# MILK COMPOSITIONS AND BLOOD METABOLITES OF HOLSTEIN DAIRY COWS DURING IMPORTANT STAGES OF LACTATION

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## ABSTRACT

The aim of our study was to determine selected parameters of Holstein dairy cows in the blood plasma, changes of milk during important stage of lactation. Fifteen dairy cows from selected agricultural farm were divided into three groups as follow: group I: 3-4 weeks after calving (the beginning of lactation), group II: 3-4 months after calving (the middle of lactation), group III: 2-3 weeks before calving (the dry period).Concentrations of selected parameters of energy profile: glucose (GLU), cholesterol (CHOL) and nitrogenous profile: bilirubin, urea, total proteins (TP) in blood plasma were measured. Significant differences among groups of dairy cows were evaluated by statistical programme Sigma Plot 11.0. Differences among the groups at $p<0.05$ and $p<0.01$ using test ANOVA one way were considered as significant. In conclusion, we detected some significant differences, especially concentration of bilirubin was higher statistically significant at the beginning of lactation (6.50±5.89 μkat.l$^{-1}$; $p<0.05$) in comparison to group during dry period (0.92± 0.29 μkat.l$^{-1}$; $p<0.05$). Consequently, concentration of total proteins was significantly higher in the middle lactation (87.60±6.54 g.l$^{-1}$; $p<0.05$) in comparison with beginning lactation (71.40±4.98 g.l$^{-1}$; $p<0.05$). Concerning energy profile, the cholesterol concentration was significantly higher in the middle lactation (3.54 ± 0.73 mmol.l$^{-1}$; $p<0.05$) in comparison to dry period (2.58 ±0.39 mmol.l$^{-1}$; $p<0.05$) and following the beginning of lactation (1.92±0.49 mmol.l$^{-1}$; $p<0.05$. In addition, we detected significant differences of glucose concentration in the middle lactation (3.97±0.19. mmol.l$^{-1}$; $p<0.01$) and at the beginning of lactation (2.84 ±0.51 mmol.l$^{-1}$; $p<0.01$) and following group of dry period (3.86±0.25 mmol.l$^{-1}$; $p<0.05$). Based on the analysis of milk indicators, an another statistically difference ($p<0.05$) was detected for lactose in the dairy cows at the beginning of lactation (4.71 ± 0.06 g.100g$^{-1}$; $p<0.05$) in comparison to the middle of lactation (5.00 ± 0.09 g.100g$^{-1}$; $p<0.05$). Futhermore, Fat/Protein ratio was lower than optimum in both groups, which lead to sub-clinical acidosis. The present observation confirm, that some symptoms leading to sub-clinical diseases, besides worsening the technological quality of the cow milk.

**Key words:** blood plasma, milk, dairy cows, Fat/Protein ratio, biochemical parameter

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## INTRODUCTION

The quality of milk and its nutritional value influenced by many factors (Tančin et al., 2006). Specifically, the nutrition and health status are major factors in determining cow milk characteristics. Metabolic perturbations, lack of nutrition and sub-clinical disorders could be detected by measuring some metabolic parameters in the blood, urine and milk (Slanina et al., 1992). At the beginning of lactation, dairy cows have to cope with the high energy and protein demands for milk synthesis at the time when nutrient intake is low. In an effort to obtain the energy necessary for milk production, the cows use up their bodily reserves, predominataly fats. An energy deficit at the beginning of lactation negatively impacts, health efficiency and reproduction performance of dairy cows (Lubojacká et al., 2005). Mobilizing energy and protein from body tissue stores and repartition 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 (acidosis, ketosis, fat cow syndrome) and consequent production losses (Fourichon et al., 1999). A dry period is necessary for involution of the mammary gland and maximizing milk yield in the subsequent lactation in cattle (Cameron et al., 1998; Rastani et al., 2005). In resent years, the production efficiency of dairy cows has constantly been increasing, which has to led to higher demands on the supply oh their nutrients. The most difficult problems have been encountered in early lactation period when dairy cows have a negative energy balance. The main problem of that period is the limited intake capacity for dry matter, as a consequence of which dairy cows are unable to cover their energy need from feeds.

The aim of our study was to determine blood metabolites of dairy cows, basic milk composition and changes of milk, Fat/ Protein content in individual milk samples of cows during important stage of lactation.

## MATERIAL AND METHODS

Fifteen dairy cows from selected agricultural farm were divided into three groups: group I: 3-4 weeks after calving (the beginning of lactation), group II: 3-4 months after calving (the middle of lactation), group III: 2-3 weeks before calving (the dry period). Blood samples for biochemical analysis were taken from *vena jugularis* 2 hours after morning feeding. The blood plasma was separated from whole blood by centrifugation at 3000 rpm for 30 minutes and samples were stored at – 18 °C. Selected biochemical parameters in blood plasma bilirubin, urea, total proteins (TP), glucose (GLU) and cholesterol (CHOL) were analyzed using semi–automated clinical chemistry analyzer Microlab 300 Vilat Scientific, Dieren, The Netherlands) (Filipejová and Kováčik, 2009).

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: Content of fat, proteins and lactose (by infrared analyzer Milcoscan FT 120; ISO 9622:1999 Whole milk – Determination of

milk fat, protein and lactose content), Non-Fat-Solis and solids (by the MilkoScan apparatus). Consequently Fat/Protein ratio was evaluated. Samples were analysed in the Institute of Nutrition in the Animal Production Research Centre in Lužianky near Nitra. Significant differences among groups of dairy cows were evaluated by using Sigma Plot 11 statistical programme. Statistical analysis was done using one-way analysis of variance (ANOVA). Differences among the groups at $p<0.05$ and $p<0.01$ were considered as significant.

## RESULTS AND DISCUSSION

Determination of indicators of the metabolic profile in course of the breeding season helps to diagnose the metabolic problems of the animals (Verheyen et al., 2007). The metabolic profile test indicates the balance of some metabolic pathways, and together with animal, diet and body condition score assessment, is a useful tool for nutritional evaluation in dairy herds (Van Saun and Wustenberg 1997, Whitaker 2000). Changes in various biochemical constituents have been blamed for reproductive failures. Thus, serum biochemical profile might be a potential aid in characterizing these problems (Ahmad et al., 2004). In the metabolic profile test reference values are defined as mean values and ranges of standard deviation. Thus, values from blood analysis are compared with the population average or ranges of reference values (Herdt, 2000). Our results of concentrations of blood plasma parameters of dairy cows are shown in the table 1 and milk composition are shown in the table 2 and table 3.

*Tab. 1 Concentration of AST, urea, total proteins, glucose, cholesterol in blood plasma of dairy cows at the beginning of lactation, in the middle of lactation and during dry period*

| | Parameter | x | Minimum | Maximum | S.D. | CV (%) |
|---|---|---|---|---|---|---|
| **BL** | BIL(μmol.l$^{-1}$) | 6.50$^{a}$ | 2.10 | 13.30 | 5.89 | 7.32 |
| | Urea (mmol.l$^{-1}$) | 3.88 | 2.47 | 6.03 | 1.37 | 1.71 |
| | TP (g.l$^{-1}$) | 71.40$^{a}$ | 68.00 | 80.00 | 4.98 | 6.18 |
| | GLU(mmol.l$^{-1}$) | 2.84$^{Aa}$ | 2.22 | 3.70 | 0.51 | 0.64 |
| | CHOL (mmol.l$^{-1}$) | 1.92$^{a}$ | 1.40 | 2.60 | 0.49 | 2.90 |
| **ML** | BIL (μmol.l$^{-1}$) | 2.48 | 0.50 | 4.00 | 1.36 | 1.68 |
| | Urea (mmol.l$^{-1}$) | 3.61 | 1.97 | 6.47 | 1.69 | 2.09 |
| | TP (g.l$^{-1}$) | 87.60$^{a}$ | 82.00 | 98.00 | 6.54 | 8.12 |
| | GLU(mmol.l$^{-1}$) | 3.97$^{A}$ | 3.68 | 4.15 | 0.19 | 0.24 |
| | CHOL (mmol.l$^{-1}$) | 3.54$^{a}$ | 2.80 | 4.40 | 0.73 | 0.91 |
| **DP** | BIL (μmol.l$^{-1}$) | 0.92$^{a}$ | 0.70 | 1.40 | 0.29 | 0.36 |
| | Urea (mmol.l$^{-1}$) | 4.56 | 3.77 | 5.43 | 0.74 | 0.92 |
| | TP (g.l$^{-1}$) | 81.20 | 66.00 | 93.00 | 11.47 | 14.24 |
| | GLU(mmol.l$^{-1}$) | 3.86$^{a}$ | 3.70 | 4.30 | 0.25 | 0.32 |
| | CHOL (mmol.l$^{-1}$) | 2.58$^{a}$ | 1.90 | 2.90 | 0.39 | 0.49 |

*BL-beginning of lactation, ML-middle of lactation, DP-dry period BIL-bilirubin, TP-total proteins, GLU-glucose, CHOL-cholesterol, x -mean, S.D.-standard deviation, CV-coefficient of variation, signifficant differences among the groups ($p<0.05$) presented as equal letters (a-a, b-b; $p<0.05$); (A-A; $p<0.01$)*

In our observation, we detected concentration of bilirubin was higher statistically significant at the beginning of lactation (6.50±5.89 μkat.l$^{-1}$; p<0.05) in comparison to group during dry period (0.92± 0.29 μkat.l$^{-1}$; p<0.05) (Tab 1). Total bilirubin value is a sensitive indicator of liver damage. The bilirubin value found in group at the beginning of lactation exceeded the physiological range of 0.17-5.13 μmol.l$^{-1}$, which implies it may indicate steatosis of liver, because these results are similar to Pechvá et al. (2003). Bilirubin increasing after parturition may indicate especially liver load, event of damage to liver tissue, but also increased during starvation, particularly in negative energy balance (Kraft and Dürr, 2001).

Consequently, concentration of total proteins was significantly higher in the middle lactation (87.60±6.54 g.l$^{-1}$; p<0.05) in comparison with beginning lactation (71.40±4.98 g.l$^{-1}$; p<0.05), while dry period (81.20 ± 11.47 g.l$^{-1}$) was without significant differences (p>0.05) (Table 1) According to Pechová (2003) reference range of total proteins in blood plasma should be 60 - 80 g.l$^{-1}$, which implies, group in the middle lactation and dry period were increased in comparison to Pechová reference range. No significant differences in urea was detected.

Moreover, energy profile was measured. We detected the highest significant differences of glucose concentration in the middle lactation (3.97±0.19. mmol.l$^{-1}$; p<0.01) and at the beginning of lactation (2.84 ±0.51 mmol.l$^{-1}$; p<0.01) and following group of dry period (3.86±0.25 mmol.l$^{-1}$; p<0.05) (Table 1). Results of glucose concentration were similar to Slanina et al. (1992), likewise Doubek et al (2010) reference range.

In addition, the cholesterol concentration was significantly higher in the middle lactation (3.54 ± 0.73 mmol.l$^{-1}$; p<0.05) in comparison to dry period (2.58 ±0.39 mmol.l$^{-1}$; p<0.05) and following the beginning of lactation (1.92±0.49 mmol.l$^{-1}$; p<0.05) (Table 1). The cholesterol concentration in blood plasma is characteristic its content in feeding dose, when increased values of cholesterol are detected by feeding supplemental fat, moreover synthetic possibility of liver, because liver is only one organ, which is able to cholesterol synthesis (Pechová et al., 2003) .

*Tab. 2 Concentration of fats, proteins, lactose, Non-fat-solids, urea, in the milk of dairy cows during beginning a middle lactation*

| | Variable | x | Minimum | Maximum | S.D. | CV (%) |
|---|---|---|---|---|---|---|
| **BL** | **Fats** (g.100g$^{-1}$) | 1.94 | 1.32 | 2.68 | 0.65 | 1.04 |
| | **Proteins** (g.100g$^{-1}$) | 4.07 | 3.62 | 5.01 | 0.66 | 1.04 |
| | **Lactose** (g.100g$^{-1}$) | 4.71$^{a}$ | 4.65 | 4.79 | 0.06 | 0.09 |
| | **Non Fat Solids** (g.100g$^{-1}$) | 9.10 | 9.06 | 10.53 | 0.66 | 1.05 |
| | **Urea** (mmol.l$^{-1}$) | 6.07 | 4.11 | 7.67 | 1.60 | 2.55 |
| **ML** | **Fats** (g.100g$^{-1}$) | 1.61 | 1.33 | 2.37 | 0.51 | 0.82 |
| | **Proteins** (g.100g$^{-1}$) | 3.61 | 3.59 | 3.63 | 0.02 | 0.03 |
| | **Lactose** (g.100g$^{-1}$) | 5.00$^{a}$ | 4.89 | 5.09 | 0.09 | 1.15 |
| | **Non Fat Solids** (g.100g$^{-1}$) | 9.37 | 9.24 | 9.45 | 1.32 | 0.14 |
| | **Urea** (mmol.l$^{-1}$) | 5.90 | 4.22 | 7.40 | 1.62 | 2.58 |

*BL-beginning of lactation, ML-middle of lactation, a-a (p<0.05)*

The milk yield is an important economic and health factor closely connected with the health status of dairy cows, their reproduction performance, longevity and milk composition and properties (Janů et al., 2007). Concerning the milk composition, we detected higher significant differences of lactose content in the middle lactation (5.00±0.09 g .100g$^{-1}$; p<0.05) in comparison to the beginning of lactation (4.71±0.06 g.100g$^{-1}$; p<0.05) (Table 2). No significant differences in fats, proteins, Non-Fat-Solids and urea in both groups were detected.

*Tab. 3 Changes in Fat/Protein ratio and milk fat and protein content during lactation*

| Stage of lactation | Fat | Proteins | F/P ratio |
|---|---|---|---|
| BL | 1.94 | 4.07 | 0.48 |
| ML | 1.61 | 3.61 | 0.45 |

Metabolic disorders can reflected 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 increase or decrease 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 that are low in effective fiber. Mobilization of body fat tends to increase whereas lack of effective fiber will tend to decrease milk fat levels (Eicher, 2004). The changes of their mutual ratio which can suggest nutritional deficiencies. In order to evaluate nutrition, conversion of nutrients and metabolism is important to analyse milk fat to milk protein ratio. The optimum Fat/Protein ratio is 1.2 – 1.4 (Haas and Hofírek, 2004).

In this study, as it is shown in Table 3, Fat/Protein ratio was lower than optimum at the beginning of lactation (0.48), but also in the middle lactation (0.45) as well. Lower values are likely to lead to sub-clinical rumen acidosis which can endanger reproduction performance of cows and enhance a possible development of mineral metabolism disorders (Richardt, 2004). Futhermore, Richardt (2004) confirms 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.

## CONCLUSION

In 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 concentration of bilirubin was higher statistically significant at the beginning of lactation (p<0.05) in comparison to group during dry period (p<0.05). Consequently, concentration of total proteins was significantly higher in the middle lactation (p<0.05) in comparison with beginning lactation (p<0.05). Futhermore, the highest significant differences of glucose concentration in the middle lactation (p<0.01) and at the beginning of lactation (p<0.01) and following group of dry period (p<0.05). Likewise the cholesterol concentration was significantly higher in the middle lactation (p<0.05) in comparison to dry period (p<0.05) and following the beginning of lactation (p<0.05). Concerning the milk composition, we detected higher significant differences of lactose content in the middle lactation (p<0.05) in

comparison to the beginning of lactation ($p<0.05$). The present observation confirm that important stage of lactation, especially critical biological stages of dairy cows change blood metabolites and influence milk composition.

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