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Full Length Research Paper

Estimation of herd-basis energy status in clinically healthy Holstein cows: Practical implications of body condition scoring and shortened metabolic profiles

Radiša Prodanović[1], Danijela Kirovski[2], Horea Šamanc[1]*, Ivan Vujanac[1], Vojin Ivetić[3], Božidar Savić[3] and Branislav Kureljušić[3]

[1]Department of Farm Animal Diseases, Faculty of Veterinary Medicine, University of Belgrade, Bulevar oslobođenja 18, 11000, Belgrade, Serbia.
[2]Department of Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Belgrade, Bulevar oslobođenja 18, 11000, Belgrade, Serbia.
[3]Scientific Veterinary Institute of Serbia, Vojvode Toze 14, 11000, Belgrade, Serbia.

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The objective of the study was to estimate herd-basis energy status (ES) using body condition scoring (BCS) and shortened metabolic profiles in multiparous clinically healthy dairy cows and to evaluate if such profiles can be informative in herd investigation to indicate on metabolic herd problems. A total of 180 Holstein cows from commercial herd were clinically examined and assigned to one of 3 equal groups: dry cows (15 days before expected calving), puerperal (10 days after calving) and cows at day 60 of lactation. Blood were sampled and analyzed for total protein, albumin, urea nitrogen (UN), β-hydroxybutyrate (BHBA), glucose and total bilirubin. Liver samples by percutaneous biopsies were obtained at puerperal period and tested for lipid content. The cows had a mean BCS of 3.92±0.03 at dry period, 3.08 ± 0.03 at puerperal period and 2.82 ± 0.02 at day 60 of lactation. Average total protein, albumin and glucose concentrations were within the physiological range, whereas UN was higher than physiologically accepted, at all examined periods. BHBA levels increased after calving and 61.66% of puerperal cows had BHBA levels above 1 mmol/L. Average total bilirubin concentration after calving was higher than recommended. Concentrations of UN, BHBA and total bilirubin were positively correlated, whereas concentrations of total protein and glucose were negatively correlated with fatty liver degree. Albumin concentration was not correlated with fatty liver degree. In conclusion, although clinically healthy the examined cows were not in adequate ES during the transition period. Consequently, 55% of the cows had moderate to severe fatty liver. A sufficient number of highly significant correlations suggest that employed metabolic profiles may offer an indirect means of estimation energy status on a herd basis.

Key words: Cow, energy status, metabolic profile, body condition score.

INTRODUCTION

The metabolic profile, a series of specific blood analytical tests, is a useful tool to indicate if homeostatic mechanisms are capable of keeping blood parameters within the physiological range under a different feeding and housing systems (Ingraham and Kappel, 1988; Gross et al., 2011). Furthermore, blood metabolite analysis, in conjunction with monitoring of health and nutritional status, can reveal subclinical disorders and indicate on their etiology (Mohamed et al., 2004; Oetzel, 2004). If metabolic profile application is well planed and if it is coupled with body condition scoring (BCS), ration and management environment evaluations, then it can be routinly used to detect metabolic problems in dairy cattle (Kida, 2002; Stengärde et al., 2008).

Various metabolic blood and milk traits, hormones, electrolytes and enzyme activities have been shown to be related to energy balance in dairy cows (Reist et al., 2002, Kida, 2003). Unfortunately, the use of many of them on a herd basis has been questioned relative to its validity and sensitivity in defining a problem as well as its total cost. Thus, most commonly used indicators of energy status are non-esterified fatty acid (NEFA) and ketone body (BHBA) concentrations during both dry and puerperal period, and total protein, albumin, glucose and urea nitrogen concentrations during puerperal period (Reist et al., 2002; Oetzel, 2004; Stengärde et al., 2008). In addition, concentrations of these parameters have been shown to be in correlation with BCS changes during peripartal period (Kim and Suh, 2003).

Several studies have shown that over conditioned dry cows have greater depressions of feed intake during the peripartal period and deeper negative energy balance than cows with a lower body condition (Hayirli et al., 2002; Rukkwamsuk et al., 1999). High BCS before calving, as well as marked losses in body condition after calving are associated with metabolism related diseases, like fatty liver, decreased fertility and increased culling rates (Hayirli et al., 2002; Šamanc et al., 2010). Moreover, peripartal energy balance disturbances are considered to be major contributing factors to the dairy cow`s metabolic problems in herds (Bobe et al., 2004). Therefore, severe loss of body condition around the calving period should be avoided with proper control of energy balance (Kim and Suh, 2003).

Based on such observations, this study aimed to estimate herd-basis energy status in clinically healthy dairy cows using BCS and metabolic profiles. However, estimation of energy-status in free-stall herds is difficult due to lack of assessments of individual cow energy ingested. In Serbia, herds are rather large, and therefore, to evaluate if employed profiles can be reliable in herd investigation to indicate on metabolic herd problems correlations between fatty liver degree and a used parameters were computed.

MATERIALS AND METHODS

The present study was performed in Holstein cows on a commercial farm in the central part of Serbia, at latitude 44°49'14'' North, longitude 20°27'44'' East, during spring season when average daily temperature ranged from 20 to 25°C. This cattle population, with 1000 cows and an average milk production of 6800 L per cow annually, was enrolled in the Serbian official milk recording scheme (Central Database) which means that animals are subjected to Program of Animal Health Protection Monitoring. The cows were kept in free-stall housing systems and grouped according to stage of productive-reproductive cycle: far off dry cows (from day 60 to day 15 before calving), close up dry cows (from day 15 before calving to calving), early lactation cows (from calving to day 100 after calving), mid lactation cows (from day 100 to day 200 after calving) and late lactation cows (from day 200 after calving to drying off). Cows included in this study were between the second and fourth lactation, aged from 3 to 7 years. Ingredients and chemical compositions of the dry and early lactation cow diets are listed in Tables 1 and 2.

A total of 180 Holstein cows, divided into three groups (n = 60 per group), were investigated. The first group included dry cows (15 days before expected calving); the second group included puerperal cows (10 days after the calving) and the third group included early lactating cows (day 60 of lactation). Cow selection criteria included only animals considered healthy by a veterinary clinical examination. This clinical visit was carried out on the day before blood sampling and it included general condition and BCS. However, in free-stall herds, inadequate disease recognition in early lactation cows can also be a problem. BCS were determined using a 5-point scale system recommended by Elanco Animal Health Bulletin AI 8478. Physiological ranges of BCS for high-yielding Holstein cows are: 3.25 to 4.00 (dry period), 3.25 to 3.75 (puerperium) and 2.50 to 3.25 (day 60 of lactation) (Šamanc et al., 2010).

Blood samples were taken from each group by jugular venipuncture 4 h after the morning feeding. The concentrations of blood glucose and BHBA were measured using commercial test bands (Precision *Xtra*, Abbott Diabetes, GBR). Samples were allowed to clot spontaneously (approximately 15 min) on ice and were subsequently centrifuged at 1,000 g for 20 min. The serum was decanted and stored at −18°C until analyzed. Serum total protein, albumin, urea nitrogen and total bilirubin concentrations were measured by photometric method using a commercial test package (Bio-Medica, Serbia).

Liver samples were obtained from each puerperal cow, one hour after blood sampling, via percutaneous biopsy following the method of Hojovcova and Kacafirek (1967). The biopsy was performed as previously described by Šamanc et al. (2010). The liver sample was expelled onto a clean wipe, blotted free of blood, and placed into storage vials that contained 10% buffered phormaldehyde solution. For histopathological determination of lipids, sections were made using a freezing microtome and stained with Sudan III. Lipid content in hepatocytes was determined through computer image analysis (Software Q Win). Cows were divided into three groups based on the degree of lipid accumulation in the liver: mild fatty liver (<10% fat), moderate fatty liver (10 to 25% fat), and severe fatty liver (>25% fat) (Gall et al., 1983). The animal-related component of the study was approved by the Ethical Committee of the Faculty of Veterinary Medicine, University of Belgrade in accordance with the National Regulation on Animal Welfare.

For the sets of obtained experimental data indicators of descriptive statistics were determined. Results are presented as $\bar{X}$±SE. The Kolmogorov-Smirnov test for normality and Levene′s test for homogeneity of variances were used. The data for all samples was homogenous (Cv ≤ 30%) and in all cases the data

*Corresponding author. E-mail: samanc@vet.bg.ac.rs. Tel: ++381 11 2685081. Fax: ++381 11 2685936.

Table 1. Ingredients of cow's diets.

Ingredient (kg)	During dry period	Until day 100 of lactation
Alfalfa hay	-	3.43
Grass hay	1.50	-
Wheat straw	0.60	-
Corn silage 44 % DM	-	9.50
Corn silage 33 % DM	10.0	-
Corn silage 33.94 % DM	-	9.00
Alfalfa haylage 51.79 % DM	2.50	-
Alfalfa haylage 47.40 % DM	-	5.00
Brewer's grain 21.00 % DM	-	5.00
Corn grain	0.98	2.50
Barley grain	0.50	1.50
Soybean grits	0.30	1.30
Soybean meal 44%N	1.10	1.13
Wheat flour	0.50	1.30
Sugar beet pulp	-	1.82
DextroFat SC	0.10	0.40
Optigen II, 41 % N	-	0.14
Dextrose monohydrate	0.04	0.10
Dicalcium phosphate 18% P	-	0.27
Magnesium oxide	-	0.05
Sodium bicarbonate	-	0.15
Sodium chloride (iodized)	-	0.07
Calcium carbonate	0.04	0.03
Milkinal trocken 3%	0.08	-
Beta carotene	0.004	-
TOTAL	18.24	42.69

distribution was in accordance with the normal distribution model. Variance was homogenous. In the study, the results of hypothesis testing on the differences of the means are described by the t-test for independent samples. The Pearson correlation coefficients between fatty liver degree and the various blood traits at the puerperal period were also computed. Statistical analysis of the experimental results was performed by STATISTICA v.6 packet.

RESULTS AND DISCUSSION

Results of the content of lipids in the liver in puerperal cows (n = 60) showed that 45% of cows had mild fatty liver (5.88 ± 0.53% fat), 45% had moderate fatty liver (20.25 ± 0.77% fat), and 10% had severe fatty liver (34.00 ± 2.05% fat).

The cows had a mean BCS of 3.92 2.82 ± 0.02 at day 60 of lactation. BCS in the dry period was significantly higher ($p < 0.001$, respectively) than at puerperium and day 60 of lactation, and BCS at day 60 of lactation was significantly lower ($p < 0.001$) than at puerperium. Furthermore, a mean BCS at dry period and day 60 of lactation were within the physiological ranges, while at puerperium was under recommended physiological range for this phase of reproductive cycles (Kim and Suh, 2003; Šamanc et al., 2010).

Analyses of distributions of individual BCS for all examined periods make a complete picture of body condition in the population, since it makes a point on the direction of deviations from physiological values ("shift to the left" refers to values that are lower than physiological; "shift to the right" refers to values that are higher than physiological). At dry period, 26.66% of the cows had BCS higher (obese cows) than recommended (Figure 1), while 56.66% of the puerperal cows had BCS lower than physiologically accepted (Figure 2). At day 60 of lactation there were no cows with BCS out of the physiological range (Figure 3).

Obtained results for BCS indicate that cows failed to properly respond to transition from late pregnancy to early lactation, due to the fact that more than one half of the puerperal cows were underfed. The most probably reason is inadequate nutritional status during the dry

Table 2. Chemical composition of cow's diets.

Chemical composition	During dry period	Until day 100 of lactation
DM kg	9.82	23.63
Net energy of lactation (NEL) MJ	65.50	163.03
Crude protein (CP) %	15.03	16.05
Rumen undegradable protein (RUP) %	5.09	5.06
Crude fat %	3.82	4.78
Acid detergent fibre (ADF) %	25.31	22.08
Neutral detergent fibre (NDF) %	40.59	35.48
Ca %	0.64	0.90
P %	0.42	0.52
Na %	0.13	0.36
Cl %	0.17	0.29
Mg %	0.27	0.34
K %	1.30	1.18
S %	0.22	0.22
Mn ppm	129.36	82.40
Cu ppm	39.81	25.64
Zn ppm	168.24	96.90
Co ppm	1.03	0.54
J ppm	3.06	1.64
Fe ppm	228.38	220.53
Se ppm	1.14	0.70
Vit A IU/kg	41 234.28	21 273.58
Vit D IU/kg	4 487.10	3 445.30
Vit E IU/kg	117.79	69.35

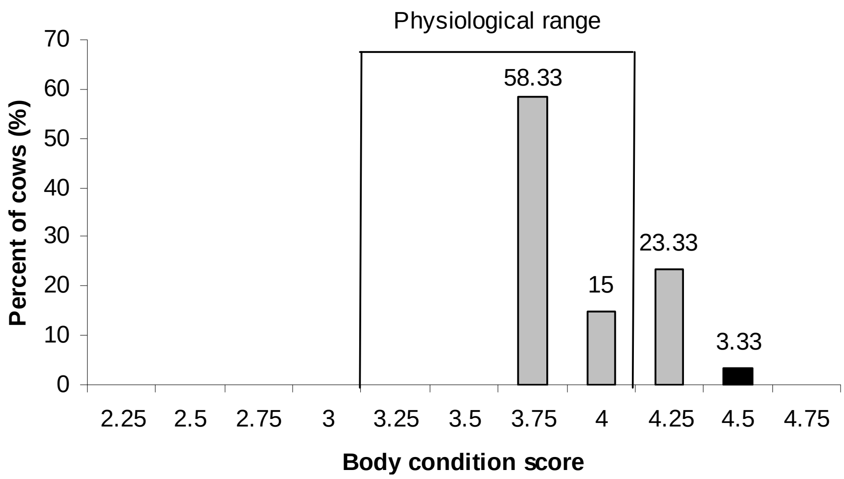

Figure 1. Distribution of individual BCS in cows at dry period.

period, since 26.66% of the cows had a BCS higher than recommended. However, obesity in dry cows is not always associated with disturbances in energy metabolism (Bobe et al., 2004; Šamanc et al., 2010a).

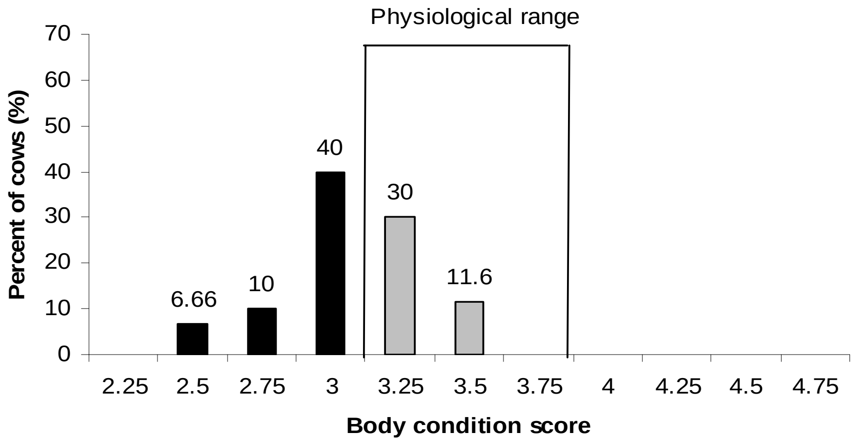

Figure 2. Distribution of individual BCS in cows at puerperium.

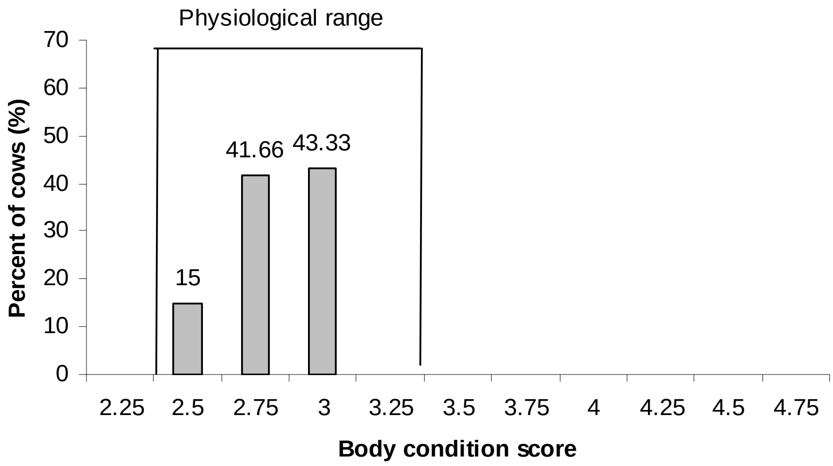

Figure 3. Distribution of individual BCS in cows at day 60 of lactation.

There is agreement in the literature that evaluation of BCS is recommended because it reflects the nutritional status and energy balance in dairy cows (Kim and Suh, 2003). Results for measured metabolite parameters (Table 3) are in accordance with the examined data on the evaluated body score.

Average total protein, albumin and glucose concentrations at puerperium were significantly lower compared to the dry period and day 60 of lactation (Table 3). Nevertheless, at all examined periods, average total protein, albumin and glucose values were within the physiological range (Kaneko, 1989). Consistent with the findings of Reist et al. (2002), physiological ranges for total protein and albumin concentrations tended to be lower in fresh cows when they might have been used to provide amino acids for milk production.

Although albumin concentration was not correlated with fatty liver degree, decreased hepatic synthesis during this period could also contribute to the low serum concentrations of albumin. However, nutrition, infection, stage of lactation, parity, season of production, and hydration, influence the proteinogram of blood serum in cattle (Cozzi et al., 2010).

The average value obtained for urea nitrogen (UN) concentration was significantly higher at puerperium than at both dry period and day 60 of lactation (Table 3). On the contrary, in the literature, an opposite trend has been commonly observed (Kim and Suh, 2003; Oetzel, 2004).

Table 3. Concentrations ($\bar{x}$±SE) of total protein, albumin, urea, glucose, BHBA and total bilirubin.

Metabolic parameter	Dry period	Puerperium	day 60 of lactation
Total protein (g/ L)	71.34 ± 1.06	67.05 ± 1.34*[aaa]	74.51 ± 1.05*
Albumin (g/L)	39.15 ± 0.70	35.59 ± 0.63***[aaa]	39.59 ± 0.63
Urea (mmol/ L)	7.78 ± 0.35	8.79 ± 0.31*[aaa]	7.25 ± 0.28
Glucose (mmol/ L)	3.04 ± 0.06	2.41 ± 0.05*** [aaa]	3.18±0.04
BHBA (mmol/ L)	0.60 ± 0.05	1.08 ± 0.07*** [aaa]	0.62 ± 0.02
Total bilirubin (µmol/ L)	6.69 ± 0.19	8.62 ± 0.39***[a]	7.76 ± 0.19***

Legend: * $p<0.05$; *** $p<0.001$; compared to dry period: [aaa] $p<0.001$; compared to day 60 of lactation.

Furthermore, average UN values that were higher than optimal values for dairy cows (4.38 to 5.84 mmol/L, according to Oetzel (2004)) may reflect the adequacy of nutrition (Rukkwamsuk et al., 2010).

According to Laven et al. (2007), among a wide variety of interrelated parameters, urea nitrogen concentration is influenced by carbohydrate amount and rumen degradability and can also be a good indicator of the cow's energy supply. It has been proven that high protein/low energy diets increase blood urea nitrogen (Oetzel, 2004; Park et al., 2010). High blood urea nitrogen may be a risk factor for lipomobilization (Oetzel, 2004; Park et al., 2010) and thus for a functional condition of the liver, too. Our study results, related to UN concentrations and lipid content in the liver of puerperal cows, confirm that.

There was a significant positive correlation between urea nitrogen and the content of lipid in the liver 10 days after calving ($r = 0.562$; $p < 0.01$). Additionally, it is known that excessive ammonia absorbed by the liver decreases the ability of the liver to convert propionate to glucose (Overton et al., 1999), thus linking fat accumulation to impaired gluconeogenesis in the liver (Drackley et al., 2001). Significant negative correlation between glucose concentrations at puerperium and lipid content in the liver is assessed in our study, as well ($r = 0.251$; $p<0.05$).

Nevertheless, average glucose concentration was within the physiological range at day 10 after calving (2.41 ± 0.05 mmol/L) probably due to the fact that milk production of the examined cows was not in keeping with the genetic potentials of those animals. This could also explain why there were no clinically visible disruptions in the health conditions of cows. However, glucose concentration, because of its homeaostatic regulation, is generally deemed as an insensitive marker of ES in cattle (Reist et al., 2002; Cozzi et al., 2010). At the puerperal period, average BHBA level was higher than physiologically accepted value (Table 3). Thirty seven puerperal cows from our study (61.67%) had levels of BHBA equal or higher than physiologilaly accepted and this may imply an abundance of subclinical ketosis (≥1 mmol/L, according to Ospina et al., 2010). It is known that in response to negative energy balance, body energy reserves are consumed for maintenance and milk production and this is why there is a greater possibility for the ketogenesis process to become intensified (Gustafsson et al., 1995; Stengärde et al., 2008). If more NEFA arrive at the liver, than needed for energy purposes, the excess may be oxidized incompletely and generate ketone bodies as well as, be converted to triacylglycerol (TAG) for deposition (Hanigan at el., 2004). Under normal conditions, TAG are secreted from the liver as very-low-density lipoproteins (VLDL). Inadequate secretion of VLDL, however, contributes to the development of fatty liver (Bobe et al., 2004). Significant positive correlation of BHBA values and lipid content in the liver at day 10 after calving ($r = 0.662$; $p < 0.001$) strongly support the validity of this view. Additionaly, significant difference that was estabished between BCS at dry and puerperal period ($p < 0.001$) also indicates on intensive lipomobilisation after calving which is followed with significant increase of BHBA concentration in puerperal cows.

Finally, it was observed that the average value of total bilirubin concentration at dry period was significantly lower than at puerperium and day 60 of lactation ($p < 0.001$, respectively). Kaneko (1989) recommends a puerperal reference value for total bilitubin of < 8.55 µmol/L. According to this reference value, aproximately one third of the cows had equal or higher values of total bilirubin on day 10 after calving, reflecting the metabolic adaptation in early lactation, as discussed in previous sections. Although bilirubin values are more specific to bile flow problems than liver cell damage, it was shown that there is a strong association between fatty liver and total bilirubin concentration in the blood (Kalaitzakis et al., 2006). The outcomes of this study confirm this statement, especially since there is a significant positive correlation between total bilirubin concentrations and fat content in the liver of puerperal cows ($r = 0.692$; $p < 0.001$).

Rosenberger (1995) also reported that the slightest degree of fattiness of the liver results in an elevated concentration of total bilirubin in the blood of the animal. Bilirubinemia values of up to 6.84 µmol/L can be found in cows during fasting or the period around calving, which is when a mild form of fatty infiltration of the liver occurs. Values over 8.55 µmol/L are considered pathological and a consequence of fatty infiltration and/or degeneration of liver cells. In such cases, liver excretory function is weakened (Rosenberger, 1995).

The results for metabolic profile obtained at day 60 of lactation indicate that cows have still experienced energy deficit characterised by high circulating concentrations of urea nitrogen and total bilirubin (Kaneko, 1989; Oetzel, 2004).

Conclusions

Based on the results presented in this paper it can be concluded that the cows at the observed commercial farm were not properly prepared for lactation. Although there were no clinically visible signs of health disorders, blood metabolites and BCS obtained from the examined animals, have shown that cows suffered from pronaunced negative energy balance during the postpartal period.

Consequently, 55% of the puerperal cows had moderate to severe fatty liver.

The results contain a sufficient number of highly significant correlations between metabolites and fatty liver degree to point out that employed metabolic profiles may offer an indirect means of estimation energy status on a herd basis. It is also clear that these metabolites are more useful as indicators of the extent to which energy intake fails to meet requirements, than as a means of assessing quantitatively magnitude of an energy surplus.

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