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CHANGES IN MILK COMPOSITION AS A RESULT OF METABOLIC DISORDERS OF DAIRY COWS.

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ABSTRACT

The aim of our study was the determination of blood parameters and changes in milk composition of dairy cows in relation to metabolic disorders and their evaluation. Thirty dairy cows from selected agricultural farm were divided into three groups as follow: group BL: 3-4 weeks after calving (the beginning of lactation), group ML: 3-4 months after calving (the middle of lactation), group DP: 2-3 weeks before calving (the dry period). Concentrations of selected parameters of energy profile (glucose, cholesterol, triglycerides); nitrogenous profile (urea, total proteins); hepatic profile (aspartate aminotransferase, bilirubin) in blood serum were measured. Content of fat, proteins and lactose, Non Fat Solids, urea, freezing point, Somatic Cell Count, Fat/Protein ratio in milk were evaluated. Cholesterol concentration was significantly higher in ML (5.33±1.17 $mmol.l^{-1}$; $p<0.001$) in comparison to BL (3.46±0.92 $mmol.l^{-1}$; $p<0.001$) and DP (2.70±0.71 $mmol.l^{-1}$; $p<0.001$). Concentration of triglycerides was significantly lower in ML (0.03±0.01$mmol.l^{-1}$; $p<0.001$) in comparison to BL (0.07±0.02 $mmol.l^{-1}$; $p<0.001$) and DP (0.09±0.04 $mmol.l^{-1}$; $p<0.001$). Albumin concentration in DP (36.90±2.99 $g.l^{-1}$; $p<0.05$) was significantly higher in comparison to BL (32.80±4.07 $g.l^{-1}$; $p<0.05$). AST concentration was significantly higher in ML (1.61±0.47$\mu mol.l^{-1}$; $p<0.001$) in comparison with DP (1.01±0.18$\mu mol.l^{-1}$; $p<0.001$) and BL (1.39±0.25$\mu mol.l^{-1;}$ $p<0.05$). Acquired results of milk composition were without significant confirmation ($p>0.05$). Fat/Protein ratio was lower than 1.1, in BL and ML, which cause rumen acidosis. The present observation confirmed that specific changes of milk composition lead to metabolic disorders.

Keywords: milk composition, metabolic disorder, fat/protein ratio, biochemical parameter

INTRODUCTION

Production cattle diseases are a set of metabolic disorders and organ diseases, which are closely related with high production of animals. Metabolic disorders are caused by the action of negative environmental factors and mainly due to an imbalance between intake and expense of nutrients and metabolites required for the optimal production and reproduction. They are characterized by a disruption in homeostasis of the organism, reduced production, poorer quality of products – milk, meat, fertility disorders and premature decommissioning of farming. Incidence of production diseases is in individual breeding varied and depending on the genetic affiliation, production level, level of nutrition, housing technology, the level of veterinary treatment and prevention. The critical period is dry period, calving and beginning of lactation. Only healthy dairy cows are able to achieve maximum conversation of nutrient from diet, high production and good fertility **(Illek, 2006).**

Milk production, reproduction, and health are the principal factors affecting the profitability of a dairy herd. In recent years, the dairy industry agenda in many countries has been dominated by health-related problems. One prerequisite for effective health management is the accurate knowledge of factors that affect the health status of a cow, such as parity, season or health history, and of the relationship between health problems and other economically important traits, such as milk production, reproduction, and length of productive life **(Emanuelson and Oltenacu, 1998).**

The milk quality is influenced by many factors acting together and influences each other. The basic requirement for quality of milk is that milk as a raw material meets the basic hygiene and health levels, poses any risk to the consumer, fulfil nourishment parameters and has unaltered technological property which allow its processing as high quality finish products **(Vasil', 2006)**.

A variety of milk components can be used as indicators of metabolic status of cow. The most relevant milk variables describe the supply of energy, protein, mineral and acid base balance, their normal concentration and their trend to change in relation to different types of metabolic disorders. Combined results of variation in milk composition may be useful for early identification of health problems. The earlier these health problems can be identified, the higher the chance for successful health management. In addition, aspects of practicability and economics are considered as well as such of the influence of the level of milk sampling **(Hamann and Krömker, 1997)**.

The aim of our study was to focus on the determination of blood parameters and changes in milk composition of dairy cows in relation to metabolic disorders and their evaluation.

MATERIAL AND METHODOLOGY

Animal Management

Dairy cows were free housing and drinking through drinkers *ad libidum*. Dairy cows were feeding two times per a day, particularly, total mixed ration (TMR).

TMR consisted of:

BL: silage 20 kg. d^{-1}, Lucerne hay 11.5 kg. d^{-1}, Meadow hay 2 kg. d^{-1}, DOVP 7.2 kg. d^{-1}, brewer´s grain 5 kg. d^{-1}.

ML: corn silage 20 kg. d^{-1}, Lucerne hay 11.5 kg. d^{-1}, Meadow hay 2 kg. d^{-1}, DOVP 9.7 kg. d^{-1}, brewer´s grain 5 kg. d^{-1}.

DP: corn silage 12 kg. d^{-1}, hay 6 kg. d^{-1}, barley straw 3 kg. d^{-1}, Meadow hay 3 kg. d^{-1}, G3B 0.15 kg. d^{-1}.

Biological material

Thirty dairy cows of Holstein breed from selected agricultural farm from western part of Slovakia in winter were examined. Dairy cows were divided into three examined groups: BL (the beginning of lactation) 3-4 weeks

after calving, ML (the middle of lactation) 3-4 months after calving and DP (the dry period) 2-3 weeks before calving. Blood samples for biochemical analysis were taken from *vena jugularis* by vacutainer tubes (8 ml) 2 hours after morning feeding and conserved cooled in ice box during transportation to the laboratory. The blood serum was separated from whole blood by the centrifugation at 3000 rpm for 30 minutes and samples were stored at – 18 °C until further analysis.

Biochemistry assay

In this experiment, energy profile: glucose (GLU), cholesterol (CHOL) and TG (triglycerides); nitrogenous profile: urea, total proteins (TP); hepatic profile: aspartate aminotransferase (AST) and bilirubin (BIL) were analysed in blood serum, using semi–automated clinical chemistry analyzer Microlab 300 (Vilat Scientific, Dieren, The Netherlands) by commercial kits DiaSys **(Filipejová and Kováčik, 2009a)**.

Milk assay

Milk samples were taken individually. Samples of milk were cooled down until 6 °C was reached. Samples were kept at the same temperature during the determination of milk quality parameters: fat content, proteins and lactose (by infrared analyzer MilkoScan FT 120; ISO 9622:1999 Whole milk – Determination of milk fat, protein and lactose content), Non-Fat-Solids (by the MilkoScan apparatus; STN EN ISO 13366-1:2008), urea (photocolorimetric device with Ehrlich's reagent, 530 nm wavelength), freezing point (ISO 5764:2002 Milk – Determination of freezing point), Somatic Cell Count (by Somatocount 150 fi Bentely Instuments on principle of flow cytometry STN EN ISO 13366 1:2008). Consequently Fat/Protein ratio was evaluated. Samples were analysed in the Institute of Nutrition in the Animal Production Research Centre in Lužianky near Nitra.

Statistical analysis

Significant differences among groups of dairy cows were evaluated by Sigma Plot 11.0 (Jandel, Corte Madera, USA) statistical programme. Statistical analysis was done using one-way analysis of variance (ANOVA) by Holm- Sidak method. Differences among the groups at $p<0.05$ and $p<0.001$ were considered as significant.

RESULTS AND DISCUSION

Investigation into nutritional problems in herd of dairy cows requires consideration of several aspects; particularly feeding, breeding and rearing management and production level. These examinations should be supported by laboratory examinations like metabolic profile tests. As with order examinations, time of observation and discussion play a dominant role in making diagnosis. Another advantage of blood sampling is that this procedure is giving an opportunity to visit the farm and to discuss feeding of animal thoroughly **(Kováč a Mudroň, 2002)**. Lactation has a great impact on the intensity of metabolism and on metabolic parameters in the blood **(Milinković-Tur et al., 2005).**

Energy profile

Energy metabolism of dairy cows is characterized by carbohydrate and lipid metabolism. Among the most important indicators of energy metabolism of cattle belong glucose and cholesterol and triglycerides **(Pechová and Pavlata, 2005)**.

In order to evaluate the energy metabolism, we detected the concentration of glucose, cholesterol and triglycerides. Changes of glucose concentrations (Table 1) reflect the load energy metabolism in the early phase of lactation. This relates to the disproportion between nutrient intake, particularly energy and an increasing need for nutrients for milk production **(Reist, 2002)**. Average blood glucose concentrations did not drop below the physiological values (3.0-3.9 $mmol.l^{-1}$) noticed by **Vrzuľa et al. (1990)**. The results indicated no significant ($p>0.05$) differences among the groups.

Consequently, significant differences of cholesterol concentration was significantly higher in ML (5.33±1.17 $mmol.l^{-1}$; $p<0.001$) in comparison to BL (3.46±0.92 $mmol.l^{-1}$; $p<0.001$) and DP (2.70±0.71 $mmol.l^{-1}$; $p<0.001$) (Table 1). Similar results were obtained in our previous study (**Filipejová and Kováčik, 2009b).**

Concentration of cholesterol showed slightly increased activity above physiological value of 5.2 $mmol.l^{-1}$ only in ML **(Vrzguľa et al., 1990; Pechová et al., 2003; Kováč et al., 2001)**.

Table 1 Energy profile of dairy cows

	BL (n=10)	ML (n=10)	DP (n=10)
Glucose	($mmol.l^{-1}$)		
x	3.58	3.83	3.85
minimum	2.87	3.60	3.43
maximum	4.32	4.12	4.47
S.D.	0.45	0.21	0.37
CV (%)	0.46	0.21	0.38
Cholesterol	($mmol.l^{-1}$)		
x	3.46^{A}	5.33^{B}	2.70^{C}
minimum	1.30	3.50	1.60
maximum	4.60	7.00	3.90
S.D.	0.92	1.17	0.71
CV (%)	0.94	1.21	0.73
Triglycerides	($mmol.l^{-1}$)		
x	0.07^{A}	0.03^{B}	0.09^{C}
minimum	0.03	0.01	0.02
maximum	0.11	0.05	0.19
S.D.	0.02	0.01	0.04
CV (%)	0.02	0.01	0.04

BL-beginning of lactation, ML-middle of lactation, DP-dry period, x-mean, S.D.-standard deviation, CV-coefficient of variation, A,B,C different letters within the row mean significant differences among the groups at level $p<0.001$.

Triglycerides as a main component of lipoproteins play an important role in metabolism of dairy cows as a source of dietary energy and fat transporter. They get into the blood by absorption from the gut and mainly synthesized in the liver. Triglycerides determine the degree of hyperlipoproteinemia **(Balabánová et al. 2009).** Concentration of triglycerides was significantly lower in ML (0.03±0.01$mmol.l^{-1}$; $p<0.001$) in comparison to BL (0.07±0.02 $mmol.l^{-1}$; $p<0.001$) and DP (0.09±0.04 $mmol.l^{-1}$; $p<0.001$) (Table 1). Obtained results of

triglycerides are lower than those presented by **Slanina et al. (1992)** and used reference range (0.17- 0.51 mmol.l^{-1}). Low blood levels of lipoproteins indicate impaired lipomobilisation, fat production and release of lipoproteins as transport forms of fat in the liver. This situation occurs when liver function is impaired or during steatosis as well as prolonged lack of energy ration in feed **(Balabánová et al. 2009)**.

Nitrogenous profile

Nitrogenous metabolism is affected in severe liver disease, where more than one third of parenchyma is dysfunctional. Reduced metabolic liver function was reflected in reduced synthesis of proteins, mainly albumins and further reduced urea synthesis, is accompanied by increased of concentration of ammonia **(Pechová et al., 2003)**.

Concerning the nitrogenous profile, average total proteins concentration (Table 2) are similar to changes of total proteins concentrations noticed by **Doornenbal et al. (1988)** and **Šlosárková et al. (2010).** However, albumin concentration (Table 2) in DP (36.90±2.99 g.l^{-1}; $p<0.05$) was significantly higher in comparison to BL (32.80±4.07 g.l^{-1}; $p<0.05$) and ML (36.30±3.62 g.l^{-1}) was without significant difference ($p>0.05$), in spite of that, in reference range (30-42 g.l^{-1}) reported by **Pechová et al. (2003)**.

Table 2 Nitrogenous profile of dairy cows

	BL (n=10)	ML (n=10)	DP (n=10)
Total proteins		(g. l^{-1})	
x	78.90	81.20	77.10
minimum	70.00	73.00	56.00
maximum	88.00	90.00	85.00
S.D.	5.86	6.61	9.11
CV (%)	6.02	6.79	9.36
Albumins		(g. l^{-1})	
x	32.80^{a}	36.30	36.90^{b}
minimum	25.00	28.00	33.00
maximum	38.00	41.00	42.00
S.D.	4.07	3.62	2.99
CV(%)	4.19	3.72	3.08
Urea		(mmol.l^{-1})	
x	3.91	4.97	4.08
minimum	2.18	2.87	1.92
maximum	6.31	5.77	5.18
S.D.	1.21	0.94	1.12
CV(%)	1.24	0.97	1.15

BL-beginning of lactation, ML-middle of lactation, DP-dry period, x-mean, S.D.-standard deviation, CV-coefficient of variation, different letters within the row mean significant differences among the groups at level ($p<0.05$) (a,b).

Urea as a final product of degradation of the protein that is synthesized in the urea cycle in the liver is income indicators in nitrogen metabolism and also is an indicator of liver and renal function **(Balabánová et al., 2009)**. Likewise, average urea concentrations of our study were correspondent with results reported by **Vrzguľa et al. (1990)** and **Slanina et al. (1992).** The results indicated no significant ($p>0.05$) differences among the groups.

Hepatic profile

Enzymatic diagnosis is used in the diagnosis of liver disease. The principle is based on the determination of enzyme activity in the blood plasma or tissues. The most common analyse enzymes activity in the blood plasma or serum. Diagnostic values of determination of enzymes are limited specificity of enzymes and their half-life **(Pechová and Pavlata, 2005)**. The total bilirubin value is a sensitive indicator of liver damage **(Lubojacká et al., 2005)**. Activity of AST enzyme and bilirubin is shown in Table 3.

Table 3 Hepatic profile

	BL (n=10)	ML (n=10)	DP (n=10)
AST		(μkat.l^{-1})	
x	1.39^{a}	1.61^{A}	1.01$^{b, B}$
minimum	1.04	1.04	0.77
maximum	1.90	2.57	1.30
S.D.	0.25	0.47	0.18
CV (%)	0.26	0.48	0.18
Bilirubin		(μmol.l^{-1})	
x	4.88	4.58	2.19
minimum	2.10	2.50	0.70
maximum	13.30	13.60	5.30
S.D.	4.43	4.33	1.75
CV (%)	4.55	3.21	1.80

BL-beginning of lactation, ML-middle of lactation, DP-dry period, AST-aspartate aminotransferase, x-mean, S.D.-standard deviation, CV-coefficient of variation, different letters within the row mean significant differences among the groups at level $p<0.05$ (a,b); and level $p<0.001$ (A, B).

AST is an ubiquitous enzyme found in liver, heart, skeletal muscle and intestinal mucosa. It is a cellular enzyme present in mitochondria **(Pechová and Pavlata, 2005)**. Increased AST activity in the serum is a sensitive marker of liver damage **(Meyer and Harvey 1998)**. There are two main isoenzymes: mitochondrial and cytosolic, which prevails in the total concentration in the blood plasma because it has a longer half-life (**Kramer and Hoffman, 1997**).

In our observation we detected significant differences in AST concentration was significantly higher in ML (1.61±0.47μmol.l$^{-1;}$ $p<0.001$) in comparison with DP (1.01±0.18μmol.l^{-1}; $p<0.001$) and BL (1.39±0.25 μmol.l$^{-1;}$ $p<0.05$). AST activity in blood serum in BL and ML was not in accordance with reference range (0.22-0.50 μmol.l^{-1}) reported by **Slanina et al. (1992)** and **Vrzguľa et al. (1990).** According to **Pechová et al. (2003)** reference range of AST is 0.72-1.41 μkat.l^{-1}. AST activity increased in blood plasma with fatty liver **(Pechová et al., 2003).** Increased AST activity in the serum is a sensitive marker of liver damage, even if the damage is of a subclinical nature **(Meyer and Harvey, 1998).**

Bilirubin is a bile pigment that has an important role as an antioxidant. Bilirubin as low molecular antioxidant examined **Capcarová and Kolesárová (2010).** Furthermore, concentration of bilirubin in blood is an indicator of hepatic metabolism. A slight increase occurs in the liver dystrophy, characterized by significant increase in liver failure and multiple is usually detected significantly increase in haemolytic jaundice **(Pechová and Pavlata, 2005)**.

Consequently, without significant differences among the groups the content of bilirubin ranged (0.17-5.13

µmol.l^{-1}). Decreased bilirubin after calving indicates load and possibly damage of liver tissue, however increased in starvation, it means in negative energy balance **(Kraft and Dürr, 2001)**.

Milk components as indicator of the health status of dairy cows

At the beginning, dairy cows have to cope with the energy and protein demands for milk synthesis at the time when nutrient intake is low. Mobilising energy and protein from body tissue stores and reparation of nutrients away from extra mammary tissues are the primary alternatives to supply sufficient nutrients for milk production during the first weeks of lactation. Excessive body reserves, especially fat, can cause a series of metabolic disorders and consequent production losses **(Fourichon, et al., 1999)**.

Changes in some milk variables are specifically related to the metabolic status of cow, some others are associated with udder health status and some milk variables indicate both the systemic and mammary gland condition. Factors predisposing to general or mammary gland diseases should be evaluated in combination with the major milk components (fat and protein) by measuring urea in milk as indicators of a balanced diet **(Haman and Krömker, 1997)**.

The effect of production diseases on milk composition confirmed that there is a close relationship between blood values and milk constituents, reduction in milk proteins during metabolic alkalosis **(Illek et al. 1994),** reduction in milk fat during rumen acidosis and reduction in lactose in all metabolic disorders **(Bergamini 1987)**.

In this study some parameters of milk composition were analysed. Results of milk composition are presented in Table 4 and Table 5.

Milk fat content is one of the most variable components, which can be influenced by feeding. Milk fat composition can be modified readily by changing the feeding regimen. The most significant changes in milk fat quality relate to rheological properties, which are influenced by numerous aspects of character and quality of manufactured dairy products **(Palmquist and Beaulieu, 1993)**. As it shown in the Table 4, milk fat content was without significant (p>0.05) confirmation. According to **STN (570529)** fat concentration should be minimum 3.3 g.100g$^{-1,}$ which implies, that milk fat content was decreased in both analyzed groups as necessary to monetize that the value indicate 3.6 g.100 g-^{1}. Lower milk fat content is frequently used in farms as a indicator of sub-acute ruminal acidosis and to predict the effectiveness of diet structure for chewing **(Merterns, 1997; De Brabander and De Boever, 1998).** Low milk fat content is caused by a lack of major precursor-acetic acid in rumen that is produced in insufficient quantity **(Illek, 2008)**. The protein content in milk is genetically determined and significantly affected by the level of nutrition and rumen fermentation. Apart from, fat content is easy influenced by nutrition, but in case the protein content of milk is more complex and the range of change is smaller **(Illek, 2003)**.

Results of milk proteins (Table 4) content showed no significant differences (p>0.05).

Table 4 Milk composition

	BL (n=10)	ML (n=10)
Fats	**(g.100g^{-1})**	
x	2.98	2.72
minimum	0.57	0.66
maximum	7.20	6.17
S.D.	2.33	1.72
CV (%)	2.39	1.76
Proteins	**(g.100g^{-1})**	
x	3.15	3.21
minimum	2.80	2.43
maximum	3.58	3.95
S.D.	0.24	0.53
CV (%)	0.24	0.55
Lactose	**(g.100g^{-1})**	
x	4.74	4.35
minimum	3.48	1.65
maximum	5.10	4.99
S.D.	0.47	1.00
CV (%)	0.47	1.03
Non Fat Solids	**(g.100g^{-1})**	
x	8.56	8.29
minimum	7.67	4.83
maximum	9.17	9.16
S.D.	0.44	1.27
CV (%)	0.45	1.31
Urea	**(mmol.l^{-1})**	
x	5.59	5.83
minimum	3.14	3.35
maximum	8.17	8.36
S.D.	1.65	1.70
CV (%)	1.69	1.75
Freezing Point	**(-m.°C)**	
x	523	520
minimum	511	482
maximum	537	532
S.D.	7.10	14.65
CV (%)	7.30	15.05
Somatic Cell Count (10^{3}.ml^{-1})		
x	217.00	410.10
minimum	4.00	13.00
maximum	1250.00	1364.00
S.D.	414.40	393.20
CV	425.30	552.40

BL-beginning of lactation, ML-middle of lactation, x-mean, S.D.-standard deviation, CV- coefficient of variation

According to **STN (570529)** milk protein content should be minimum 2.80 g.100g^{-1}, but basic protein content of milk is to monetize 3.2 g.100g^{-1} **(Foltýs and Kirchnerová, 2009)**. Generally, if milk protein content increased and milk fat content decreased it leads to sub-clinical acidosis. On the other hand, if milk protein decreased and milk fat content increased it leads to another metabolic disorder-ketosis most often in the beginning of lactation **(Pavlata et al., 2008)**.

Lactose content in milk is closely dependent on the health status of each cow and decreases with increasing lactation

period**.** Composition of feeding ration and nutrition has less influence on the lactose content **(Damodaran et al., 2007)**.

Concerning the lactose content (Table 4), differences were not significant ($p>0.05$). According to **STN (570529)** lactose content should be 4.60 $g.100g^{-1}$. Decrease lactose content, which is accompanied by increased with content of somatic cell count **(Kováč et al., 2001)** and what was confirmed in our study, is associated mainly with the inflammatory processes of the mammary gland or unsatisfactory long-term nutrition of dairy cows due to disruption of metabolism **(Gajdůšek, 1993)**. Lactose content considerably increased in metabolic disorder-ketosis **(Pavlata et al., 2008)**.

Moreover, changes that occur in Non Fat Solids are primarily due to changes in the protein and occasionally the lactose content of milk. Good quality hay tends to increase Non Fat Solids, but poor quality hay may reduce both intake and Non Fat Solids. A decline in Non Fat Solids, protein, and lactose content is associated with sub-clinical and clinical mastitis **(Harris and Bachman, 2003)**. Regarding to Non Fat Solids**,** results of presented study showed no significant differences ($p>0.05$).

Monitoring of urea in milk (Table 4) is an indicator of metabolic nitrogen balance of cows **(Jílek et al., 2006),** which characterise their health, reproductive ability and longevity **(Hanuš et al., 2000; Hanuš et al., 2008)**. The main factors influencing the level of urea in the organism and subsequently in milk are intake of energy and crude protein in feed, while a higher protein concentration the urea level increases and higher energy intake of urea concentration in milk decreases **(Hering et al., 2008)**. If the ration is well balanced, the urea level in milk is in the normal range (2.5-5.00 $mmol.l^{-1}$) **(Eicher, 2004)**. Increased urea concentration in milk can indicate worse milk quality **(Hanuš et al., 1993a; Hanuš et al. 1993b)**, accompanied by rumen alkalosis, indigestion, lack of production volatile fatty acids and bacterial protein **(Frank and Swenson, 2002)**. In our experiment, urea content was increased in both groups. Acquired results of milk urea were without significant confirmation ($p>0.05$) can leads mentioned problems.

Freezing point (Table 4) is not only an indicator of possible adding water to milk, but also an indicator of technological relevance, (if no adding water to milk) unsatisfactory results of freezing point of the milk are caused by unbalanced ration in protein and energy, respectively deficiencies in mineral nutrition of dairy cows **(Kadlec**, **1999).** Regarding to freezing point results, no significant differences ($p>0.05$) were found.

Milk Somatic Cell Count (SCC) is a key measure of milk quality, reflecting the health status of the mammary gland and the risk of non-physiological changes to milk composition. It is also the key component of national and international regulation for milk quality, udder health and the prevalence of clinical and sub-clinical mastitis in dairy herds **(Van Schaik et al. 2002)**. In case of, milk Somatic Cell Count (Table 4) no significant difference ($p>0.05$) was detected. Obtained results increase risk of incidence of mastitis, mainly in ML.

Significant correlations among milk parameters were mentioned in a previous study **(Filipejová et al. 2010)**.

Table 5 Fat/Protein ratio

Stage of lactation	Fat	Proteins	F/P ratio
BL	2.98	3.15	0.95
ML	2.72	3.21	0.85

Metabolic disorders can reflect chemical-technological characteristics of milk and thus we focused on the changes of milk fat and protein content in individual milk samples of Holstein cows during lactation. Milk fat can be increased or decreased depending on ration composition. It is not uncommon for two metabolic disorders and/or nutritional problems to act in opposition to one another within the same group of cows. For example early lactation cows have a tendency to mobilize body reserves while ingesting rations are low in effective fibre. Mobilization of body fat tends to increase whereas lack of effective fibre will tend to decrease milk fat levels **(Eicher, 2004)**. In order to evaluate nutrition, conversion of nutrients and metabolism is important to analyse milk fat to milk protein ratio. **Richardt (2004)** considers the F/P ratio to be a very important indicator of animal health. The optimum Fat/Protein ratio is 1.2 – 1.4 **(Haas and Hofírek, 2004). Richardt (2004)** confirmed that the F/P ratio higher than 1.5 can indicate sub-clinical ketosis whereas the F/P ratio lower than 1.1 can mean suspected rumen acidosis. In our experiment, the both groups had F/P ratio lower than 1.1, which implies rumen acidosis.

CONCLUSION

Deficiency in dairy cow's nutrition may influence many biochemical and physiological processes. In this study, blood metabolites and milk composition of Holstein dairy cows were analysed. We detected some significant differences in cholesterol ($p<0.001$) and triglycerides concentration ($p<0.001$). There were significant differences in albumin ($p<0.05$) and AST concentration ($p<0.001$), as well. Based on our results, we can conclude, that decreased triglycerides concentration ($p<0.001$) and increased AST concentration ($p<0.001$), may indicate inflammation of liver. Furthermore, in dairy cows could occur lack of energy in feeding ration, what suggest increased urea concentration in milk, while concentration of proteins in milk was increased.

However, decreased content of milk fat and increased proteins content of milk, can lead to metabolic disorder – acidosis, what was confirmed Fat/Protein ratio was lower than 1.1, which implies rumen acidosis in both groups. No significant differences ($p>0.05$) were detected among parameters of milk composition.

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