

Effects of body condition score and parity on oxidative stress indicators of periparturient Iranian dairy buffaloes

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Abstract: This experiment was carried out to investigate the effects of parity and body condition score (BCS) pre-calving on oxidative stress indices of 43 milking buffaloes in transition period. The experiment was performed based on completely randomized design with unequal replications, using 3*4 factorial experiments comprising four levels of BCS (2.5-2.99; 3-3.49; 3.5-3.99 and 4-4.5) at two weeks before calving and three groups of parity (1; 2-5 and >5). Blood samples were collected from experimental animals in three stages including: 14 days before parturition, parturition day and 14 days after parturition. The results showed that the serum aspartate transaminase (AST) and alkaline phosphatase (ALP) activities in the highest BCS (4-4.5) group were significantly higher than that of other BCS groups; while the serum gamma-glutamyl transaminase (GGT) activity was decreased with increasing BCS. In addition, blood glutathione peroxidase (GPx) activity and serum malondialdehyde (MDA) content in the lowest level of BCS (2.5-3) were significantly lower than that of other groups. The higher serum catalase activity as well as total antioxidant capacity (TAC) was observed in the BCS of 3.5-3.99. At periparturient period, MDA level and GPx and catalase activities at calving day and alanine aminotransferase (ALT) and AST at 14 days after calving were increased significantly. Moreover, it is observed that multiparous buffaloes have significantly higher oxidative stress indicators than

primiparous animals. Based on the results of this study, activity of oxidative stress factors in dairy buffaloes with a higher BCS at parturition significantly increased in 14 days after calving, which indicates an increase in the metabolism of lipids and proteins in these conditions.

Keywords: Body condition score, Buffalo, Oxidative stress, Transition period

Introduction

The periparturient (transition) period, from three weeks before to three weeks after calving, is critically important to health, production, and fertility of dairy cows. Dairy cows are exposed to several physiological challenges during this period, which might result in greater oxidative stress and metabolic disorders (Drackley, 1999; Bernabucci et al. 2005). In this period, daily dry matter intake decreases up to 30% and drastic metabolic and endocrine adjustments for postpartum milk production induce negative energy balance (NEB). Therefore, cows mobilize stored lipids of adipose tissues to satisfy the increased energy requirement for milk production, and preferentially use lipids as energetic substrate, also experience several metabolic challenges characterized by decrease in responsiveness of tissues to insulin and increase in liver gluconeogenesis (Ingvarsen and Andersen, 2000; Reynolds et al. 2003; Pires et al. 2013). These metabolic challenges resulted in increase of oxygen consumption and enhancing reactive oxygen species (ROS) production, reduce the antioxidant capacity and inflammatory responses (Bernabucci et al. 2005; Chan et al. 2002; Castillo 2005). Roche et al. (2009) showed that the higher body condition score (BCS) at calving and the decrease in post-calving BCS, can affect milk production and metabolic status of dairy cows, Several studies reported that oxidative stress is one of the most important factors for many calving related abnormalities (Lykkesfeldt and Svendsen, 2007; Celi, 2011). The incidence of oxidative stress especially as a result of high BCS or milk yield in dairy cows previously has been observed by other researchers (Bernabucci et al. 2005; Castillo 2005). Cows with high milk yield have higher concentrations of ROS than lower yielding animals (Castillo, 2005; Lohrke et al. 2004). It is reported that in last weeks of pregnancy and at the beginning of lactation, high energy demand intensify maternal

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metabolism due to augmented mitochondrial activity in maternal tissues (Arousseau et al. 2006). The imbalance between the rate of ROS production and their neutralization lead to the oxidative stress and increase antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (Lean, 2014). Therefore, production of ROS during late gestation and early lactation is intensified and it is reasonable to assume that the high BCS combined with high milk yield in the previous lactation might result in higher metabolic and oxidative stress (Castillo, 2005; Pedermera et al. 2010).

Body condition score system based on evaluation of the outer appearance of the animal interacts with its body fat reserves and therefore is directly influenced by energy balance (Anitha et al. 2010; Anitha et al. 2011). In dairy and beef cattle determination of body condition score by measuring the thickness of subcutaneous fat in skeletal areas using ultrasound was proposed by Bruckmaier and Blum (1992) and Domecq et al. (1995), which is more accurate than the visual assessment system and is recommended for research studies. By using the same technique, Anitha et al. (2011), suggested for Murrah buffaloes a body condition scoring system based on fat reserves and 1 to 5 scores. Buffalo has special nutritional and physiological features and in this regard, little research has been done. Therefore, the objective of this study was to evaluate the effects of pre-calving body condition score as well as number of parity on oxidative stress indicators in periparturient period of lactating buffaloes.

Materials and Methods

Forty three buffaloes (Iranian water buffalo: Azeri ecotype), of different body condition score and parity, were selected from North-West Buffalo Rearing and Breeding Centre, West Azerbaijan, Iran, for the study (Table 1).

Ultrasonography and body condition score assessment

Body condition score determined by subcutaneous fat thickness followed by ultrasonic method (Anitha et al. 2010), The BCS and ultrasonography measurements were obtained independently for the buffaloes at 15 days before estimated calving date. A Chison Q9 (China) ultrasound machine with a 5 MHz convex transducer was used to determine the amount of subcutaneous fat at five body locations through a coupling gel on each buffalo (Anitha et al. 2011). Body locations were selected based on the skeletal checkpoints used for body condition scoring and ease of obtaining and reading ultrasonography measurements (Table 2).

Blood collection and biochemical tests

Table 1 Buffaloes with different body condition score and parity

Factor	Body condition score				Parity		
	2.5-2.99	3-3.49	3.5-3.99	4-4.5	1	2-5	>5
Number of buffaloes in each group	10	11	13	9	12	22	9

Blood samples were collected from expected 14 days prior to calving, on the calving day and 14th days after calving. Approximately 6 ml of blood was collected into sterile test tubes by jugular vein puncture and allowed to clot by placing the test tubes in a slanting position. After one hour, the serum was separated and centrifuged at 3000 rpm for 5 minutes to get clear serum. The serum was stored at -20°C until determination of biochemical parameters. Glutathione peroxidase (GPx, Ransel, Randox Co. UK), superoxide dismutase (SOD, Ransod, Randox Co. UK), total antioxidant capacity (TAC, Randox, Co. UK), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), catalase enzymes activity by using Pars Azmoon kit (Tehran, Iran) and malondialdehyde (MDA) level was evaluated as thiobarbituric acid reactive substances (TBARS) by the method of Nair and Turner (1984) with slight modification. Statistical analysis of the experiment was carried out based on completely randomized design with unequal replications, using 3*4 factorial experiments comprising four levels of pre-calving BCS (2.5-2.99; 3-3.49; 3.5-3.99 and 4-4.5) and three groups of parity (1; 2-5 and >5). The effects of age and weight were considered as a covariate. The results were analysed with generalised linear model (GLM) procedure software of SAS 9.2 (2011). Moreover, detailed means were compared by Duncan's multiple range tests. Mean differences were statistically significant at $P < 0.05$.

Results and Discussion

The results of the effects of BCS at calving on oxidative stress indicators of milking buffalo are shown in Table 3. This result showed that the GPx activity and MDA level in dairy buffaloes with 2.5-2.99 BCS were significantly lower than that of other groups. The different BCS did not affect SOD activity in dairy buffaloes. The effect of BCS on ALT activity was significant, but there was not clear trend, that the higher and lower activity of the ALT was 3-3.49 and 2.5-2.99 BCS, respectively. Aspartate transaminase (AST) and ALP activity in the buffaloes with the higher BCS (4-4.5) was significantly higher than other BCS groups, while the gamma-glutamine transaminase activity was decreased with increasing BCS and activity of this enzyme in skinny buffaloes (2.5- 2.99 BCS) were significantly higher than other groups. The higher activity of catalase enzyme and TAC was observed in the 3.5- 3.99 BCS.

GPx: glutathione peroxidase, SOD: superoxide dismutase, ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP:

Fig. 1 Showing the skeletal checkpoints for body condition score

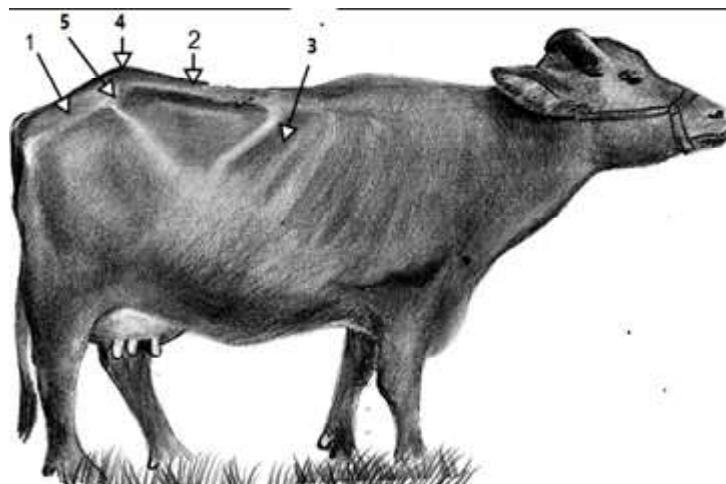


Fig. 2 The subcutaneous fat thickness in Lumbar area of the 4-4.5 BCS Buffalo



Fig. 3 The subcutaneous fat thickness in Lumbar area of the 2.5-2.99 BCS Buffalo

Table 2 Body condition score of buffaloes based by subcutaneous fat thickness

Score	Subcutaneous fat thickness (mm)				
	Between sacral crest and hooks	Lumbar area	Between 12th and 13th ribs	Between sacral crest and hooks	Between hooks and pins
2.5-2.99	2.1-3.0	2.6-3.5	2.1-3.5	2.1-3.0	2.1-3.0
3-3.49	3.1-4.0	3.6-5.0	3.6-5.0	3.1-4.0	3.1-4.0
3.5-3.99	4.1-6.0	5.1-7.0	5.1-7.0	4.1-6.0	4.1-5.0
4-4.49	>6.0	>7.0	>8.0	>6.0	>5.0

Table 3 Effects of body condition score on oxidative stress indicators at calving

Factors	Body Condition Score				SEM	P value
	2.5 - 2.99	3 - 3.49	3.5 - 3.99	4 - 4.5		
GPx (UL ⁻¹)	26.9 ^b	31.0 ^a	30.9 ^a	31.0 ^a	2.2	0.029
SOD (U mL ⁻¹)	1.78	1.62	1.73	1.87	0.1	0.292
ALT (UL ⁻¹)	49.6 ^c	64.4 ^a	60.3 ^{ab}	54.4 ^{bc}	6.5	0.014
AST (UL ⁻¹)	144 ^{ab}	123.5 ^b	125 ^b	163 ^a	18.5	0.021
ALP (UL ⁻¹)	279 ^{ab}	237 ^b	268 ^b	292 ^a	23.4	0.028
GGT (UL ⁻¹)	37.9 ^a	31.9 ^{ab}	29.2 ^b	29.5 ^b	4.00	0.046
Catalase (UL ⁻¹)	62.04 ^{ab}	67.18 ^{ab}	78.50 ^a	53.67 ^b	0.38	0.012
MDA (mol L ⁻¹)	0.91 ^b	0.99 ^a	1.06 ^a	1.01 ^a	0.06	0.001
TAC (mmol L ⁻¹)	0.50 ^b	0.53 ^b	0.64 ^a	0.47 ^b	0.07	0.010

^{a-c}: Means on the same row with different superscripts differed significantly (p<0.05)

alkaline phosphatase, GGT: gamma glutamyl transferase, MDA: malondialdehyde and TAC: total antioxidant capacity.

The oxidative stress indicators in transition period of buffaloes have been shown in Table 4. Base on the results, activity of GPx at 14 days of prepartum and calving day was higher than that of 14 days postpartum, and SOD and GGT activity at 14 days prepartum were significantly higher than that of other interval. The ALT activity significantly decreased in calving day than that of the 14 days pre- and post-calving. However, AST activity at 14 days of postpartum was significantly higher than calving and post-calving levels. The activity of catalase and MDA levels at calving day significantly increased compared to the pre- and post-calving, whereas, the Alkaline phosphatase activity and TAC content was not affected during transition period of the buffaloes.

The relationship between parity and oxidative stress factors in milking buffaloes displayed in Table 5. Table data shows that GPx, AST, ALP, GGT and catalase activities as well as TAC in multiparous buffaloes were significantly more than that of primiparous buffaloes; while, SOD, ALT and MDA was not significantly affected by parity.

The results of current study showed that the serum AST and ALP activities in the group with highest BCS (4-4.5) were significantly higher than that of other BCS groups; while the serum GGT activity was decreased with increasing BCS. In addition, GPx activity and serum MDA content was lowest level at BCS (2.5-2.99) were significantly lower than that of other BCS groups. The higher serum catalase activity as well as TAC was observed in the BCS of 3.5-3.99. During periparturient period, MDA level, GPx and catalase activities were higher on calving day and ALT and AST were significantly higher on 14th days after calving.

According to our findings, Festilä et al. (2012) and Roche (2009) stated that increasing the BCS in the dairy animals leads to an increase in many plasma oxidative stress markers. Dairy cows with a higher BCS and in early lactation period that loss their BCS faster, the concentration of active enzymes in oxidative stress control has loss (Bernabucci et al. 2005). Therefore, cows with a high BCS at calving and cows that are more lower their BCS at postpartum susceptible to oxidative stress, and are infected with mastitis, metritis and endometritis (Cebra et al. 1997; Bertoni et al. 2008). Bernabucci et al. (2005) reported that cows with higher BCS, before and after calving, had higher ROS, and after calving, had active thiobarbituric acid more than normal and lean cows.

Table 4 The variations of oxidative stress indicators at periparturient period of dairy buffaloes

Factors	Periparturient Period			SEM	P value
	-14	Calving day	+14		
GPx (UL ⁻¹)	31.7 ^a	31.0 ^a	27.1 ^b	2.4	0.012
SOD (U mL ⁻¹)	1.91 ^a	1.62 ^b	1.72 ^{ab}	0.14	0.016
ALT (U L ⁻¹)	59.2 ^{ab}	51.2 ^b	60.3 ^a	4.9	0.028
AST (U L ⁻¹)	131.5 ^b	129.0 ^b	156.8 ^a	15.9	0.046
ALP (U L ⁻¹)	284	260	264	12.8	0.256
GGT (U L ⁻¹)	37.13 ^a	29.10 ^b	30.10 ^b	4.3	0.017
Catalase (U L ⁻¹)	55.8 ^b	85.9 ^a	55.8 ^b	19.5	0.000
MDA (mol L ⁻¹)	0.93 ^b	1.09 ^a	0.97 ^b	0.08	0.000
TAC (mmolL ⁻¹)	0.53	0.56	0.51	0.02	0.710

^{a-c}: Means on the same row with different superscripts differed significantly (p<0.05)

Table 5 Relationship between number of parity and oxidative stress factors in milking buffaloes

Factors	Number of Calving		SEM	P value
	Primiparous	Multiparous		
GPx (UL ⁻¹)	28.7 ^b	32.1 ^a	3.3	0.002
SOD (U mL ⁻¹)	1.69	1.80	0.07	0.234
ALT (U L ⁻¹)	57.2	57.7	0.48	0.882
AST (U L ⁻¹)	128.7 ^b	151.2 ^a	15.9	0.047
ALP (U L ⁻¹)	225 ^b	308 ^a	58.1	0.000
GGT (U L ⁻¹)	27.2 ^b	37.1 ^a	7	0.000
Catalase (U L ⁻¹)	43.26 ^b	89.34 ^a	23.6	0.000
MDA (mol L ⁻¹)	1.00	0.98	0.01	0.504
TAC (mmolL ⁻¹)	0.27 ^b	0.81 ^a	0.37	0.000

^{a-c}: Means on the same row with different superscripts differed significantly (p<0.05)

Therefore, cows that lost much of their body scores were more susceptible to oxidative stress. Bernabucci et al. (2005) reported no significant difference in GPx between different groups of BCS but SOD levels in fat and thin cows were higher than normal cows.

At calving, SOD and GPx enzymes represent the major antioxidant components in protecting the cells against increased ROS. Superoxide dismutase catalyzes the dismutation of superoxide radical to hydrogen peroxide, which is further metabolized to water by GPx enzyme. The higher erythrocyte SOD activity on the day of parturition indicates higher oxidative stress and lower antioxidant status (Bernabucci et al. 2005; Castillo et al. 2005). During our experiment, the SOD activities were gradually elevated, which was probably caused by a response of the organism to higher superoxide generation, especially after calving. Although the SOD activity increases after calving in dairy cows (Gaál et al. 2006), several recent studies have shown that the antioxidant capacity in periparturient dairy cows is insufficient to counteract the increase in ROS supply (Bernabucci et al. 2005; Castillo et al. 2005). In the present study, reduction in GPx activity after two weeks of calving could be supported by increasing postpartum oxidative stress. The results are in correspondence to that of reported by Festilã et al. (2012) who recorded a decrease of mean blood GPx in dairy cows after 14 days of calving as a loss of homeostatic control in the postpartum period.

Higher alkaline phosphatase activity is an indicator of liver damage. Dairy cows with higher BCS may have greater liver damage due to increased liver function in the metabolism of free fatty acids released from adipose tissue in the negative energy balance (Roche 2013; Roche et al. 2013). The study of Bertoni et al. (2008) showed higher levels of ALP at calving and AST after two weeks of calving in dairy cows. They suggested that higher ALP at calving could be due to higher metabolism of the liver for the synthesis of special materials of fat for making colostrum at calving.

The activity of aminotransferases in blood is very important. Aminotransferases act as a catalyst in connecting the metabolism of amino acids and carbohydrates. Accordingly, changes in their activity in the blood can be a consequence of their increased activity in cells (primarily liver), but also a reflection of cell structure damage. In the liver AST, ALT and GGT showed higher activity and are most often determined, if there is a suspicion of acute and chronic liver disease (Bernabucci et al. 2005; Castillo et al. 2005). Determining AST and GGT activities in dairy animals mostly connected with fatty liver syndrome (Cebra et al. 1997), low appetite and the appearance of ketosis in dairy cows during early lactation (Steen, 2001). Increased AST activity in the serum is a sensitive marker of liver damage, even if the damage is of a subclinical nature (Meyer and Harvey, 1998). Unlike AST, horse, pig, and ruminant liver cells do not show high ALT activity, and the increased activity of that enzyme in the

serum during liver damage, even in necrosis, is insignificant (Saleh et al. 2007).

Gamma-glutamyl transferase is a membrane bound enzyme in organs with a specific function in secretion and resorption. In the plasma, it is significant as a sign of hepatobiliary system diseases connected with cholestasis and is used in diagnosing liver disease. Its activity is relatively high in livers of cows, horses, sheep and goats (Tenant 1997).

Saleh et al. (2007) reported slight changes in the activity of AST and GGT enzymes during pregnancy and early lactation, while the activity of ALT decreases significantly in the seventh and eighth month of pregnancy and at the beginning of lactation. Sharma et al. (2011) found significant differences in the activity of GGT and AST. In late pregnancy, GGT showed a significant decrease in activities, while the activity of AST in this period was much higher than in the first week after calving.

Malondialdehyde readily reacts with thiobarbituric acid producing a red pigment that can be measured spectrofluorometrically and the increased MDA concentrations after calving indicate imbalance between oxidants and antioxidants levels (Castillo et al. 2005; Bouwstra et al. 2010). In the present study, the highest MDA production by dairy buffaloes was determined after two weeks of parturition. These results are in accordance to those of Mudron and Konvicna, (2006) and Sharma et al. (2011) in dairy cows after parturition.

Total antioxidant capacity (TAC) is a single measure that aims to describe the dynamic equilibrium between pro-oxidants and antioxidants in the plasma compartment (Ghiselli et al. 2000). Nevertheless, interpretation of the changes in serum antioxidants status depends upon the conditions under which the serum antioxidant status is determined. An increased TAC value in serum is not necessarily a desirable condition if it is due to an adaptive response to increased oxidative stress. Similarly, the decrease in serum TAC is not necessarily an undesirable condition when the production of reactive species (reflected in MDA values) decreases (Castillo et al. 2005).

Abrupt increase in metabolic rate during the transition period and early lactation, thus a significant increase in oxygen requirements as energy demand increases due to high-energy metabolism in cows that produce high levels of ROS (Dobbelaar et al. 2010). Sharma et al. (2011) reported that freshly calved cows had more oxidative stress factors than that of postpartum cows, which had lower levels of antioxidants in their blood.

In the present investigation, it is observed that multiparous buffaloes have significantly higher oxidative stress indicators than primiparous animals; which was in line with Omidi et al. (2017). They found that oxidative stress was significantly lower in the primiparous cows compared to multiparous cows. They have suggested that increase of metabolic rate after calving is in

accordance with increase of milk production in multiparous cows may be responsible for the elevation of respiratory electron transfer and increasing ROS production.

Conclusions

Based on the results of study, it can be concluded that the activity of oxidative stress indices in dairy buffaloes with higher BCS at calving was higher after 14 days of calving, indicating the intensity of physiological changes and increased energy and protein metabolism. Moreover, higher number of parity resulted in higher oxidative stress. Therefore, considering the importance of the BCS in the production and health of dairy animals, it is necessary to study further the determination of the favorable body condition score for dairy buffaloes in different physiological conditions, especially before calving period.

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