

MDA and GSH-Px activity in transition dairy cows under seasonal variations and their relationship with reproductive performance

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Abstract

Introduction: The purpose of the current study was to evaluate the blood glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) levels under seasonal variations in dairy cows during transition period, and to assess the relationship between chosen reproductive parameters, GSH-Px, and MDA. **Material and Methods:** Holstein cows calving in January were assigned into winter group (n = 42), while cows calving in August were assigned into summer group (n = 42). Blood samples were collected from the jugular vein 21, 14, and 7 days before calving, at calving (0 day), and 7, 14, and 21 days after calving. Reproductive parameters obtained from farm records were evaluated. **Results:** In both groups of cows, GSH-Px activity decreased from 21 days before calving to day 0, and it gradually continued to increase until 21 days after calving. GSH-Px activity was higher in winter group compared to summer group during the transition period ($P < 0.05$). MDA levels in both groups increased over time starting from 21 days before calving to 0 day, but it gradually decreased thereafter. MDA levels were higher in summer group compared to winter group during the transition periods ($P < 0.05$). Summer group of cows showed higher intervals of calving-to-oestrus, calving-to-conception, and higher insemination index ($P < 0.01$). Negative correlation was recorded between GSH-Px and MDA during all examination days ($P < 0.01$). MDA levels correlated with calving to conception interval on day 21 before calving and day 0 ($P < 0.01$) and insemination index on day 0 and 21 days after calving ($P < 0.01$). GSH-Px activity was negatively correlated with calving to conception interval on day 21 before calving, day 0, and 21 days ($P < 0.01$) after calving. Negative correlation on day 21 before calving and day 0 was also determined between GSH-Px and insemination index ($P < 0.01$). **Conclusion:** This study showed that blood oxidant and antioxidant levels have affected the fertility parameters in cows under seasonal variations.

Keywords: cow, glutathione peroxidase, malondialdehyde, reproductive performance, season.

Introduction

Transition period has been defined as the most critical period in relation to health status of dairy cows during the lactation cycle. Transition dairy cows show dramatic changes in energy balance, alteration of the immune system, and oxidant/antioxidant balance (25, 40). These alterations can affect the health and fertility of dairy cows (26). In this period, the metabolic and oxygen requirements of dairy cows increase with foetal development and lactation (18, 39). Increased requirements can result in increased production of reactive oxygen species (ROS) negatively affecting the

oxidative balance during transition period (1, 39). ROS lead to damage of biological macromolecules of cells and disruption of metabolism and physiology. These alterations in cells also lead to metabolic disorders and development of diseases in dairy cows (24, 33). Antioxidants can be defined as substances that delay, prevent, or remove the oxidative damage to target molecules (20).

Under physiological conditions, ROS are neutralised by the antioxidant system (30). Imbalance between increased ROS production and reduced capacity of antioxidant system induces oxidative stress (5, 8 18). Oxidative stress and antioxidant status

depend on the stage of lactation (33), nutrition (17), disease (30), and seasonal variations (27). It has also been reported that heat stress, depending on the climatic changes, induces the free radicals production and reduction in plasma antioxidant activity (19, 28).

Some studies considering the effect of heat stress on reproductive performance and health status of cows are available (13, 14). Heat stress reduces the duration and intensity of oestrus, decreases the conception rates, and increases the number of inseminations (14, 42). During warm season, there is an increase in the frequency of ovarian cysts and pregnancy losses (12, 14). The effect of heat stress on the fertility of dairy cows is associated with several mechanisms including feed intake, rumen physiology, acid-base balance, and imbalance of oxidant/antioxidant activity and hypothalamo-hypophyseal-ovarian axis (12, 14).

The purpose of the study was to evaluate the blood glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) levels in transition dairy cows under seasonal variations. Possible relations of these oxidant-antioxidant parameters with fertility were also investigated.

Material and Methods

Study design and animals. The present study was conducted at a commercial dairy farm in Western Thrace (41° 8' 11" N, 24° 52' 57" E) with Holstein cows housed in free stall resting barns. All animals were fed a mixed *ad libitum* ration containing grass, corn silage, and commercial concentrate twice a day. Cows were milked with automatic milking system, twice daily. The age of the cows (n = 84) ranged from 3 to 6 years. All cows were pregnant and clinically healthy. Average ambient temperature and relative humidity during the study are shown in Table 1.

Table 1. Annual climatological data during study (mean ± SD)

Months	Air temperature (°C)		Precipitation (mm)
	Max.	Min.	
January	10.3 ± 0.17	6.1 ± 0.87	69.6 ± 0.75
February	13.3 ± 0.86	5.7 ± 1.51	27.0 ± 1.21
March	16.1 ± 0.47	6.4 ± 1.04	134.0 ± 1.51
April	18.2 ± 0.21	9.8 ± 1.28	181.6 ± 0.88
May	23.8 ± 1.21	12.6 ± 1.74	46.0 ± 1.08
June	29.8 ± 1.31	17.4 ± 1.07	85.4 ± 1.17
July	33.6 ± 0.28	19.6 ± 1.17	18.6 ± 1.62
August	34.3 ± 0.89	20.4 ± 1.08	32.0 ± 1.04
September	26.2 ± 0.54	16.5 ± 1.24	83.6 ± 1.19
October	20.7 ± 1.2	11.7 ± 1.54	42.6 ± 1.36
November	15.2 ± 0.15	7.9 ± 1.47	32.8 ± 1.27
December	12.2 ± 1.23	5.4 ± 1.15	127.5 ± 1.42

The animals were divided into two equal groups according to calving season. The cows calving in January were assigned into winter group, while cows calving in August were assigned into summer group.

Collection of blood samples and biochemical analysis. A total of 9 ml of blood were collected from the jugular vein into tubes with K₂EDTA (for MDA analysis) and into tubes with heparin (for GSH-Px analysis) at 21, 14, and 7 days before calving (-21, -14, -7), at calving (0), and 7, 14, and 21 days after calving (7, 14, 21). Blood samples were collected early in the morning, always at the same time, with the same environmental conditions. MDA levels were analysed with the method described by Uchiyama and Mihara (43). GSH-Px activity was also determined by the method of Hefamen *et al.* (19). The results of MDA and GSH-Px were expressed as µmol/L and units/mg Hb, respectively.

Evaluation of reproductive performance. Reproductive performance was assessed based on the calving-to-oestrus interval, calving-to-conception interval, and insemination index (service/conception).

Statistical analysis. Before performing the statistical analysis, data were examined for normality as parametric test assumptions. Descriptive statistics for each variable were calculated and presented as mean standard deviation (± SD). Pearson's correlation coefficient was used to determine the correlation between MDA, GSH-Px, and fertility parameters except insemination index. Spearman correlation coefficient was used to determine the correlation between MDA, GSH-Px, and insemination index since insemination index was not normally distributed. To test the differences in each parameter between time sampling in winter and summer group, two-way ANOVA with repeated measures design with group (winter, summer) as between-subject factor, and days from calving as within-subject factor were used after performing Mauchly's sphericity test to check the sphericity assumption. When a significant difference was revealed, any significant terms were compared by simple effect analysis with Bonferroni adjustment. The calving-to-oestrus interval and calving-to-conception interval were evaluated with a *t*-test. Insemination index was also analysed by the Mann-Whitney *U* test. P value of < 0.05 was considered to indicate statistical significance. SPSS for Windows 14.1 (Licence No. 9869264) was used for analyses of the data.

Results

MDA levels in both groups of cows increased over time starting from day -21 to day 0 but it gradually decreased thereafter (Table 2). MDA levels of winter group ranged from 3.21 ± 0.29 to 3.3 ± 0.17 µmol/L while that of summer group ranged from 3.59 ± 0.17 to 3.4 ± 0.05 µmol/L. MDA level was also higher in

summer group compared to winter group during the transition period ($P < 0.05$).

Table 2. The values of MDA ($\mu\text{mol/L}$) in summer and winter groups (mean \pm SD)

Days before and after calving	Group	
	Winter (n = 42) X \pm SD	Summer (n = 42) X \pm SD
-21	3.21 \pm 0.29 ^{e,B}	3.59 \pm 0.17 ^{c,A}
-14	3.19 \pm 0.12 ^{e,B}	3.51 \pm 0.05 ^{d,A}
-7	3.31 \pm 0.09 ^{d,B}	3.62 \pm 0.1 ^{c,A}
0	3.69 \pm 0.28 ^{a,B}	4.1 \pm 0.21 ^{a,A}
7	3.54 \pm 0.21 ^{b,B}	3.87 \pm 0.18 ^{b,A}
14	3.4 \pm 0.13 ^{c,B}	3.6 \pm 0.03 ^{c,A}
21	3.3 \pm 0.17 ^{d,B}	3.4 \pm 0.05 ^{c,A}

^{a-c} means with different small letters in the same column are significantly different ($P < 0.05$)

^{A-B} means with different capital case letters in the same row are significantly different ($P < 0.05$)

In both groups of cows, GSH-Px activity decreased from day -21 to day 0, and it gradually continued to increase until 21 days after calving (Table 3). The GSH-Px activity in winter group ranged from 3.16 ± 0.03 to 2.1 ± 0.021 U/g Hb, while that in summer group ranged from 2.97 ± 0.16 to 1.8 ± 0.08 U/g Hb.

GSH-Px activity was also higher in winter group compared to summer group during the transition period ($P < 0.05$).

Table 3. The values of GSH-Px activity (units/mg Hb) in summer and winter groups (mean \pm SD)

Days before and after calving	Group	
	Winter (n = 42) X \pm SD	Summer (n = 42) X \pm SD
-21	3.16 \pm 0.03 ^{a,A}	2.97 \pm 0.16 ^{a,B}
-14	3.19 \pm 0.08 ^{a,A}	2.78 \pm 0.11 ^{b,B}
-7	2.7 \pm 0.11 ^{b,A}	2.0 \pm 0.21 ^{c,B}
0	1.7 \pm 0.12 ^{c,A}	1.52 \pm 0.08 ^{c,B}
7	1.85 \pm 0.04 ^{c,A}	1.63 \pm 0.12 ^{c,B}
14	1.98 \pm 0.16 ^{d,A}	1.69 \pm 0.29 ^{c,B}
21	2.1 \pm 0.021 ^{c,A}	1.8 \pm .08 ^{d,B}

^{a-f} means with different small letters in the same column are significantly different ($P < 0.05$)

^{A-B} means with different capital case letters in the same row are significantly different ($P < 0.05$)

Compared to winter group, summer group had a longer calving to the first oestrus interval, a longer calving to conception interval, and a higher insemination index ($P < 0.01$ for all) (Table 4). Correlations between the parameters in summer and winter groups are shown in Table 5.

Table 4. Fertility parameters of summer and winter cows (mean \pm SD)

Fertility parameters	Group	
	Winter (n = 42) X \pm SD	Summer (n = 42) X \pm SD
Calving to oestrus interval	34.73 \pm 8.35 ^B	46.40 \pm 6.88 ^A
Calving to conception interval	99.6 \pm 29.12 ^B	145.83 \pm 47.8 ^A
Insemination index	1.73 \pm 0.91 ^B	2.93 \pm 0.91 ^A

^{A-B} means with different small letters in the same row are significantly different ($P < 0.01$)

Table 5. The correlation coefficients between MDA, GSH-Px, and fertility parameters in winter and summer groups

Days before and after calving	MDA and GSH-Px	MDA			GSH-Px		
		Calving to first oestrus interval	Calving to conception interval	Insem. index	Calving to first oestrus interval	Calving to conception interval	Insem. index
-21	0.66**	ns	0.44**	ns	ns	0.504**	0.53**
-14	0.591**	ns	ns	ns	ns	ns	ns
-7	0.542**	ns	ns	ns	ns	ns	ns
0	0.682**	ns	0.366**	0.353**	ns	0.37**	0.469**
7	0.608**	ns	ns	ns	ns	ns	ns
14	0.433**	ns	ns	ns	ns	ns	ns
21	0.51**	ns	ns	0.282**	ns	0.34**	ns

* $P < 0.05$, ** $P < 0.01$; ns – not significant, n = 84)

Discussion

The results obtained in the present study showed that both groups of cows showed higher oxidative stress and lower antioxidant activity until calving compared to cows in postpartum period. In addition, summer group of cows had higher oxidative stress and lower antioxidant activity during transition period. It has been demonstrated that heat stress negatively affects the oxidative balance of cows during the transition period. While blood antioxidant activity showed negative correlation, oxidant activity had positive correlation with fertility parameters.

MDA is the last product of lipid peroxidation and therefore changes of MDA concentrations can be used as a biomarker of oxidative stress (8). In findings of the present study, MDA levels gradually increased in both groups from -21 to calving days. Thereafter, it continuously decreased after calving in both groups. Although MDA levels gradually decreased after calving, blood MDA levels were still quite higher compared to levels before calving. The results contained by research groups regarding MDA levels in prepartum and lactation period are inconsistent. While some authors reported no significant changes in plasma MDA levels during periparturient period (9, 34), others described increased MDA levels around calving (5, 6). Sharma *et al.* (37) noted that plasma MDA level was significantly higher in early lactating cows as compared to advanced pregnant cows. Konvicna *et al.* (22) reported that the highest serum levels of MDA were found in the first week after parturition compared to antepartum and postpartum times. In addition, some authors have also defined a similar transient increase in MDA levels in dairy cows in the same period after calving (29, 36). Observations of the current study are consistent with previous reports, and data obtained from the study may show that calving and lactation affects the blood MDA levels as a cause of oxidative stress. In prepartum period, metabolic requirements of cows increase for foetal development and colostrum synthesis. This demand also requires energy and oxygen, which can result in increased ROS production. With the onset of lactation, energy, and oxygen requirements reach much higher levels than prepartum. Higher MDA levels are also available in the postpartum period.

GSH-Px and SOD enzymes are the major antioxidant defence components in protecting the cells against increased ROS (1, 22). Data regarding the relationship between GSH-Px activity and lactation stage have been obtained in previous studies (5, 8, 22, 33). However, previous results are controversial. In the present study, GSH-Px activity gradually decreased in both groups from -21 to calving days. Although GSH-Px activity gradually increased after calving, blood levels of GSH-Px activity were significantly lower after calving compared to those before calving. Some authors noted that antioxidant capacity in the

peripartum period of cows is insufficient to neutralise the increase in ROS (5, 8). Konvicna *et al.* (22) observed that the mean GSH-Px activity of cows in the first week after calving was lower compared to the cows between six and nine weeks after calving. Aitken *et al.* (2) described that GSH-Px activity has increased during early lactation. Pilarczyk *et al.* (33) reported that the highest serum activity of GSH-Px was found during the first two months of lactation and the lowest activity was in dry cows. Cigliano *et al.* (10) also reported that GSH-Px activity was lower in early lactation than in mid-late lactating cows. Festila *et al.* (16) described that the mean blood GSH-Px activity in dairy cows was higher in advanced gestation compared to the lactation, and after calving GSH-Px activity has started to increase again. However, these different values of GSH-Px activity were not statistically significant. Similar to the findings of Festila *et al.* (16), Sharma *et al.* (37) did not observe any significant differences in blood GSH-Px activity between advanced pregnant and early lactating cows. The current study showed that GSH-Px activity had variations during transition period similar to reports by Konvicna *et al.* (22), Aitken *et al.* (2), Pilarczyk *et al.* (33), and Cigliano *et al.* (10). The highest GSH-Px activity was found before calving and gradually increased following the calving. It is possible to explain the changes in GSH-Px activity as increasing oxidative stress during the calving period and gradually decreasing oxidative stress during the early lactation period.

Exposure to heat stress during the hottest season causes the oxidant/antioxidant imbalance (23, 28). Heat stress also causes a reduction in plasma antioxidant activity increasing the production of free radicals and decreasing endogenous synthesis of antioxidants (23, 24). Measuring the antioxidant and oxidant activities is very important to evaluate the effect of summer high ambient temperature (35). Stress causes an increase in the glucocorticoid levels. It is also associated with decrease in functions of the immune system cells (41). High levels of glucocorticoids have decreased blood glutathione and erythrocyte superoxide dismutase activity in rats (7, 31), goats (3), and cows (28). Some authors pointed out the increased oxidant and decreased antioxidant activity during the summer months (6, 23, 28). Heat stressed cows had lower total antioxidants after calving during summer than those calving in winter (42). A decrease in blood GSH-Px activity has also been observed in high ambient temperature during summer (6, 35). Similar to GSH-Px, blood MDA levels are affected by heat stress during the hottest season. In the study presented here, we investigated blood GSH-Px and MDA levels in cows with different calving seasons. Compared to winter and summer seasons, blood GSH-Px activity was significantly higher in winter group than that in summer group during the transition period. Cows calving in summer had a higher blood MDA levels than those calving in winter. This finding is consistent with previous reports (6, 28, 35).

In contrast, some authors have found that cows have higher blood oxidant levels and lower antioxidant activity during winter compared to summer period (38). The same authors also explained that maintenance of cows on pasture field in summer period has increased antioxidant activity. However, heat stress during the summer period significantly affects bovine physiology causing a decrease in feed intake and increasing body temperature. This can result in increased energy demand and lipolyses of body reserves, which leads to elevated free radicals production and reduced antioxidant activity (8, 23). The data obtained in the current study indicated that blood GSH-Px activity and MDA levels in cows would be influenced by seasonal variations (temperature and humidity) during the transition period and the summer group had more oxidative stress compared to cows in winter.

Because of the stimulating effect of heat stress on the activity of the hypothalamic-pituitary-adrenal axis, marked increase in serum cortisol levels was reported in cows (21, 28). However, increased levels of glucocorticoids block the follicular development and ovulation (11). Some studies showed that heat stress reduced the duration and intensity of oestrus, reduced the conception rates, and increased the number of inseminations (15, 42). Wolfenson *et al.* (44) also indicated that oestradiol production by granulosa cells is lower during summer season as a result of impaired dominant follicle development, which leads to an impaired corpus luteum formation with a low progesterone production. Moreover, heat stress leads to increased free radical production and decreased antioxidant activity (28). The elevation of free radicals has affected strongly all body systems including reproductive performance of males and females (28). Higher oxidant levels in serum and follicular fluid was associated with infertility in women (32). In addition, it has been reported that oxidative stress is negatively correlated with ovarian oestradiol-17 β concentrations (4, 26) and this situation may result in ovarian dysfunction. In the present study the calving-to-oestrus interval, the calving-to-conception interval, and the insemination index (rate) were higher in cows calving in summer with high levels of MDA and low GSH-Px activity. It was found that cows calving in winter had better reproductive performance than those calving in summer. Additionally, at different times of the transition period in both groups, significantly positive correlations between fertility parameters and MDA levels were observed, while negative correlations were found between fertility parameters and GSH-Px activity. It is possible to say that higher oxidant level and lower antioxidant activity cause poor reproductive performance. Blood MDA and GSH-Px activity at calving time also seem to affect the fertility parameters in both groups as well. Similar to our results, Turk *et al.* (42) reported that cows in summer group had higher number of services per conception and longer calving-to-conception interval than winter cows. These

authors also found a negative correlation between antioxidant activity and calving-to-conception interval, similarly to our results. Findings obtained in the current study may suggest that blood oxidant and antioxidant levels can affect fertility parameters. Fertility parameters of summer cows are more affected by the heat stress and oxidant-antioxidant imbalance during the transition period.

In conclusion, compared to postpartum period, both groups of cows had oxidative stress until calving. However, summer group of cows had higher oxidative stress and lower antioxidant activity during transition period. Reproductive performance is associated with GSH-Px activity and MDA levels. We suggest that GSH-Px and MDA would be useful in evaluation of the fertility of dairy cows.

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