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# EVALUATION OF COW'S ENERGY STATUS CHANGES DURING EARLY LACTATION BASED ON THE CONCENTRATIONS OF ORGANIC MILK INGREDIENTS

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**Summary:** The aim of the study was to evaluate the energy status of cows (n=132) based on the concentration of organic milk constituents during early lactation. Cows were divided into three groups according to the stage of lactation (group A, n=43, 15-30 DIM; group B, n=33, 31-45 DIM; group C, n=56, 46 to 60 DIM). Concentrations of milk fat and protein and the milk fat: protein ratio tended to decrease, and the concentration of urea had increased towards to the end of the evaluated period. Statistically significant differences were found in concentrations of milk fat (41.58±37.15 vs. 37.15±3.63 and 36.11±4.57 g/L, respectively), urea (2.86±0.50 vs. 3.48±0.69 and 3.61±0.56 mmol/L, respectively) in all three groups, and milk fat : protein ratio in the group A compared to the other two groups, and also in lactose concentration between groups A and B (47.14±01.29 vs. 47.71±1.4 g/L). The ratio of the concentration of milk fat and protein, as well as relations between urea and protein showed a strong energy deficit in all tested cows, with more or less pronounced deficit or relative surplus of protein. Such condition negatively affects health status and milk production of evaluated cows, as well as their reproductive performance.

Key words: cows, lactation, energy status.

## INTRODUCTION

Disruptions in energy status are one of the most important group of health disorders of dairy cows in a narrow period of transition from the dry to an early lactation period. This group of disorders has a particular importance for high-yielding cows, which had an extremely large load of metabolism during early lactation (Drackley, 1999, Šamanc et al., 2005a, Horvat et al., 2007). Due to the growing milk production and inability to enter sufficient amount of energy through diet negative energy balance (NEB) regularly appears during early lactation. Sinovec (2003) and Šamanc et al. (2005, 2006) suggest that, although glycogen body proteins participate in compensation of

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energy deficit, the main source of energy in this period is body fat reserves, i.e. lipomobilisation. Lipomobilisation is particularly pronounced in animals that were not adequately prepared to initiation of lactation or those animals that start the lactation in obese condition (Kampl, 2005; Goff, 2006). Fats mobilized from body depots are passing through the liver, thus limiting its synthetic and detoxifying capacity, which, together with NEB, can adversely affect the further cow's productive and reproductive characteristics (Sinovec, 2003, Šamanc et al., 2005a, Horvat et al., 2009).

Given the above mentioned, the period of early lactation represents the most critical period in the production and reproductive cycle of high lactating dairy cows, so special attention to should be given the diagnosis of health disorders in this period. This applies particularly to subclinical health disorders. Valid indicators of the metabolic status of cows in early lactation are body condition score, concentration of biochemical blood parameters and metabolically active hormones, and the concentration and ratio of organic milk ingredients (Jovičin et al., 2005, Kampl, 2005, Šamanc et al., 2006, Savić et al., 2010, 2011).

Estimation of energy status of cows on the basis of determination of the concentration and the ratios of organic milk ingredients is a method which, has the advantage over these other because of its simplicity, reliability and economy (Jonker and Kohn, 2001, Marenjak et al., 2004, Kampl, 2005 Šamanc et al., 2006, Horvat et al., 2007, 2009) and is widely used in practice. The parameters for the evaluation in this method are the concentrations of fat, protein and urea in milk, as well as the ratio of urea and protein, and fat and protein (fat to protein ratio, FPR). A more detailed description of this method, as well as the characteristics of the physiological processes that underlie are given in our previous papers (Savić et al., 2010, 2011), and review paper by Kirovski et al, (2012). The target group for the assessment of energy status by using this method are cows between 15 and 60 days of lactation, which is considered as a critical period for future productive and reproductive performance of the cows.

The results of our previous study (Savić et al., 2010, 2011), as well as research by other authors (Kampl, 2005, Šamanc et al., 2006, Horvat et al., 2007, 2009) suggest significant deviation values of these parameters in the individual animals compared to the average group results. These deviations can be, inter alia, associated with the stage of lactation, i.e. current day of lactation in each tested animal and its distance from the upper or lower limit of the interval from the 15<sup>th</sup> to 60<sup>th</sup> day of lactation, as well as the individual characteristics of the each animal. So, the aim of this paper is to form groups of cows as accurately as possible in the stage of lactation within a given interval, and to assess the differences in the energy status and milk yield between the groups.

## MATERIAL AND METHODS

The study was conducted at the industrial type dairy farm, during the period June-July 2009. Tested Holstein cow (n=132) were in the first (n=84) and second (n=48) lactation. All 132 cows were in early lactation (15-60 days in milk, DIM), and were divided into three groups based on lactational stage. The first group (group A, n=43) were cows with 15-30 DIM, second (group B, n=33) 31-45 DIM, and the third (group C, n=56) cows with 46-60 DIM. All cows were kept under usual farm conditions (free-stall system) and fed a diet adapted to the given productive category and period of the year.

Milk samples were taken during regular milking. In all samples, the concentrations of milk fat, protein. lactose and urea were determined, while data on daily milk yield were taken from the farm records. Concentrations of fat, protein and lactose were determined on Bentley 150 Infrared Milk Analyzer, and urea concentration on Bentley Chemspec150 Urea Analyzer for Milk. Based on the obtained data, the ratios of urea and protein and FPR were calculated. The results were analyzed by descriptive statistics and presented in tables. Statistical significance of differences between the studied parameters was determined by t-test at the level of p <0.05 and p <0.01. Ratios of urea and protein, and milk fat and protein are shown graphically.

#### **RESULTS AND DISCUSSION**

The values of the concentration of organic milk ingredients and milk yield of tested groups of cows are shown in Table 1.

Table 1. Values of the concentration of organic milk ingredients and milk yield of tested groups of cows

Parameter	Group	M±SD	CV	IV	Statistical significance of differences
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					p<0.05	p<0.01
Milk fat (g/L)	А	41.58±5.35	12.86	30.40-52.70		
	В	37.15±3.63	9.77	29.50-47.30		A:B, A:C
	С	36.11±4.57	12.65	24.30-45.10		
Proteins (g/L)	А	30.58±2.04	6.69	26.40-36.30		
	В	30.27±2.08	6.87	25.60-33.60		
	С	29.91±2.46	8.21	23.00-35.30		
FPR	А	1.36±0.19	13.74	0.97-1.82		
	В	1.23±0.11	9.15	1.04-1.45		A:B, A:C
	С	1.21±0.15	12.02	0.82-1.70		
Urea (mmol/L)	А	2.86±0.5	17.53	1.73-4.76		
	В	3.48±0.69	19.66	1.95-4.74		A:B, A:C
	С	3.61±0.56	15.52	2.26-4.58		
Lactose (g/L)	А	47.14±1.29	2.74	41.60-49.20		
	В	47.71±1.04	2.18	45.80-49.40	A:B	
	С	47.59±1.34	2.81	43.00-50.80		
Milk yield (L)	А	27.89±8.4	30.10	8.40-55.70		
	В	31.19±7.92	25.37	14.20-45.90		
	С	30.90±7.04	22.79	14.90-43.10		

The data presented in Table 1 indicate significant discrepancies of investigated parameters within all three groups, which could be interpreted to individual variations. The exception in terms of deviations is found only in the lactose concentration, which is in a relatively narrow range in all groups, as evidenced by the very low coefficient of variation.

Kirovski et al. (2012) reported that average physiological concentrations of organic milk ingredients in Holstein cows are between 32 and 36 g/L for milk fat, about 30.6 g/L for protein, and between 2.0 and 6.0 mmol/L for urea, respectively. According to these authors, urea concentrations above 4 mmol/L, protein below 32 g/L and fat content over 45 g/L, respectively, are taken as the limit value indicating the presence of metabolic disorders. Mulligan et al. (2006) reported that the large percentage of cows in early lactation with a protein concentration below 3.05%, and those with FPR above 1.50 indicates the presence of a serious health disorders at the herd level. The average concentration of fat in all the examined group of cows was within the physiological range and consistent with the breed characteristics. Average concentration of fat had a declining trend, which could be interpreted as a consequence of decrease of lipomobilisation in the later stages of surveyed period, i.e. improving the energy status of the examined cows (Drackley, 1999, Goff, 2006). The percentage of cows with milk fat concentration above the critical threshold of 45 g/L was the highest in the group A (27.91%), while in the other groups was negligible.

The average protein concentration in all three groups of cows was on the lower limit of the physiological range. In all groups of cows large individual variations are noticeable, as well as a large number of cows (from 51.52% in group B, up to 58.14% in group A) with values below the critical limit of 3.05%, reported by Mulligan et al. (2006). Similarly to milk fat, protein concentration also decreases with the progress of lactation, indicating a lack of supply of protein through the ration, as well as their reduced utilisation due to prolonged NEB. The protein concentration in all three groups of cows is consistent with the values found in our previous studies (Savić et al., 2010, 2011). The present data on the average values of the FPR indicate its declining trend with the progress of lactation, which is in line with the expected trend of the energy balance (Šamanc et al., 2006). Only within the group A we found a certain number cows (25.58%) with the values above the critical threshold of 1.50, which suggests a strong lipomobilisation (Mulligan et al., 2006). This finding is in line with the expected most unfavorable energy balance in this group of cows, so its adversely effect on their health and milk production in the later stages of lactation, and reproductive performance can be expected.

The concentration of urea in all groups of cows was within the physiological range. Urea concentration above the physiological limit indicates an imbalance of energy and protein in the diet, which is particularly pronounced in the groups B and C (30.30% and 30.36%, respectively). There is a noticeable trend of increasing concentrations of

urea with the progress of lactation, which may be explained by increased ration intake, but with insufficient energy density, so the rumen microflora is not able to bind the liberated ammonia (Kampl, 2005; Savic et al., 2010).

Stability of lactose concentration among all groups of cows and its maintenance within narrow limits indicate a strong homeorrhetic control of its synthesis. Metabolic priority of the mammary glands and milk synthesis in the early lactation was indicated by Stamatović et al. (1983), who found significantly higher levels of glucose in blood samples from v. subcutanea abdominis in relation to ones from v. auricularis magna during early lactation.

The average milk yield had trend of a slight increase towards the middle of the surveyed period, and then stagnation, rather than the upward trend continues. Such trend can be attributed to the inadequate formulation of ration, which do not meet needs of animals for a given stage of lactation, so the peak of milk production was achieved significantly earlier than usual. Extremely large individual variations in milk yield within each group of cows were observed, which, when taken into account that all cows were fed identical regardless of production, certainly affects the degree of satisfying their individual needs and individual energy status of each cow. A similar trend was observed in our previous studies and by other authors (Šamanc et al., 2006, Horvat et al., 2009; Savić et al., 2010, 2011).

The present data on concentrations of milk fat and urea, as well as the FPR, together with the previously set forth interpretation, indicate a trend of energy status stabilisation with the progress of lactation. In all three parameters statistically significant differences between the first and the other two groups were found, which can be associated with intensive lipomobilisation and extremely NEB in the first group of cows.

Decline of the milk fat concentration with the progress of the lactation may be, in addition to a restoration of impaired energy balance and decreasing of lipomobilisation intensity, also linked to the ration composition, based on the use of large amounts of green forage (Horvat et al., 2009). Additional factor, especially pronounced in decreasing of protein concentration with the progress of lactation, can be emptying of body reserves, which culminates at the end of the surveyed period, and leads to exhaustion of the organism.

The statistical significance of differences in the lactose concentration between groups A and B can be seen as a result of hypoglycemia, which is usually present in early lactation cows (Šamanc et al., 2005), causing mammary gland does not get enough precursors for the synthesis of lactose. Also, impact of individual variations in the concentration of lactose, which is the highest for cows first group, should be taken into account. The results are also shown graphically. The ratio of the concentrations of urea and protein in three groups of cows is shown in Figures 1, 2 and 3.

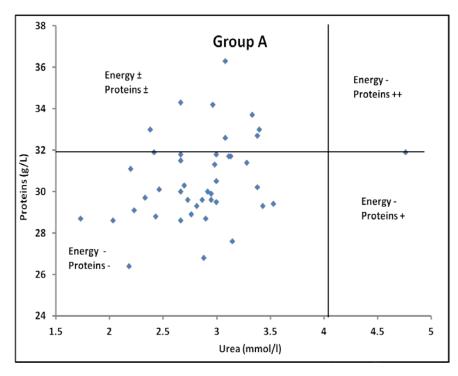


Figure 1. Urea to protein ratio in milk of cows from group A

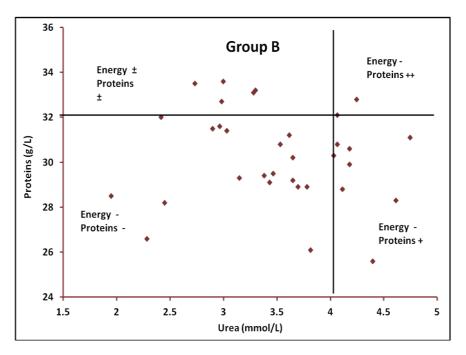


Figure 2. Urea to protein ratio in milk of cows from group B

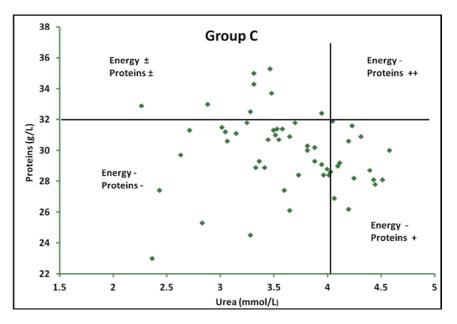


Figure 3. Urea to protein ratio in milk of cows from group C

The data presented in Figures 1, 2 and 3 indicate the existence of a combined deficit of energy and protein in the majority of cows from all three surveyed groups. Deficit of energy and protein was particularly pronounced in the group A, while in groups B and C situation was somewhat better, which can be attributed to the increased food intake, as well as adapting to the increasing energy needs. Low energy content in the diet, with easily digestible protein and a small amount of celulose leads to development of energy deficit and relative surplus of protein in small number of cows from groups B and C, indicated by their position within a chart. Only a few cows from all three groups were adequately supplied with energy and protein (upper left quadrant), which can be interpreted by individual variation in milk production and utilization of available nutrients from ration, i.e. their better adaptability to present conditions. The ratio of the concentrations of milk fat and protein in three groups of cows is shown in Figures 4, 5 and 6

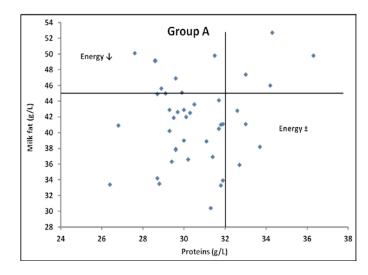


Figure 4. Milk fat:protein ratio in milk of cows from group A

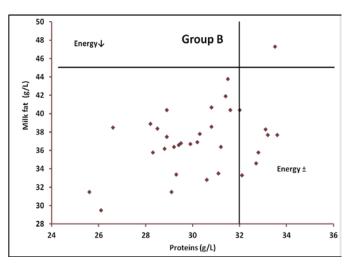


Figure 5. Milk fat:protein ratio in milk of cows from group B

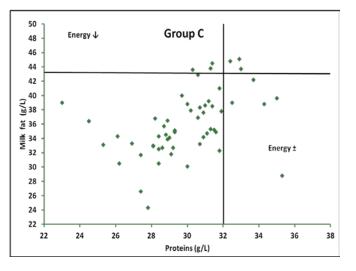


Figure 6. Milk fat:protein ratio in milk of cows from group

Displayed concentration ratio of milk fat and protein also indicates the energy deficit in the ration. In cows of the group A share of those with milk fat concentration above 45 g/L was the highest, indicating also intensive lipomobilisation. In the other two groups, the number of these cows was significantly lower. In all three groups of we found cows with milk fat concentrations below 30 g/L. This phenomenon was most likely formed as a result of ration containing low cellulose content, so a smaller amount of acetic acid as a precursor of milk fat was absorbed from the rumen. Also, the energy deficit in the ration adversely affects the abundance of cellulolytic microflora, which further inhibits the synthesis of lower fatty acids, primarily acetic. Reduced synthesis of acetic acid, besides its effect on milk fat content, will adversely affect the synthesis of estrogen, and so the emergence of the first postpartum estrus and ovulation.

## CONCLUSION

The data presented in this paper indicate serious shortcomings in preparing of ration for cows, ranging from the dry period and preparation for the upcoming lactation, which is why cows during early lactation are prone to occurence of metabolic disorders. Large variations in milk yield within the surveyed groups of cows, with the data presented about their energy status indicate inadequate management of nutrition, which leads to insufficient utilization of genetic potential for milk production. Exhaustion of organism by high milk production, combined with the unbalanced ration, will inevitably result in the clinical and subclinical health disorders, decline in milk production, extension of service period and low reproductive perfomances, which will all together adversely affect the replacement rate and economic feasibility of production.

In this regard, it is necessary to perform essential corrections of ration, and to form a more narrow group of cows according to stage of lactation and milk yield, better adjusted to their needs. Determination of the relationship of organic milk constituents, if rutinely conducted over a period of time, could be used as a simple and reliable method for monitoring the effects of the ration correction.

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### PROCJENA KRETANJA ENERGETSKOG STATUSA KRAVA TOKOM RANE LAKTACIJE NA OSNOVU KONCENTRACIJE ORGANSKIH SASTOJAKA MLIJEKA

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**Izvod:** Cilj istraživanja bio da se na osnovu koncentracije organskih sastojaka mlijeka procijeni energetski status krava (n=132) tokom perioda rane laktacije, podijeljenih u tri grupe prema stadijumu laktacije (grupa A, n=43, 15-30 dana; grupa B, n=33, 31-45 dana; grupa C, n=56, 46-60 dana laktacije). Koncentracije mliječne masti i proteina i odnos mliječna mast:proteini imali su tendenciju opadanja, a koncentracija uree tendenciju porasta prema kraju ispitivanog perioda. Statistički značajne razlike ustanovljene su u koncentracijama mliječne masti (41.58 $\pm$ 5.35 naprema 37.15 $\pm$ 3.63, odnosno36.11 $\pm$ 4.57 g/L), uree (2.86 $\pm$ 0.50 naprema 3.48 $\pm$ 0.69, odnosno 3.61 $\pm$ 0.56 mmol/L) i odnosu mliječna mast:proteini između prve u odnosu na druge dvije grupe, kao i u koncentraciji laktoze između prve i druge grupe krava (47.14 $\pm$ 1.29 naprema 47.71 $\pm$ 1.04). Na osnovu odnosa koncentracije mliječne masti i proteina, kao odnosa uree i proteina ustanovljeno je da kod svih ispitanih krava postoji jak deficit energije, uz više ili manje izražen deficit ili relativni suficit proteina. ovakvo stanje se nepovoljno odražava na zdravlje i proizvodnju mlijeka ispitanih krava, kao i na njihove reproduktivne karakteristike.

Ključne riječi: krave, laktacija, energetski status.

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