group 7 ± 2 days (one large pen, straw bedding), fresh II group next 21 ± 2 days (one line of individual boxes, mattress). Dry group TMR: maize silage 6 kg, alfalfa silage 21 kg, grass hay 2 kg, soybean extracted meal 0.6 kg, wheat 0.2 kg, mineral feeds 0.2 kg, brewer´s grain 1 kg. Crude protein: 15.4 %, crude fiber 22.4 %, NEL 5.12 MJ, Ca 82 g. Close-up group TMR: maize silage 11 kg, alfalfa silage 13 kg, wheat straw 0.6 kg, brewer´s grain 2 kg, molasses 0.4 kg, concentrate (anion salts 20 %, soybean extracted meal 15 %, rape extracted meal 10 %, wheat, barley, energy additive, mineral feeds) 3.5 kg. Crude protein:

Table 4. Health and production parameters of herd on Farm 2

	Daily milk yield to 50th day of lactation	Culling to 50th day of lactation	Metritis	Calcium 1. day of lactation	NEFA 1. day of lactation	BHB 10. day of lactation	Clinical score	Milk score	Biochemical score
	l	% of animals	% of animals	mmol/l	mmol/l	mmol/l	Points	Points	Points
March 2008	34.5 ± 4.1	6	1	7	1.75 ± 0.22	0.76 ± 0.23	1.28 ± 0.26	2.93 ± 0.82	2.32 ± 0.73	2.86 ± 0.78
March 2009	39.3 ± 2.7	2	9		2.03 ± 0.17	0.49 ± 0.14	0.74 ± 0.18	1.78 ± 0.57	1.53 ± 0.48	1.61 ± 0.69
Significance	*				*	*	*	* *	*	*

P ≤ 0.05 = *, P ≤ 0.01 = **

Classification: NC PPM was adequately (better) for substitution of classical system of care.

14.9 %, crude fiber 17.4 %, NEL 6.28 MJ, Ca 96 g.

February 2009 – partially use of the NC PPM (91 parturitions, blood samples – 22 cows):

1030 H cows, drying 53 ± 9 days before calving, dry period 38 ± 5 days (one group, outside shed plus yard), close-up period 15 ± 2 days (14 outside parturition pens, straw bedding), fresh I group 6 ± 2 days (one large pen, straw bedding), fresh II group next 22 ± 2 days (one line of individual boxes, mattress). One TMR for dry and close-up group: maize silage 14 kg, bean silage 7 kg, wheat straw (particles 8–10 cm) 3 kg, brewer´s grain 2 kg, molasses 0.4 kg, concentrate ($CaCO_3$ 4 %, soybean extracted meal 15 %, rape extracted meal 10 %, wheat, barley, energy additive, mineral feeds) 2 kg. Crude protein: 13.1 %, crude fiber 25.6 %, NEL 5.82 MJ, Ca 155 g.

Results of control biochemical analysis of 12 animals in fresh I group in February 2009: diagnosis – ruminal alkalosis (ruminal fluid: pH 6.82 ± 0.22 log mol c), compensated metabolic alkalosis (venous blood: pH 7.366 ± 0.003 log mol c, BE + 6.4 ± 0.3 $mmol.l^{-1}$, HCO^3 29.1 ± 1.5 $mmol.l^{-1}$, pCO_2 7.66 ± 0.85 kPa).

Main causes of problems: high content of calcium in ration, high content of crude fiber, long particles of straw, separation and subsequent separation of TMR, insufficient nutrition of submissive animals, suspected antinutritive substances in the bean silage, low production of ruminal microbial protein, environmental stress from mixing cows in parturition pens.

Classification: inadequately for substitution of classical system of care as a result of listed compromise practices

Farm 2

March 2008 – classical nutrition and management (30 parturitions, blood samples – 12 cows):

400 Holstein cows, drying 55 ± 4 days before calving, dry period 34 ± 4 days (one group, outside shed plus yard), close-up period 21 ± 5 days (2 inside parturition pens, straw bedding), fresh I group 8 ± 2 days (one large pen, straw bedding), fresh II group next 20 ± 3 days (two line of individual boxes, straw bedding). Dry group TMR: maize silage 9 kg, alfalfa silage 12 kg, alfalfa hay 2 kg, barley straw 1.5 kg, mineral feeds 0.2 kg, brewer´s grain 1 kg. Crude protein: 13.7 %, crude fiber 27.7 %, NEL 5.1 MJ, Ca 122 g.

Close-up group TMR: maize silage 13 kg, alfalfa silage 11 kg, alfalfa hay 0.5 kg, brewer´s grain 2 kg, fresh beet pulp slices 1.5 kg, glycerol 0.6 kg, concentrate (soybean extracted meal 25 %, rape extracted meal 12 %, wheat, barley, maize, mineral feeds) 4 kg. Crude protein: 17.7 %, crude fiber 18.7 %, NEL 7.5 MJ, Ca 135 g.

March 2009 – use of the NC PPM (32 parturitions, blood samples – 12 cows):

390 Holstein cows, drying 52 ± 5 days before calving, dry period 40 ± 4 days (one group, outside shed plus yard), close-up period 12 ± 2 days (4 inside parturition pens, straw bedding), fresh I group 21 ± 4 days (one large pen, straw bedding), fresh II group next 21 ± 5 days (two line of individual mattress).

The same TMR for dry and close-up group: maize silage 10 kg, alfalfa silage 4 kg, pea silage 2.5 kg, wheat straw (particles 3–5 cm) 3 kg, brewer´s grain 1.5 kg, fresh beet pulp slices 1.5 kg, concentrate (soybean extracted meal 65 %, wheat, maize, energy additive, mineral feeds) 1.5 kg. Crude protein: 14.8 %, crude fiber 22.8 %, NEL 5.87 MJ, Ca 79 g.

CONCLUSION

Thus, preventive medicine procedures combined with individual diagnostic and therapeutical intervenes are basic features of modern veterinary medicine performed by private practitioners in high-producing dairy herds.

REFERENCES

1. **Drackley, J. K., Janovick-Guretzky, N. A., 2007:** Rethinking Energy for Dairy Cows. In Proceedings of the Four-State Nutrition and Management Conference, Dubuque, 19–34.

2. **Grummer, R. R., Brickner, A., Silva-del-Rio, 2007:** High Forage or High Grain for Dry Cows: What is Best for Animal Health and Production? In: Proceedings of the 13th International Conference Production Diseases in Farm Animals, Leipzig, 187–195.

3. **Grummer, R. R., 2008:** Feeding Strategies to Reduce Metabolic Disorders in Periparturient Dairy Cattle. In: Proceedings of the 1st US-CZ Dairy Plan 2008, V. Jenikov, 12–25.

4. **Noordhuizen, J., Cannas, J., 2008:** Veterinary Herd Health & Production Management and Quality Risk Management programmes in dairy practice: what´s new? In: Proceedings of the XXV. Jubilee World Buiatrics Congress, Budapest, 91–98.

5. **Nordlund, K. V., 2008:** A Changing Perspective of Monitoring and Managing Transition Cows. In: Proceedings of the 1st US-CZ Dairy Plan 2008, V. Jenikov, 26–31.

6. **Nordlund, K. V., Bennet, T., 2009:** WisGraph 8.0 Operations and TCI™, AgSource, Verona, 1–8.

7. **Skřivánek, M., Doubek, J., Šlosárková, S. *et al.*, 2001:** Prerequisites of full-value production in high-producing dairy cows. In: Proceedings of the 3rd Middle-European Buiatrics Congress "Health problems of Ruminants", Milovy, 325–328.

8. **Skřivánek, M., Šlosárková, S., Vacek, M., 2006:** Prerequisites of dairy cows full-value production and their realization in a modern farm. In: Proceedings of the international conference "Milk Day 2006: Health, welfare, performance and production quality in dairy cows", Czech Agricultural University Prague, 13–16.

9. **Skřivánek, M., Šlosárková, S., Doubek, J., 2007:** Methods to control the peripartal period in dairy cows. In: Proceedings of the 13th International Conference Production Diseases in Farm Animals, Leipzig, 386.

10. **Skřivánek, M., Šlosárková, S., 2008:** Monitoring of Health Parameters in Dairy Herd. In: Proceedings of the International Conference "Milk Day 2008: Prerequisite of Effective Milk Production", Czech Agricultural University Prague, 62–64.

11. **Skřivánek, M., Šlosárková, S., Fleischer, P., Doubek, J., 2008:** Intensive care – a Prerequisite to Controlling the Transition Period in Dairy Cows. In: Proceedings of the XXV. Jubilee World Buiatrics Congress, Budapest, 151.

HEALTH STATUS OF BEEF COWS AND THEIR CALVES IN THE CZECH REPUBLIC

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ABSTRACT

The objective of this study was to assess the health status and the most common health problems occurring in beef cattle in the Czech Republic.

Thirty nine beef herds of different breeds were monitored from January 1st to December 31st, 2007. The health status of 4556 animals (2556 cows and 2249 of their calves) was analyzed. Farm management data were collected by means of a questionnaire completed by the farmers. Herd characteristics were evaluated, as well as the course of calving, losses in calves due to different diseases and in different periods post partum.

The conception rate was approximately 90 %. Total losses of calves were 10.5 % on the average. Most calf deaths occurred during the first week of life (61 %). Betwen two and four weeks the losses were 27 % and up to six months of age, 10 %. The most common cause of death was diarrhea (65.6 %), followed by respiratory diseases (9.4 %), omphalitis (10 %) and low viability (4 %). In the calves that survived the early stage of life, diarrhea was most often diagnosted (39.7 %) followed by respiratory diseases (13.8 %), omphalitis (13 %), sepsis (5.6 %), low viability (12.5 %) and others (15.6 %). Adult animals suffered most frequently from metabolic disorders (17.2 %), respiratory diseases (18 %), puerperium disorders (16.8 %) and diseases of the hoof. A performance check at the age of 120 days revealed that the calves suffering from diarrhea showed a lower body weight by 12.6 kg than those calves at the same age from the same herd that did not suffer from diarrhea.

Key words: beef cattle; herd management; calf; diarrhea; respiratory diseases; metabolic disorders

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EVALUATION OF THE EFFECTS OF RATIONS NATURALLY CONTAMINATED WITH DIFFERENT MYCOTOXINS ON HOLSTEIN CROSSBRED DAIRY COWS IN SOUTHEAST ASIA AND THE EFFICACY OF A MYCOTOXIN DEACTIVATOR

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INTRODUCTION

Mycotoxins are highly toxic secondary metabolites produced by fungi mainly belonging to Fusarium, Aspergillus and Penicillium species under a wide variety of environmental conditions in the field and during harvest and storage. The most common mycotoxins are trichothecenes (deoxynivalenol, T-2 toxin), zearalenone, ochratoxins, aflatoxins and fumonisins. As an attempt to prevent these hazardous substances to enter the food chain preventive and counteracting strategies that aim the decrease of mycotoxin contamination in feedstuffs and food are applied. As these prevention techniques act in a very limited way with unsatisfactory results, detoxification strategies were developed. These strategies can be physical, chemical and biological methods. Physical and chemical methods are too costly and often destroy or remove essential nutrients and reduce palatability. Biological methods comprise binding by adsorptive materials as well as microbial inactivation by specific microorganisms or enzymes.

Several scientific reports exist proving the impact of mycotoxins in ruminants. They increased the incidence of disease and reduced production efficiency in cattle (3, 7, 1), stimulated a trend towards decreased grain consumption (640 ppb deoxynivalenol in a grain mix) (9), negatively modulated milk production (500 to 900 ppb deoxynivalenol) (10) elevated rumen ammonia concentration and reduced the duodenal flow of microbial protein in cows fed DON (2) and impacted the metabolic parameters and the immunity of dairy cows (5). Due to their complex diet, dairy cows may be exposed to a varying number of mycotoxins, originating from different feed materials such as roughage and concentrates and this may result in unexpected health risks. The objectives of this trial were to determine the impact of mycotoxins on lactating cow performance and on milk parameters and to evaluate the effects of adding different levels of Mycofix® Plus (MPL), a mycotoxin deactivator, to the ration on the studied factors.

MATERIALS AND METHODS

This study was carried out at the Rajamangala University of Technology in Thailand. Twenty-four, multiparous Holstein Friesian crossbred dairy cows with an average body weight of 420 kg and an average daily milk production of 13.7 kg were allocated to four dietary treatments with six animals per treatment. Diets were fed as a total mixed ration.

The dietary treatments consisted of the contaminated TMR diet plus various levels of a mycotoxin detoxifying product (0, 15, 30 or 45 g/head/day). Mycotoxin analysis showed a total contamination with 38 ppb aflatoxin B1, 541 ppb zearalenone, 720 ppb deoxynivalenol, 701 ppb fumonisins, 270 ppb T-2 toxin and 74 ppb ochratoxin A as shown in Table 1.

Table 1. TMR mycotoxin concentration

Mycotoxin	Aflatoxin B_1(AfB_1)	Zearalenone (ZEA)	Deoxynivalenol (DON)	Fumonisin B_1(FB_1)	T-2 Toxin (T-2)	Ochratoxin A (OTA)
Concentration [µg. kg^{-1}]	38	541	720	701	270	74

In order to obtain the multiple parameters needed for the study, an exact schedule had to be followed. For the study of the milk production parameters the measurement of the individual milk yield was done daily. Weekly, the individual milk composition (including milk urea nitrogen and somatic cell count (SCC)) was examined. Once a month, the milk was analysed for AfM_1 contamination.

RESULTS AND DISCUSSION

Milk yield and milk protein were significantly higher ($P < 0.05$) in cows fed the supplemented diets compared with those fed the control diet, but there were no significant differences among supplemented cows. Milk production was numerically higher for cows in Treatment 4 group (Table 2). There were no differences among treatments for milk fat, lactose, solids-not-fat, and total solids; however, milk fat percentage tended to be higher for supplemented compared with non-supplemented cows. This trend indicates that rumen fermentation was positively affected by the Mycofix® Plus supplementation. The higher level of milk fat in the animals fed Mycofix® Plus probably resulted from the better utilization of dietary NDF fiber (data not shown) from which the precursors for mammary lipid synthesis are derived. In addition, the high milk fat concentrations in this study could have been due to the decreased acetate:propionate

Table 2. Milk production and milk quality data

Parameters	Treatment 1 Negative Control	Treatment 2	Treatment 3	Treatment 4
Milk yield [$kg.cow^{-1}.day^{-1}$]	12.6^a	14.7^b	14.7^b	14.9^b
Fat [$g.kg^{-1}$]	34.1	37.2	37.2	36.4
Protein [$g.kg^{-1}$]	31.0^a	34.2^b	34.3^b	36.1^b
AfM_1 [$\mu g.kg^{-1}$]	0.7	nd	nd	nd
Somatic Cell Count [$x10^3$ $cell.ml^{-1}$]	547^a	385^b	346^c	346^c

^{a,b,c} – numbers with different superscripts differ significantly ($P < 0.05$)

ratio in the rumen (4) which is known to result in higher milk fat concentration (8). This finding was consistent with Whitlow et al. (10) who found that average milk production was correlated with the level of DON contamination of feedstuffs (500 to 900 ppb).

It was reported that feeding TMR naturally contaminated with DON (32 to 36 ppb) for 56 days to lactating cows did not cause a reduction in milk production (5). These results are in contrast to those of the current experiment, in which a reduction in milk production was observed in cows fed contaminated TMR containing 720 ppb of DON for 90 days. Korosteleva (5) fed TMR with lower source of contaminated feedstuffs as compared to the current study in which a wider blend of naturally contaminated feedstuffs was used for the TMR. A blend would more likely contain combinations of mycotoxins that can act synergistically to decrease feed intake. It is also likely that a feeding period of 56 days was not long enough for decreasing milk production of cows, as compared to the current study which lasted 90 days. Increased milk production of supplemented cows in the current study was probably due to the increased feed intake (data not shown), which provided supplemented animals more nutrients in comparison with non-supplemented animals.

Somatic cell count was significantly lower ($P < 0.05$) in supplemented compared with non-supplemented cows and decreased linearly with the increasing level of supplementation. Milk SCC is a widely used marker for both udder health and milk quality (6). Kelly and McSweeney (4) reported that the principal physiological function of somatic cells is to defend the udder from infections. The lower SCC in cows fed Mycofix® Plus compared with those of the control diet illustrates the better udder health of the supplemented groups.

AFM_1 was found in the milk of the cows which were not fed Mycofix® Plus, whereas this AfB_1 milk metabolite was not detected in the milk of supplemented cows. AFM_1 concentration (0.7 ppb) found in the milk of the non-supplemented cows was higher than both the 0.5 ppb and the 0.05 ppb limits imposed by the Food and Drug Administration (FDA) and the EC, respectively.

CONCLUSION

The results of this trial confirm that mycotoxin contaminated lactation rations can adversely affect performance of lactating dairy cows. Evidences were given that mycotoxins can be deactivated with the right feed additive, which helps cows to achieve the milk production they are genetically and nutritionally capable of delivering.

REFERENCES

1. **Coulombe, R. A., 1993:** J. Dairy Sci,. 76, 880–891.
2. **Dänicke, S. K., Matthaus, P., Lebzien, H., Valenta, K., Stemme, K. H., Ueberschar, E., Razzazi-Fazeli, J., Böhm and G. Flachowsky, 2005:** J. Anim. Physiol. Anim. Nutr. (Berl.), 89, 303–315.
3. **Joffe, A. Z., 1986:** J. Wiley and Sons, Inc., NY.
4. **Kelly, A. L. and McSweeney, P. L. H. , 2002:** Adv. Dairy Chem., 1, 494–519.
5. **Korosteleva, S. N., Smith, T. K., Boermans, H. J., 2007:** J. Dairy Sc,. 90, 3867–3873.
6. **O'Brien, C. N., Guidry, A. J., Douglass, L. W., Westhoff, D. C., 2001:** J. Dairy Sci., 84, 1791–1799.
7. **Pier, A.C., 1992:** J. Anim. Sci., 70, 3964–3970.
8. **Sutton, J. D., Knight, R., McAllan, A. B., Smith, R. H., 1983:** Br. J. Nutr., 49, 419–432.
9. **Trenholm, H. L., Thompson, B. K., Hartin, K. E., Greenhalgh, R., McAlister A. J., 1985:** J. Dairy Sci., 68, 1000–1005.
10. **Whitlow, L. W., Nebel, R. L., Hagler, W. M., Jr., 1994:** ed. Plenum Press, New York, NY.