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INTERNAL MILIEAU OF DAIRY COWS AT THE BEGINNING OF LACTATION AND ITS INFLUENCE ON COMPOSITION OF RAW MILK

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ABSTRACT

The aim of this work was to evaluate selected blood biochemical parameters and milk composition of dairy cows at the beginning of lactation and to observe the correlations between blood and milk parameters. In total, 15 Holstein cows at the beginning of lactation were chosen. Blood and milk samples were collected. Energetic (glucose - GLU, d-beta-hydroxybutyrate - D-BHB, triglycerides - TG), nitrogenous (total proteins - TP, UREA), hepatic (aspartate aminotransferase - AST, alanine aminotransferase - ALT, gamma-glutamyltransferase - GGT, alkaline phosphatase - ALP, bilirubin - BILI, cholesterol - CHOL) and mineral (sodium - Na, potassium - K, chlorides - Cl^-, calcium – Ca, phosphorus – P, magnesium - Mg) profiles were determined in the blood serum. Levels of lactose, fat, proteins and minerals (sodium - Na, potassium – K, calcium – Ca, phosphorus – P, magnesium - Mg) were determined in milk. Most of the parameters outside physiological limits were found among mineral and hepatic profile. Levels of calcium, phosphorus and sodium were decreased in comparison to reference values. Average concentration of urea was also lower. On the other hand, increase of aspartate aminotransferase and gamma-glutamyltransferase were observed. Levels of lactose (4.82 $g.100g^{-1}$), fat (4.21 $g.100g^{-1}$), protein (3.14 $g.100g^{-1}$) and calcium (4.82 $g.l^{-1}$) in milk complied with Slovak national standard (STN 57 0529). In our study, ratio of fat to protein lower than 0.75 was observed in 13 % of cows (risk of ketosis) and higher than 1.4 in 40 % (NEB). Strong negative correlation between serum cholesterol and milk fat (-0.716; $P<0.01$) and middle strong negative correlation between cholesterol and milk protein (-0.397; $P<0.01$) were observed.
ALT affected negatively amount of phosphorus in milk (-0.417; $P<0.001$), which complied with demineralization of the organism and following restriction of liver detoxification activity. On the other hand, strong positive correlation was observed between serum ALT and milk sodium (0.738; $P<0.05$), what points to close relationship between hepatic enzymes and mineral composition of raw milk.

Keywords: Dairy cows, metabolic test, milk quality, beginning of lactation

INTRODUCTION

The quality of milk and its composition is influenced by several factors. One of the key factors is the nutrition of dairy cows. According to many authors **(Kirst, Jakobi, 2002; Kirchnerová et al., 2002; Kováčik, 2006)** nutrition of dairy cows can affect the production and quality of milk. High-producing dairy cows, bred for high milk production, require sufficient nutrition and possible nutritional deficiencies are strongly reflected in a reduction of milk production, but also in a decrease of its technological properties (**Kirchnerová et al., 2002; Hanuš et al., 2002)**.
During the dairy cow production cycle, the transition period is critical due to the several endocrine and metabolic changes that accompany parturition and the initiation of lactation **(Grummer et al., 2004; Smith et Risco, 2005)**.
Lactation has a great impact on the intensity of metabolism and on metabolic parameters in the blood **(Filipejová et Kováčik, 2009; Milinković-Tur et al., 2005)**. Economically most important diseases of high-producing dairy cattle include metabolic disorders - ketosis, acidosis, and others. E.g. metabolic alkalosis causes a reduction in cattle tissue oxygen consumption, energy deficit, acid-base and water-electrolyte balance disturbances **(Nicpoń et Hejłasz, 2005)**.
The diagnosis of metabolic disorders is very difficult. Several methods have been developed to detect them. One of the most important methods is metabolic profile test (MPT) **(Stengärde, 2008)**. Using MPT is possible in preclinical stages, even before the appearance of clinical signs of disease **(Andrews, 2000)**. Their early diagnosis and preventive measures could prevent or at least alleviate clinical symptoms of disorders and hence their impact on the economy of farming **(Kantíková et Balážik, 2003)**.
The gist of MPT is the determination of the diagnostically relevant metabolites of body fluids **(Slanina, 1993)** of a representative group of individuals at different stages of the reproductive cycle. Metabolic test consists of various indicators, eg. hematocrit, albumin, globulin, glucose, total cholesterol, aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), calcium, inorganic phosphorus or magnesium, etc. **(Kida , 2002)**.
The aim of this work was to evaluate selected blood biochemical parameters and milk composition of dairy cows at the beginning of lactation and to observe the correlations between blood and milk parameters.

MATERIAL AND METHODS

Blood sampling and analysis

In total, 15 Holstein cows at the beginning of lactation were chosen.
Blood samples collection for biochemical analysis was performed 2 hours after the morning feeding by *vena caudalis mediana* puncture. Blood was captured directly into centrifuge tubes and centrifuged. Energetic (glucose - GLU, d-beta-hydroxybutyrate - D-BHB, triglycerides - TG), nitrogenous (total proteins - TP, UREA), hepatic (aspartate aminotransferase - AST, alanine aminotransferase - ALT, gamma-glutamyltransferase - GGT, alkaline phosphatase - ALP, bilirubin - BILI, cholesterol - CHOL) and mineral (sodium - Na, potassium - K, chlorides - Cl^-, calcium – Ca, phosphorus – P, magnesium - Mg) profiles were determined in the blood serum. Analyses were provided in biochemical and hematological laboratory at the Department of Animal Physiology of SUA through commercial sets DiaSys (Diagnostic Systems GmbH, Germany) on the devices Rx Monza (Randox Laboratories Ltd., United Kingdom) and Easy Lyte Plus (Medica corp., Bedford, USA).

Milk sampling and analysis

Milk samples were collected at morning milking and stored at 6°C. Levels of lactose, fat and protein were determined at Institute for Animal Breeding and Product Quality using infrared absorbance analysator Milkoscan FT 120. Amount of minerals (sodium - Na, potassium – K, calcium – Ca, phosphorus – P, magnesium - Mg) was determined at the Department of Chemistry (SUA in Nitra) using atomic absorption spectrometry.

Statistical analysis

GraphPad Prism 5 was used to conduct statistical analysis. Data presented are given as mean and standard deviation (SD). Correlations between blood and milk parameters were determined (significance at levels $P<0.05$, $P<0.01$, $P<0.001$).

RESULTS AND DISCUSSION

Blood parameters are presented in Table 1. Results were compared to reference values according to **Merck Sharp & Dohme Corp, (2012)** and **Slanina (1992)**. Values outside reference limit are marked.
Most of the parameters outside physiological limits were found among mineral and hepatic profile. Levels of calcium, phosphorus and sodium were decreased in comparison to reference values. Average concentration of urea was also lower. On the other hand, increase of aspartate aminotransferase and gamma-glutamyltransferase were observed.

Table 1 Blood parameters of dairy cows at the beginning of lactation compared to reference intervals according to 2 sources – Slanina (1992) and Merck Sharp & Dohme Corp (2012).

Parameter	Unit	Mean	SD	Slanina (1992)	Merck Sharp & Dohme Corp, (2012)
Ca	$mmol.l^{-1}$	**1.89**	0.43	2.25-3.00	2.10-2.80
P	$mmol.l^{-1}$	**1.77**	0.46	1.60-2.26	1.80-2.60
Mg	$mmol.l^{-1}$	0.98	0.28	0.80-1.07	0.60-0.90
Na	$mmol.l^{-1}$	**131.5**	31.19	136-150	136-144
K	$mmol.l^{-1}$	4.53	0.34	4.00-5.80	3.60-4.90
Cl	$mmol.l^{-1}$	100.1	2.73	-	99-107
UREA	$mmol.l^{-1}$	**3.24**	0.83	3.32-6.66	3.60-8.90
TP	$g.l^{-1}$	73.86	9.70	68-84	67-75
GLU	$mmol.l^{-1}$	3.2	0.61	2.20-4.10	2,20-5.60
AST	$\mu kat.l^{-1}$	**2.29**	0.87	-	1.00-2.08
ALT	$\mu kat.l^{-1}$	0.41	0.19	-	0.12-0.58
GGT	$\mu kat.l^{-1}$	**0.7**	0.28	-	0.10-0.29
ALP	$\mu kat.l^{-1}$	1.57	0.64	-	0.30-2.55
CHOL	$mmol.l^{-1}$	3.22	1.20	1.20-5.20	1.60-5.00
D-BHB	$mmol.l^{-1}$	0.76	0.46	-	-
BILI	$\mu mol.l^{-1}$	5.52	1.95	-	0-27
TG	$mmol.l^{-1}$	0.23	0.06	0.17-0.80	-

There is significant relationship between serum concentrations of minerals pre- or postpartum and the peripartum diseases **(Van Saun et al., 2006)**. Low blood calcium concentration (< 2.0 $mmol.l^{-1}$), immediately postcalving, is a risk indicator for subclinical hypocalcemia **(Oetzel, 2004)**. Sodium level lower than 139 $mmol.l^{-1}$ and potassium level higher than 4.7 $mmol.l^{-1}$ are also connected with high probability of some postpartum diseases presence **(Kováč et al., 2012)**. For diagnostics of subclinical ketosis amount of ketone bodies is used. **Quiroz-Rocha (2009)** observed D-BHB levels between 216 and 1177 $\mu mol.l^{-1}$. A serum BHBA concentration of 1.4 mmol/L or greater during the first weeks postpartum is often used to define hyperketonemia and was shown to be associated with impaired subsequent health, production, and reproduction in dairy cows **(LeBlanc et al., 2005; Duffield et al., 2009; Ospina et al., 2010)**. In our work, average level of D-BHB was in this range, but in 20 % of cows it was over 1200 $\mu mol.l^{-1}$. Together with increased level of AST it points to higher lipomobilisation and liver cell damage. Results of our previous study were very similar **(Tušimová et al., 2014)** as well as results presented by **Polák and Jančová (2012)**, who observed increase of AST, GGT and also ALT. It might be caused by lipolysis and demineralization of the organism and following restriction of liver detoxification activity.
Results of milk analyses are summarized in Table 2. Levels of lactose (4.82 $g.100g^{-1}$), fat (4.21 $g.100g^{-1}$), protein (3.14 $g.100g^{-1}$) and calcium (4.82 $g.l^{-1}$) in milk complied with Slovak national standard **(STN 57 0529)**. Requirements for sodium, potassium, phosphorus and magnesium are not set.

Table 2 Dairy cows´ raw milk composition compared to Slovak national standard STN 57 0529.

Parameter	Unit	Mean	SD	STN 57 0529
Lactose	$g.100g^{-1}$	4.82	0.22	min. 4.60
Fat	$g.100g^{-1}$	4.21	1.77	min. 3.30
Protein	$g.100g^{-1}$	3.14	0.51	min. 2.80
Na	$g.l^{-1}$	0.29	0.1	-
K	$g.l^{-1}$	1.59	0.23	-
Ca	$g.l^{-1}$	1.34	0.36	min. 1.20
P	$g.l^{-1}$	0.89	0.21	-
Mg	$g.l^{-1}$	0.09	0.02	-
F:P ratio	-	1.32	0.57	-

The ratio of fat to protein (F:P) in milk was evaluated as an indicator of negative energy balance (NEB) **(Heuer et al., 2001)**. **Dirksen (1994)** observed ratio F:P over 1.4 indicates NEB and according to **Duffield et al. (1997)** 0.75 is the critical upper limit of ketosis presence. In our study, average value of F:P ratio (1.32) was approaching the 1.4 limit. Ratio of F:P lower than 0.75 was observed in 13 % of cows (risk of ketosis) and higher than 1.4 in 40 % (NEB).
Correlations between biochemical parameters of blood and milk composition (Table 3) showed several significant dependences.

Table 3 Correlations between biochemical parameters of blood (column) and milk (row) components. Significant correlations are marked (*P<0.05, ** P<0.01, ***P<0.001).

Parameter	Lactose	Fat	Proteins	Na	K	Ca	P	Mg	F:P ratio
Ca	0.395	-0.397	-0.237	0.551	0.225	0.407	-0.338	0.126	-0.270
P	-0.134	0.088	0.268	**-0.668***	-0.008	-0.739	**0.456****	-0.316	-0.006
Mg	0.126	-0.216	-0.217	**0.648****	0.171	0.607	**-0.34***	0.457	-0.144
Na	0.115	0.206	0.402	-0.242	-0.3	-0.29	0.285	0.326	0.062
K	-0.147	0.139	0.156	**0.549***	0.03	0.558	**-0.268***	0.15	0.239
Cl	-0.315	0.336	0.467	**-0.611***	-0.211	-0.6	**0.706***	0.095	0.063
UREA	0.34	-0.257	0.206	0.252	-0.135	0.164	0.05	0.207	-0.274
TP	0.32	-0.007	0.122	-0.208	-0.519	-0.206	0.034	-0.019	-0.044
GLU	0.07	0.135	0.46	-0.08	0.07	-0.048	0.267	0.223	0.09
AST	-0.331	0.067	-0.267	-0.073	-0.058	0.157	-0.081	0.335	-0.025
ALT	0.098	-0.311	-0.48	**0.738***	0.219	0.831	**-0.417*****	0.565	-0.272
GGT	0.379	-0.249	-0.14	-0.348	0.187	-0.364	-0.094	-0.244	-0.094
ALP	-0.303	0.154	0.238	-0.095	0.495	-0.067	0.458	0.146	0.009
CHOL	0.702	**-0.716****	**-0.397****	0.414	0.099	0.324	-0.365	0.256	**-0.601***
D-BHB	-0.338	0.272	-0.336	0.026	-0.019	-0.063	-0.074	-0.19	0.258
BILI	-0.164	0.231	0.144	-0.198	-0.147	-0.171	0.342	-0.069	0.087
TG	0.039	-0.025	-0.279	-0.369	-0.224	-0.358	-0.153	-0.433	-0.003

Strong negative correlation between serum cholesterol and milk fat (-0.716; P<0.01) and middle strong negative correlation between cholesterol and milk protein (-0.397; P<0.01) as well as between cholesterol and F:P ratio (-0.601; P<0.05) were observed. Therefore, level of cholesterol significantly affects milk composition and its nutritional value.

Most affected were levels of minerals in milk – sodium and phosphorus – mainly by mineral profile of blood – phosphorus, magnesium, potassium and chlorides. Similar results were observed by **Jelínek et al. (1996)** in sheep milk. ALT affected negatively amount of phosphorus in milk (-0.417; P<0.001), which complied with already mentioned demineralization of the organism and following restriction of liver detoxification activity. On the other hand, strong positive correlation was observed between serum ALT and milk sodium (0.738; P<0.05).

CONCLUSION

Our results showed decreased levels of minerals in blood, what might be related to some peripartum diseases. On the other hand, hepatic enzymes concentrations (AST, GGT) were increased. It could be caused by demineralization of the organism and following restriction of liver detoxification activity and liver cells damage. Even the milk composition complied with Slovak national standard, the ratio fat: protein showed the possibility of some disorders (NEB, ketosis). Levels of minerals in milk were affected predominantly – sodium and phosphorus – mainly by mineral profile of blood – phosphorus, magnesium, potassium and chlorides, but also by serum ALT, what points to close relationship between hepatic enzymes and mineral composition of raw milk.

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