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Original scientific paper

THE EFFECT OF BODY CONDITION SCORE OF HEIFERS ON GLUCOSE AND β-HYDROXYBUTYRATE CONCENTRATIONS AND GLUTHATIONE PEROXIDASE ACTIVITY

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Abstract: The aim of this study was to establish if there are differences in glucose and β-hydroxybutyrate (BHB) concentrations as well as glutathione peroxidase (GPx) activity related to the body condition in heifers. Based on the body condition (BC), heifers were divided into two groups: 1. BC =3.75 and 2. BC ≥4.0. Whole blood samples from 23 heifers, taken between day 1 and day 10 after parturition, were used in the study. Glucose and BHB concentrations were determined immediately after sampling, while GPx activity was determined 24 hours after that. Heifers with higher body condition had significantly higher average BHB concentration and GPx activity. Average glucose concentrations did not differ between groups.

Key words: glucose, glutathione peroxidase (GPx), ketone bodies, body condition

INTRODUCTION

Intensive livestock production requires use of nutrients of high quality that will completely satisfy basic and productive needs of breeding animals, maximizing the genetic capacity of individuals. Nevertheless, this type of production is associated with many metabolic disturbances in animals. In dairy cows, these disturbances are especially pronounced, since dietary nutrient and energy are primarily used for mammary gland needs and then for the rest of the processes in organisms.

As a consequence of inadequate nutrition in cows, metabolic disturbances occur with ketosis being the most frequent one.

Ketosis is the metabolic disturbance that appears in high yielding dairy cows, most frequently in early lactation i.e. peripartum period. Due to inadequate carbohydrate and fat metabolism, animal experiences negative energy balance that is characterized by decline of blood glucose concentration and liver glycogen reserve depletion. All glucose obtained as energy source is redirected to mammary gland for lactose synthesis. Organism tries to compensate the loss of glucose by increasing the degree of liver gluconeogenesis, primarily from oxaloacetate that is the main gluconeogenic precursor. Redirecting of oxalacetate from citric acid cycle into gluconeogenesis provoke acetyl-CoA accumulation, that originates from β fatty acid oxidation as a consequence of negative energy balance. Because of that, metabolism is mainly redirecting to ketone bodies synthesis (*Mihailović and Jovanović*, 2008). This is esspecially pronaunced in cows with high body condition since in this cows, due to higher fatty depots, β oxidation is more intensive and consequently degree of ketogeneisis is increased (Cincović, 2013).

In recent studies, more attention is given to the role of oxidative stress in pathogenesis of

different metabolic disturbances typical for perupartum period (*El Deeb and El Bahr*, 2017). It is considered that higher degree of β oxidation of fatty acids affect the increase production of free radicals. The gluthatione peroxidase enzyme (GPx) has important role in antioxidative protection and it contains selenium in the form of selenocysteine in its own catalytic center (Forstrom at al., 1978). Gluthatione peroxidase is enzyme that reduces hydrogen peroxyde and peroxides of free fatty acids protecting cells from oxidative damages.

Due to all previously mentioned, the aim of this study was to establish the effect of body condition on glucose and β-hydroxybutyrate levels in blood of heifers as well as GPx activity.

MATERIAL AND METHODS

With the aim to establish effect of body condition (BC) of heifers on glucose and β-hydroxybutyrate levels, as well as gluthatione peroxidase activity, estimation of body condition was performed by Virginia system modified by Edmenson, using 1 to 5 scale (Edmenson, 1989). According to that, 23 hefers were classified in one of the two groups. First group (n=5) includes heifers with optimal body condition (BC=3.75), while the second group (n=18) includes obese heifers with the BC score ≥4.0. Body condition scoring was performed 3 weeks before expected calving. In order to avoid variations related to the body condition estimation, scoring was performed by 4 persons simultaneously. Average age of calved heifers was 25.5 months.

Collecting and examination of blood samples of heifers was done from day 1 to day 10 after parturition. Blood was obtained by *v. coccigea media* puncture. Blood analyses were done using test strips on which blood drop was applied. Glucose and β-hydroxybutyrate concentrations were recorded on apparatus "FREESTYLE PRECISION NEO Blood Glucose and Ketone Monitoring system".

Glutathione peroxidase activity (GPx) from whole blood of cows was determined using method described by Günzler at al. (1974) on spectrophotometer Cecil 2000. The principle of this measurement is based on spectrophotometric registration of NADPH utilization in coupled enzyme reaction. Method was obtained in a way that 500 µl of potassium phosphate buffer, 200 µl of glutathione (GSH), 50 µl of glutathione reductase (GR), 10 µl of whole blood hemolyzed with Drapckin reagent (21 time of dilution) and 490 µl of redistilled water was added in mentioned order in each tube. Then a 10-minute preincubation in thermostat on 37°C was obtained, followed by addition of 200 µl of nicotinamide adenine dinucleotide phosphate (NADPH) and 550 µl of tertial butylhydroxyperoxide (TBH). After addition of NADPH and TBH, tube content is poured into the cuvette. By placing of cuvette in spectrophotometer, utilization of NADP begins, and the absorbance is recorded in 30 seconds intervals at wavelength of 366 nm.

Solutions of GR, GSH and NADPH are always freshly prepared, with the usage of redistilled water as a solvent for GR and GSH. As a solvent for NADPH, 0.1 % $NaHCO_3$ was used. Composition and final concentrations of reagents are presented in Table 1.

Table 1. Composition and volume of reagents used for spectrophotometric determination of GPx activity

Reagents	Volumen (µl)	Final concentration
Potassium phosphate buffer (400 mmol/L, pH 7)	500	100 mmol/l
GSH (604 mmol/L)	200	4 mmol/L
Gluthatione reductase (GR)	50	6 mmol/L
Whole blood (dilution. 21x)	10	0.375 U/mL
NADPH	200	0.3 mmol/L
TBH	550	1.575 mmol/L
Redestilated water	490	

Results obtained in this study are grouped in certain statistical series and processed using certain statistical methods in MS Excel 2007 program. Calculated mean (arithmetic mean – Xmean, relative measures of variability (coefficient of variation – Cv) and absolute measures of variability (standard deviation – SD) were statistical methods that were used. Analyses of statistical significance were obtained by Student t-test. Obtained results are presented by figures.

RESULTS

Glucose, BHB concentrations and GPx activity were determined with the aim to compare results between heifers of optimal body condition and obese heifers.

By determination of glycemia, no significant difference was observed (p=0.71) between glucose concentration means obtained in heifers with optimal body condition and obese heifers (Figure 1).

In heifers with optimal body condition, mean value of glucose concentration was 3.16±0.51 mmol/L with interval of values from 2.8-3.9 mmol/L and coefficient of variation 16%.

In obese heifers, mean value for glucose concentrations was 3.19±0.66 mmol/L, with interval of values from 1.5-4.6 mmol/L and coefficient of variation 20%.

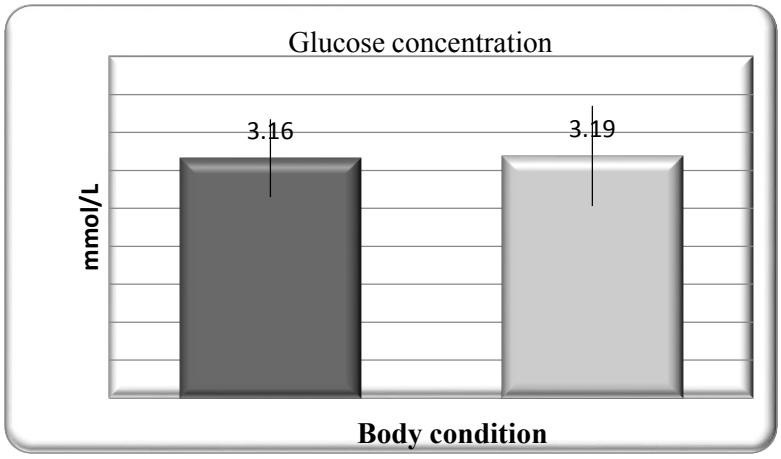

Figure 1. Values of glucose concentrations in blood of heifers of optimal and increased body condition

In heifers with optimal body condition, mean value of BHB concentration was 0.4±0.16 mmol/L, with interval of values from 0.2-0.6 mmol/L. In obese heifers, mean value for BHB concentrations was 0.67±0.22 mmol/L, with interval of values from 0.4-1.1 mmol/L. By determining the statistically significant difference, significant difference was established (p=0.01) between mean values of BHB concentrations in heifers with optimal body condition and obese ones. Obese heifers had significantly higher mean BHB values (Figure 2).

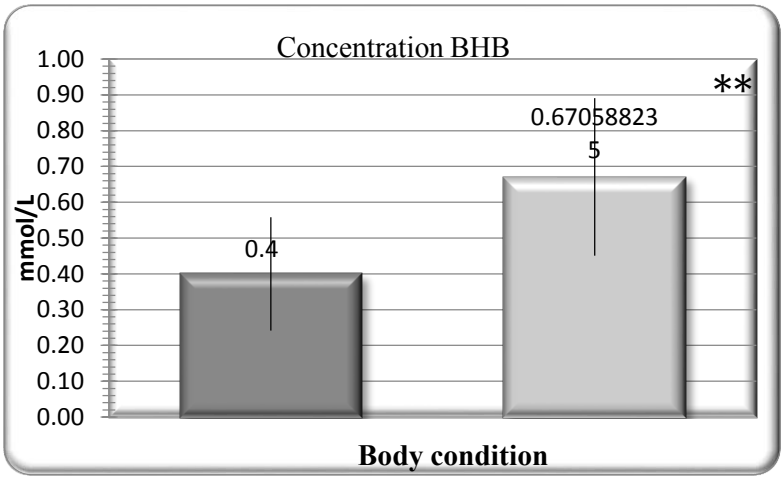

Figure 2. Values of β hydroxybutirate (BHB) concentrations in the blood of heifers of optimal body condition and increased body condition (** p=0.01)

In heifers with optimal body condition, average GPx activity was 326±30 μKat/L, with intervals of values from 292-361 μKat/L. In obese heifers, average GPx activity was 423±140 μKat/L, with intervals of values from 225-687 μKat/L.

By determining the statistically significant difference, significant difference was established (p=0.02) between average values of GPx activity of heifers with optimal body condition and obese heifers. In obese heifers, there was significantly higher GPx activity (Figure 3).

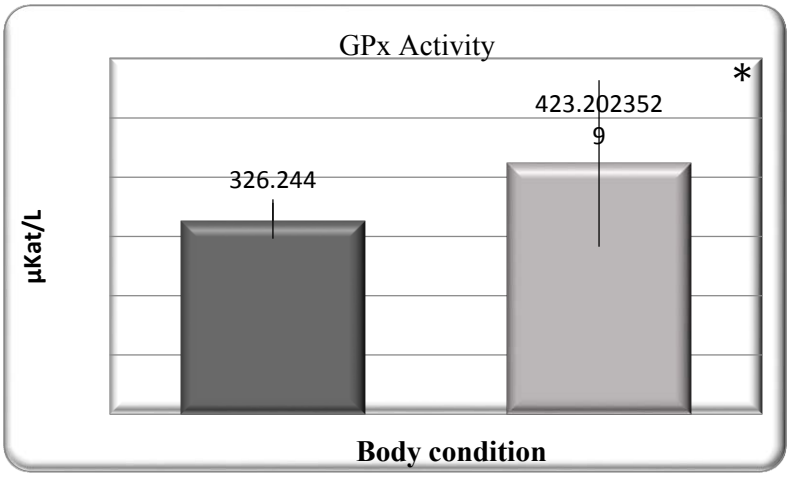

Figure 3. GPx activity in whole blood in heifers with optimal body condition and increased body condition (* p=0.02)

DISSCUSION

Ketosis is a common metabolic disturbance in high yielding dairy cows. Causes that contribute to the appearance of ketosis might include inadequate nutrition related to lactation phase, hormone disbalance, poor body condition, high milking and many others.

Body condition scoring is obtained in all phases of productive and reproductive cycles, and favorable score in calving period is 3.25-3.75. Over-conditioned cows in calving and early lactating period, characterized by negative energy balance, are more exposed to the risk of ketosis appearance, due to the release of free fatty acids and acetyl CoA deposition. In order to avoid these risks, it is necessary to maintain body condition of cows in certain range, meaning 3.25-3.75 at calving, 2.5-3.25 in early lactation, 2.5-3.5 in mid lactation, 3-3.5 in late lactation and 3.25-3.75 in dry period (Edmenson, 1989). As reported by Gillund at al. (2001), appearance of ketosis stage in obese cows after parturition is common. Based on our results, it is rare and can be explained by lower milk production in first calving cows. Due to less milk production, negative energy balance is less pronounced then in cows who achieved maximum milking.

Danger of ketosis stage appearance increases with increased age of first calving cows. It is speculated that first calving cows younger than 20 months of age belong to group with the lowest risk. Since average age of examined heifers was 25.5 months, they can be classified in moderate risk group (Van Der Drift, 2013).

In animals with an optimal body condition, average glucose concentration value was 3.16 mmol/L, while in obese heifers was 3.19 mmol/L. Both vales are within reference range that is 2.3-3.5 mmol/L (Stojić, 2010).

BHB value over 0.7 mmol/L two weeks after calving, combined with higher concentration of free fatty acids in blood, indicates on negative energy balance (Celeska et al., 2010). Values of BHB concentrations in examined heifers with

optimal body condition were within reference range, while in obese heifers, six animals (33.3%) had increased values. In this period, negative energy balance starts to develop, with consequently increased ketone body synthesis. In these 6 animals lower glucose concentration was also observed.

There is no data within available literature that related to relationship between ketone bodies level and glutathione peroxidase enzyme activity, as well as explanation for its increased activity in heifers with higher BHB levels. This result can indicate that studying oxidative stress and antioxidative capacity in animals with negative energy balance need more attention.

REFERENCES

1. Cincović M. (2013): *Patofiziološka procena peripartalnog metaboličkog stresa kod visokoproduktivnih krava upotrebom endokrinih i metaboličkih kriterijuma*. Doktorska teza, Poljoprivredni fakultet, Univerzitet u Novom Sadu.
2. Celeska I., Ulčar I., Stojkovski V., Dovenski T., Mitrov D., Džadžovski I. (2010): *Effect of lactation on energy metabolism in dairy cows from different categories*. Mac Vet Rev 33(2):15−21.
3. Edmenson A.J., Lean I.J., Weaver L.D., Farver T., Webster G. (1989): *A body condition scoring chard for Holstein dairy cows*. Journal of Dairy Science 72:68−78.
4. El Deeb W.M., El Bahr S.M. (2017): *Biochemical Markers of Ketosis in Dairy Cows at Post-paturient Period: Oxidative Stress Biomarkers and Lipid Profile*. Am J of Biochemy Mol Biol 7(2): 86−90.
5. Forstrom J.W., Zakowski J.J., Tappel A.L. (1978): *Identification of the catalytic site of rat liver glutathione peroxidase as selenocysteine*. Biochemistry 17:2639–2644.
6. Gillund P., Reksen O., Grohn Y.T., Karlberg K.J. (2001): *Body Condition Related to Ketosis and Reproductive Performance in Norwegian Dairy Cows*. Dairy Sci 84:1390–1396.
7. Günzler W.A., Steffens G.J., Grossman A., Kim S.M.A., Otting F., Wendel A., Flohe L., (1974): *The aminoacid sequence of a bovine glutathione peroxidase*. Physiol Chem 365:195.
8. Mihailović M. i Jovanović I.B. (2007): *Biohemija*. Naučna KMD, Beograd.
9. Stojić V. (2010): *Veterinarska fiziologija*. Naučna KMD, Beograd.
10. Van Der Drift S.G.A. (2013): *Ketosis In Dairy Cows: Etiologic Factors, Monitoring, Treatment. Dissertation*. Faculty of Veterinary Medicine, Utrecht University, the Netherlands.

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