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INFLUENCE OF LIPOLYSIS AND KETOGENESIS TO METABOLIC AND HEMATOLOGICAL PARAMETERS IN DAIRY COWS DURING PERIPARTURIENT PERIOD

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The aim of this study was to examine the characteristics of the metabolic profile and complete blood count in cows in the periparturient period on the basis of the intensity of lipolysis and ketogenesis (concentration of non esterified fatty acid – NEFA and beta-hydroxybutyrate – BHB). Based on median values of NEFA and BHB cows were divided into 3 groups: cows physiologically burdened with catabolism (NEFA and BHB levels above the median one week after parturition), cows significantly burdened with catabolism (NEFA and BHB levels above the median one week before and after parturition) and cows that are not burdened with catabolism (NEFA and BHB below the median, i.e. the control group). The median value of NEFA was 0.27 mmol/L one week before parturition and 0.61 mmol/L one week after it. The median value of BHB was 0.51 mmol/L one week before parturition and 0.99 mmol/L one week after it. A significant group effect was shown for each week separately, so that cows physiologically burdened with catabolism and/or cows significantly burdened with catabolism compared to the control group have the following features of the metabolic profile and complete blood count: higher concentrations of NEFA and BHB (weeks: -1, 1, 2, 4, 8), lower concentrations of glucose (weeks: 1, 4), lower concentration of cholesterol (week 8), lower concentrations of total protein (weeks: 1, 2), lower concentrations of urea (weeks: 1, 2, 4, 8) and a higher concentration of bilirubin (weeks: -1, 1, 2, 4, 8), increased levels of AST (weeks: -1, 1) and ALT (weeks: -1, 2), lower value of Ca (week -1), lower hemoglobin concentration (week -1), lower white blood cell count (week 4), a larger number of neutrophils (weeks: -1, 1, 2) and a higher number of lymphocytes (week 4). Using the method of factor analysis and principal components showed that NEFA, BHB and glucose are the major components that affect the metabolic profile and blood count, making 71.8% of the variability of all parameters. Cows with hypoglycemia, hypocalcemia, hypoalbuminemia, hyperbilirubinemia, decreased hemoglobin concentration and/or red blood cell count and neutrophil to lymphocyte *ratio >1 showed significantly higher concentrations of NEFA and BHB compared to cows with parameters within normal ranges. Complete ROC (receiver operating characteristics) analysis showed that NEFA and BHB are important diagnostic indicators that allow the separation of cows with parameters out of the reference values from cows with normal values of parameters in the metabolic profile and blood count (0.5<AUC<0.87). Reliability of identification of cows with parameters that are out of the reference value increases with increasing concentrations of NEFA and BHB, which confirms that intense lipolysis and ketogenesis start a series of compensatory processes in the metabolism in cows. The results confirm that NEFA and BHB significantly affect the value of numerous metabolic and hematological parameters in the periparturient period.*

Key words: blood tests, dairy cows, beta-hydroxybutyrate, metabolic profile, non esterified fatty acids, ROC analysis

INTRODUCTION

In the period close to calving dairy cows enter a state of negative energy balance and expressed catabolism. Catabolism is the result of reduced food intake and increased needs for the coming lactation. In order to satisfy the needs in energy, cows mobilize the most potent energy metabolites, such as fat. Therefore, stimulation of lipolysis occurs in the period around calving, as well as suppression of *de novo* production of fat and decreased esterification of free fatty acids. These adaptive processes have resulted in increased concentrations of nonesterified fatty acids (NEFA) in the bloodstream. NEFA can be used for energy purposes in its entirety, but due to the extremely large share of fat in energy metabolism, fatty acids partially oxidise and then create ketone bodies (beta-hydroxybutyrate, BHB) (Ingvarsten, 2006, Reist et al., 2002). In addition to these, the period around calving is characterized by numerous metabolic and hematological changes such as decreased glycemia, decreased cholesterol concentrations, low concentrations of urea, elevated bilirubin concentration, reduced calcemia (Cincović et al., 2011); reduced number of red blood cells and hemoglobin, reduced white blood cell count but increased number of neutrophils, etc. (Belić et al.,2011). These changes occur as a result of negative energy balance and /or direct effects of NEFA and BHB in cells and tissues (Doepel et al., 2002; Lacetera et al., 2005; Rafia et al., 2011).

The aim of this study was to examine the parameters of the metabolic profiles and blood counts in healthy cows, which are classified based on the intensity of lipid mobilization and ketogenesis the week before and after partus, and examine the usefulness of NEFA and BHB in the evaluation of metabolic and hematological changes during early lactation.

MATERIALS AND METHODS

Animals and *experimental design*

The test included 29 cows of Holstein-Friesian breed. Blood from 29 healthy cows was examined by routine analysis. Cows were kept in a free system, haused on deep litter without outlet. Nutrition and care were identical for all cows.

Blood sampling

Blood samples were taken from the jugular vein, one week before partus, and then in the first, second, fourth and eighth week after partitution.

To avoid the prandial influence on the metabolites, blood was taken from 12 to 3 p.m. After transport to the laboratory, selected biochemical parameters such as: NEFA, BHB, glucose, total protein, albumin, urea, bilirubin, cholesterol, ALT, AST and Ca were quickly determined.

Blood analysis

The analysis of blood included: red blood cell count, hemoglobin concentration, leukocyte count, neutrophil count and lymphocyte count. Hematologic analyses were carried out on the counter Hemavet 950cv.

Blood serum biomarkers determination

Biochemical parameters were determined by conventional biochemical methods (kits Randox, UK and Pointe Scientific, USA) with Analyser Rayto RT-1904cv.

Statistical analysis

A. After data collection, the median value for NEFA and BHB was determined. Based on that, all cows were divided into those that are burdened with catabolism (NEFA and BHB values above the median) and cows not burdened with catabolism (NEFA and BHB values below the median). Detailed comparison of the values of NEFA and BHB in the week before and after partum showed that three theoretical groups of observations could be formed – see Table 1. ANOVA analysis was used to determine the difference between groups in general and in each week separately. For separate weeks LSD test was performed.

B. Influence of NEFA and BHB in the variation of blood values and metabolic profile – Principal component analysis is appropriate when you have obtained measures on a number of observed variables and wish to develop a smaller number of artificial variables (called principal components) that will account for most of the variance in the observed variables. The principal components may then be used as predictor or criterion variables in subsequent analyses. This method was used to assess the influence of lipolysis and ketogenesis on the variations of metabolites and blood counts. For this purpose Statgraphics software was used.

C. Application of NEFA and BHB in the clinical assessment of metabolic profile and blood count – As a frequent laboratory finding in the periparturient period can be stated: hypoglycemia, hypocalcemia, hyperbilirubinemia, hypoalbuminemia, anemia and stress leukogram (neutrophile to limphocyte ratio >1). Difference in the mean of NEFA and BHB concentration between groups with and without listed changes was confirmed by t-test. In order to determine whether NEFA and BHB can be used as predictors of changes in metabolic profile and blood count fractionation of NEFA and BHB was performed. The diagnostic performance and the accuray of NEFA and BHB to discriminate cases with listed changes from normal cases is evaluated using Receiver Operating Characteristic (ROC) curve analysis. The test was evaluated on the basis of area under the ROC curve (AUC) in the following manner so that the value of AUC = 0.5 was considered insignificant, a value $0.5<AUC\leq0.7$ as significant, $0.7<AUC\leq0.9$ very significant, $0.9<AUC<1$ highly significant and AUC=1 perfect. ROC curve for each selected parameter was constructed using 145 samples (29 cows x 5 weeks). Based on the ROC curve the concentration of NEFA and BHB was determined, which would predict listed changes in the metabolic profile and blood picture in more than 90% of the samples.

Table1. Groups of cows based on the intensity of lipolysis and ketogenesis

Group	Biological characteristics	Description	No. of cows
1	Control - not burdened by catabolism	Prepartum: NEFA and BHB below the median Postpartum: NEFA and BHB below the median	10
2	Group with the physiological postpartal catabolism	Prepartum: NEFA and BHB below the median Postpartum: NEFA and BHB above the median	8
3	Group significantly burdened with catabolism	Prepartum: NEFA and BHB above the median Postpartum: NEFA and BHB above the median	11
Σ			29

RESULTS

In the week before partus the median value of NEFA was 0.27 mmol/L, and BHB 0.51 mmol/L. In the week after partus median value for the concentration of NEFA was 0.61 mmol/L, while the median value for the BHB was 0.99 mmol/L.

The results presented in Figures 1 to 16 show the values of selected parameters within hematological and metabolic profiles in cows in three experimental groups during the nine weeks of observation. The graphs show results as mean ± SD.

Using ANOVA significant differences in three experimental groups (F_{NEFA}= 55.5, $p<0.001$; F_{BHB} = 64.23, $p<0.001$) were shown. Cows loaded with catabolism have a significantly higher concentration of NEFA and BHB in relation to the control group during the five experimental weeks. The concentration of NEFA in the group of cows with physiological catabolism was not significantly different from the control group in the week before calving. The concentration of BHB in the control group and the group of cows with postpartal catabolism is equalized in the eighth postpartum week.

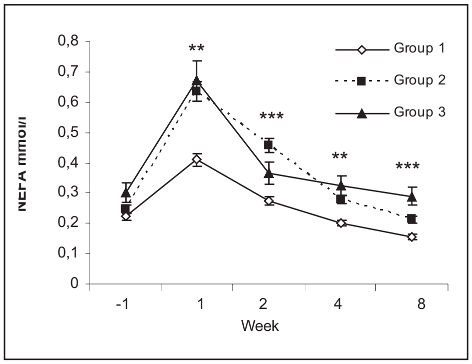

Figure 1. The concentrations of NEFA (mmol/L)

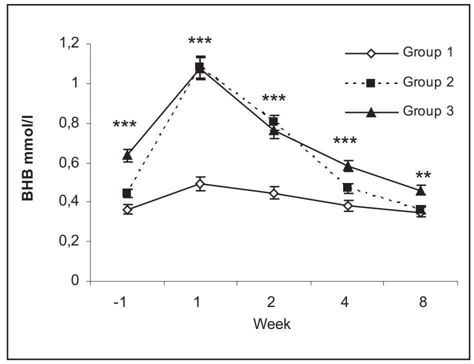

Figure 2. The concentrations of BHB (mmol/L)

Blood glucose (Figure 3) was not significantly different in the three observed groups (F=1.59, p>0.05). Looking at groups within the same week, we concluded that cows burdened with catabolism have significantly lower concentrations of glucose in the first (2.4±0.41 and 2.31±0.35 compared to 2.8±0.23 mmol/L) and fourth week postpartum (2.91±0.28 and 2.92±0.36 compared to 3.3±0.25 mmol/L).

The influence of group on the concentration of cholesterol (Figure 4) was not statistically significant (F=0.15, p>0.05). Comparing groups within each particular week, we concluded that the week before calving and the first and fourth week after calving do not show a statistically significant difference between groups. In the fourth week after parturition there is a significant group effect so that

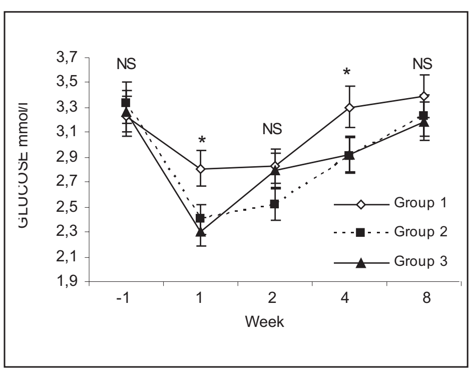

Figure 3. Concentrations of glucose (mmol/L)

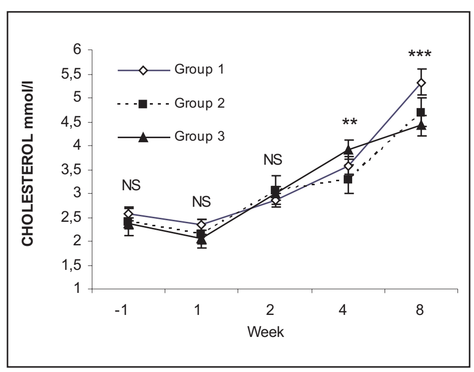

Figure 4. Concentrations of cholesterol (mmol/L)

cows with postpartal physiological catabolism showed the lowest cholesterol concentration (3.09±0.3 compared to 3.58±0.32 and 3.91±.26 mmol/L). In the eighth week postpartum cows with a burdened catabolism have a lower concentration of cholesterol than the control group (4.69±0.28 and 4.61±0.29 compared to 5.32±0.37 mmol/L).

The influence of group on protein concentration was significant (F=4.05; p<0.05) (Figure 5). Cows significantly burdened with lipolysis and ketogenesis have significantly lower concentrations of protein than the control group of cows in the first and second week after parturition (74.13±3.9:66.5±4.5 and 78.4±3.9:72.37±4.4 g/L). The concentration of albumin was not significantly different between groups, but cows significantly burdened with catabolism showed a tendency to lower albumin concentration in the week before and the first and second week after partum (Figure 6).

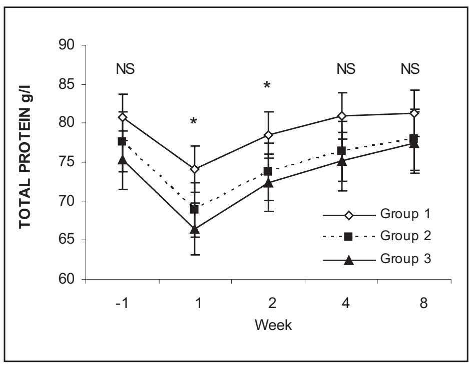

Figure 5. Concentrations of total protein (g/L)

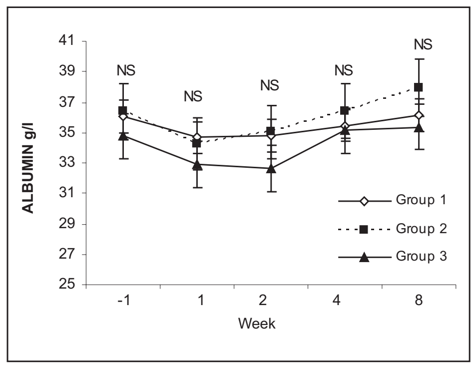

Figure 6. Concentrations of albumin (g/L)

The concentration of urea (Figure 7) significantly depends on the influence of group (F=24.3, p<0.001). Cows with physiological or intensive lipoliysis and ketogenesis showed significantly lower concentration of urea compared to control group in first, second, fourth and eight postpartal weeks.

The concentration of bilirubin (Figure 8) was significantly deference in function of group (F=23.32, p<0.001). Cows significantly burned with NEFA and BHB showed higher concentration of bilirubin in week before and first, second, fourth and eight week after partus compared to other two groups. Bilirubin concentration showed tendency to be higher (p<0.1) in cows with physiological catabolism compared to control group in firs and fourth group after partum.

AST activity was significantly higher in cows significantly burned with catabolism in the week before (82.03±4.11 compared to 71.2±5.03 and 74.1±6.11 IU/L) and week after partum (92.18±6 compared to 77.5±6.03 and 77.14±5.51 IU/L). In the same group ALT activity was higher in the week before (27.33±5.65 compared to 17.17±4.78 and 9.21±5.5 IU/L) and second week after partum (28.32±4.99 compared to 20.3±4.6 and 21.1±5.01 IU/L).

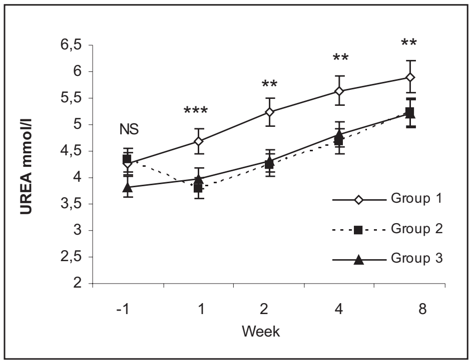

Figure 7. Concentrations of urea (mmol/L)

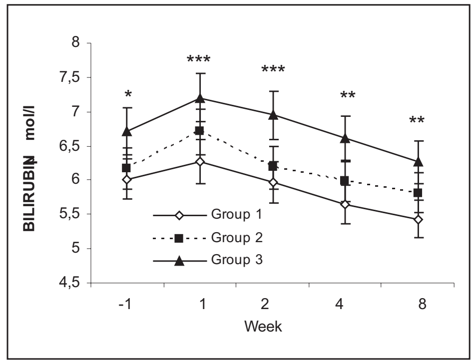

Figure 8. Concentrations of bilirubin (μmol/L)

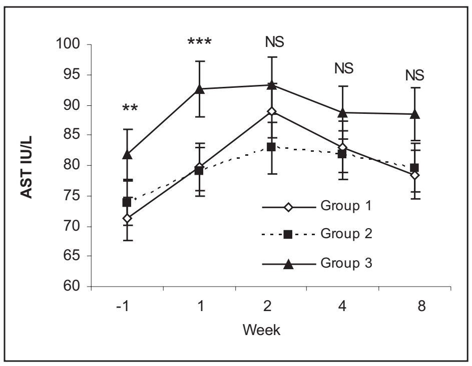

Figure 9. Concentrations of AST (IU/L)

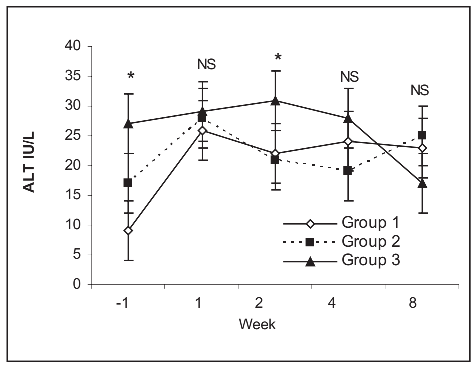

Figure 10. Concentrations of ALT (IU/L)

The concentration of calcium (Figure 11) is not significantly dependent on the influence of group. However, the LSD test showed that the calcium level in the week before calving has been significantly lower in cows that are significantly burdened with catabolism (2.09±0.09 compared to 2.29±0.08 and 2.34±0.073 mmol/L).

The influence of group on red blood cell count and hemoglobine concentration was not significant (Figure 12 and 13). Cows significantly burdened with catabolism had lower hemoglobin value in the week before partum compared to the control (98±4.5:105±4.7 g/L, $p<0.05$). There was no significant effect to total

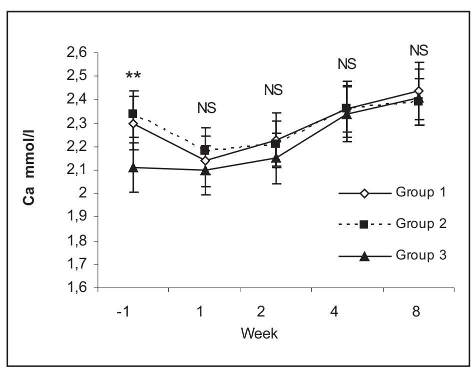

Figure 11. Concentrations of Ca (mmol/L)

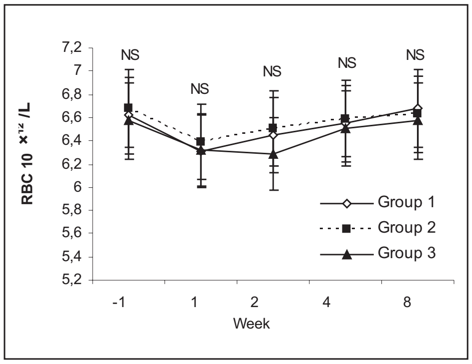

Figure 12. Number of erythrocytes

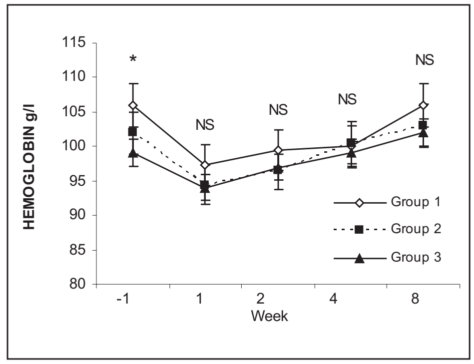

Figure 13. Hemoglobin concentration

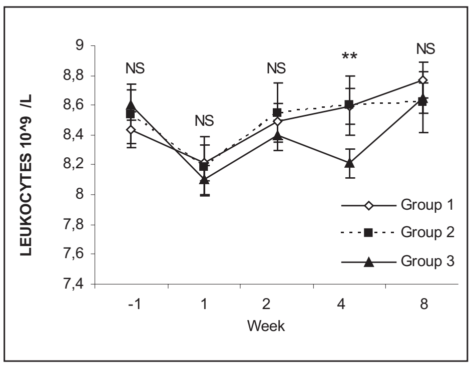

Figure 14. Number of leukocytes

leukocyte and lymphocyte number (Figures 14 and 15), whereas neutrophile (Figure 16) number significantly differed between groups ($F=14.14$, $p<0.001$). Cows that are significantly burdened with catabolism have a tendency towards a reduced number of leukocytes ($p<0.1$), and significantly lower number of leukocytes in the fourth week after partum (8.2 ± 0.15 compared to 8.52 ± 0.2 and 8.54 ± 0.21 10^9/L). Also, this group of cows has a significantly higher number of neutrophils in the week before partum compared to other groups (4.09 ± 0.16 compared to 3.95 ± 0.17 and 3.89 ± 19 10^9/L). A significant difference in the number of neutrophils in the first and second week after

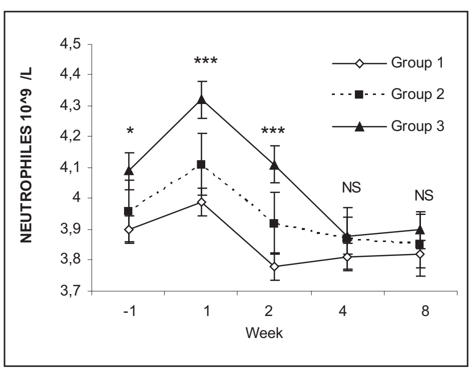

Figure 15. Number of neutrophils, 10^9/L

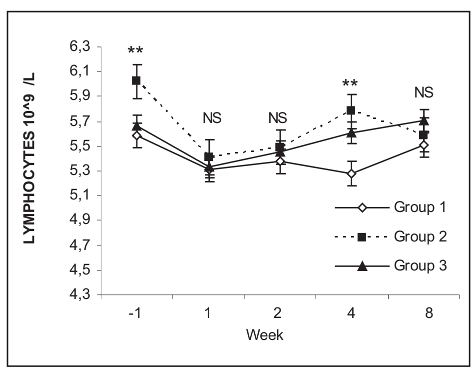

Figure 16. Number of lymphocytes, 10^9/L

parturition exists between all three groups, so that cows that with a significantly burdened catabolism have the largest number of neutrophils.

The question is what factors may represent a high percentage of variability of metabolic profile parameters and complete blood count, as well as which variables they include. Using both classical and principal components factor analysis was determined important factors. The first important factor explaining 71.8% of the variability of all parameters is shown on the Scree plot (Figure 17). This factor significantly correlated with high values of NEFA (<0.01), BHB (<0.01) and glucose (<0.01) (Table 2). This implies that the use of these three parameters of the metabolic profile can predict the variation of other parameters in over 70% of cases. This finding confirms the influence of NEFA and BHB on the metabolic profile and hematological parameters. The second important factor which could explain 14.45% of variation is correlated with cholesterol (<0.01) and bilirubin (<0.01) concentration.

Table 2. Factor Score Coefficients – displays the coefficients used to calculate the factor scores

	Factor 1	Factor 2
NEFA	0.895519	0.304169
BHB	0.771006	0.45395
Glucose	-0.760845	0.192284
Cholesterol	0.0773579	-0.89172
Bilirubine	0.362545	0.800408

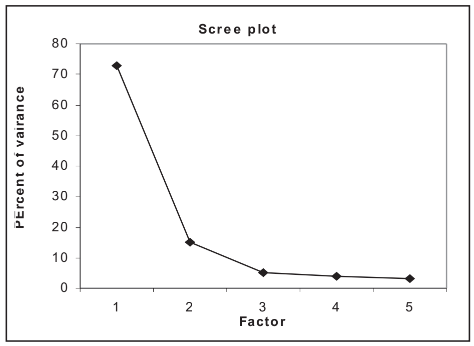

Figure 17. Scree plot – plots the eigenvalue or percent of total variance attributable to each factor

Cows with hypoglycemia, hypocalcemia, hyperbilirubinemia, hypoalbuminemia, anemia and stress leukogram showed higher concentrations of NEFA and BHB (Figure 18 and 19). Analyzing the ROC curves it was concluded that the value of NEFA and BHB are a

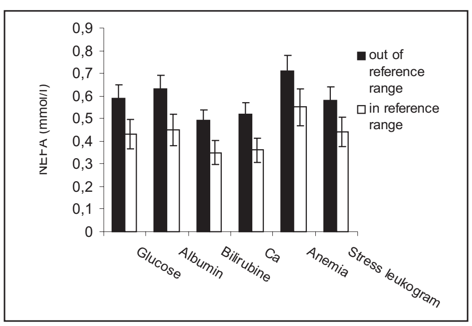

Figure 18. Concentration of NEFA is significantly higher (<0.01) in cows whose parameters are out of the reference range

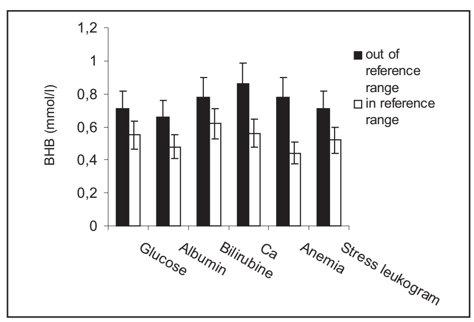

Figure 19. Concentration of BHB is significantly higher (<0.01) in cows whose parameters are out of the reference range

significant predictor of changes in the metabolic profile and blood count ($0.5<AUC\leq0.7$ is a significant, $0.7<AUC\leq0.9$ is very significant) (Table 3). With increasing values of NEFA and BHB significantly increases the frequency of samples with parameters out of the reference range. These results confirm the influence of lipid mobilization and ketogenesis in the evaluation of metabolic profile and blood count.

Table 3. ROC Analysis – Area under ROC curve and cut off value of NEFA and BHB at 90% specificity (90% correct identification of cows whose parameters are out of reference)

	AUC NEFA	Specificity 90% NEFA*	AUC BHB	Specificity 90% BHB*
Hypoglicemia	0.85	0.85	0.69	1.05
Hypoalbuminemia	0.80	0.93	0.66	1.14
Hyperbilirubinemia	0.87	0.79	0.70	1.09
Hypocalcemia	0.69	0.88	0.60	1.23
Anemia	0.66	0.99	0.55	1.26
Stress leukogram	0.74	0.87	0.60	1.21

DISCUSSION

Metabolic parameters of lipolysis and ketogenesis (NEFA and BHB) are useful indicators for measuring peripartal stress in cows. The results of Hochenberg et al. (2007) indicate that the values of NEFA above 0.5 mmol/L and values of BHB above 1.2 mmol/L exist in cows that were significantly burdened with catabolism in the period close to calving. These authors found that cows with higher concentrations of NEFA have a lower concentration of IGF-I (which is an indicator of anabolism), while cows with higher values of BHB lose body condition considerably. Cows which are significantly subjected to catabolic processes in the period clos to calving, have a higher concentration of BHB and NEFA (Busato et al., 2002), which matches our assumption that NEFA and BHB are good indicators of catabolism and stress. In our experiment, there is a considerable difference in the concentration of NEFA and BHB between the three groups of cows in the week before and after partus, respecting the conditions of the experiment given in materials and methods. The classification of cows based on the median value in the week before and after partus proved usable, because the cows that are burdened with catabolism have a significantly higher concentration of NEFA and BHB compared to the control even after the first postpartum week. Significant changes in metabolic profile and blood count in cows with NEFA and BHB values above the median in the week before and after parturition may be due to decompensatory process resulting from prolonged catabolism.

NEFA concentration increases in the week after calving as a result of energy deficit and changes in hormonal status of cows (Drackley et al., 2005, Bertoni et al., 1998). Elevated NEFA concentrations in cows may be associated with reduced capacity to adapt (NEFA concentration increased due to reduced pituitary-adrenal response) and increased stress sensitivity (increased concentrations of NEFA due to epinephrine activity) (Beerda et al., 2004; Underwood et al., 2003). NEFA is used for energy purposes and is a precursor for the synthesis of BHB in the hepatocytes (Guo et al., 2008). The concentration of BHB is a significant indicator of the health and productivity of cows. Cows with high values of BHB have lower reproductive capacity, significant loss in body condition, produce a small amount of milk and suffer from extreme metabolic changes (Kessel et al., 2008, Huszenicza et al., 2006). The increased value of BHB indicates the possibility of fatty liver, which can affect the process of metabolic adaptation (Šamanc et al., 2011).

A typical metabolic profile of cows a week after calving is characterized by a reduced concentration of glucose, protein, albumin, cholesterol, calcium and a high concentration of bilirubin and liver enzymes, which matches the previously obtained results (Cincović et al., 2011; Đoković et al., 2010; Park et al, 2010; Đoković et al., 2009). As for the blood count in the peripartal period, it is characterized by a reduced number of erythrocytes and leukocytes, reduced hemoglobin concentration and an increased number of neutrophils, which is in agreement with previously obtained results (Belić et al, 2011; Mirzadeh et al., 2010; Klinkon and Zadnik, 1999).

The concentration of glucose is significantly lower in the first week postpartum, because of the reduced food intake and increased utilization of glucose for milk production (Doepel et al., 2002). The physiological connection between lipolysis/ketogenesis and glucose concentrations can be explained by the fact that the liver is the main organ responsible for processes of gluconeogenesis, so in the case of greater accumulation of ketones, the ability of liver cells for gluconeogenesis decreases (Đoković et al., 2009). Reduced blood glucose level supports lower concentrations of cortisol, which confirms the reduced adrenal capacity and reduced adaptability to stress in cows (Forslund et al., 2010). Cholesterol concentrations did not differ significantly due to catabolism, which matches the results of previous authors (Busato et al., 2002). There is a negative correlation between NEFA and cholesterol, and their proportion can be used to estimate the risk of mastitis, metritis and retention of the placenta (Kaneene et al., 1997). Increased bilirubin concentration is a result of stressing the hepatocytes with ketones (Đoković et al., 2009). The concentration of urea depends on the intake of food. The variation of urea may be affected by NEFA, but only at the level of 14% (Rastani et al., 2006), and that is the reason why urea does not vary outside its reference values, as we have presented in the results. Reduced concentrations of total protein and albumin particular can also be linked to the biosynthetic function of the liver, which is reduced by the presence of ketone bodies in the hepatocytes (Šamanc et al., 2011). Reduced concentrations of proteins may occur as a result of increased use of amino acids in processes of gluconeogenesis in the period close to calving. In favor of this assumption there is an elevated concentration of AST, which is a significant indicator of catabolism of proteins and their use for gluconeogenesis (Seal and

Reynolds, 1993). A high concentration of AST along with a high concentration of NEFA is a significant indicator for the exclusion of cows from a herd (Gehrke and Markiewicz, 2009). Increased activity of AST is a valid indicator of liver condition in the peripartal period, important in the assessment of subclinical ketosis in which hepatocyte function is damaged and averages 116.23±54.90 U/L (Radojičić et al., 2008), although AST activity is very variable (Kasagić et al., 2011). Hypocalcaemia is a common finding in the period close to calving (Cincović et al., 2011) and occurs as a consequence of reduced food intake, initiation of lactation, altered parathyroid gland function and altered metabolism in the period around calving (Larsen et al., 2001). Hypocalcemic cows show higher concentration of NEFA and worse metabolic adaptation (Reinhardt et al., 2011). Reduced concentration of calcium along with the elevated NEFA predisposes dairy cows to develop dislocations of the abomasum (LeBlanc et al., 2005), and leads to a number of immunological dysfunctions (Kimura et al., 2006).

Reduced hemoglobin concentration and reduced number of red blood cells can be attributed to partus and to the beginning of lactation (Sattarand Mirza, 2009). There is not much data about the direct influence of lipolysis and ketogenesis on red blood cell parameters, but the results have shown that cows diagnosed with ketosis have significantly lower hemoglobin concentration, with the presence of anisocytosis and poikilocytosis (Belić et al., 2010). The effect of lipolysis and ketogenesis to the number and functional status of the white cells is much more familiar. Cows burdened with catabolism have fewer leukocytes in the peripartal period (Hochenberg et al., 2007), which is consistent with our results. Increased concentration of ketones interferes with cell proliferation in the bone marrow (Hoeben et al., 1999), the function of lymphocytes *in vitro* (Franklin et al., 1991) and leukocyte chemotaxis *in vitro* (Suriyasathaporn et al., 1999), as well as respiratory activity of polymorphonuclear leukocytes (Hoeben et al., 1997). Also, a similar effect of NEFA on immune cells has been discovered (Lacetera et al., 2004a). Increased ratio of neutrophils and lymphocytes occurs when animals are under stress, primarily due to the effects of cortisol (Bertoni et al., 2003). Cows that have excessive lipid mobilization and ketogenesis as well as reduced concentrations of glucose have a significantly higher ratio of neutrophils and lymphocytes (Belić et al., 2011).

Prepartal NEFA value which indicates the risk of peripartal disease is from 0.2 to 0.5 mmol/L, while in the postpartal period that value is from 0.57 to 1 mmol/L (Oetzel, 2004; Chapinal et al., 2011; Ospina et al. 2010). Cows that are healthy can show considerable variation in metabolic profile and blood count if they have concentrations of NEFA within this range, so a health assessment based on metabolic profiles and blood counts must be interpreted through lipid mobilization and ketogenesis in early lactation.

Based on the model of NEFA and BHB median value, effects of NEFA and BHB on variations in metabolic parameters and blood counts and differences in concentration and cut off values of NEFA and BHB in cows that have significant changes in metabolic profile and blood count (out of range), we conclude that lipolysis (NEFA) and ketogenesis (BHB) have a significant influence on the metabolic profile and blood count in cows during the periparturient period.

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UTICAJ LIPOLIZE I KETOGENEZE NA METABOLIČKE I HEMATOLOŠKE PARAMETRE KOD MLEČNIH KRAVA TOKOM PERIPARTALNOG PERIODA

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SADRŽAJ

Cilj ovog rada je bio da se ispitaju karakteristike metaboličkog profila i krvne slike krava u peripartalnom periodu na osnovu intenziteta lipolize i ketogeneze (koncentracija NEFA i BHB). Na osnovu vrednosti medijane NEFA i BHB krave su podeljene u 3 grupe: krave fiziološki opterećene katabolizmom (NEFA i BHB iznad medijane u nedelji posle partusa), krave značajno opterećene katabolizmom (NEFA i BHB iznad medijane u nedelji pre i posle partusa) i krave koje nisu opterećene katabolizmom (NEFA i BHB ispod medijane). Srednja vrednost NEFA bila je 0.27 mmol/l u nedelji pre partusa i 0.61 mmol/l u nedelji posle partusa. Srednja vrednost BHB iznosila je 0.51mmol/l u nedelji pre partusa i 0.99 mmol/l u nedelji posle partusa. Dokazan je signifikantan uticaj grupe za svaku nedelju posebno, tako da krave fiziološki opterećene katabolizmom i/ili značajno opterećene katabolizmom u odnosu na kontrolnu grupu imaju: višu koncentraciju NEFA i BHB (nedelje: -1, 1, 2, 4, 8), nižu koncentraciju glukoze (nedelje:1, 4), nižu koncentraciju holesterola (nedelja 8), nižu koncentraciju ukupnih proteina (nedelje:1, 2), nižu koncentraciju uree (nedelje:1, 2, 4, 8) i višu koncentraciju bilirubina (nedelje: -1, 1, 2, 4, 8), višu aktivnost AST (nedelje: -1, 1) i ALT (nedelje: -1, 2), nižu vrednost Ca (nedelja -1), nižu koncentraciju hemoglobina (nedelja -1), manji broj leukocita (nedelja 4), veći broj neutrofila (nedelje: -1, 1, 2) i veći broj limfocita (nedelja 4). Faktorskom analizom i metodom glavnih komponenti utvrđeno je su NEFA, BHB i glukoza najznačajnije komponente koje utiču na metabolički profil i krvnu sliku čineći 71.8% varijabilnosti svih parametara. Krave kod kojih je utvrđena hipoglikemija, hipokalcemija, hipoalbuminemija, hiperbilirubinemija, snižena koncentracija hemoglobina i/ili broj eritrocita i odnos neutrofila i limfocita >1 imaju značajno višu koncentraciju NEFA i BHB u odnosu na krave sa vrednostima ovih parametara u okviru referentnih. Kompletnom ROC (receiver operating characteristics) analizom je dokazano da su NEFA i BHB značajni dijagnostički indikatori koji omogućavaju razdvajanje krava sa parametrima izvan referentnih vrednosti od krava čiji su parametri u okviru referentnih vrednosti (0.5<AUC< 0.87). Pouzdanost identifikacije krava sa parametrima izvan referentneih vrednosti raste sa porastom koncentracije NEFA i BHB (intenzivniji katabolizam), što potvrđuje da intenzivna lipoliza i ketogeneza pokreću niz kompenzatornih procesa u metabolizmu krava. Dobijeni rezultati potvrđuju da NEFA i BHB značajno utiču na vrednost metaboličkih i hematoloških parametara u peripartalnom periodu.