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# REFERENCE VALUES AND FREQUENCY DISTRIBUTION OF METABOLIC PARAMETERS IN COWS DURING LACTATION AND IN PREGNANCY*

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*SUMMARY: Biochemical parameters in 40 Holstein-Friesian cows have been studied. Cows were in the second and third lactation. Blood was sampled during four productive periods as follows: in the dry period (2-3 weeks before delivery), the postpartum period (0-2 weeks postpartum), in the middle of lactation (15-17 weeks postpartum) and late lactation (2-3 weeks before the start of drying). Concentrations of glucose, protein, cholesterol, bilirubin, urea, NEFA, BHB, ALT, AST, Ca, P and Mg were testes by using standard biochemical methods. The parameters of the metabolic profile of Holstein-Friesian cows correspond to the reference values in cattle. Parameters are within the normal (Gaussian) distribution. Periparturient changes in the concentrations of metabolites are characterized by negative energy balance, with the development of liver failure and reducing the concentration of calcium. Frequency distribution of values of metabolic profile suggests that clinically healthy animals during the entire period of lactation may show signs of metabolic changes, which is probably caused by high demand for milk production, due to load stress and inadequate nutrition.*

***Key words:*** *metabolic profile, milk cows, the reference values, frequency distribution of parameters.*

## INTRODUCTION

Forty years ago in England it was recommended that the purpose of evaluation of metabolic status in dairy cows is examination of the metabolic profile. Then the so-called Compton test was adopted which includes the determination of hematocrit,

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the concentration of glucose, urea, phosphorus, copper, zinc, magnesium, total protein, albumin, globulin, beta-carotene and vitamin A in the blood. Development of clinical medicine introduced other important parameters such as bilirubin, nonesterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), triglycerides, cholesterol, and many others. Determination of values for these parameters in the blood is of great importance in terms of evaluation of nutrition, productivity and potential susceptibility to development of various diseases (Payne et al., 1970).

Each of these parameters has a specific meaning in clinical practice. The concentration of glucose, NEFA, BHB, cholesterol and triglycerides indicates the energy status of the organism. The concentration of bilirubin and protein with the previous parameters can indicate the degree of fattening of the liver and liver failure and the risk of ketosis. Lipolysis is a characteristic response of cows to different, in particular acute stressor (Cincović et al., 2010). The concentration of urea suggests the supply of the body protein, but also points to the health of the kidneys or liver. Calcemia indicates the risk of puerperal paresis and phosphoremia the possibility of developing locomotor disorders (Kaneko et al., 2008). It is known that at the beginning of lactation leads to important changes in the metabolism of cows. Periparturient period in dairy cows leads to reduced intake of food dry matter (Grummer et al., 2004), while the other grows daily milk production. Such physiological changes have led cows in a state of negative energy balance and lipolysis. Negative energy balance leads to numerous changes in the movement of values of metabolic parameters. On the other hand, most diseases occur in the periparturient period and are the consequences of that negative energy balance (James et al., 2005). The parameters of metabolic profile are important in assessing the conditions for feeding and care and its impact on the health of cows (Krnić et al., 2006).

The aim of this paper is to examine the reference values for selected parameters of metabolic profile, and to present the frequency distribution of values of parameters depending on the period of lactation.

## MATERIAL AND METHODS

Biochemical parameters were examined in 40 Holstein-Friesian cows. Cows were in the second and third lactation and grown under the same conditions of diet (standard recipe meals based on corn silage, TMR) and care (free rearing system on deep litter). Blood was collected in four main productive periods as follows: in the dry period (2-3 weeks before delivery), the postpartum period (0-2 weeks postpartum), in the middle of lactation (15-17 weeks postpartum) and late lactation (2 - 3 weeks before the start of drying). Blood was taken from the jugular vein after the morning milking. After sampling the blood was immediately centrifuged (2000 rpm) and serum disposed in handy fridge, while the laboratory analysis was carried out during the day. Concentrations of glucose, protein, cholesterol, bilirubin, urea, NEFA, BHB, ALT, AST, Ca, P and Mg were tested by using standard biochemical methods. Biochemical analysis was performed by using standard methods of biochemical tests on blood analyzer Rayto RT1904C, with production of standardized kits RANDOX UK (NEFA) and POINT SCIETIFIC USA (other parameters).

## RESULTS

The results presented in Table 1 show the average values of metabolic parameters in four product categories of cows. The results show that cows after delivery showed a number of adaptive mechanisms, which are reflected in the concentrations of certain metabolites. Due to metabolic stress concentration of glucose was lower and concentrations of NEFA and BHB higher in cows after birth compared to the other three studied groups. The concentration of calcium was significantly lower in the post-parturition. These parameters are outside the normal range after delivery. The concentrations of protein, bilirubin and urea were also significantly different in cows after delivery, but do not exceed physiological values. Table 2 shows the number of cows which were established by these changes. In 10 cows NEFA concentration was high above the physiological data, while the higher concentration of BHB in 34 cows. The concentration of glucose was lower in 17 cows, and calcium concentrations in 20 cows. Charts 1-12 show the frequency distribution of cows with different values of parameters, in order to get the impression of the situation in the population.

Table 1. The average values of blood biochemical parameters according to stage of lactation
*Tabela 1. Prosečne vrednosti biohemijskih parametara krvi shodno stadijumu laktacije*

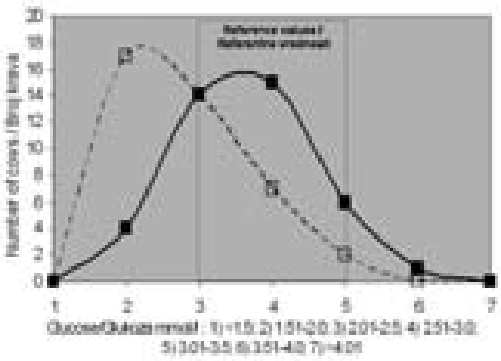
| | Precalving *Zasušene* | Fresh cows *Početak laktacije* | Mid lactation *Sredina laktacije* | End lactat. *Kraj laktacije* | Ref.value *Ref.vredn. literatura* |
|---|---|---|---|---|---|
| Glucose *Glukoza*, mmol/l | 2.55 | 1.91* | 2.46 | 2.31 | *2.2-3.3* |
| Proteins *Proteini*, g/l | 78.44 | 77.13* | 80.16 | 80.43 | *60-80* |
| Hoelsterol mmol/l | 4.21 | 4.13 | 3.72 | 3.62 | *1.3-3.6* |
| Bilirubin µmol/l | 4.23 | 5.71* | 5.55 | 4.82 | *0.9-6.9* |
| Urea mmol/l | 3.22 | 2.36* | 3.01 | 3.06 | *1.7-6.7* |
| NEFA mmol/l | 0.22 | 0.47** | 0.25 | 0.15 | *0.1-0.35* |
| BHB mmol/l | 0.41 | 0.98** | 0.43 | 0.35 | *0.3-0.5* |
| ALT U/I | 26.8 | 28.5 | 27.74 | 28.03 | *10-40* |
| AST U/I | 83.31 | 88.97 | 79.56 | 82.27 | *78-132* |
| Ca mmol/l | 2.5 | 1.98* | 2.4 | 2.37 | *2-3* |
| P mmol/l | 1.93 | 1.91 | 1.84 | 1.82 | *1.6-2.3* |
| Mg mmol/l | 0.94 | 1.01 | 1.07 | 1.12 | *0.7-1.2* |

*p<0.05, ** p<0.01.

Table 2.The number of cows whose metabolic parameter values are outside the reference (+ turn to the right, - turn left)
*Tabela 2. Broj krava čije su vrednosti metaboličkog parametra van referentnih (+ skretanje u desno, - skretanje u levo)*

| | Precalving *Zasušene* | | Fresh cows *Početak laktacije* | | Mid lactation *Sredina laktacije* | | End lactat. *Kraj laktacije* | |
|---|---|---|---|---|---|---|---|---|
| Vrednost izvan referentnih | + | - | + | - | + | - | + | - |
| Glukoza mmol/l | 0 | 1 | 0 | 17 | 1 | 1 | 0 | 1 |
| Proteini g/l | 0 | 1 | 1 | 5 | 2 | 0 | 1 | 2 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Hoelsterol mmol/l | 0 | 0 | 2 | 0 | 3 | 0 | 5 | 0 |
| Bilirubin µmol/l | 3 | 0 | 9 | 0 | 4 | 0 | 3 | 0 |
| Urea mmol/l | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 |
| NEFA mmol/l | 2 | 0 | 10 | 0 | 2 | 0 | 1 | 0 |
| BHB mmol/l | 0 | 0 | 34 | 0 | 1 | 0 | 0 | 0 |
| ALT U/I | / | / | / | / | / | / | / | / |
| AST U/I | / | / | / | / | / | / | / | / |
| Ca mmol/l | 1 | 2 | 3 | 20 | 1 | 3 | 4 | 3 |
| P mmol/l | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 |
| Mg mmol/l | 1 | 0 | 1 | 2 | 1 | 3 | 2 | 1 |



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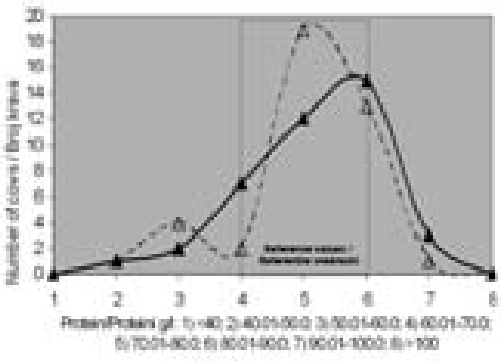

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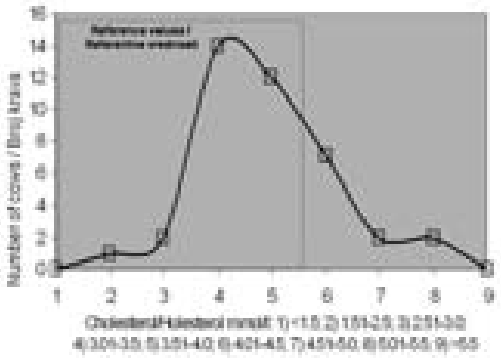

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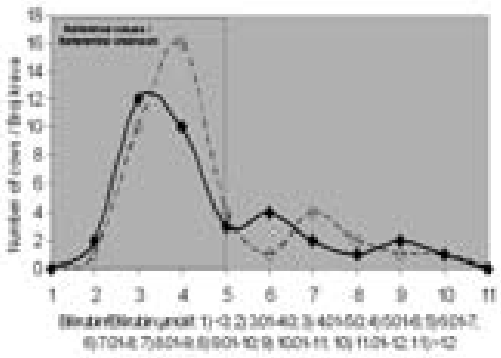

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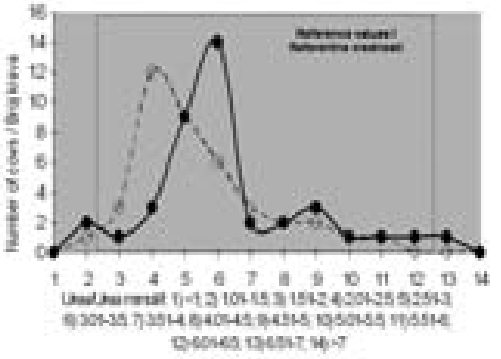

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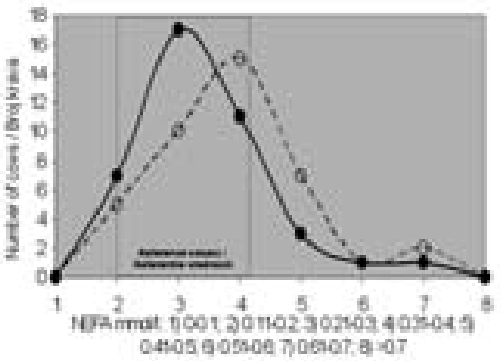

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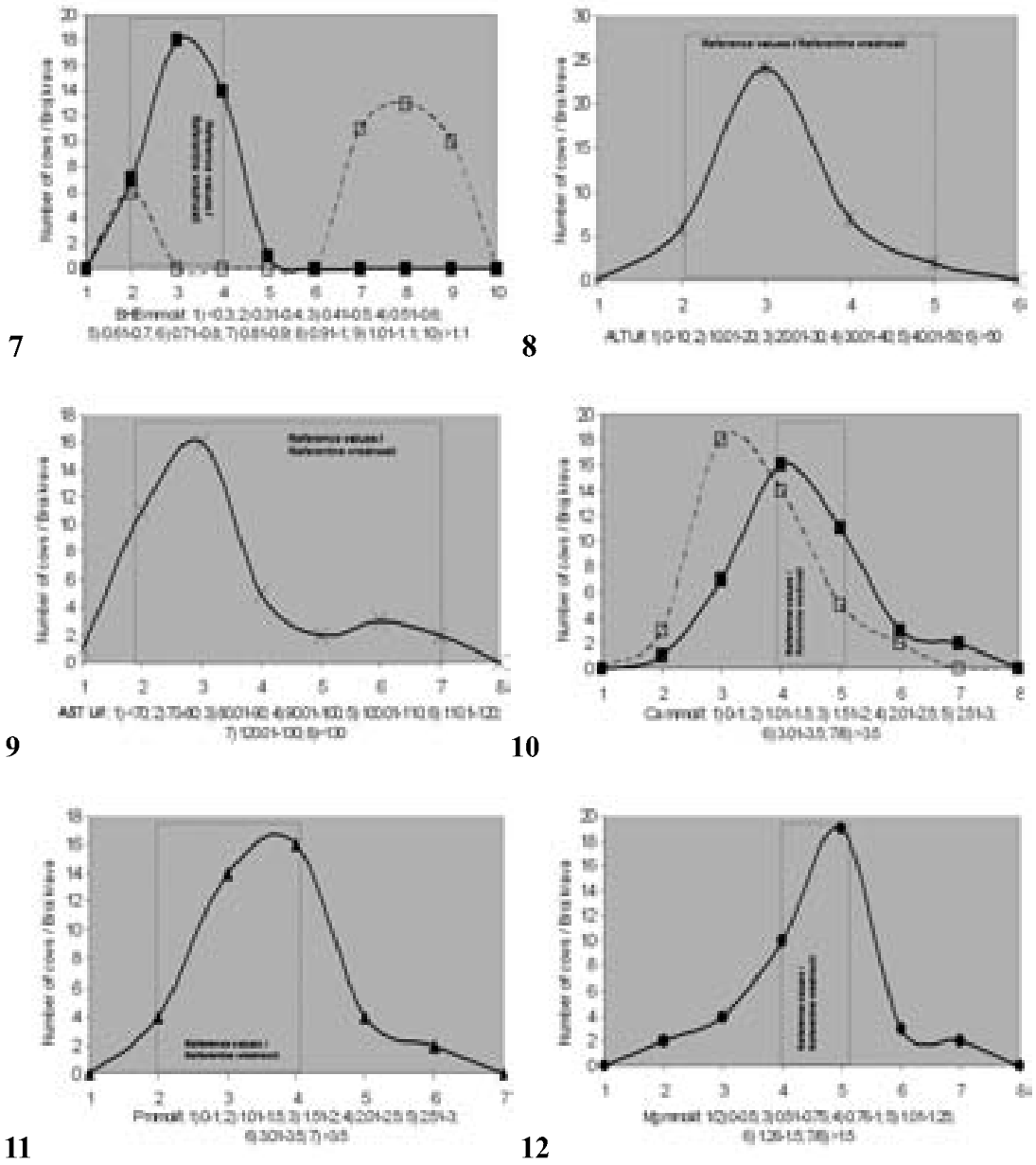

Graph 1-12: Frequency distribution of average values of the parameters examined in 40 cows (solid line), with a distinctive distribution of trends in the periparturient period for the parameters whose values differ significantly from the average population (dashed line), a rectangle shows the reference values obtained by analysis of the literature.

*Grafici 1-12: Distribucija frekvencije prosečnih vrednosti parametara kod 40 ispitivanih krava (puna linija), sa naglašenom distribucijom kretanja u peripartalnom periodu za parametre čije se vrednosti signifikantno razlikuju za ovaj period (isprekidana linija), pravougaonik pokazuje referentne vrednosti dobijene anlizom literature.*

## DISCUSSION

Our results are in agreement with the results of most researchers in our region and beyond. Thus, Jovanović et al. (1987) found that 10-15 days before parturition the

concentration of glucose is about 2.71 mmol / l, 10 days after calving 2.4 mmol / l, the second month of lactation 2.6 in the fifth month of lactation 2.7 mmol / l. Reduced blood glucose in the periparturient period (Table 1, Graph 1) resulted from the negative energy balance, due to reduced food intake and very intense lactation (Table 1). Because of the negative energy balance volume of lipid for energy purposes increased, which is reflected in increased lipolysis and ketogenesis. So the periparturient period, especially in the days after calving increases the concentration of NEFA and BHB, and the obtained values are in accordance with the results of previous studies (Kovačević et al., 2002; Hachenberg et al., 2007, Park et al., 2010). Bilirubin concentration was slightly higher in the puerperal period compared to the rest of lactation, and in the range of previously obtained results (Šamanc, 1992). Bilirubin concentration correlated with the degree of fatness of liver. It is important to note (Graph 4) that, looking at the average level of the tested cows, a number of animals still show a slightly higher concentration of bilirubin in relation to the reference value. This situation is due to the stress, inadequate nutrition and energy load of cows in our population. Cholesterol (Table 1; Graph 3) agrees with previously obtained results (Cincović et al., 2010), and the relationship of cholesterol and NEFA can be used to assess fatty liver in periparturient period. Hypoalbuminemia, hypocholesterolemia and hypoglycemia with ketosis cows show reduced biosynthetic function of liver (Đokovic et al., 2007).

Protein concentration (Table 1, Graph 2) belongs to the low variable indicators, because disorder occurs only in the decompensated stages of various diseases. The concentration of proteins in different studies on dairy cows ranged from 60-80 g / l (Jovanovic et al., 1987; Kupezinski and Chudoba-Drozdovski, 2002). The concentration of urea (Table 1, Graph 5) was slightly lower in the periparturient period compared to other parts of lactation, possibly due to the decrease of dry matter intake in food and altered liver metabolism during this period, which agrees with the result in lower proteinemia. Similar results were obtained by Važić et al. (2010). Transaminases up (Table 1, Graph 8 / 9) are in the expected range, and period of lactation has no significant effect on their value in healthy cows.

Stojević et al. (2005) found low values of these enzymes in serum. The concentration of ions was within the expected values and a somewhat wider range of distribution than the current reference value. Similar values can be found in recent studies obtained from Đoković et al. (2010). In the early puerperium Calcemia is smaller than in other periods of lactation. Calcemia less than 2 is common in periparalnom period. Decrease of calcemia below 1.8 mmol / l may be a predisposing factor towards the development of puerperal paresis, abomasal dislocation and other (Goff et al., 2000).

## CONCLUSION

The parameters of the metabolic profile of Holstein-Friesian cows correspond to the reference values for a given type of animal. Periparturient changes in the concentrations of metabolites are characterized by negative energy balance, with the development of liver dysfunction and reduced Calcemia. Frequency distribution of values of metabolic profile suggests that clinically healthy animals may show signs of metabolic changes, which is probably caused by high demand for milk production, load stress and inadequate nutrition.

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## DIURNALNA VARIJACIJA METABOLITA KRVI KOD KRAVA TOKOM TOPLOTNOG STRESA

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### Izvod

Ispitivani su biohemijski parametri kod 40 krava Holstajn-Frizijske rase. Krave su bile u drugoj i trecoj laktaciji. Krv je uzorkovana u okviru četiri osnovna produktivna perioda i to: u zasušenju (2-3 nedelje pred porođaj), u postpartalnom periodu (0-2 nedelje postpartum), u sredini laktacije (15-17 nedelja postpartum) i u kasnoj laktaciji (2-3 nedelje pred početak zasušenja). Standardnim biohemijskim metodama ispitana je koncetracija: glukoze, proteina, holesterola, bilirubina, uree, NEFA, BHB, ALT i AST, Ca, P i Mg. Parametri metaboličkog profila Holštaj-frizijskih krava odgovara referentnm vrednostima za datu vrstu, a parametri se nalaze u okviru normalne (Gausove) distribucije. Peripartalne promene u koncentraciji metabolita odlikuju se negativnim energetskim bilansom, sa razvojem insuficijencije jetre i smanjenjem kalcemije. Distribucija frekvencija vrednosti metaboličkog profila ukazuje da klinički zdrave životinje tokom kopletnog perioda laktacije mogu pokazivati znake metaboličke izmene, koja je najverovatnije posledica velikih zahteva za proizvodnjom mleka, opterećenosti stresom i neadekvatne ishrane.

**Ključne reči:** metabolički profil, mlečne krave, referentne vrednosti, distribucija frekvencije parametara.

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