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Original article

Oxidative stress in cows according to calving season: passive calf immunity and its relationship with colostrum quality

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Abstract

This study details the relationship between maternal plasma oxidant-antioxidant enzymes with colostrum quality, serum gamma glutamyl transferase (GGT), immunoglobulin G (IgG) and IgM concentrations of calves in the different calving seasons. Holstein breed cows between two and eight lactations and their calves were enrolled in the study. Holstein cows calving in winter (n=45) and their calves (n=45) were assigned to the winter group, while cows calving in summer (n=45) and their calves (n=45) were assigned to the summer group. Samples for malondialdehyde (MDA) and glutathione peroxidase (GSH-Px) were collected on day -21±3 before expected calving and also on calving day (Day 0). IgG and the specific gravity of the colostrum were determined after calving. Serum GGT and IgG and IgM were measured before the feeding, with colostrum, of calves (0 hours) and also in the 24th hour following the feeding of colostrum. Plasma MDA levels at -21±3 and 0 days in the summer cows were determined to be higher. GSH-Px activity was higher in the winter cows. IgG levels and the specific gravity of the colostrum were also higher in the winter cows. Calf IgG levels at the 24th hour of life were higher in the winter cows. In the winter group, IgM levels at 0 and 24 hours were also higher. While MDA was negatively correlated with IgG, IgM, GGT, IgG and the specific gravity of colostrum, GSH-Px activity had a positive correlation with IgG, IgM, GGT, IgG and the specific gravity of colostrum. The observed differences in plasma MDA, GSH-Px, calf serum IgG and IgM levels, and colostrum quality between both groups suggest a possible seasonal effect. The relationship between maternal oxidant-antioxidant enzymes, colostrum quality, and passive calf immunity revealed that these enzymes could be used as indicators in the evaluation of calf health and colostrum quality.

Key words: calf, colostrum, cow, immunoglobulin, calving season

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Introduction

Oxidative stress is the result of the production of reactive oxygen species occurring faster than the antioxidant mechanism that neutralizes them (Chigerwe et al. 2013). Increased production of free radicals and reactive oxygen species and decreased antioxidant defense mechanisms can lead to damage of biological macromolecules and the disruption of metabolism and physiology. These alterations in cells also lead to metabolic disorders and the development of diseases in dairy cows (Pilarczyk et al. 2012). Some physiological and pathological conditions such as pregnancy, calving, lactation, and disease are associated with oxidative stress (Gaal et al. 2006, Abuelo et al. 2015) could change according to the season, lactation stage and nutrition (Pilarczyk et al. 2012). The periparturient period, which includes dramatic changes in the metabolic, physiological, nutritional, and oxidant/antioxidant balances, is one of the most stressful periods. Periparturient dairy cows show increased metabolism, energy, and oxygen requirements and also overproduction of reactive oxygen species (ROS) in order to meet the energy demands for fetal development, colostrum production, and the physical effort of calving (Albera and Kankofer 2011). In several studies, oxidative stress in women and their newborns during pregnancy, parturition, and the postpartum period has been reported (Pressman et al. 2003, Myatt and Cui 2004). Insufficient maternal antioxidants and increased ROS production during the intrauterine period are associated with pregnancy complications such as fetal death, intrauterine growth restriction, and increased risk of diseases (Rodriguez-Rodrugiez et al. 2018). A high correlation has additionally been identified between maternal and fetal (cord blood) plasma levels of antioxidants and oxidative stress markers. Oxidative stress is generally transferred from the mother to the fetus via the placenta (Luo et al. 2006). It has been reported that maternal status during late pregnancy can have carryover effects on several health and production variables of neonatal calves. In particular, the calf immune system starts developing in utero from the 42nd day of gestation and is affected by maternal conditions during pregnancy (Abuelo 2020). Prenatal exposure to maternal parameters of metabolic stress may adversely affect some metabolic and inflammatory responses of the calves (Ling et al. 2018). Prenatal conditions such as heat and metabolic and oxidative stress have the potential to significantly affect the productivity, immune, and metabolic function of calves (Monteiro et al. 2012).

Good-quality colostrum is essential for the health and survival of newborn calves. Colostrum quality is related to levels of immunoglobulins (Igs) and can vary depending on factors such as age, vaccination, and the dry period length of cows (Godden 2008). Colostrum is a source of antioxidants such as lactoperoxidase, superoxide dismutase, catalase and glutathione peroxidase (Przyblska et al. 2007). However, it has been identified as a source of ROS affecting passive transfer as well (Abuelo et al. 2014).

In veterinary medicine, antioxidative and oxidative statuses have been studied during pregnancy, lactation, and calving in both cows, ewes, and mares and their newborns (Gaal et al. 2006, Rizzo et al. 2008). Calves exposed to high maternal oxidant status also had greater blood concentrations of haptoglobin and TNFα. This situation could be associated with adverse health outcomes (Abuelo 2020). Additionally, the effects of maternal metabolic and immunological changes on colostrum or calf immunology are known (Ling et al. 2018). However, there are no studies to our knowledge investigating the effects of oxidative status in cows on passive calf immunity and colostrum quality.

In this study, we hypothesized that maternal oxidative status leads to changes in colostrum quality and the immune responses of newborn calves. The purpose of this study was to evaluate the relationship between the oxidant-antioxidant enzymes of maternal blood with colostrum quality, serum GGT, and IgG and IgM concentrations of calves in different calving seasons.

Materials and Methods

Animals and study procedures

The study was carried out on a commercial dairy farm (41°8′11″ N, 24°52′57″ E) with Holstein breed cows between two and eight lactations and their calves. No ethical approval was needed for the routine veterinary procedures used in the study. The animals were allocated to groups related to calving season. Cows (n=45) calving in the winter (December, January, February) and their calves (n=45) were assigned to the winter group, while cows calving (n=45) in the summer (June, July, August) and their calves (n=45) were assigned into the summer group. All cows and calves underwent similar management conditions. All the cows had ad libitum access to water and were fed with total mix ratio (TMR). The diets were formulated according to the NRC nutrient requirements (Table 1). TMR was offered three times daily. All calves came from full-term gestations and were delivered by eutocic birth with little or no assistance. Calves were separated from their dams directly after calving. All calves also received 3 L of first-milked colostrum within the first three hours of life. Eight hours later, 1 L of colostrum was offered to the newborn calves. Repeat colostrum

Table 1. Dietary components for cows.

Component	Ingredient. % of DM
Vetch hay	-
Corn flake	7.09
Alfalfa hay	12.89
Corn silage	54.12
Wheat straw	2.06
Soybean pulp 8% CP	3.22
Concentrated feed	20.62
Limestone	1.57
Salt	0.26
Ammonium chloride	-
Dicalcium phosphate	0.44
Magnesium oxide	0.44
Magnesium sulphate	0.25
Sodium bicarbonate	0.7
Calcium sulphate	0.1
Mineral-vitamin mix[1]	0.2

[1] Contained a minimum of 4.3%Mg, 8% S, 6.1% K, 2.0% Fe, 3.0% Zn, 3.0% Mn, 5000 mg/kg Cu, 250 mg/kg of I, 40 mg/kg of Co, 150 mg/kg Se, 2200 kIU/kg of vitamin A, 660kIU/ kg of vitamin D_3, and 7700 IU/kg of vitamin E.

Table 2. Seasonal values for climatological data during the study.

Season	Air temperature (°C)		Precipitation (mm)
	Max.	Min.	
Spring	19.36	9.6	120.53
Summer	32.56	19.13	45.33
Fall	20.7	12.03	53
Winter	11.93	5.73	74.7

feedings were given at 12 hour intervals for 3 days. All cows and calves were considered as clinically healthy based on daily clinical examinations during the study period. Seasonal values for climatological data during the study are shown in Table 2.

Laboratorial analyses

In both groups, maternal blood samples for malondialdehyde (MDA) and glutathione peroxidase (GSH-Px) were taken by venipuncture of the jugular vein into heparinized vacutainer tubes on day -21±3 before expected calving and also on calving day. Plasma was separated by centrifugation and stored at -20°C until biochemical analysis. Plasma MDA concentration measurements were conducted spectrophotometrically (Hitachi, Japan) according to Placer et al. (1966) modified by Matkovics et al. (1988). MDA concentration was expressed as µmol/L. GSH-Px activity was measured using the kinetic method using a commercially available kit (RANSEL, Randox Laboratories) according to the method of Paglia and Valentine (1967) as described in a previous study (Bernabucci et al. 2005). The results of GSH-Px activity were expressed as U/mL. Colostrum samples from cows were collected immediately after calving. The concentration of IgG in colostrum samples was determined using a semi--automated single radial immunodiffusion method (Mancini et al. 1965) using commercial kits (ID Ring BOV IgG; ID Biotech, Issoire, France) and expressed as mg/dL. The specific gravity of the colostrum was measured by use of a colostrometer and expressed in g/mL. Serum gamma glutamyl transferase (GGT) and immunoglobulin G (IgG) and immunoglobulin M (IgM) levels were measured before the feeding of calves with colostrum (0 hours) and also in the 24th hour following the feeding of colostrum. Serum IgG and IgM levels of calves were determined with a commercially available ELISA test kit (Bovine IgG and IgM ELISA Quantitation kit, Bethyl Laboratories Inc.) and expressed in mg/100 mL. Levels of serum GGT were measured using an autoanalyzer (BT 3000 plus, Rome, Italy) expressed in U/L.

Table 3. Blood malondialdehyde (MDA) values and glutathione peroxidase (GSH-Px) activity in different season groups (mean±SEM).

Parameters	n	Days		P value		
		-21±3	0	Group	Time	Group*Time
MDA (μmol/L)						
Winter	45	3.44±0.04^{b,B}	3.90±0.03^{a,B}	<0.001	<0.001	0.005
Summer	45	3.90±0.03^{b,A}	4.23±0.03^{a,A}			
GSH-Px (U/mL)						
Winter	45	3.35±0.04^{a,A}	1.71±0.02^{b,A}	<0.001	<0.001	<0.001
Summer	45	2.75±0.06^{a,B}	1.38±0.02^{b,B}			

Mean values within a row (a-b) and column (A-B) with different superscript letters differ significantly (p<0.05).

Table 4. IgG levels and specific gravity of colostrum (mean±SD).

Parameters	Groups		p
	Summer (n=45)	Winter (n=45)	
IgG levels (mg/dL)	4568.89±1298.17	4944.67±1343.20	0.019
Specific gravity (g/mL)	1043.31±10.04	1052.29±9.24	<0.001

SD: Standard deviation

Table 5. Calf serum GGT, IgG and IgM values in different seasons (mean±SEM).

Parameters	n	Hours		p value		
		0	24	Group	Time	Group*Time
GGT (U/L)						
Winter	45	32.65±0.37^{a}	206.25±1.56^{b}	0.001	<0.001	0.096
Summer	45	29.92±0.236^{a}	199.47±1.95^{b}			
IgG (mg/100 mL)						
Winter	45	786.44±13.29^{b}	2295.88±40.80^{a,A}	0.004	<0.001	<0.001
Summer	45	776.12±13.18^{b}	2099.82±38.53^{a,B}			
IgM (mg/100 mL)						
Winter	45	14.81±0.21^{b,A}	601.87±6.95^{a,A}	0.004	<0.001	0.013
Summer	45	12.46±0.17^{b,B}	573.65±7.64^{a,B}			

Mean values within a row (a-b) and column (A-B) with different superscript letters differ significantly (p<0.05).

Statistical analysis

Before performing the statistical analysis, data were examined using the Shapiro-Wilk test for normality and the Levene test for homogeneity of variances as parametric test assumptions. Descriptive statistics for each variable were calculated and presented as "mean±standard error of the mean".

The Pearson correlation coefficient was used to determine the correlation between MDA, GSH-Px, GGT, IgG and IgM, and colostrum IgG and specific gravity. The Mann-Whitney U test was used to evaluate the difference between the winter and summer groups regarding lactation, IgG, and colostrometer variables.

All data were analyzed using the MIXED procedure of the Statistical Package for the Social Sciences version 22.0 software program (IBM Corp., Armonk, NY USA). The effect of group, day of sampling, and their interaction with the MDA, GSH-Px, GGT, IgG, and IgM parameters were analyzed using the following model with repeated measures:

$$Y_{ijk} = \mu + G_i + D_j + (G \times D)_{ij} + e_{ijk}$$

Where Y_{ijk} is the dependent variable; μ is the overall mean; G_i is the effect of the group (i = winter and summer groups); D_{ij} is the effect of day of sampling (j = -21 and 0 days or 0 and 24 hours); $(G \times D)_{ij}$ is the interaction between group i and the day of sampling j; and e_{ijk} is the residual error.

Animals within the groups were assessed as a random effect, while group, period, and day of sampling

Table 6. Correlation coefficients between MDA, GSH-Px and, IgG and specific gravity of colostrum.

Groups	Days	Parameters	IgG	Specific Gravity
Winter	-21±3	MDA	-0.738**	-0.592**
		GSH-Px	NS	NS
	0	MDA	-0.582**	-0.585**
		GSH-Px	0.615**	0.503**
Summer	-21±3	MDA	-0.586**	-0.600**
		GSH-Px	0.715**	0.567**
	0	MDA	-0.669**	-0.528**
		GSH-Px	0.618**	0.560**

* p<0.05, ** p<0.01; NS-not significant

Table 7. Correlation coefficients between MDA, GSH-Px, and calf serum GGT, IgG and IgM levels.

Groups	Parameters	Days	Hours					
			0			24		
			GGT	IgG	IgM	GGT	IgG	IgM
Winter	MDA	-21±3	NS	-0.487**	-0.619**	NS	NS	-0.396**
		0	NS	-0.481**	-0.326**	NS	-0.364*	NS
	GSH-Px	-21±3	NS	NS	NS	NS	0.375*	-0.582**
		0	NS	0.470**	0.317*	NS	0.428**	NS
Summer	MDA	-21±3	NS	-0.501**	NS	-0.567**	-0.598**	-0.446**
		0	NS	-0.582**	NS	-0.635**	-0.584**	-0.362*
	GSH-Px	-21±3	NS	0.520**	0.362*	0.468**	0.513**	0.318*
		0	NS	NS	0.400**	NS	0.326*	NS

* p<0.05, ** p<0.01; NS-not significant

and their interaction were assessed as a fixed effect. When a notable difference was revealed, any significant terms were compared by simple effect analysis with Bonferroni adjustment. A p-value of less than 0.05 was considered to be significant in all analyses.

Results

Plasma MDA levels at -21±3 and 0 days in summer cows were determined higher as compared within winter cows. Plasma MDA levels were higher at 0 days than during prepartum days (Table 3) in both groups. At -21±3 and 0 days, GSH-Px activity was higher in winter cows than summer cows. In contrast with MDA, plasma GSH-Px activity was lower at 0 days than during prepartum days (Table 3).

IgG levels and the specific gravity of colostrum were also higher in winter cows than in summer cows (Table 4, p<0.05).

Although calf serum GGT levels changed over time in both groups (p<0.05, Table 5), no significant differences were observed between the two groups at -21±3 and 0 days (p>0.05). Calf serum IgG and IgM levels also changed over time in both groups (p<0.05). Calves born in the winter had higher serum IgG levels at the 24th hour as compared with those born in the summer (p<0.05, Table 5). Serum IgM levels at the 0 and 24th hours for calves born in summer was lower versus those born in winter (Table 5, p<0.05).

The results of the correlation analysis are shown in Tables 6 and 7. MDA values at various time points were negatively correlated with serum IgG and IgM, GGT, and IgG and the specific gravity of colostrum in different calving seasons, while GSH-Px activity at various time points had positive correlations with serum IgG and IgM, GGT, and IgG and the specific gravity of colostrum.

Discussion

MDA is a product of lipid peroxidation, and MDA changes can be used as an indicator of oxidative stress. GSH-Px activity is also a major antioxidant defense component for protecting cells against increased ROS. Many studies have reported increased oxidant parameters and decreased antioxidant levels in cows at high ambient temperatures (Çolakoğlu et al. 2017, Hady et al. 2018). The results obtained in this study suggest that summer cows had higher MDA levels and lower GSH-Px activity. This is consistent with the findings of previous studies considering the interaction of antioxidative and oxidative balance with high ambient temperature. In the present study, although ambient temperatures were not as high as those reported in other studies, blood MDA and GSH-Px activity were also affected by the ambient temperature.

Similarly, blood oxidant and antioxidant balance is known to have dynamic changes during the transition period (Nakov et al. 2016). Lista et al. (2010) reported that lipoperoxide concentration increases in the last period of pregnancy and the highest concentrations are observed during the delivery. Gaal et al. (2006) indicated that no differences were found between the concentrations of precalving, calving, and postcalving free radicals and MDA. Many authors have also recorded a tendency for a transient increase of lipid peroxidation in dairy cows in the same period after calving (Mudron and Konvicna 2006, Sharma et al. 2011). Hady et al. (2018) demonstrated increased levels of MDA around calving. In our study, we did not define blood MDA levels in the lactation period but, when compared to the prepartum period, higher MDA levels associated with stress at calving increased in this period as well.

Previous results regarding blood GSH-Px activity in cows during precalving, calving, and postcalving periods are controversial. Some authors reported increased SOD and GSH-Px activity in the blood after parturition versus in the period of advanced gestation (Marseglia et al. 2015, Nakov et al. 2016). Conversely, Gaal et al. (2006) found no differences between the precalving, calving, and postcalving GSH-Px activity of cows. On the other hand, some authors have demonstrated a more significant decrease in GSH-Px and SOD levels in early lactation than during advanced pregnancy (Sharma et al. 2011). Festila et al. (2012) highlighted higher blood SOD and GSH-Px levels in cows in advanced gestation versus two stages of lactation. The same authors also reported a decrease in mean blood GSH-Px in dairy cows in the first week after calving. Costantinescu et al. (2015) reported that the highest serum activity of GSH-Px occurred during late gestation and the lowest activity occurred in early lactation. Similarly to previous results, ROS were increased immediately after calving, while GSH-Px activity started to decrease during the same in the present study. Increased blood MDA and decreased GSH-Px activity indicate that the cows had more stress around calving as compared with during the advanced pregnancy period.

The colostrum contains nutrients, cytokines, hormones and antimicrobial and growth factors. Additionally, colostrum is an antioxidant and oxidant source. Igs are the most important antimicrobial factors of colostrum. The quality of colostrum is mainly related to Ig levels. The determination of colostrum quality is important for good neonatal management and the reduction of the risk of neonatal diseases (Przybylska et al. 2007, Albera and Kankofer 2011). The quality of colostrum (IgG content) can be determined directly by laboratory techniques (RID) or indirectly by a colostrometer (specific gravity). The specific gravity of colostrum has been shown to have a high correlation with IgG concentrations (Bartier et al. 2015). Many factors such as breed, age, season, the health status of the cow, and colostrum volume are associated with colostrum quality (Godden 2008). The effect of season on colostrum quality is still controversial. Some studies have reported no effect of calving season on colostrum quality (Indra et al. 2012, Yaylak et al. 2017), while others reported lower colostrum quality in the summer than in other seasons (Morin et al. 2001, Genç and Çoban 2017). It is generally accepted that hot seasons may reduce colostrum quality (Trifković et al. 2018). In the present study, the IgG levels and specific gravity of the colostrum of the winter group were found to be significantly higher than in the colostrum of the summer group, in agreement with previous reports (Morin et al. 2001, Trifković et al. 2018). Warm environmental conditions during late pregnancy reduce colostrum quality. The effects of warm environmental conditions on colostrum quality are associated with several mechanisms including reduced dry matter intake (Das et al. 2016) and impaired mammary growth before parturition (Trifkovic et al. 2018).

The passive immune transfer can be determined by measuring serum IgG and IgM concentrations directly and serum GGT activity indirectly (Godden 2008). In the findings of the present study, serum IgG and IgM and GGT levels gradually increased in both groups from 0 h to 24 hours after calving, similar to the findings of Aydogdu and Guzelbektas (2018) as well as Çakıroglu et al. (2010). This is an indication that colostrum is sufficient for calf management and there is no absorption disorder.

Prenatal heat stress during late pregnancy alters the immune function of calves (Strong et al. 2015). Additionally, Trifkovic et al. (2018) reported that, during the summer season, dams and calves had a compromised immunity. The same authors also observed lower blood IgG during the first week of life. Some authors have suggested that the blood serum IgG levels of calves decreased with increased ambient temperature (Genç and Çoban 2017). Conversely, total IgG concentrations in colostrum were not significantly different between heat stress calves and cooling calves (Monteiro et al. 2014). The data obtained in the current study indicate that serum Ig levels in calves are influenced by seasonal variations. Summer calves at 24 hours had lower IgG levels. The calves also had lower IgM levels at 0 and 24 hours. However, there were no differences between summer and winter calves in terms of serum GGT levels. GGT is also associated with liver damage and the level of GGT is increased in diseases. The findings obtained in the current study may suggest that, while serum Ig levels are affected by seasonal temperature, GGT levels are not affected unless there is severe liver damage.

The correlation analysis revealed a statistically significant relationship between the MDA concentration of cows and colostrum quality at -21±3 days before calving and on the calving day in both groups. Additionally, a significant positive correlation was observed between GSH-Px activity of cows and colostrum quality on the same days in both groups. Colostrum quality is affected by certain factors, especially the health status of the cow (Godden 2008).

No information was available regarding the relationship between cows' MDA or GSH-Px activity and colostrum quality in previous studies. This makes it difficult to compare the results of this present study. It is possible to suggest that increased blood MDA and decreased blood GSH-P x activity are associated with a decrease in colostrum quality.

Oxidative stress may affect the immune function (Abuelo et al. 2015). Oxidative stress causes a reduction in serum IgG levels during lead intoxication (Ercal et al. 2000). The physiological increases in serum 8-isoprostane levels, a stable lipid marker of oxidative stress, have been associated with decreased serum IgG antibody in periodontal diseases. Oxidative stress changes Th cell and antigen-presenting cell responses and disrupts Th-1 cell and plasma cell functions, reducing IgG antibody responses (Singer et al. 2009, 2015). As a result of the effects of free radicals, the tertiary structures of proteins such as IgG and albumin, which have a large number of disulfide bonds, deteriorate and are unable to perform their normal functions (Freeman and Crapo 1982). Ling et al. (2018) reported that maternal metabolic stress in late gestation may adversely affect some metabolic and inflammatory responses of calves. Calves born to cows with a higher oxidative stress index during late gestation had a significantly higher serum concentration of ROS and nitrogen species and showed lower body weight at birth. Maternal exposure to stress during the late gestation period impairs the immune system in calves before calving (Tao et al. 2012). It has been observed that antioxidant activity of the colostrum is positively correlated with the serum IgG concentration of calves (Abuelo et al. 2014). However, no previous studies have assessed the relationship between maternal oxidative status and serum Ig levels in calves. The correlation analysis revealed a statistically significant relationship between the MDA concentration of cows and the serum Ig levels of their calves in both groups. Additionally, a significantly positive correlation was observed between the GSH-Px activity of cows and the serum Ig levels of their calves in both groups. These results suggest that, while maternal MDA levels contribute to suppressed serum Ig levels of calves, maternal increased GSH-Px activity is associated with high serum Ig levels of calves.

Conclusions

In conclusion, differences in plasma MDA, GSH-Px activity, calf serum IgG and IgM levels, and IgG and the specific gravity of the colostrum in both groups revealed a possible seasonal effect. Summer cows had higher oxidative stress, resulting in a negative effect on their colostrum quality and passive immune transfer to their calves. Additionally, the relationship between maternal oxidant-antioxidant enzymes, colostrum quality, and passive calf immunity also revealed that these enzymes could be used as indicators in the evaluation of calf immunity and colostrum quality.

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