



# Influence of Season and Lactation Stage on Oxidative, Haematological, and Metabolic Profile Parameters in Holstein Dairy Cows

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**Abstract** | Seasonal variations and lactation are among stressors that have a significant impact on the blood metabolites of dairy cattle. The current study aimed to compare the impact of season, different lactation stages, and their combined effect on the hematobiochemical and oxidative stress variables in Holstein dairy cows. Forty (40) multiparous Holstein dairy cows with the age range of 5-7 years were enrolled in this study. This study was conducted during the Winter (January 2020) and Summer (August 2020). Cows were divided according to production period and season into early-lactating Summer group (n=10), mid-lactating Summer group (n=10), early lactating Winter group (n=10), and mid-lactating Winter group (n=10). One blood sample was withdrawn from each cow for hematological, selected serum biochemical, and oxidative stress parameters estimation. Regarding season, in Summer, significant elevation in triglyceride and malondialdehyde (MDA) were recorded. In Winter, total protein was significantly higher. Regarding the lactation stage, early lactation was associated with a significant decrease in glucose, cholesterol, and total antioxidant capacity (TAC) and a significant increase in non-esterified fatty acids (NEFA) and MDA compared to the mid-lactating stage. Significant increases in leukocytes and lymphocytes were recorded in the Summer season compared to the Winter season regardless of the lactation stage. Season and stage of lactation have influenced some of the blood biochemical, hematological, and oxidative stress parameters. Mild heat stress might influence the metabolites in the early lactation stage.

**Keywords** | Oxidative biomarkers, NEFA, Lactation stage, Season, Cow

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## INTRODUCTION

In Egypt, the dairy cattle industry constitutes a cornerstone of the national economy. With an approximate headcount of about 4,410,000 head at 2019 census, they are the primary providers of milk and other dairy products nationally (Habeeb *et al.*, 2019). One of the foremost stressors impacting animals is lactation (Salem, 2017). The early-lactation stage is historically associated with the highest incidence of ailments, in this period, negative energy balance is expected and risk for the nutritional deficit is maximized to meet the demand for milk production (Zollitsch *et al.*, 2004). When energy

demands exceed, animals utilize the body reserves to compensate (Mulligan *et al.*, 2006), and subsequently, reduction in glucose, fat mobilization, and ketone bodies formation will ensue (Colgrave, 2017).

To assess animal metabolic profiles, estimation of blood constituents is usually performed (Štolcova *et al.*, 2020). A blood profile is a relatively cheap and essential tool for the diagnosis, treatment, and prognosis of post-parturient diseases (Ndlovu *et al.*, 2007).

The season might alter blood metabolites as photoperiod might influence the physiologic responses Ganaie *et al.*,

Temperature extremes during the summer or winter could be a significant stressor and the temperature-humidity index (THI) can determine the extent of its effect on productivity (Gantner et al., 2011). Temperature between 5-25°C is ideal for dairy cattle (Roth, 1998), the thermoregulatory mechanism will be disrupted when temperature extremes are reached (Khadjeh and Papahn, 2002; Al-Bakry, 2019).

Serum biochemistry is a simple tool to evaluate different physiologic responses. Lactation stages in dairy cattle can be divided into early, mid, and late lactation stages; the early-lactation stage is known to be associated with metabolic alterations (Cozzi et al., 2011), however, other reports showed that mid and late lactation stages have a significant impact on blood metabolites (Kim et al., 2020). Oxidative stress is a state in which free radicals and reactive oxygen species surpass the ability of the antioxidant system to scavenge them (Halliwell, 2006). When Reactive oxygen species (ROS) and other free radicals accumulate in the body, tissue damage is expected (Kumar et al., 2015). Oxidative stress has been implicated in numerous diseases affecting ruminants (Salem et al., 2016; Abdel-Saeed and Salem, 2019) and non-ruminant animals (Kubesy et al., 2017, 2020).

Recent studies proposed oxidative stress in association with transitioning cows especially multiparous ones (Yehia et al., 2020), however, this topic is still understudied in different lactation stages. The current study aimed to compare the impact of season, different lactation stages, and their combined effect on the hematobiochemical and oxidative stress variables.

## MATERIALS AND METHODS

### ETHICAL APPROVAL

The research procedures conducted in this study were approved by the Institutional Animal Care and Use Committee with the document serial number Vet-CU-01102020210, Faculty of Veterinary Medicine, Cairo University, Egypt.

### STUDY PERIOD AND LOCATION

This study was conducted in Summer (August, 2020) and Winter (January, 2020). Samples were collected from the same dairy farm located in Fayoum Governate (Egypt).

### ANIMALS

Forty (40) lactating highly producing multiparous Holstein dairy cows (28-37 kg/day, milk fat 3.45-4.31%, protein 3.13-3.6%) were enrolled in this study with the age range

of 5-7 years, weight range was 550-700 kg, and BCS of 3.75-4.5. Milking of the cows was done twice daily; the photoperiod was natural with access to water ad libitum. Animals were kept in loose shaded barns and access to the yard to allow roaming within the yard; fan and mist systems were supplied during the Summer season. The barns were divided into sections according to the production stage and milk yield.

Studied animals were allocated according to the day in milk (DIM) and season into four different groups as follows:

Group I (n=10): (14-21DIM) in summer; group II (n=10): (60-90 DIM) in summer; group III (n=10): (14-21 DIM) in winter; group IV (n=10): (60-90 DIM) in winter.

Different cows were used in each season/stage of production. Enrolled cows in the study have a similar milk production range, Body condition score (BCS) using a five-point scale, and parity. Animals were healthy.

### ENVIRONMENTAL TEMPERATURE, RELATIVE HUMIDITY (RH), AND THI

A digital hygrometer-thermometer device was daily used for measuring RH and temperature at 7:00 a.m., 1:00 p.m., and 7 p.m. daily throughout the experiment period. The THI was calculated according to the following equation (McDowell et al., 1976):  $THI = 0.8AT + RH \times (AT - 14.1) + 46.4$ , where AT is air temperature (°C), and RH is relative humidity.

### SAMPLING AND ANALYSIS

One blood sample was collected from each animal, via coccygeal vein puncture. Each sample was then divided into two portions. The first sample was collected on an EDTA tube for complete blood count (CBC) via an automated veterinary hematology analyzer. The second part was kept in a plain tube for serum separation. Serum was stored under -20°C till use. Serum was used to estimate total antioxidant capacity (TAC) and malondialdehyde (MDA) (Bio diagnostic, Egypt), cholesterol, triglycerides, total protein, albumin, and glucose (Spectrum diagnostic, Egypt) and non-esterified fatty acid (NEFA) (Randox, UK) using specific test kits.

### STATISTICAL ANALYSIS

Data represented as mean  $\pm$  SE. Comparison of data set was done using two way ANOVA, SPSS statistic program version 16.0 with a P-value of  $\leq 0.05$  considered of significant value.

### ENVIRONMENTAL TEMPERATURE (°C), RH, AND THI MEASUREMENTS

In the winter, the temperature ranged from 6 to 22°C, January monthly average environmental temperature 14°C. The humidity percentage for the same season ranged from 26 to 94%, being January's monthly average humidity of 59% and the monthly average temperature-humidity index (THI) 66. The average monthly temperature in summer (August) was 30°C (range 24-38°C), the average humidity percentage was 56% (range 17-89%), and THI 79.

### SERUM BIOCHEMISTRY AND OXIDATIVE STRESS

Season, stage, and their combined effect on serum biochemistry and oxidative stress variables were presented in Tables 1 and 2.

### EFFECT OF SEASON

Regarding season effects, significant changes were observed in total protein, triglycerides, and MDA concentrations. A significant increase in triglycerides and MDA concentrations were observed in summer compared to winter. Total protein concentration showed a significant increase in winter compared to summer as shown in Tables 1 and 2.

### EFFECT OF LACTATION STAGE

Regarding lactation stage effects, significant changes were observed in glucose, NEFA, cholesterol, MDA, and TAC concentrations. A significant decrease in cholesterol, glucose and TAC associated with a significant increase in NEFA and MDA were recorded in the early lactation stage compared to the mid-lactation stage (Tables 1 and 2).

### SEASON-STAGE COMBINED EFFECT

Regarding season stage interaction, significant interactions were observed in both glucose and triglycerides. Triglycerides showed a considerable decrease in the winter mid-lactation group when compared to the summer mid-

lactating group. Glucose showed a substantial increase in Summer and Winter mid-lactating groups compared to Summer early lactating groups as shown in Tables 1 and 2.

### HEMATOLOGIC PARAMETERS

The influences of season, lactation stage, and season-stage interaction on hematologic parameters are presented in Table 3. Regarding season, changes were observed in leukocytes and lymphocytes; a significant increase in total leukocytes and lymphocytes in both groups of the Summer season as compared to Winter but did not affect red blood cells (RBCs), Packed cell volume (PCV), Hemoglobin (HB), platelets, and neutrophils. The lactation stage and season-stage combined effect showed a non-significant effect on haematologic parameters.

### DISCUSSION

This study showed that the season and stage of lactation have an impact on some hematology, biochemistry, and oxidative stress parameters.

THI is an appropriate tool for the evaluation of heat stress in dairy cows (Soumya et al., 2016). THI can be categorized into mild (72-80), severe (80-85), and deadly stress zones (>85) (Kohli et al., 2014), this categorization reflect outside zone (Giri et al., 2017). The Summer in the current study was in the upper limits of mild stress zones. The reported values were considered comfortable to animals during winter and stressful during summer. Blood biochemical parameters of animals are influenced by seasonal differences (Massányi et al., 2009). The summer season has a more intensive impact than the winter (Al-Saeed et al., 2009).

The season influenced triglycerides, MDA, and protein regardless of the lactation stage. Triglyceride concentration was significantly higher during the Summer, and their effect together was also reported as triglyceride level was

**Table 1:** Serum biochemical parameters estimated in different lactation stages and season and their combined effect on these parameters.

Parameter/ unit	Glucose (mmol/L)		NEFA (mmol/l)		Cholesterol (mmol/L)		Triglyceride (mmol/L)		Total protein (gm/L)		Albumin(gm/L)	
	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter
Early lactation	1.61± 0.18	2.17 ± 0.16	0.70 ± 0.13	0.42 ± 0.17	3.14 ± 0.06	3.40 ± 0.35	0.42 ± 0.01	0.43 ± 0.03	46.5 ± 2.60	69.3 ± 1.40	25.6 ± 1.70	10.2 ± 0.50
Mid lactation	2.92 ± 0.17	2.45 ± 0.30	0.25 ± 0.04	0.26 ± 0.05	4.85 ± 0.33	3.47 ± 0.24	0.47 ± 0.06	0.27 ± 0.03	65.5 ± 1.80	68.5 ± 4.50	27.5 ± 0.90	11.4 ± 0.60
Season	0.71		0.225		0.231		0.033*		0.001*		0.074	
Stage	0.001*		0.014*		0.011*		0.208		0.092		0.777	
Season* stage	0.041*		0.229		0.65		0.026*		0.281		0.628	

lower in the Winter mid-lactation stage. The combined season-stage effect validates the season impact on triglyceride level, especially during the mid-lactation stage. Triglyceride tends to decline until calving and remained at the same level throughout the postpartum period in both seasons, though, it is higher in Summer compared to Winter (Moreira et al., 2015). Controversially, a lower level of triglyceride in Summer with a tendency for a decrease in triglyceride level with the advance of pregnancy was also reported (Ahmed and Abdalla, 2012).

**Table 2:** Oxidative stress parameters measured in different lactation stages and seasons and their combined effect on these parameters.

Parameter/unit	TAC (mM/L)		MDA (nmol/ml)	
	Summer	Winter	Summer	Winter
Season stage				
Early Lactation	0.71±0.16	1.30±0.03	5.18±1.01	2.45±0.30
Mid lactation	1.55±0.33	1.60±0.21	2.58±0.63	1.91±0.47
Season	0.161		0.009*	
Stage	0.022*		0.041*	
Season*Stage	0.234		0.069	

Triglycerides are lipids that circulate and utilize by the cells to create ATP (Kaneko, 2008), and NEFA. They are linked to lipid mobilization and the extent of negative energy balance (Ospina et al., 2010), along with uptake by mammary glands as precursors for milk fat formation (Bauman and Griinari, 2003). The higher concentration of triglycerides in Summer may be associated with the expected lower milk fat production in this season (Kabil et al., 2015), and the reduction of triglyceride uptake by the mammary gland with lower food availability and feed intake during this season (Dar et al., 2019).

Serum protein level elevated in winter, and this finding agreed with previous reports (Dar et al., 2019; Chandrashekhar et al., 2017). However, this finding contradicts Cozzi et al. (2011) who reported high protein levels in summer as a response to heat stress. Moreover, higher total protein concentration during summer in medium and high producing cattle was reported (Das et al., 2014).

The total protein content in blood plasma is one of the nitrogen metabolism indicators and differs during different seasons of the year (Chudoba-Drozowska, 1984). Heat stress has been proven to enhance the catabolism of amino acids for energy synthesis (Abeni et al., 2007). A mild stress zone was reported during Summer in this study, this zone might be implicated in lower total protein as previously shown in goats (Sejian et al., 2013).

The lactation stage has no apparent effect on total protein

in the current study as previously reported (Casamassima et al., 2007). However, in previous studies protein is reduced toward the late-lactation stage (Cavestany et al., 2005).

The MDA level was significantly higher during the Summer as compared to the winter regardless of the lactation stage. Lipid peroxidation was found to be more intense during the Summer in cows (Bernabucci et al., 2002; Rathwa et al., 2017) and on the exposure of animals to the sun compared to in shade (Aengwanich et al., 2011). It could be linked to heat stress, and the latter is accountable for increased production of free radicals, which leads to oxidative stress (Ghosh et al., 2013; Maan et al., 2013; Chaudhary et al., 2015). MDA is the product of cell membrane lipids oxidation (Elsayed et al., 2020), and in the face of cell membrane peroxidation, an elevation in free radicals and consequently, MDA is expected (Salem et al., 2020).

NEFA and MDA were higher in the early-lactation stage while glucose, cholesterol, and TAC were higher in the mid-lactation one, regardless of the season. Throughout the lactation stage, secretory cells of the mammary gland use up to 80% of the blood circulating metabolites for milk production (Abd-El-Naser et al., 2014). Therefore, total protein, triglycerides, and non-esterified fatty acids are vital markers for metabolic status in lactating animals (Das et al., 2016). In the present study, glucose concentration was significantly decreased during the early-lactation stage regardless of the season and was higher in the mid-lactation stage in both, summer and winter, compared to early-lactating summer. The state of hypoglycemia detected during lactation has been previously reported (Wu et al., 2019; Djokovic et al., 2019). However, De Castro Dias et al. (2017) previous study has detected no changes in glucose blood levels throughout different lactational stages. Fast uptake of glucose by the mammary gland in early lactation could be accounted for reduction (Djokovic et al., 2019; Regmi and Pande, 2018).

Cholesterol concentration was lower in the early-lactation stage, this finding agrees with previous reports (Cozzi et al., 2011; Abd-El Naser et al., 2014; Ruginosu et al., 2011). Cholesterol is influenced by pregnancy and the number of lactations (Nath et al., 2005). Cholesterol decreases immediately after calving and increases gradually throughout lactation (Ruginosu et al., 2011). The season has no apparent effect on cholesterol in the current study as previously reported (Cozzi et al., 2011). However, elevated cholesterol has been reported under heat stress (Garcia et al., 2015).

**Table 3:** hematological parameters measured in different lactation stages and season and their combined effect on these parameters.

Parameter/ unit	RBCs (10 <sup>12</sup> /L)		PCV(l/l)		HB (gm/L)		Platelet (10 <sup>9</sup> /L)		WBCs (10 <sup>9</sup> /L)		Lymphocyte%		Neutrophil%	
	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter
Early lactation	6.58 ± 0.37	6.72 ± 0.16	0.29 ± 0.01	0.28 ± 0.02	103.3 ± 3.20	100.2 ± 5.7	291.00 ± 35.69	238.25 ± 20.29	19.99 ± 1.53	14.48 ± 1.43	58.06 ± 4.61	52.66 ± 3.92	25.33 ± 1.76	24.00 ± 1.15
Mid lactation	6.54 ± 0.79	6.42 ± 0.17	0.29 ± 0.02	0.28 ± 0.03	104.0 ± 5.0	100.4 ± 5.6	268.00 ± 35.00	240.50 ± 17.85	17.23 ± 0.76	11.66 ± 1.32	63.40 ± 4.24	50.66 ± 0.66	24.93 ± 1.73	23.33 ± 1.76
Season	0.980		0.659		0.602		0.154		0.003*		0.041*		0.393	
Stage	0.651		0.926		0.946		0.677		0.082		0.66		0.751	
Season*Stage	0.724		0.830		0.971		0.670		0.986		0.353		0.937	

NEFA showed an increase in concentration in the early lactation stage, due to utilization of tissue reserves and intense mobilization of lipids to support the milk yield at this phase could be linked to this elevation (Mishra et al., 2016). Negative energy balance (NEB) occurs with an increase in fat mobilization and production of lipid peroxides (Trevisan et al., 2001). NEFA was not affected by the season in the current study. Similar findings have been reported previously (Moreira et al., 2015).

MDA level was significantly higher in the early-lactation stage than the mid-lactation stage and TAC was significantly lower in the early-lactation stage than the mid-lactation stage regardless of the season. Nearing parturition, the antioxidant defense mechanisms are reduced and therefore, oxidative stress may occur (Waller, 2000; Gitto et al., 2002). MDA was higher in 2-week prepartum till 3-week postpartum (Elshahawy and Abdullaziz, 2017) and in the first two days of lactation (Wu et al., 2019) and postpartum cows at week 3 of parturition (Yehia et al., 2020).

Decreased levels of TAC were reported in the first week of lactation (Elshahawy and Abdullaziz, 2017) and were higher at week 8 of lactation. The stress of heavy pregnancies and the start of parturition and lactation were related (Castillo et al., 2003, 2006). In another animal model, TAC showed a significant reduction in the early lactation stage compared to the late lactation stage (El-Tarabany et al., 2018).

The vital guide of physiologic alterations in the animal body is hematologic values, seasonal variation could play havoc on the animal physiological response (Giri et al., 2017). In this study, there were no recorded alterations in hematological parameters except for WBCs and lymphocytes, which agreed with other reports (Farooq et al., 2017). Seasonal impact on leucocytes and lymphocytes was noticed regardless of the lactation stage, which agreed with other reports (Wegner et al., 1976; Abdelatif and Alameen, 2012). Stress-induced elevation in leukocytes and “capture-stress” could be implicated (Sajjad et al.,

2012). Elevated release of epinephrine/corticosteroids in response to high temperature may increase TLC (Müller et al., 2019). Elevated environmental temperature contributes to neuroendocrine alterations factored by deviation in immunity (Lacetera et al., 2005). Lymphocytes were reported to elevate with higher environmental temperature (Narayan et al., 2007). The elevation in lymphocytes could be implicated in overall elevation in WBCs count (Farooq et al., 2017). The stage appears to have a minimal effect on hematological parameters. Hematological values did not differ throughout lactation in a report that dealt with goats, however, a reduction in WBCs in the early-lactation stage when compared to other stages was found (El-Tarabany et al., 2018).

## CONCLUSIONS AND RECOMMENDATIONS

The effect of season and lactation stage on hematobiochemical and oxidative stress biomarkers was strongly exhibited in the present study. The close monitoring of dairy cows during the transition phase is tremendously important to avoid metabolic problems and economic losses, especially during the Summer and early-lactation stage. Further extensive investigations are needed to pinpoint the influence of heat stress on dairy production and health and the most appropriate managemental tools to minimize these consequences.

## NOVELTY STATEMENT

The Novelty of this study is of significant value in Egypt. Studies concerning summer season effects on Holstein dairy farms in Egypt are scant, therefore extensive investigations were needed to clearly pinpoint the influence of heat stress on dairy production, health and the most appropriate managemental tools to minimize these consequences. Additionally, oxidative stress biomarkers and season-stage interactions not widely studied in the past. Further investigations were required to ovoid economic losses and metabolic diseases during this vital stage.

All authors contributed equally to the manuscript.

### CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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