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Original study

Relationship between some variables of protein profile and indicators of lipomobilization in dairy cows after calving

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Abstract

The objective of this study was to determine the concentrations of the main indicators of lipomobilization and selected variables of protein profile in dairy cows after calving, including immunoglobulins and acute phase proteins, as well as to evaluate the relationships between the altered lipid metabolism and changes in protein profile. Into the evaluation we included 54 clinically healthy dairy cows of a Slovak spotted breed, low-land black spotted breed and their crossbreeds in the period of 1-2 weeks after parturition. Blood samples were analysed for non-esterified fatty acids (NEFA, mmol/l), β-hydroxybutyrate (BHB, mmol/l), total proteins (TP, g/l), albumin (Alb, g/l), immunoglobulin G (IgG, g/l), haptoglobin (Hp, g/l) and serum amyloid A (SAA, mg/l). In cows with concentrations of NEFA above 0.35 mmol/l (n=20) we found significantly lower mean serum concentrations of total proteins, albumin and IgG than in cows with serum NEFA concentrations below 0.35 mmol/l (n=34) (P<0.001). On the other hand, cows with higher values of NEFA showed significantly higher mean concentrations of BHB, Hp and SAA (P<0.001). The concentrations of NEFA significantly negatively correlated with the values of TP (P<0.001), albumin (P<0.01) and IgG (P<0.001). Significant positive correlations were found between the concentrations of NEFA and BHB, Hp, as well as SAA (P<0.001). Similar correlations were also found between the values of BHB and the variables of protein profile except for albumin. This study indicates strong relationships between NEFA and selected variables of protein profile in cows after parturition.

Keywords: dairy cows, non-esterified fatty acids, acute phase proteins, lipomobilization, postcalving period

Abbreviations: Alb: albumin, BHB: beta-hydroxybutyrate, ELISA: enzyme linked immunosorbent assay, Hp: haptoglobin, IgG: immunoglobulin G, IgM: immunoglobulin M, NEFA: non-esterified fatty acids, SAA: serum amyloid A, TP: total proteins

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Introduction

The time around parturition (transition period) is the most important and difficult period for high-yielding dairy cows, which is characterized by a high incidence of metabolic, infectious, and reproductive disorders (Goff & Horst 1997, Kelton *et al*. 1998). Roughly it spans from 3 weeks before to 3 weeks after parturition, and is defined as the change from a gestational non-lactating to a non-gestational lactating state (Mulligan & Doherty 2008, Contreras & Sordillo 2011). High-yielding dairy cows suffer from negative energy balance during the first weeks of lactation because of energy expenditure due to milk production and limited feed intake (Nielen *et al*. 1994). Under negative energy balance or low concentrations of insulin, the secretion of hormone-sensitive lipase is stimulated, triggering lipolysis with the subsequent release of fatty acids in its non-esterified form (non-esterified fatty acids – NEFA) to the bloodstream through a process known as lipid mobilization (Herdt 2000, Melendez *et al*. 2009). The NEFA are converted to more available energy substrates, the liver transforms them into ketone bodies. The most common ketone body in dairy cows is beta-hydroxybutyrate (BHB). NEFA and BHB are the most important blood indicators to assess the degree of negative energy balance and lipid mobilization (González *et al*. 2011).

The metabolic changes occurring in transition dairy cows may have consequences on cow's health causing severe economic losses for dairy farmers due to drop in milk yield and increase in culling rates (Goff & Horst 1997, Jorritsma *et al*. 2001). Studies in human medicine demonstrated that high concentrations of NEFA are linked to metabolic and inflammatory diseases, induce inflammation and affect immune function (Zhang *et al*. 2006, Yaqoob & Calder 2007). Increases in blood lipid content and higher concentrations of serum NEFA have been associated with an increased incidence of periparturient diseases (retained foetal membranes, displacement of the abomasums) also in cattle and predispose dairy cows to inflammatory-based diseases (mastitis, metritis, lameness) (Dyk *et al*. 1995, Sordillo *et al*. 2009). In veterinary medicine, the complex pathophysiology of changes in blood lipids and how they affect dairy cow's immunity during the transition period are not yet completely understood. The relationships between some variables related to energetic metabolism and the activation of acute phase response in dairy cows after calving were evaluated previously (Bossaert *et al*. 2012, Tóthová *et al*. 2013). However, studies dealing with the evaluation of the association between the concentrations of serum NEFA in cows after calving and changes in protein metabolism are rather scarce. Therefore, this study was aimed at the determination of main blood indicators of lipomobilization and selected variables of protein metabolism in dairy cows shortly after calving, and to describe the possible relationships between the altered lipid metabolism and changes in protein profile, including immunoglobulins and acute phase proteins.

Material and methods

Animals and sample collection

The study was carried out on 54 multiparous high-yielding dairy cows (from second to fifth calving) of a Slovak spotted, low-land black spotted breed and their crossbreeds from three conventional dairy farms (from 340 to 420 dairy cows in a herd) under similar husbandry,

management and feeding conditions (corn silage, hay and concentrates). The average herd milk production at these farms ranged from 7 105 to 8 350 litres per cow. The age of the monitored cows ranged from 3.5 to 8 years and they were in the period of 1-2 weeks after parturition. The daily milk yield of the evaluated animals ranged from 28 to 36 L. The animals were housed in free-stalls, and fed twice a day diets for lactating cows with free access to water in automatic drinking troughs. The cows were milked twice a day. Before sample collection, the cows were examined clinically using standard general clinical examination procedures (Jackson & Cockcroft 2002). None of the cows developed clinical signs of diseases during the time of observation (2 weeks after calving).

The analyses of evaluated parameters were performed in blood serum. Blood for the investigations was taken by direct puncture of *v. jugularis*. Blood samples were collected into plastic tubes with serum clot activator (Meus, Piove di Sacco, Italy). The separated serum was stored at –20 °C until analysed for indicators of lipomobilization NEFA (mmol/l), BHB (mmol/l), and selected variables of protein metabolism – total proteins (TP, g/l), albumin (Alb, g/l), immunoglobulin G (IgG, g/l), and acute phase proteins – haptoglobin (Hp, g/l) and serum amyloid A (SAA, mg/l).

Laboratory analyses

The concentrations of NEFA were assessed using commercial diagnostic kits (Randox, Crumlin, UK) by enzymatic colorimetric method. The values of BHB, TP, and Alb were determined using commercial diagnostic kits (Randox, Crumlin, UK) on automatic biochemical analyser ALIZE (Lisabio, Pouilly en Auxois, France). The concentrations of IgG were analysed by enzyme linked immunosorbent assay (ELISA) using commercially available diagnostic kits (Cusabio, Wuhan, China). Haptoglobin was assessed using commercial colorimetric kits (Tridelta Development, Maynooth, Ireland) in microplates, based on Hp-haemoglobin binding and preservation of the peroxidase activity of the bound haemoglobin at low pH. Serum amyloid A was analysed by sandwich ELISA method using commercial kits (Tridelta Development, Maynooth, Ireland). The reading of absorbencies and the consecutive calculation of final concentrations of IgG, Hp, and SAA were performed on automatic microplate reader Epoch (BioTek, Winooski, VT, USA).

The obtained results from evaluated cows were divided into two groups according to the measured concentrations of NEFA and presence of lipomobilization: Group A (n=34) – cows with serum concentrations of NEFA below 0.35 mmol/l and Group B (n=20) – cows with serum concentrations of NEFA above 0.35 mmol/l.

Statistical analyses

Arithmetic means and standard deviations for each evaluated variable and group of cows were calculated using descriptive statistical procedures. Unpaired Student's t-test was used to compare and to evaluate the significance of the differences in means between the groups of cows. Relationships between the concentrations of the evaluated variables in the monitored cows were calculated by linear regression and Pearson correlation test, including the correlation coefficient and significance of the correlation. All statistical analyses were done using the programme GraphPad Prism V5.02 (GraphPad Software Inc., La Jolla, CA, USA).

Results

The results of the concentrations of selected variables of energetic and protein metabolism characterized by average values and standard deviations, as well as the evaluation of significance of differences in means between two groups of cows are given in Table 1, and on Figure 1 and 2. The analyses of relationships between monitored variables in cows are presented in Table 2.

The mean value of NEFA in cows of Group A was significantly lower than in cows of Group B ($P<0.001$, Table 1). The values of BHB in cows with serum concentrations of NEFA above 0.35 mmol/l were significantly higher compared to cows with concentrations of NEFA below 0.35 mmol/l ($P<0.001$). On the other hand, the concentrations of total serum proteins, albumin and IgG were significantly lower in cows with NEFA values above 0.35 mmol/l ($P<0.001$). While in cows with serum NEFA concentrations below 0.35 mmol/l (Group A) the median of IgG concentrations was 25.25 g/l and the individual values ranged from 17.60 to 31.75 g/l, in cows with NEFA values above 0.35 mmol/l (Group B) the median IgG concentration was 15.95 g/l,

Table 1
Comparison of the concentrations of selected parameters of energetic and protein metabolism between the two groups of dairy cows (mean ± SD)

Parameters	Group of cows		*P*
	A (n=34)	B (n=20)	
NEFA, mmol/l	0.18 ± 0.08	0.87 ± 0.34	<0.001
BHB, mmol/l	0.53 ± 0.19	0.74 ± 0.18	<0.001
TP, g/l	84.6 ± 5.9	78.2 ± 5.5	<0.001
Alb, g/l	40.2 ± 2.6	37.0 ± 3.3	<0.001
IgG, g/l	25.0 ± 3.5	16.3 ± 2.7	<0.001
Hp, g/l	0.14 ± 0.06	0.72 ± 0.44	<0.001
SAA, mg/l	10.3 ± 14.2	107.8 ± 29.5	<0.001

Group A: cows with serum concentrations of NEFA below 0.35 mmol/l, Group B: cows with serum concentrations of NEFA above 0.35 mmol/l, *P*: significance of the differences of means between the groups of cows

Table 2
Regression analysis of the relationship between the concentrations of selected parameters of energetic and protein metabolism in cows

Parameters		NEFA, mmol/l	Hp, g/l	SAA, mg/l	TP, g/l	Alb, g/l	IgG, g/l
NEFA, mmol/l	R	-	0.5878	0.8110	−0.4358	−0.3953	−0.6382
	P	-	<0.001	<0.001	<0.001	<0.01	<0.001
BHB, mmol/l	R	0.4479	0.6024	0.4792	−0.3483	−0.2383	−0.4527
	P	<0.001	<0.001	<0.001	<0.01	>0.05	<0.001

R: correlation coefficient; *P*: significance of the correlation

and the measured values ranged from 12.10 to 23.30 g/l (Figure 1). More detailed analysis of individual IgG concentrations showed that while in Group A 50 % of measured values ranged from 21.95 to 27.63 g/l, in Group B this range was from 14.60 to 17.28 g/l.

An opposite trend was found in the concentrations of evaluated acute phase proteins, with significantly higher values of Hp and SAA in cows with serum concentrations of NEFA above 0.35 mmol/l ($P<0.001$, Table 1). The median concentration of Hp in cows with NEFA concentrations below 0.35 mmol/l (Group A) was 0.13 g/l, with the individual values ranged from 0.01 to 0.26 g/l (Figure 2a). The median of Hp concentrations in cows of Group B was higher (0.82 g/l), and the measured concentrations showed wider range of individual values (from 0.13 to 1.71 g/l). Similar trend was observed in the concentrations of SAA with approximately 10 fold higher mean value in cows with higher values of NEFA. The median concentration of SAA in cows from Group A was 4.70 mg/l, and the individual values ranged from 0.30 to 64.50 mg/l (Figure 2b). The median of SAA concentrations in cows from Group B was higher (109.00 mg/l), and the individual values ranged from 62.00 to 166.00 mg/l.

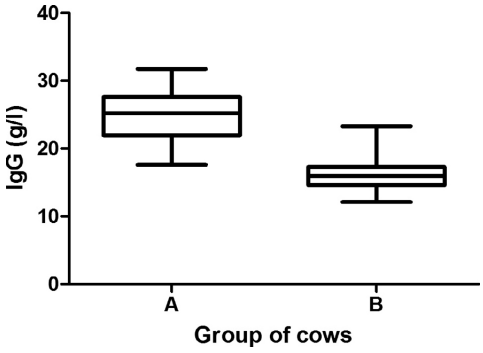

Figure 1
Concentrations of IgG in serum of cows with NEFA concentrations below 0.35 mmol/l (A) and above 0.35 mmol/l (B). The plots show the median (line within box), 25th and 75th percentiles (box), minimum and maximum values (whiskers).

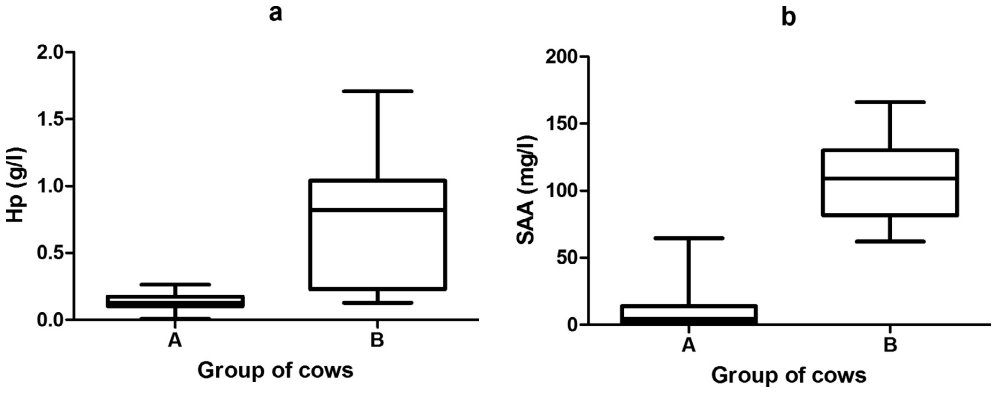

Figure 2
Concentrations of Hp (a) and SAA (b) in serum of cows with NEFA concentrations below 0.35 mmol/l (A) and above 0.35 mmol/l (B). The plots show the median (line within box), 25th and 75th percentiles (box), minimum and maximum values (whiskers).

By the assessment of the correlations between the evaluated variables (Table 2) we recorded a significant negative correlation between the concentrations of NEFA and total proteins ($P<0.001$), albumin ($P<0.01$) and IgG ($P<0.001$). On the other hand, the concentrations of NEFA in cows after parturition significantly positively correlated with the values of BHB, Hp and SAA ($P<0.001$). Similar correlations were also found between the values of BHB and the variables of protein profile except for albumin ($P>0.05$).

Discussion

Studies in human medicine demonstrated that high concentrations of NEFA induce low-grade inflammation and affect immune function (Hansson 2005, Zhang *et al*. 2006). In dairy cows, metabolic status can influence the incidence of peripartum diseases, including diseases that are not directly of metabolic origin (metritis, uterine diseases, mastitis) (Kaneene *et al*. 1997, Melendez *et al*. 2009). Geishauser *et al*. (2000) and LeBlanc *et al*. (2005) showed that peripartum cows with marked fat mobilisation have an increased risk for displaced abomasum. The mechanism of association between metabolic changes and inflammatory diseases around calving is not entirely clear, but may be mediated through diminished immune-cell functions (Gilbert *et al*. 1993). Our study showed in dairy cows with higher serum NEFA values significantly lower concentrations of immunoglobulin G, and high concentrations of NEFA, as well as BHB, correlated negatively with the diminished concentrations of IgG. Similarly, Mösch (2011) demonstrated that the increased concentrations of NEFA on the 3rd day of lactation were associated with decreased concentrations of IgG. Moreover, higher concentrations of NEFA correlated with the reduced concentrations of IgG, while the concentrations of BHB do not exhibit a correlation with the values of IgG. In the study presented by Melendez *et al*. (2009), overconditioned cows at calving had higher concentrations of NEFA and lower concentrations of IgM post partum. Lacetera *et al*. (2004) assessed the effect of various concentrations of NEFA on lymphocyte function of heifers and found inhibited IgM secretion by bovine peripheral blood mononuclear cells at concentrations of NEFA of 2, 1, 0.5 and 0.25 mmol/l. This immunosuppression modulated by NEFA was also demonstrated in ewes by Lacetera *et al*. (2002). They found significantly inhibited secretion of IgM by higher concentrations of NEFA, while higher concentrations of BHB do not inhibited the IgM secretion. According to Gilbert *et al*. (1993) and Kaneene *et al*. (1997) peripartum changes in leukocyte function probably are related to the same parturition-associated metabolic and endocrine events, as are changes in NEFA and cholesterol concentrations in serum. These authors concluded, that the extent of peripartum changes in NEFA concentrations is directly or indirectly related to the extent of peripartum reduction in leukocyte function. Contreras *et al*. (2010) indicated that changes in the concentration and composition of plasma directly affect white blood cell function due to changing of the composition of the cellular membrane of blood cells, or by altering the internal communication of white blood cells. Thus, lower concentrations of IgG in cows with higher concentrations of NEFA observed in our study, may be caused by changes in blood lipid content during periods of lipid mobilization.

The results presented in the study showed in cows with higher concentrations of NEFA significantly lower values of total serum proteins and albumin. Moreover, the concentrations of NEFA negatively correlated with the total protein and albumin concentrations. Similar

findings were reported by González *et al*. (2011), who found in cows with high lipid mobilization lower serum concentrations of total proteins, albumin, as well as urea compared to cows with low lipomobilization. According to Bobe *et al*. (2004), liver lesions caused by fatty infiltration as a consequence of lipomobilization are typically observed in high producing dairy cows during the first stage of lactation. The main indicators of hepatic lesions and function are the liver enzymes, but the serum concentrations of metabolites like glucose, proteins and urea are also indicators of hepatic functionality and decreases in their concentrations may reflect fat infiltration in animals with high lipomobilization (Adewuyi *et al*. 2005). West (1990) also reported, that fat infiltration into the liver may affect the concentrations of some blood components, including the diminished concentrations of glucose, total proteins, albumin, and urea. Moreover, González *et al*. (2011) found, similarly to our results, a significant correlation between serum NEFA values and the concentrations of total proteins. Thus, in the study found changes in the protein metabolism characterized by lower concentrations of total proteins and albumin in cows with higher NEFA values may be a consequence of alterations in liver functions in the affected animals.

An opposite trend was observed in the concentrations of evaluated acute phase proteins with higher values of Hp and SAA in cows with higher concentrations of NEFA. Parturition with following metabolic challenges constitutes a potentially stressful event for the dairy cow. One of the ways how an animal can manifest stress is in the form of activated acute phase response, including increased production of acute phase proteins by liver. The physiological processes taking place around the time of parturition, especially increase in myometrial activity, involution of the uterus, as well as degeneration and regeneration of the endometrium, may be also responsible for higher concentrations of acute phase proteins in blood serum (Regassa & Noakes 1999). On the other hand, the periparturient period is characterized by a sudden increase in energy requirements imposed by the onset of lactation and by negative energy balance (Leroy *et al*. 2008). A significant adaptation to the aforementioned negative energy balance during the transition period is the mobilization of fat from body stores and the release of non-esterified fatty acids into the blood stream. Animals may react to these disturbances in their homeostasis and changes in metabolism with a set of physiological changes, including changes in the concentration of some plasma proteins, especially acute phase proteins. According to Bernabucci *et al*. (2005) and Sordillo *et al*. (2009) increased circulating NEFA concentrations are directly associated with increased systemic inflammatory conditions, and large amounts of adipose stores during time of energy deficiency are linked with adverse health effects on the transition cow. Other researches demonstrated also a clear relationship between nutrition, inflammation and disease susceptibility, and that elevated NEFA concentrations are positive risk factors for many inflammatory periparturient diseases in dairy cows (Goff 2006, Calder 2008, Wood *et al*. 2009). Many of the changes occurring during the acute phase response may be beneficial for the host, but may also have some undesirable consequences for the transition period (Gruys *et al*. 2005). For example, lower concentrations of negative acute phase proteins, including albumin, have been associated with decreased liver function, reproductive performance, and impaired immunocompetence (Bertoni *et al*. 2008).

Presented results indicate strong relationships between the concentrations of non-esterified fatty acids, as well as BHB and selected variables related to protein metabolism

shortly after calving. The study demonstrates that metabolic changes associated with energy imbalance and fat mobilization may be related to alterations in protein metabolism and immune function. These data contribute to the better understanding of the complicated metabolic changes occurring in dairy cows after calving. However, further studies are needed to investigate in detail the association of changes in protein profile, including the acute phase response, with alterations in lipid metabolism and liver variables.

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