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Changes in white blood pictures and some biochemical parameters of dairy cows in peripartum period and early lactation

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Summary

The objective of this research was to determine the changes in the number of cells of white blood line and some biochemical parameters: concentration of glucose, concentration of non-esterified fatty acids (NEFA), activity of enzymes of aspartat aminotransferase (AST), alanin aminotransferase (ALT) and gamma glutamyltranferase (GGT), in 17 Holstein-Friesian breed dairy cows in peripartum period and early lactation (on d 15 prepartum, on the day of parturition and on d 15, 30, and 45 postpartum). The highest values of total leukocytes count, neutrophiles, monocytes and basophiles count were recorded on the day of parturition, and then decreased, so that the minimum value established on d 45 of lactation. The lymphocytes count was the lowest on the day of parturation, while the eosinophils and basophiles counts were significantly different in observed time intervals. The concentration of glucose ranged from 3.07 mmol/L on d 15 prepartum to 2.71 mmol/L on d 15 postpartum. A high concentration of non-esterified fatty acids (NEFA) in the postpartum period is a consequence of changes in energy balance and more intensive process of lypolisis in fat tissue, due to energy disbalance at the beginning of lactation. Established activity of enzymes AST, ALT and GGT was approximately the same in studied time intervals and was within physiological limits. The results of hematological and biochemical analysis in this paper do not indicate the development of postpartum ailments, because the investigated parameters were within physiological limits.

Key words: dairy cows, leukocytes profil, biochemical parameters, peripartum period, early lactation

Introduction

Dairy cows are exposed to numerous hematological and biochemical changes, particularly in late gestation and early lactation. Various factors influence a metabolic profile in animals. Reliable indicators of metabolic profile and health status of dairy cows in peripartum period are haematological and biochemical parameters. A transitional period in dairy cows is followed by physiological, metabolic and nutritional changes. The way in which these changes happen and develop have a great influence on lactation performance, subclinical and clinical postpartum ailments and reproduction disorders, thus significantly affecting profitability (Block, 2010). Various metabolic and hormonal changes that occur during this period can diminish immunological defence (Kehrli et al., 1998).

Parturition and the onset of lactation bring dairy cows into the state of increased metabolic activity. Maternal tissues start the processes of adaptation, especially mammary gland, so the nutritive matters are directed mostly towards this gland (Blum at al., 1983).

During lactation period, a quantity of energy needed for the maintenance of tissue function is considerably higher than that which animals can provide by food. The activity of mammary gland considerably increases and the animals start to use their own reserves, primarily fats from body deposits. Mobilisation of fats starts in the last week of pregnancy,

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and reaches maximum in the first weeks of lactation. This state is followed by the increase of the concentration of non-esterified fatty acids (NEFA) in the blood of dairy cows, reaching its peak in the first week of lactation (Hachenberg et al., 2007). In this period dairy cows are in the state of negative energy balance (Baird, 1982). In the period of high milk production the intensive lipid mobilisation is essential, but if it is excessive or if homeostatic control fails, the development of metabolic diseases - ketosis, milk fever, dislocation of abomasus, fatty liver and other (Goff and Horst, 1997) is perceived.

The analysis of certain blood parameters, during peripartum period indicates a physiological, nutritive, metabolic and clinical state in animals (Găvan et al., 2010). Changes in the number of cells of white blood line and certain biochemical parameters are frequent in the course of late gestation and early lactation. Total leukocytes count is significantly higher at parturition than before and after this period (Klinkon and Zadnik, 1999; Meglia et al., 2001). Parturition is followed by the increase of corticosteroids which induce neutrophilia by the increased departure of neutrophiles from the bone marrow and demargination from the walls of blood vessels (Lee and Kehrli, 1998). Stress and neuroendocrine changes have a direct influence on the neutrophiles and lymphocytes counts during a peripartum period (Kehrli et al., 1989). Quiroz-Rocha et al. (2009) did not observe significant differences in the number of cells of white blood line, except for eosinophiles in prepartum and postpartum period.

The concentration of glucose in dairy cows is low, since it is produced mostly in the process of gluconeogenesis, and great quantities are secreted in milk in the form of lactose. The value of glycaemia depends both on gluconeogenesis and the consumption of glucose by mammary gland. In a pronounced negative energy balance the intensive lypomobilisation leads mostly to decreased volume of gluconeogenesis, followed by hypoglicaemia at the beginning of lactation (Jorritsma, 2003; Doepel et al., 2002).

The mobilisation of fats from body deposits is a consequence of negative energy balance. The concentration of non-esterified fatty acids increases two times - between d 17 and 2 before calving while the peak is repeated again at calving. A good indicator of energy status in dairy cows is a concentration of NEFA and in postpartum period it can initiate development of metabolic ailments such as ketosis and fatty liver (Grummer, 1993). The concentration of NEFA in blood plasma can increase by even 446 % on the third day postpartum compared to the concentration prepartum. The increase of the concentration of NEFA up to 123 % postpartum is considered to be within physiological value (Rukkwamsuk et al., 1999).

The enzymes aspartat aminotransferase (AST) and alanin aminotransferase (ALT) represent the basis in the metabolism of aminoacids and carbohydrates. They are most present in liver, thus by their activity determination the insight into the state of this organ can be obtained. Increased levels of transaminasis in blood plasma are either a consequence of increased permeability of the membrane of hepatocytes or their deterioration. If hepatocytes deteriorate, the determination of gamma glutamyltranferase is essential (GGT) (Stojević et al., 2001). The activities of mentioned enzymes in blood plasma of dairy cows are: AST 80-130 U/L, ALT 11-40 U/L and GGT 6-17 U/L (Kaneko et al., 1997).

These parameters above mentioned make the base of metabolic profile of high producing dairy cows and on their base metabolic disorders can be discovered, particularly in their subclinical form.

The aim of this research was to determine the changes in the cell count of white blood line, in the concentration of glucose, NEFA, and to determine the levels of enzymes AST, ALT and GGT in blood plasma of dairy cows in prepartum and postpartum period (on d 15 prepartum, on the day of parturition and on d 15, 30 and 45 postpartum).

Materials and methods

The trial was carried out on 17 clinically healthy cows of Holstein-Friesian breed in 3rd lactation, on one cattle farm in Serbia. The animals were fed complete fodder mixtures *ad libidum*. The blood samples were taken from v. jugularis into test tubes VF-054 SDK (with anticoagulant EDTA) 15 days prepartum, on the day of parturition and on d 15, 30 and 45 postpartum. The values of haematological parameters - total leukocytes count, count of neutrophiles, lymphocytes, monocytes, eosinophils and basophils was determined by means of automatic haematological analyser Arcus Diatron®, Gmbh Wien, Austria. For the determination of the concentration of glucose in blood plasma Dialab test kit was used. The concentration of non-esterified fatty acids in blood plasma was determined by a commercial ELISA kit, Gentaur France Sarl. The activity of enzymes ALT, AST and GGT was determined by a biochemical analyser Prochem V, Drew Scientific, USA.

Statistical analysis was carried out by descriptive statistic and Levene's test, and depending on the nature of the data for further analysis parametric or nonparametric tests were used. Total leukocytes count, count of neutrophiles, lymphocytes, monocytes, eosinophils and basophils, and for glucose and non-esterified fatty acids concentration needed to be analyzed by Kruskal-Wallis test and Mann-Whitney test (U test). For data concernig activity of ALT, AST and GGT the analysis of variance (ANOVA), and LSD test were used. Significant differences were considered for $P<0.05$. Calculations were performed with software STATISTICA 8.0. (StatSoft, Inc. 2007) and Microsoft Office EXCEL 2007.

Results and discussion

The results for the values of haematological parameters are shown in Table 1.

Mean values of total leukocytes count in observed time intervals (15 days prepartum, on the day of parturition and on d 15, 30 and 45 postpartum) were within the limits of physiological values. The highest total leukocytes count was established on the day of parturition (9.26×10^9/L), and the lowest on d 45 of lactation (7.14×10^9/L). This difference was statistically significant ($P<0.05$), and the difference between the leukocytes count on d 15 postpartum in relation to 15 days prepartum and d 45 of lactation. By comparing the difference of the leukocytes count in other studied time intervals significant differences were not established. Mean values in the neutrophiles count in prepartum and postpartum period ranged from 2.59×10^9/L on the fifteenth day prepartum to 4.84×10^9/L on the day of parturition. The highest lymphocytes count was determined on the d 15 prepartum (2.92×10^9/L), and the lowest on the day of parturition (2.41×10^9/L) and these differences were statistically significant. The monocytes count was the highest on the day of parturition (1.38×10^9/L), and lowest on d 45 of lactation (0.80×10^9/L). The eosinophiles count in studied periods ranged from 0.69×10^9/L on the fifteenth day prepartum to 0.39×10^9/L on the forty-fifth day of lactation. On the day of parturition the basophiles count was the highest (0.13×10^9/L), henceforth having the tendency of constant fall, so that on the d 45 of lactation a confirmed count was 0.03×10^9/L.

The changes in the cell count of white blood line were established in the period around parturition, and the results obtained in this study were within the physiological values (Brun-Hansen et al., 2006). Similarly to these results, Meglia et al. (2001) confirmed a higher leukocytes count on the day of parturition than before and after calving. It

Table 1. Amount of cells of white blood line in Holstein-Friesian breed dairy cows in peripartum period and early lactation

Days	Parameters[1]					
	Leukocytes (10^9/L)	Neutrophils (10^9/L)	Lymphocytes (10^9/L)	Monocytes (10^9/L)	Eosinophils (10^9/L)	Basophils (10^9/L)
15 prepartum	7.16 ± 1.87^{a}	2.59 ± 0.70^{a}	2.92 ± 0.48^{a}	0.89 ± 0.34^{a}	0.69 ± 0.17^{a}	0.07 ± 0.01^{a}
day of parturation	9.26 ± 3.04^{ab}	4.84 ± 1.39^{b}	2.41 ± 0.59^{b}	1.38 ± 0.33^{b}	0.51 ± 0.12^{b}	0.13 ± 0.02^{b}
15 postpartum	8.58 ± 1.99^{b}	4.04 ± 1.40^{bc}	2.84 ± 0.76^{a}	1.04 ± 0.32^{a}	0.61 ± 0.19^{ab}	0.05 ± 0.02^{c}
30 postpartum	7.52 ± 1.52^{abc}	3.41 ± 1.21^{c}	2.61 ± 0.78^{ab}	0.85 ± 0.20^{ab}	0.61 ± 0.18^{ab}	0.04 ± 0.01^{d}
45 postpartum	7.14 ± 2.25^{ac}	3.03 ± 1.01^{ad}	2.89 ± 0.81^{ab}	0.80±0.25a	0.39 ± 0.11^{c}	0.03 ± 0.01^{d}

[1] Mean values ± standard deviation are showed in the columns
a, b, c, d - Values within the same column not sharing a common letter were significantly different ($P<0.05$)

Table 2. Concentration of glucose and non-esterified fatty acids (NEFA) in Holstein-Friesian breed dairy cows in peripartum period and early lactation

Days	Parameters[1]	
	Glucose (mmol/L)	NEFA* (mmol/L)
15 prepartum	3.07 ± 0.58^{a}	0.11 ± 0.02^{a}
day of parturation	2.95 ± 0.47^{ab}	0.32 ± 0.08^{b}
15 postpartum	2.71 ± 0.43^{b}	0.41 ± 0.09^{c}
30 postpartum	2.80 ± 0.63^{ab}	0.35 ± 0.07^{b}
45 postpartum	2.99 ± 0.53^{ab}	0.30 ± 0.09^{b}

[1] Mean value ± standard deviation are showed in the columns
*NEFA- non-esterified fatty acids
a, b, c, d - Values within the same column not sharing a common letter were significantly different (P<0.05)

is the result of significant increase of neutrophiles (Piccinini et al., 2004) and monocytes counts. However, lymphocytes count decreased in the same period. Alon et al. (1995) report it is possible that because lymphocytes migrate into different tissues in relation to neutrophiles, high levels of cortisol detected at parturition have no effect on adhesion molecules of lymphocytes and for that reason they are able to penetrate into the tissues. However, corticosteroids can induce neutrophilia, increasing the departure of neutrophiles from a bone marrow or their demargination from the blood vessels (Lee and Kehrli, 1998). Contrary to results in this study, Găvan et al. (2010) confirmed the highest count of total leukocytes (14.9×10^9/L) in dairy cows in 40-120 days, and lowest (11.68×10^9/L) in the period of early lactation (0-21 days). These authors report that the highest neutrophiles count in dairy cows was 30 days prepartum (7.3×10^9/L), being considerably lower in the first three weeks of lactation (5.02×10^9/L), what is also in contrast to this study. However, the authors state that in animals observed the eosinophiles count had a falling tendency from 0.99-0.21×10^9/L (30 days before calving) to 40-120 days of lactation, what conforms to this study. Meglia et al. (2005) confirmed neutrophilia, eosinopenia, lymphopenia and monocytosis in dairy cows 7 days prepartum and 7 days postpartum. The authors state that these chematological changes have no effect on phagocytosis and inflammatory processes. Nazifi et al. (2008) established the highest leukocytes count in d 50-60 prepartum (6.45×10^9/L), and lowest in d 25-30 postpartum (5.3×10^9/L). This finding does not comply with results in this research. Jain (1986) points to the effect of stress on different content of the cells of white blood line. The author says there is a significant increase in the neutrophiles count, and decrease in the lymphocytes count, what conforms to this study. Quiroz-Rocha et al. (2009) report that in the blood samples of dairy cows a week before and a week after parturition significant differences in the leukocytes count were not determined, except for eosinophiles. Klinkon and Zadnik (1999) state that peripartum period has no effect on the basophiles count, what is in contrast with thic study and the statements Jain (1986). Also, Brun-Hansen et al. (2006) state that the basophiles count in cows in lactation ranges from 0-0,1×10^9/L. Concentration of glucose and non-esterified fatty acids (NEFA) are shown in Table 2.

The changes in the mean values of the glucose in blood of animals in studied periods ranged from 2.71 mmol/L to 3.07 mmol/L. The concentration of glucose in blood was lowest on the d 15 of lactation (2.71 mmol/L), but this value was not significantly lower (P>0.05) than the values confirmed in other time intervals, except in the relation to the concentration of glucose recorded 15 days prepartum (P<0.05). The concentration of non-esterified fatty acids (NEFA) was the lowest 15 days prepartum (0.11 mmol/L) and this value is significantly lower than confirmed values in other periods. The highest concentration of NEFA was established on d 15 of lactation (0.41 mmol/L) and it was significantly higher in comparison with the values on the day of parturition (0.32 mmol/L) and on d 45 of lactation (0.30 mmol/L).

The concentration of glucose decreases in the first and second week of lactation. A temporary fall in the level of glucose in the first weeks of lactation is a consequence of increased synthesis of lac-

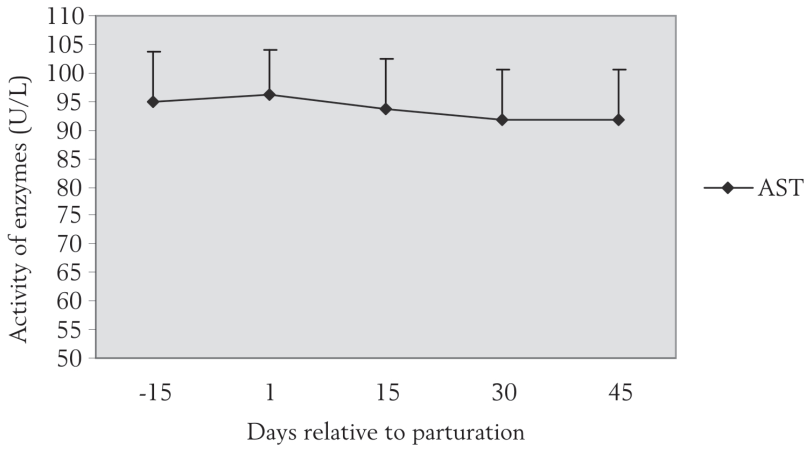

Figure 1. Activity of aspartat aminotransferase (AST) in Holstein-Friesian breed dairy cows in peripartum period and early lactation

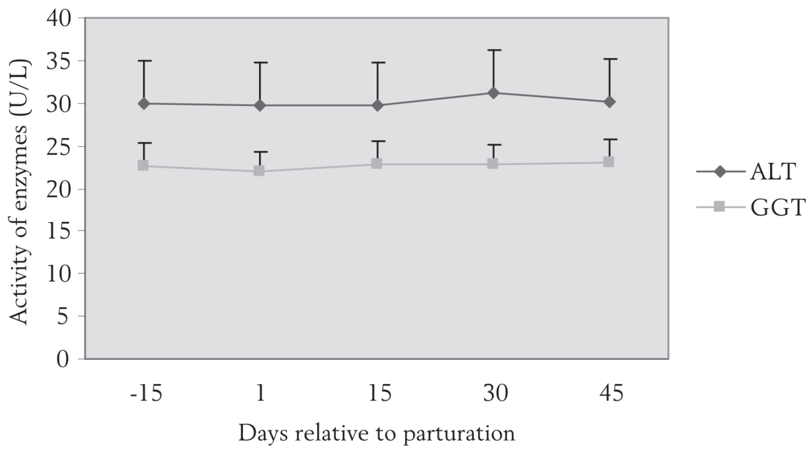

Figure 2. Activity of alanin aminotransferase (ALT) and gamma glutamyltranferase (GGT) in Holstein-Friesian breed dairy cows in peripartum period and early lactation

tose, and decreased gluconeogenesis (Doepel et al., 2002), what conforms to this study. During peripartum period, hormonal changes primarily regulate parturition, initiate lactation and adapt metabolism (Adewuyiet et al., 2005). These changes provoke hypoglycaemia postpartum (Ingvartsen, 2006), however, it is possible that some animals show gluconeogenetic effect of adrenaline and cortisol due to stress induced by calving.

A period of early lactation is followed by a negative energy balance which is a consequence of insufficient food intake and increased needs for milk production. The organism has a capacity to decrease existing energy misbalance by the process of mobilisation of fats from body deposits, but these compensatory mechanisms can lead to disturbance in metabolic balance (Šamanc et al., 2000). However, Mallard et al. (1998) state that compensative mechanisms depress mostly from 3 weeks prepartum to 3 weeks postpartum. Similarly to this study, Blum et al. (1983) report that the concentration of NEFA increases significantly postpartum and reaches its highest value two weeks postpartum. A high concentration of NEFA is a consequence of changes in energy balance and more intensive process of lipolysis in fat tissue, due to energy misbalance at the beginning of lactation (Reid et al., 1983). A considerable increase in the concentration of NEFA occurs in the calving period and is maintained by the second month of lactation until an energy balance is maintained (Kovačević, 2000). However, Grummer (1993) reports that the concentration of NEFA increases between d 17 and 2 prepartum, while the peak is reached at parturition, what is not in accordance with this research. Activity of enzymes AST, ALT and GGT are shown in Figure 1 and Figure 2.

Confirmed activities of AST, ALT and GGT did not differ significantly in studied time intervals. The values for AST ranged from 91.69±8.90 to 96.21±7.75 U/L, for ALT 29.69±5.91 to 31.14±5.84 U/L and for GGT 21.89±2.47 to 23.00±2.75 U/L, what is within the limits of reference values.

The activity of enzymes AST, ALT and GGT was approximately the same in studied time intervals and was within physiological limits. Confirmed values for AST are in accordance with the results reported by Samardžija et al. (2005), who reported that in cows with milk yield higher than 25 L the maximum activity of AST is 97.10 U/L, while in cows with lower lactation it is 69.30 U/L. Sahinduran et al. (2010) report that in dairy cows in early lactation (2-37 days) the activity of ALT was 29.57±6.32 U/L, AST 94.85±10.63 U/L and GGT 22.75±3.11 what conforms to results in this study. The authors say that the activity of these enzymes increases by even 100% in animals having ketosis. Contrary to findings in this study, Filar (1999) points to the increased activity of these enzymes what he associates with energy misbalance postpartum. The increase in the activity of GGT over 30 U/L occurs when the liver is damaged. Also, Saba et al. (1987) established high levels of AST and ALT postpartum suggesting that it is a consequence of more intensive metabolic processes caused by lactation. ALT and AST are good indicators of deficit of serum proteins, which are the results of the changes in the function of specific organs (Payne and Laws, 1978). The increase of AST activity is a sensitive indicator of impaired liver, even in subclinical state (Meyer and Harvey, 1998). GGT is an indicator of haepatobiliar diseases. The activity of GGT in serum is increased when the epithelium of bile duct is damaged, or in obstruction, in the case of cirrhosis and fat liver (Kramer and Hoffmann, 1997). GGT is a membrane enzyme situated in haepatocytes and biliary epithelium, having a major role in cell detoxication (Center, 2007).

Conclusions

The values of haematological and biochemical parameters, except for the activity of enzymes, in dairy cows, differed significantly in studied time intervals but they were within physiological values. During a transitional period, primary role of certain hormonal changes is to enable parturition and the onset of lactation while secondary role is to adapt metabolic processes to physiological events. The increase of the certain hormones level (corticosteroids) during parturition leads to the changes in haematological values. Established haematological and biochemical values in this paper do not point to the development of postpartum ailments, taking into account that they did not change extremely, but remained within physiological limits. This hypothesis is supported by insignificant changes in the level of enzymes in this paper, since in the opposite case their significant increase would point to metabolic disturbances, particularly in subclinical form.

Promjene bijele krvne slike i nekih biokemijskih parametara kod mliječnih krava u peripartalnom razdoblju i ranoj laktaciji

Sažetak

Cilj ovog rada bio je utvrditi promjene u broju bijelih krvnih stanica i pojedinih biokemijskih parametara - koncentraciji glukoze, koncentraciji nezasićenih masnih kiselina (NEFA), aktivnosti enzima aspartat aminotransferaze (AST), alanin aminotransferaze (ALT) i gama glutamattransaminaze (GGT), kod 17 mliječnih krava holštajn-frizijske pasmine u peripartalnom razdoblju i ranoj laktaciji (15 dana prije partusa, na dan partusa, 15., 30. i 45. dana nakon partusa). Najveće vrijednosti ukupnog broja leukocita, neutrofila, monocita i bazofila zabilježene su na dan partusa, a potom su se smanjivale, tako da su najniže vrijednosti utvrđene 45.

dana laktacije. Broj limfocita bio je najniži na dan partusa, dok se broj eozinofila i bazofila značajno razlikovao u ispitivanim intervalima (P<0.05). Koncentracija glukoze kretala se od 3,07 mmol/L 15 dana prije partusa do 2,71 mmol/L 15. dana nakon partusa. Visoka koncentracija nezasićenih masnih kiselina (NEFA) u postpartalnom razdoblju posljedica je promjena u bilanci energije i intenzivnijeg procesa lipolize u masnom tkivu uslijed energetskog disbalansa na početku laktacije. Utvrđene aktivnosti enzima AST, ALT i GGT bile su približno iste u ispitivanim vremenskim intervalima i kretale su se u fiziološkim granicama. Rezultati hematoloških i biokemijskih analiza u ovom radu ne ukazuju na razvoj postpartalnih oboljenja, jer su vrijednosti ispitivanih parametara bile u okviru fizioloških granica.

Ključne riječi: mliječne krave, leukocitni profil, biokemijski parametri, peripartalno razdoblje, rana laktacija

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References

1. Adewuyiet, A.A., Gruys, E., van Eerdenburg F.J.C.M. (2005): Non esterified fatty acids (NEFA) in dairy cattle, *A review. Vet* Q *27*, 117-126.
2. Alon, R., Kassner, P.D., Woldemar Carr, M., Finger, E.B., Hemler, M.E., Springer, T.A. (1995): The integrin VLA-4 supports tethering and rolling in flow on VCAM-1. *J Cell Biol 128*, 1243-1253.
3. Baird, G.D. (1982): Primary ketosis in the high-producting dairy cow: clinical and subclinical disorders, treatment, prevention and outlook. *J Dairy Sci 65*, 1-10.
4. Block, E. (2010): Transition cow research - What makes sense today?, High Plants Dairy Conference, Amarillo, Texas. *Proceedings*, 75-98.
5. Blum, J.W., Kunz, P.L., Leuenberger, H., Gautschi, K., Keller, M. (1983): Thyroid hormones, blood plasma metabolites and haematological parameters in relationship to milk yield in dairy cows. *Anim Prod 36*, 93-104.
6. Brun-Hansen, H.C., Kampen, A., Lund, A. (2006): Hematologic values in calves during the first 6 months of life. *Vet Clin Path 13*, 182-187.
7. Center, S.A. (2007): Interpretation of liver enzymes. *Vet Clin Small Anim 37*, 297-333.
8. Doepel, L., Lapierre, H., Kenneky, J.J. (2002): Peripartum Performance and metabolism of dairy Cows in Response to prepartum energy and Protein Intake. *J Dairy Sci 85*, 2315-2334.
9. Filar, J. (1999): Schorzenia przemiany weglowodanowo-tluszczowej u przezuwaczy, [Ailments of carbohydrate-lipid metabolism in ruminants], *Wyd AR w Lublinie* [in Polish].
10. Găvan, C., Retea, C., Motorga, V. (2010): Changes in the hematological profile of Holstein primiparous in periparturient period and in early to mid lactation. *Anim Sci and Biotech 43 (2)*, 244-246.
11. Goff, J.P., Horst, R.L. (1997): Physiological Changes at Parturition and their relationship to Metabolic Disorders. *J Dairy Sci 80*, 1260-1268.c
12. Grummer, R.R. (1993): Feed to avoid fatty liver and ketosis. *Hoards Dairyman 138*, 754.
13. Hachenberg, S., Weinkauf, C., Hiss, S., Sauerwein, H. (2007): Evaluation of classification modes potentially suitable to identify metabolic stress in healthy dairy cows during the peripartal period. *J Anim Sci 85*, 1923-1932.
14. Ingvartsen, K.L. (2006): Feeding- and management-related diseases in the transition cow: Physiological adaptations around calving and strategies to reduce feeding-related diseases. *Anim Feed Sci Technol 126*, 175-213.
15. Jain, N.C. (1986): *Schalms Veterinary Hematology*, 4th edition, Lea and Febiger, Philadelphia, pp 20-80, 185-207.
16. Jorritsma, R. (2003): Negative energy balance in dairy cows as related to fertility, *Dissertation*, Utrecht University.
17. Kaneko, J.J., Harvey, W.J., Bruss, M.L. (1997): *Clinical biochemistry of domestic animals*, *Fifth edition*, Academic Press, San Diego, California.
18. Kehrli, M.E., Nonnecke, B.J., Roth, J.A. (1989): Alteration in bovine lymphocyte function during the periparturient period. *Am J Vet Res 50*, 215-220.
19. Kehrli, M.E., Kimura, K., Goff, J.P., Stabel, J.R., Nonnecke, B.J. (1998): Periparturient immunosuppreression in dairy cows: nutrition and lactation effects, Production Diseases in Farm Animals, *10^{th} International Conference*, Ed. Th. Wensing, Netherlands.
20. Klinkon, M., Zadnik, T. (1999): Dynamics of red and white blood picture in dairy cows during the periparturient period. *Comparative Haematology International 9*, 156-161.
21. Kovačević, M. (2000): Funkcionalno stanje jetre i hormonalni status u krava različite uhranjenosti u graviditetu i ranoj laktaciji, *Doktorska disertacija*, Beograd.
22. Kramer, J.W., Hoffmann, W.E. (1997): *Clinical Enzymology, 5^{th} Edn. In: Kaneko JJ, Harvey JW and Bruss ML (Eds)*, *Clinical Biochemistryof Domestic Animals*, Academic Press, San Diego, 315-317. DOI: 10.1023/A:1017137803035.
23. Lee, E.K., Kehrli, M.E. (1998): Expression of adhesion molecules on neutrophils of periparturient cows and neonatal calves. *Am J Vet Res 59*, 37-43.

24. Mallard, B.A., Dekkers, J.C., Ireland, M.J., Leslie, K.E., Sharif, S., Lacey Vanjampen, C., Wagter, L., Wilkie, N. (1998): Alteration in immune responsiveness during the peripartum period and its ramification on dairy cow and calf health. *J Dairy Sci 81*, 585-595.

25. Meglia, G.E., Johannisson, A., Petersson, L., Persson Waller, K. (2001): Changes in some blood micronutritiens, leukocytes and neutrophil expression of adhesion molecules in periparturient dairy cows. *Acta Vet Scand 42*, 139-150.

26. Meglia, G.E., Johannisson, A., Agenäs, S., Holtenius, K., Persson Waller, K. (2005): Effects of feeding intensity during the dry period on leukocyte and lymphocyte populations, neutrophil function and health in periparturient dairy cows. *The Veterinary Journal, 169*, 3, 376-384.

27. Meyer, D.J., Harvey, J.W. (1998): Evaluation of hepatobiliary system and skeletal muscle and lipid disorders, *In: Veterinary Laboratory Medicine, Interpretation and Diagnosis, (Meyer, D.J., Harvey, J.W., Eds), 2nd ed.*, W.B. Sauners company Philadelphia, 157-187.

28. Nazifi, S., Ahmadi, M.R., Gheisari, H.R. (2008): Hematological changes of dairy cows in postpartum period and early pregnancy, *Comparative Clinical Pathology*, Springer-Verlag London Ltd, DOI 10.1007/s00580-008-0730-6.

29. Payne, E., Laws, L. (1978): Tissue enzyme levels as indices of protein status in sheep. *Br J Nutr 39*, 441-449.

30. Piccinini, R., Binda, E., Belloti, M., Casirani, G., Zecconi, A. (2004): The evaluation of non-specific immune status of heifers in field conditions during the periparturient period. *Vet Res 35*, 539-550.

31. Reid, I.M., Dew, S.M., Collins, R.A., Ducker, M.J., Bloofield, G.A., Morant, S.V. (1983): The relationship between fatty liver and fertility in dairy cows a farm investigation. *J Agric Sci 101*, 499-502.

32. Rukkwamsuk, T., Wensing, T., Geelen, M.J.H. (1999): Effect of fetty liver on hepatic glukoneogenesis in periparturient cows. *J Dairy Sci 82*, 500-505.

33. Saba, L., Bialkowski, Z., Janecki, T., Junkuszew, W. (1987): Ocenia niektórych wskaźników profilu metabolicznego i plodności krów w rejonach neidoborów mineralnych [Evaluation of some parametrs of metrabolic profile and fertility of cows in the regions with mineral deficiencies]. *Ann UMCS Sect DD 42*, 95-102.

34. Sahinduran, S., Sezer K., Buyukoglu, T., Albay M.K., Karakurum M.C. (2010): Evaluation of some haematological and biochemical parameters before and after treatment in cows with ketosis and comparison of different treatment methods. *J Anim Vet Adv 9* (2), 266-271.

35. Samardžija, M., Dobranić, T., Getz, I., Harapin, I., Vince, S., Prvanović, N., Đureković, I. (2005): Influence of metabolic parameters on reestablishment of ovarian cyclicity in puerperium of simmental cows, *6th Middle-European Biatricus Congress / Malinowski, Edward (ur.), Krakov*, National Veterinary Research Institute in Pulawy, 351-356.

36. Stojević, Z., Gradinski-Vrbanac, B., Milinković Tur, S., Barbić, LJ., Zdelar Tuk, M., Piršljin, J. (2001) : Praćenje hematoloških i biohemijskih pokazatelja u krvi peradi kao metoda otkrivanja kliničkih i subkliničkih bolesti u intezivnoj proizvodnji. *Zbornik Simpozijuma Peradarski dani*, Poreč, 41-42.

37. Quiroz-Rocha, G.F., Leblanc, S.J., Dufield, T.F., Wood, D., Leslie, K.E., Jacobs, R.M. (2009): Reference limits for biochemical and hematological analytes of dairy cows one week before and one week after parturition. *Can Vet J 50* (4), 383-388.

38. Šamanc, H., Nikolić, J.A., Đoković, R., Damjanović, Z., Ivanov, I., Janković, D. (2000): Blood insulin, lipids and glucose concentrations in health or ketotic cows. *Lucr. St. Med. Vet., Timisoara 33*, 19-23.