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**Relationship between body condition score, blood metabolites and oxidative stress in transition period and reproductive performance of dairy cows**

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### Βιβλιογραφική αναφορά:

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## Relationship between body condition score, blood metabolites and oxidative stress in transition period and reproductive performance of dairy cows

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**ABSTRACT.** The aim of the current study was to investigate the relationship between prepartum body condition score (BCS), blood metabolites (Glucose, beta-hydroxybutyrate; BHB), oxidative stress (Malondialdehyde; MDA, Glutathione peroxidase; GSH-Px) in transition period and some reproductive parameters in Holstein dairy cows. Fifty cows were divided into two groups [low-BCS = 2.75-3.0 (n=25); adequate-BCS = 3.25-3.75 (n=25)] according to BCS on the 21<sup>st</sup> day before expected calving. BCS was also recorded at 0 and +21 days after calving. Blood samples collected at -21, 0 and +21 days from calving for MDA and GSH-Px analysis as well as at -21, +14 and +21 days of postpartum for glucose and BHB. Data on reproductive parameters obtained from farm records were also collected. MDA concentrations were higher in group of low-BCS compared to group of adequate-BCS at -21 and 0 d related to calving ( $p<0.05$ ). GSH-Px activity was lower in group of low-BCS than adequate one during the transition period ( $p<0.05$ ). Low-BCS group also showed higher concentrations of BHB at +14 and +21 days after calving. During the transition period, low-BCS group had lower glucose concentrations compared to adequate one ( $p<0.05$ ). Adequate-BCS group of cows showed shorter intervals of calving to first estrus ( $p<0.01$ ) and calving to conception ( $p<0.01$ ). In conclusion, cows with lower BCS at the prepartum period had worse metabolic and oxidative balance during the transition period. This situation also was associated with worse reproductive performance in cows.

**Keywords:** body condition score, cow, fertility, oxidative stress, metabolites, transition period

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## INTRODUCTION

Transition period has been defined as the most critical period in relation to health status and reproductive performance of dairy cows. Transition dairy cows undergo dramatic changes including metabolic, physiological, nutritional and oxidant/antioxidant balance. This process can result in a period of negative energy balance and loss of BCS when rations do not meet the energy demand for a dairy cow at this reproductive phase (Konvicna et al., 2015, Barletta et al., 2017).

The body condition score is a simple, practical and reliable method for evaluating the energy and fat reserves in dairy cows (Roche et al., 2007, Barletta et al., 2017). BCS is associated with dry matter intake, milk yield, cow immun response and reproductive performance. Many authors agree that controlling BCS of high yielding dairy cows could minimize the effect of negative energy balance which is one of the most important reasons of poor reproductive performance (Stefanska et al., 2016). It is also well known that low BCS and high BCS loss are associated with increased incidence of anestrus and anovulatory cycles, and reduced conception rate (Ferguson et al., 1994, Barletta et al., 2017). Barletta et al. (2017) noted that the BCS at 21 d before calving was more informative for BCS loss or gain than the BCS near calving.

Oxidative stress occurs in cows when energy demand, non-esterified fatty acids (NEFA) and lipid peroxidation concurrently increase in this period (Gheise et al., 2017). Oxidative stress characterised by an increased production of free radicals and decreased antioxidant defense is the result of an imbalance between oxidant and antioxidant mechanisms (Konvicna et al., 2015). Oxidants damage to macromolecules of cells and lead to disruption of normal metabolism and physiology. These alterations in cells lead to metabolic disorders and diseases in dairy cows (Pilarczyk et al., 2012).

In humans, oxidative stress is associated with high body mass index and body weight loss (Vincent and Taylor, 2006). The oxidative stress especially as a result of high BCS in dairy cows has been also observed (Bernabucci et al., 2005).

Few studies concerning the relationship between BCS and; metabolic or oxidative balance in dairy cows are available (Laubenthal et al., 2017). However, the reproductive performance of cows has not been studied in these studies. The aim of the current study was to investigate the hypothesis that there is a relationship between prepartum body condition score (BCS), blood metab-

olites [Glucose,  $\beta$ -hydroxybutyrate (BHB)], oxidative stress [malondialdehyde (MDA), glutathione peroxidase (GSH-Px)] in transition period and reproductive parameters (calving to estrus interval, calving to conception interval and insemination index) in dairy cows.

## MATERIALS AND METHODS

The current study was carried out in a large commercial dairy farm consisting of 1000 lactating dairy cows located in Western Thrace. All cows were housed in the same building under the same environmental and nutritional conditions. All animals were fed a mixed ration containing grass, corn silage and commercial concentrate twice a day ad libitum. The ration for dry and lactating cows contains 16% and 19% of crude protein, respectively.

A total 50 pregnant Holstein cows (2-4 parturitions) were selected according to their BCS on the 21<sup>st</sup> day before expected calving date. The animals were divided according to BCS (Gheise et al. 2017) into two equal groups: low-BCS (2.75-3.0, n=25) and adequate-BCS (3.25=3.75, n=25). The BCS was recorded by the same person using a scale of 1-5 with 0.25 increments as described by Ferguson et al. (1994). And BCS was recorded again at 0 and +21 days after calving. Twin calvings, stillbirths and dystocia were excluded from the study.

For monitoring the oxidative stress, blood samples were collected from the coccygeal vein into tubes with K<sub>2</sub>EDTA (for MDA analysis) and into tubes with heparin (for GSH-Px analysis) at -21, 0 and +21 days from calving. The tubes with K<sub>2</sub>EDTA were centrifuged at  $1419 \times g$  at 4 °C for 20 min. and plasma were stored at -18 °C until analysed. Plasma MDA concentrations ( $\mu\text{mol/l}$ ) were analysed by the method of Konvicna et al. (2015). Hemolysates were quickly stored at -20 °C following the processed for preperation of hemolysate and enzyme activities were estimated with the method described by Sharma et al. (2011).

Blood samples were also taken from the coccygeal vein into tubes without anticoagulant at -21, +14 and +21 days from calving for glucose and BHB analysis. The concentrations of glucose (mg/dL) and  $\beta$ -HBA (mmol/L) were measured using commercial kits (WAKO®, USA, Cat No. 439-90901 and Cat No. 417-73501, respectively) according to the manufacturer's instructions. Blood samples were collected before morning feeding in the same environmental conditions. After blood collections, serum were separated by centrifugation for 10 min at 3,000 g and stored at -20°C until assayed.

Reproductive performance of the two groups was assessed based on the calving-to-estrus interval, calving-to-conception interval, and insemination index.

Before performing the statistical analysis, data were examined with Shapiro-Wilk test for normality and Levene test for homogeneity of variances as parametric test assumptions. Descriptive statistics for each variable were calculated and presented as “Mean  $\pm$  Standard Error of Mean”. Student t-test was used to evaluate the difference between groups for the lactation, and reproductive variables. To test the differences in BCS, MDA, GSH-Px, glucose and BHB parameters between groups, General Linear Models with the repeated measures design were used. When a significant difference was revealed, any significant terms were compared by Simple effect analysis with Bonferroni adjustment.  $P < 0.05$  was considered as significant in all analyses. SPSS® for Windows 14.1 (Licence No:9869264) was used in the analysis of the data.

## RESULTS

Mean values of the body condition score in low-BCS and adequate-BCS cows at -21, 0 and +21 days from calving were showed in Table 1. In both groups of cows, the mean BCS loss during transition peri-

od was determined as 0.25-0.75 unit. Mean BCS at -21 and 0 days from calving were significantly higher in group of adequate-BCS than group of low-BCS ( $p < 0.05$ ; Table 1).

MDA concentrations increased from -21 day to 0 days ( $p < 0.05$ ) in both groups of cows. MDA concentrations were markedly higher in cows with low-BCS compared to adequate-BCS at -21 and 0 days from calving ( $p < 0.05$ ; Table 1). However, no significant difference was observed between the two groups at +21 days from calving ( $p > 0.05$ ).

In both groups of cows, GSH-Px activity decreased from -21 days to 0 days, and it increased from 0 to +21 days ( $p < 0.05$ ). Cows with low-BCS had lower GSH-Px activity than adequate-BCS cows in all sampling dates ( $p < 0.05$ ; Table 1).

Glucose concentrations significantly varied between sampling dates, with low-BCS cows showing lower glucose concentrations ( $p < 0.05$ ; Table 2), in all dates.

No significant differences were observed between two groups in blood BHB concentrations at -21 days from calving ( $p > 0.05$ ). However, BHB concentrations were higher in cows with low-BCS than adequate-BCS at +14 and +21 days from calving ( $p < 0.05$ ; Table 2).

**Table 1.** The values of the body condition score, Malondialdehyde (MDA,  $\mu\text{mol/l}$ ) concentrations and Glutathione peroxidase (GSH-Px, units/mg Hb) activity in low-BCS ( $n=25$ ) and adequate-BCS ( $n=25$ ) cows at different sampling dates (mean $\pm$ SEM).

Parameters	Days from calving			Group	P value Time	Group*Time
	-21	0	+21			
<b>BCS</b>						
Adequate-BCS	3.40±0.02 <sup>a,A</sup>	3.47±0.04 <sup>a,A</sup>	2.90±0.03 <sup>b,A</sup>	<0.001	<0.001	<0.001
Low-BCS	2.91±0.03 <sup>b,B</sup>	3.16±0.05 <sup>a,B</sup>	2.82±0.04 <sup>b,A</sup>			
<b>MDA</b>						
Adequate-BCS	3.35±0.04 <sup>b,B</sup>	3.50±0.04 <sup>a,B</sup>	3.55±0.02 <sup>a,A</sup>	0.003	0.001	0.074
Low-BCS	3.51±0.08 <sup>b,A</sup>	3.69±0.06 <sup>a,A</sup>	3.55±0.03 <sup>b,A</sup>			
<b>GSH-Px</b>						
Adequate-BCS	2.85±0.08 <sup>a,A</sup>	1.69±0.03 <sup>c,A</sup>	2.12±0.07 <sup>b,A</sup>	0.001	<0.001	0.150
Low-BCS	2.42±0.10 <sup>a,B</sup>	1.46±0.04 <sup>c,B</sup>	1.89±0.05 <sup>b,B</sup>			

Mean values within a row (a-b) or a column (A-B) with different superscript letters differ significantly.

**Table 2.** Serum glucose (mg/dL) and BHB (mmol/L) concentration of low-BCS ( $n=25$ ) and adequate-BCS ( $n=25$ ) at different sampling dates (mean $\pm$ SEM).

Parameters	Days from calving			Group	P value Time	Group*Time
	-21	+14	+21			
<b>Glucose</b>						
Adequate-BCS	58.76±0.76 <sup>a,A</sup>	53.15±1.05 <sup>b,A</sup>	56.78±0.66 <sup>a,A</sup>	<0.001	<0.001	0.019
Low-BCS	49.63±2.18 <sup>ab,B</sup>	47.47±1.08 <sup>b,B</sup>	53.47±0.77 <sup>a,B</sup>			
<b>BHB</b>						
Adequate-BCS	0.14±0.01 <sup>b,A</sup>	0.41±0.06 <sup>a,B</sup>	0.16±0.01 <sup>b,B</sup>	0.016	<0.001	0.109
Low-BCS	0.16±0.01 <sup>c,A</sup>	0.69±0.09 <sup>a,A</sup>	0.27±0.05 <sup>b,A</sup>			

Mean values within a row (a-b) or a column (A-B) with different superscripts differ significantly.

**Table 3.** Reproductive parameters of low-BCS (n=25) and adequate-BCS (n=25) dairy cows.

Fertility parameters	Groups		
	Adequate-BCS	Low-BCS	p
Calving to estrus interval	40.22±0.2	48.47±0.3	<0.001
Calving to conception interval	112.76±4.42	144.79±10.05	<0.05
Insemination index	2.10±0.12	3.01±0.25	<0.05

Table 3 shows the reproductive parameters of cows with low-BCS and adequate-BCS. The calving-to-estrus intervals ( $p<0.001$ ) and calving-to-conception intervals ( $p<0.05$ ) were quite long in cows with low-BCS (Table 3); cows with low-BCS had also higher insemination index ( $p<0.05$ ), compared to cows with adequate-BCS.

## DISCUSSION

Results obtained from the current study showed that cows with low-BCS at prepartum period had higher oxidative stress, lower antioxidant activity, higher BHB and lower glucose levels during the transition period. All these metabolic and oxidative imbalances in cows with low-BCS manifest as poor fertility.

During the transition period, body fat reserves are mobilized to meet the cow's energy requirements. Adipose tissue plays an important role in the maintenance of metabolic homeostasis (Alharthi et al., 2018). The mobilization of body fat reserves results in body condition score loss and increased concentrations of NEFA in blood. The intensified process of NEFA oxidation in the liver results in increased production of reactive oxygen species (ROS) and oxidative stress during the transition period (Folnozic et al., 2015, Turk et al., 2015).

Oxidative stress and antioxidant status depend on the stage of lactation, nutrition, disease, season and environmental stresses such as heat stress (Pilarczyk et al., 2012, Turk et al., 2015, Çolakoğlu et al., 2017). Additionally, obesity is related to oxidative stress, increased oxidant and decreased antioxidant activity in humans (Roh et al., 2017). Visceral fat in human is a source of several pro-inflammatory cytokines. The enhanced expression of these cytokines in obese patients can induce a proinflammatory environment and facilitate oxidative damage (Furukawa et al., 2004). Similar relationships have been observed in animals such as dog and cows (Bernabucci et al., 2005, Pasquini et al., 2013). A correlation between high body mass index, body weight loss and oxidant stress is also known in humans (Vincent and Taylor, 2006), cows (Bernabucci et al., 2005, Laubenthal et al., 2017) and dogs (Pasqui-

ni et al., 2013). BCS is associated with lipid mobilization and the imbalance of oxidative status in transition cows (Bernabucci et al., 2005). Increasing BCS was associated with enhanced oxidative stress (Laubenthal et al., 2017). However, previous results on the relation between BCS and oxidant markers are contradictory. Gheise et al. (2017) reported that the concentration of plasma MDA was affected by pre-calving BCS in dairy cows. Bernabucci et al. (2005) reported that cows with high BCS ( $>3.0$ ) before calving and with more BCS losses had higher reactive oxygen metabolites (ROM) in the circulation. In contrast, Alharthi et al. (2018) used cows with higher BCS than our study and indicated that BCS prior to parturition had no effect on the concentration of ROM. O'Boyle et al. (2006) have not reported any significant differences in total lipid hydroperoxide levels between high BCS ( $\geq 3.5$ ) and normal BCS (2.5-2.7) in mild lactation cows. In the present study, we evaluated adequate and low-BCS cows and blood MDA levels were significantly higher in low-BCS cows compared to adequate-BCS cows. These discrepancies may be related to oxidative stress markers, lactation time and different BCS points used in groups of the studies. In addition, the results of the study presented here are in agreement with previous studies (Bernabucci et al., 2005, Çolakoğlu et al., 2017, Alharthi et al., 2018) showing that the MDA levels were higher at calving and early lactation periods compared to prepartum period. During the calving time and early lactation, increased energy and oxygen requirements may lead to increased ROS production. In addition to this metabolic and oxidative imbalance, the low BCS further adversely affect cow's general health.

ROS are neutralised by the antioxidant system such as GSH-Px, SOD (Festila et al., 2012). In a previous study (Alharthi et al., 2018), cows with low-BCS ( $\leq 3.25$ ) had higher expression levels of enzyme SOD1 than cows with high BCS ( $\geq 3.75$ ). Bernabucci et al. (2005) reported that cows with high BCS ( $>3.0$ ) before calving and those with more BCS losses had lower SOD activity in circulation. Gheise et al. (2017) reported that plasma SOD and GSH-Px activities were affected by pre-calving BCS and that high BCS cows had lower plasma SOD and GSH-Px activities than



cows with medium BCS. GSH-Px enzyme is an indicator of oxidative stress (Festila et al., 2012). O'Boyle et al. (2006) have also found that cows with high BCS ( $\geq 3.5$ ) had lower total antioxidant potential than cows with normal BCS (2.5-2.7). However, authors have found any significant differences in GSH-Px activities between cows with normal BCS and high BCS in mild lactation (O'Boyle et al., 2006). The present study results showed low GSH-Px activity in cows with low-BCS during the transition period. Additionally, higher GSH-Px activity, in both groups, was found at prepartum period than after calving, similar to reports by Colakoglu et al. (2017) and Festila et al. (2012). As in MDA concentrations, the discrepancies in relative GSH-Px activity may be related to sampling time and BCS point used in groups of the studies. Our findings may suggest that low-BCS cows are more exposed to oxidative stress during the transition period, which manifested as higher MDA and lower GSH-Px activity.

Serum BHB and NEFA during the transition period are energy metabolites and changes of NEFA and BHB concentrations can be used as indicators of energy balance (Chapel et al., 2017). BHB concentrations are influenced by BCS. Some authors reported that cows with high pre-calving BCS had higher plasma BHB concentrations that could be associated with the higher fat mobilizations (Gheise et al., 2017). Walsh et al. (2007) also demonstrated that cows with high BCS had higher levels of BHB at 15 days pp. Bernabucci et al. (2005) found that cows with high BCS have higher BHB levels but they did not find any significant differences in BHB levels between cows with low and medium BCS. However, other studies (Folnozić et al., 2015) have described that BHB concentrations were numerically higher in cows with adipose BCS than in cows with optimal BCS, but the differences were not significant. In the present study, we evaluated low and adequate-BCS cows and found that low-BCS cows had higher BHB concentrations at pp 14 and 21 days. Findings obtained from this study may also suggest that not only high BCS but also low-BCS can cause high BHB levels. Moreover, it was suggested that BCS was associated with postpartum BHB levels.

Glucose, as a primary metabolic source of energy, is essential for the fetal growth and milk production. Folnozić et al. (2015) observed that adipose cows had higher glucose concentration than cows with optimal body condition on the day of calving. However, other authors (Bernabucci et al., 2005) have not found any significant differences in glucose concentrations

among cows with low ( $<2.5$ ), medium (2.6-3.0) and high BCS ( $>3.0$ ). Delfino et al. (2018) have not also found any significant differences in glucose concentrations between high ( $>3.5$ ) and low BCS ( $\leq 3.5$ ). Gheise et al. (2017) have not found any significant differences in glucose concentrations between medium and high BCS. In our present study, the adequate-BCS cows showed a significantly lower mean glucose concentration during the transition period, compared to low-BCS. It could be suggested that low and adequate-BCS cows have different energy requirements and energy utilization during the transition period.

Roche et al., (2007) have reported that reproductive performance was affected by BCS. In contrast, some authors reported that pre-calving BCS was not significantly associated with reproductive performance (Buckley et al., 2003). Godara et al. (2016) found that postpartum interval to estrous was significantly affected by pre-calving BCS. Chapel et al. (2017) have shown that cows with BCS $>3.75$  had worse reproductive performance than optimal BCS (3.5-3.75). Additionally, BHB concentrations are related to decreased reproductive performance (Walsh et al., 2007). Ovarian activity and the immune system are also adversely affected by the lower concentrations of glucose and higher concentrations of BHB (Lucy et al., 2014). Leroy et al. (2006) further suggested direct toxic effects of BHB on ova maturation. The elevation of free radicals as well as BCS and BHB levels have a negative effect on all body systems including the reproductive performance (Megahed et al., 2008). Higher oxidant levels in serum and follicular fluid was associated with infertility in women (Ozkaya and Nazıroğlu, 2010). In addition, it has been reported that oxidative stress is negatively correlated with ovarian oestradiol-17 $\beta$  concentrations (Appasamy et al., 2008) and this situation may result in ovarian disfunction. The results of the present study were in accordance with these studies. We found that the calving-to-estrus interval, the calving-to-conception interval and the insemination index (rate) were higher in low-BCS cows that exhibited high levels of MDA and BHB, low levels of GSH-Px and glucose.

## CONCLUSION

It is known that either high or low-BCS affects cow health, oxidative balance, and fertility. Our findings suggest that low-BCS cows had worse oxidative status, metabolic profiles and lower reproductive performance than adequate-BCS cows. Accordingly, different feeding strategies such as antioxidant sup-

plements should be utilized for low-BCS cows to improve their metabolic and oxidative status.

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## CONFLICTING OF INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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