

MONITORING THYROID HORMONES, SOME OF OXIDATIVE STRESS MARKERS AND BIOCHEMICAL CHANGES DURING THE EARLY AND MID STAGE OF LACTATION IN DAIRY COWS

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ABSTRACT

This study aimed to compare changes in biomarkers of nutrient metabolism, thyroid hormones and oxidative stress parameters present during the early (EL) and mid-lactation (ML) stages in dairy cows reared under the hot summer conditions at Assiut city, Upper Egypt. Blood serum of twenty nine multiparous clinically healthy lactating Holstein Fresien cows (16 early lactating and 13 mid lactating cows) belonged to a private farm located under the same temperatures and living conditions were used in this study. Cows of ML had significantly lower values ($P < 0.05$) than those of EL stage for glucose and cholesterol concentrations and also non-significantly differences in total protein, albumin and triglycerides ($P > 0.05$). Similarly, non-significance differences ($P > 0.05$) were found between the two lactation stages for thyroid hormones (Triiodothyronine, Thyroxine). For oxidants/antioxidant parameters, ML cows had significantly decreased values ($P < 0.05$) for Superoxide dismutase and significantly increased values ($P < 0.05$) for Glutathione S- Transfereas activities than those of EL as well as non-significantly differences ($P > 0.05$) for Malondialdehyde concentrations and catalase activity. Pearson's correlation coefficients between the metabolic parameters, thyroid hormones and oxidative indices in early and mid-stages of lactation showed different positive and also negative correlations. Can be concluded that this study gives evidence that the lactating cows undergo a substantial oxidative stress that related to metabolic and physiological adaptation during early lactation to mid lactation stages under the under the influence of high temperature conditions at Upper Egypt.

Key words: Thyroid hormones- Lactation- Oxidative stress- antioxidant- dairy cow.

INTRODUCTION

The lactation periods in dairy cows are especially critical and present considerable physiological challenges to homeostasis by imposing significant metabolic stressors that may contribute to the onset of diverse disorders (Castillo *et al.*, 2005). Lactation causes negative energy balance and high mobilization of lipids from body fat reserves immediately before and after parturition as well as during the first stage of lactation in dairy cows (Reist *et al.*, 2002), either in ewes leading to decline in animal productive and reproductive performance (Morgante *et al.*, 2012). Due to these metabolic stresses which severely influence animal welfare causing so called production diseases like ketosis, hypocalcaemia (Ceciliani *et al.*, 2018) or fatty liver and lipogenesis in the liver (Šamanc *et al.*, 2010) rather than imbalance in hepatic

carbohydrate (Goff and Horst, 1997). This conditions results in free radical generation exceeding than body's antioxidant production capacity and oxidative stress development (Lamp *et al.*, 2015; Koch *et al.*, 2016; Ceciliani *et al.*, 2018 and Putman *et al.*, 2018) and disrupting inflammatory and immune functions (Contreras and Sordillo, 2011). Therefore, the evaluation of blood redox homeostasis has increasingly contributed to the knowledge of the processes involved in reproductive and metabolic status of dairy herds (Bernabucci *et al.*, 2002; Castillo *et al.*, 2012).

Adaptation of the endocrine system during the lactation period, primarily the thyroid gland is the key factor in maintaining metabolic balance Since, thyroid hormones play an important role in the ketosis pathogenesis (Djokvic *et al.*, 2014), their impairment is an indicative parameter for these metabolic stresses.

Environmental conditions during hot summer in Egypt imposing direct or indirect effects on physiology, metabolism, hormonal, and immunity

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system (Marai *et al.*, 1999), hence, heat stress may be responsible for oxidative stress observed in lactating dairy cows (Nardone *et al.*, 2006) which involves changes in post absorptive lipid, carbohydrate, and amino acid utilization (Lamp *et al.*, 2015).

Therefore, the goal of this study was to document changes in biomarkers of nutrient metabolism, thyroid hormones and oxidative stresses present during the early and mid-lactation stages in dairy cows reared under the hot summer conditions in Assiut city, Upper Egypt.

MATERIALS AND METHODS

Animals:

This study was carried out on 29 multiparous cows (4-6 years) belonged to a private farm at Assiut city feeded Barseem Hegazzy, silage, dried grass and concentrate mixture. (5Kg/head/day). Water was supplied ad libitum, kept under ambient temperature range from 40°-45°. Animals were clinically healthy, with history of no metabolic or reproductive disturbances showing negative milk California Mastitis Test (CMT) APH, (1978). Where they were divided into 2 groups; the first (N=16) was in early lactation period, 20-100 days (EL) and the second (N=13) was in mid lactation period 100-150 days (ML).

Sampling:

Blood samples were collected into dry, clean centrifuge tubes without anticoagulant from jugular vein of all cows before morning feeding, left to clot at 4°C then centrifuged at 3000 rpm for 20 min to obtain clear serum and kept in clean dry 1.5 ml tubes (Eppendorf tubes) and stored at - 20 °C until used for biochemical analysis.

Biochemical analysis:

Serum proteins, glucose and lipids:

Total proteins (TP), albumin (Alb), glucose (Gluc), total cholesterol (Chol) and triglycerides (Trig) were determined by colorimetric methods using available commercial test kits after the method described by Henry *et al.* (1974).

Thyroid hormones:

Enzyme Immunoassay (EIA) was used for the quantitative determination of concentrations of Triiodothyronine (T3), and Thyroxine (T4) in blood serum using commercial test kits according to Wistom (1976) and manufacture instructions.

Malondialdehyde (MDA):

MDA levels were determined by colorimetric method according to Ohkawa *et al.* (1979), based on thiobarbuturic acid (TBA) reactivity.

Superoxide dismutase (SOD):

Concentration of (SOD) was measured according to Marklund and Marklund (1974). Briefly, 50µL of serum, 75mM of tris-HCL buffer, 30 mM of EDTA and 2mM of pyrogallol were added and absorbance waves were recorded at 420nm for 30 min. The activity of SOD is expressed as U/ml of serum.

Catalase activity (CAT)

The (CAT) activity was measured in serum by the method of Beers and Sizer (1952). Decomposition of H₂O₂ was followed directly by the decrease in absorbance at 240 nm, and the difference in absorbance per min was taken as a measure of the CAT activity. CAT activity was expressed as U/L of serum.

Glutathion S- transferases (GST):

The of activity (GST) was measured according to the method of (Habig *et al.*, 1974).

Statistical analysis:

The packaged SPSS program for windows version 10.0.1 (SPSS, Chicago, IL, USA) was used for statistical analysis according to SPSS (1999). Data were analyzed using one-way analysis of variance (ANOVA) and expressed as mean ± standard error (SE). Differences between groups were determined by means of Student's *t*-test. Pearson's correlation (*r*) was performed on the paired data obtained by the individual cases. Significance level was set at P<0.05.

RESULTS

Mean values (±SE) of the biochemical parameters are shown in Table 1. Significance differences was shown between two stages of lactation, where the mid lactation stage had a lower values than the early lactation stage for glucose and total cholesterol concentrations (P<0.05). Non-statistical significance differences (P>0.05) were found between the two lactation stages for total protein, albumin and triglycerides.

Similarly, non-significance differences (P>0.05) were found between the two lactation stages for thyroid hormone (T3 and T4) as shown in table 2.

Mean values (±SE) of oxidants and antioxidant parameters are shown in Table 3. A significance differences was shown between two stages of lactation, where the mid lactation stage had a lower values (P<0.05) for SOD and higher values (P< 0.05) for GST activities than the early lactation stage. Non-significance differences (P>0.05) were found between the two lactation stages for MDA concentrations and catalase activity.

Pearson's correlation coefficient between all parameter measured in early stage of lactation. Were summarized in (Table 4).

Pearson's correlation coefficient between all parameter measured in mid stage of lactation. Were summarized in (Table 5).

Pearson's correlation coefficients at confidence interval 95% between the metabolic parameters, thyroid hormones and oxidative indices in early and mid-stages of lactation illustrated different positive and also negative correlations table 4, 5.

Table 1: Mean values of some biochemical parameters during two different stages of lactation.

Parameters	Early lactation	Mid lactation	P value
Total Proteins (g/dl)	9.151 ± 0.398	8.444 ± 0.468	0.2571
Albumin (g/dl)	3.358 ± 0.171	3.103 ± 0.278	0.4230
Glucose (mg/dl)	35.19 ± 2.602	27.18 ± 2.402	0.0350*
Cholesterol (mg/dl)	147.0 ± 10.96	112.7 ± 11.00	0.0373*
Triglycerides (mg/dl)	27.54 ± 1.704	23.37 ± 1.674	0.0964

Data are expressed as mean±SE of 29 samples
Statistical significant value= (P<0.05)

Table 2: Mean values of thyroid hormones during two different stages of lactation.

Parameters	Early stage	Mild stage	P value
T3 (ng/dl)	80.48 ± 5.108	83.20 ± 5.152	0.7136
T4 (µg/dl)	6.388 ± 0.526	6.746 ± 0.610	0.6584

Data are expressed as mean±SE of 29 samples
Statistical significant value= (P<0.05).

Table 3: Mean values of oxidant /antioxidant parameters during two different stage of lactation.

Parameters	Early lactation	Mid lactation	P value
MDA (nmol/mL)	16.60 ± 1.550	14.95 ± 2.211	0.5347
SOD (U/L)	82.99 ± 3.021	67.89 ± 5.174	0.0138*
CAT (U/L)	334.2 ± 53.17	279.5 ± 39.67	0.4357
GST (U/L)	1.536 ± 0.278	2.471 ± 0.283	0.0270*

Data are expressed as mean±SE of 29 samples
Statistical significant value= (P<0.05).

Table 4: Pearson's correlation coefficients for the all determining parameters in early stage of lactation.

	Alb	Gluc	Chol	Trig	T3	T4	MDA	SOD	CAT	GST
TP	0.445	0.522	-0.282	0.162	0.195	-0.105	0.310	-0.211	-0.09	-0.030
Alb		0.234	-0.025	0.344	0.171	0.120	0.214	-0.571	-0.456	-0.045
Gluc			0.295	0.487	0.123	-0.026	0.459	-0.108	0.352	0.224
Chol				0.169	-0.041	-0.217	0.113	-0.030	0.553	0.102
Trig					0.196	0.475	0.483	-0.083	0.425	0.134
T3						-0.087	0.226	-0.260	0.021	0.095
T4							-0.225	0.166	0.078	-0.395
MDA								0.033	0.256	0.468
SOD									0.278	0.104
CAT										0.272

Pearson's correlation coefficients at confidence interval 95%

Table 5: Pearson's correlation coefficients for the all determining parameters in mid stage of lactation

	ALB	Gluc	Chol	Trig	T3	T4	MDA	SOD	CAT	GST
TP	0.578	0.027	0.599	-0.170	0.306	0.363	-0.057	0.054	-0.277	0.241
ALB		0.128	0.447	0.138	0.261	0.105	-0.270	-0.036	-0.150	0.390
Gluc			0.359	0.136	0.073	0.381	-0.172	0.227	-0.318	0.272
Chol				-0.37	0.257	0.291	-0.239	0.126	-0.245	0.196
Trig					0.364	0.236	0.537	0.207	0.249	-0.031
T3						0.666	0.303	0.303	0.384	-0.255
T4							0.032	0.445	-0.111	-0.294
MDA								-0.116	0.607	-0.097
SOD									0.105	0.057
CAT										-0.19

Pearson's correlation coefficients at confidence interval 95%

DISCUSSION

This study highlighted the changes of metabolic, thyroid hormones and oxidative stress biomarkers between the early and mid-lactation period in dairy cows reared under summer high ambient temperature conditions. The present study indicated also that the metabolic and thyroid hormone indices are linked to changes of pro-oxidative and anti-oxidative parameters during the early and mid-lactation periods.

Significance differences in the biochemical parameters were shown between the two stages of lactation, where the mid lactation stage had a lower values than the early lactation stage for glucose and cholesterol concentrations. The changes in glucose level during the two stages of lactation might be explained by different factors such as reduced energy intake, because of the reduction in dry matter intake and increased thermoregulation and the negative effect of the heat on gluconeogenesis, as an endocrine acclimation to hot conditions. Lower values of glucose in cows in hot conditions were also observed by Ronchi *et al.* (2000), where they found a reduction of liver activity, so that the decrease in glucose concentration was explained by the negative effects of that reduction on gluconeogenesis. In addition decreased blood glucose levels may be attributed to intake of low energy diet (Bremmer *et al.*, 2000), when high rate of glucose utilization in the mammary gland is required (Nazifi *et al.*, 2008). But in our study.

Total cholesterol level in present study was higher in the early lactation than mid lactation stage which nearly the results of Piccione *et al.* (2009), where changes in lipid metabolism were found during pregnancy and lactation in most mammals (Roche *et al.*, 2009). Conversely, triglycerides show non-significant $p > 0.05$ change between two lactation

stages, where endocrine profile changes, lipolysis and lipogenesis are regulated to increase lipid reserve during pregnancy, and subsequently, these reserves are utilized following parturition and the initiation of lactation (Roche *et al.*, 2009 and Nazifi *et al.*, 2002).

The changes in glucose and cholesterol levels are not associated with significant changes in serum total proteins and albumin, which may reflect the consistency of hepatic effort during this period and adaptation of the studied cows to their nutritional environment. A similar finding was reported in a previous study (Castillo *et al.*, 2005).

Thyroid hormones (T3, T4) provide a major mechanism that important to acclimation for lactation. Thyroid hormones had direct and indirect effects on most metabolic processes in the body and they needed to maintain metabolic rate and to modulate oxygen consumption and mitochondrial dynamics in all cells of the body (Berghout and Weirsinga, 1998). The current study recorded non-significant changes in T3 and T4 level between the early and mid-stage of lactation. The absence of variations in concentrations of these hormones may be associated with metabolic adaptation of lactating cows to lactation stress and especially under warmed climate in an attempt to reduce endogenous heat production (Kaneko, 1997).

Serum levels of total proteins, albumin, glucose, total cholesterol, and triglycerides are indicators of hepatic function (Djoković *et al.*, 2011). Positive correlations were found between the some bio-energetic parameters levels in early and mid lactation stages may indicates that fat infiltration of the liver could develop in early or mid lactating cows. Possible changes in the liver function may have a harmful effect on their metabolism and a negative effect on milk production or reproduction

by an increase of total serum protein during the lactation (Chamberlin *et al.*, 2013).

With progress in lactation, high levels of dietary starch has been shown to induce oxidative stress in dairy cows, possibly due to cellular changes related to oxidative phosphorylation (Gabai *et al.*, 2004). These situations may induce no clinical signs, but may consume energy and protein for production of immune responses, scavenging of free radicals and decrease the nutrient availability for production purposes, impairing the performance of the animal for the whole lactation period (Sordillo and Aitken, 2009).

Under physiological conditions, oxidants are neutralized by the antioxidant system (Omidi *et al.*, 2017). Imbalance between increased oxidants production and reduced capacity of antioxidant system induces oxidative stress (Castillo *et al.*, 2005, Gong and Xiao., 2016). Oxidative stress and antioxidant status depend on the stage of lactation, seasonal variations (Pilarczyk *et al.*, 2012), and disease (Omidi *et al.*, 2017). It has also been reported that heat stress, depending on the climatic changes, induces the free radicals production and reduction in plasma antioxidant activity (Megahed *et al.*, 2008).

The present study revealed no significant different ($p>0.05$) between two stages in mean value of serum MDA, however the observed note is higher than reported by others in clinically healthy lactating cows This coincide with Mohebbi-Fani *et al.* (2016), where they observed that the levels of MDA were not different between farms and among different lactation stages. These results are nearly compatible with Colakoglu *et al.* (2017), recorded high level of MDA in summer group compared to winter group cows. In addition Yattoo *et al.* (2014) observed significantly elevated levels of oxidative stress indices in lactating animals under different seasons than non-lactating animals indicating production stress in addition to climatic stress in lactating animals thus climate change and demand for milk production predisposes animals to oxidative stress.

The current study, proved statistically significant increase in SOD at early stage of lactation in contrast with Cigliano *et al.* (2014) observed higher level of concentration in mid lactation cow than early lactation.

The SOD initiates the antioxidant process, transforming ROS into hydrogen peroxide, which is neutralized by catalase, further it is to be considered that SOD enzyme is transient in its action and actively shows a spurt on spontaneous induction of stress which may possible until other homeostasis enzyme have removed the association ROS and vice versa (Petersen and Enghild, 2005). In the current

study, there were non-significant changes in catalase activity between the two groups, which agree with the results of Sharma *et al.* (2011). However, we noticed significant differences between the mean values of SOD in the two stages of lactation cows. Higher serum SOD activity in early lactation might be due to physiological upgrading of this enzyme in an attempt to neutralize/mitigation of superoxide radical challenges and adaptation of animals to oxidative stress in an attempt to improve the antioxidant status (Anil and Meenaxi, 2018).

Glutathione S- transferases (GST) are selenium-independent enzymes that also utilize GSH for the reduction of different electrophilic substrates such as hydroperoxides or lipid peroxides (Wilce and Parker, 1994). This mechanism may be involved in the detoxification of ROS (Scha"ffer *et al.*, 1988). The level of GST can be regarded as a marker of cell vulnerability to stress factors (Nørgaard *et al.*, 2008). In the current study, early lactation cows had lower GST levels than mid lactation cows. This result may adjusted to the fact that GST is consumed in early lactation because it is used to increase lipid mobilization from the adipose tissue, and increases blood non steroid fatty acid (NEFA) and beta-hydroxybutyrate (BHB) in early lactation cows (Jindal and Ludri, 1994).

The possibility that metabolic activity may determine oxidant status is supported by the detected correlations depending on the physiological condition. Correlations between most metabolites are dependent on energy balance (Castillo *et al.*, 2005). The positive correlation between circulating thyroid hormones concentrations and metabolic variables is well known in cattle and provide some indication of postpartum disease risk and can be useful as a herd monitoring tool (Đoković *et al.*, 2016). The positive correlations between thyroid hormones and some metabolites during mid lactation as indicator of energy coincide with Capuco *et al.* (2001) and Cassar-Malek *et al.* (2001), but incompatible with Nixon *et al.* (1988), where they mentioned negative correlation. This is supported by the fact that classifying cows according to metabolites levels can be of considerable help in detecting cows that would show certain metabolic changes in early lactation or develop a metabolic disease and, thus, produce less milk (Ospina *et al.*, 2010, Cincović *et al.*, 2012), due to poor adaptation to homeorhetic processes. Correlations among thyroid hormones and biochemical parameters suggest a relationship between thyroid hormones and predictors of lipomobilization. Monitoring of thyroid hormones, especially T3, could represent an important tool to evaluate the metabolism adaptation in dairy cows and in order to understand when regulatory mechanisms break through the physiological limits predisposing the cows to metabolic problems (Fiore *et al.*, 2017).

Liperoxide concentrations showed positive correlations with lipid metabolism indicators. The increase in the oxidative metabolism implies peroxidation of fatty acids leading to formation of lipid peroxides (Kankofer, 2001). The positive correlations detected between MDA and the metabolic parameters indicate that these metabolites are implicated in the formation of oxidative stress during the early lactation period. This is supported by the negative correlations between the metabolic activity and the antioxidant parameters during the same period. Similar results were obtained by Castillo *et al.* (2005) and Boudjellaba *et al.* (2018) who found correlations between the metabolic indices and the oxidant/antioxidant markers (MDA, SOD, CAT and GST).

As a conclusion, this study gives evidence that the lactating cows undergo a substantial oxidative stress that related to metabolic and physiological adaptation during early lactation to mid lactation stages under the hot summer conditions at Assiut city, Upper Egypt. And further investigation must be done to know how the stage of lactation responds dairy cows to heat stress and how the first change is occurring.

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رصد هرمونات الغدة الدرقية، بعض ضغوط الأوكسدة والتغيرات البيوكيميائية خلال مرحلة الحليب الأولى والوسطى في الأبقار الحلابة

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هدفت هذه الدراسة إلى مقارنة التغيرات في المؤشرات الحيوية لعملية التمثيل الغذائي وهرمونات الغدة الدرقية والإجهاد التأكسدي الذي يحدث خلال المرحلة المبكرة والمتوسطة من الحليب في أبقار الألبان التي تربي تحت ظروف الصيف الحارة بمدينة أسيوط ، صعيد مصر. في هذه الدراسة تم استخدام مصل دم عدد ٢٩ من الأبقار هولشتاين فريزين متعددة الولادات وسليمة ظاهريا (١٦ بقرة حلابة في وقت مبكر و ١٣ بقرة حلابة في المرحلة المتوسطة) وتابعة لمزرعة خاصة وكانت تقع تحت نفس درجات الحرارة وظروف المعيشة. أظهرت الدراسة بعض الفروق بين الأبقار في مرحلتي الحليب للمؤشرات البيوكيميائية ، حيث كانت مرحلة منتصف الحليب لها قيم أقل من المرحلة المبكرة لكل من تركيز الجلوكوز والكوليسترول ($P < 0.05$). وكانت هناك فروق غير معنوية ($P > 0.05$) بين مرحلتين الحليب للبروتين الكلي والألبومين والدهون الثلاثية. وبالمثل لم يكن هناك فروق معنوية ($P > 0.05$) بين المرحلتين لتركيز هرمونات الغدة الدرقية. وبالنسبة لعلامات الإجهاد التأكسدي كانت مرحلة منتصف فترة الحليب تحتوي على قيم أقل ($P < 0.05$) بالنسبة لنشاط سوبر اوكسيد ديسميوتيز والقيم عليا ($P < 0.05$) لنشاط الجلوتاثيون اس- ترانسفيريز مقارنة بمرحلة الحليب المبكرة ولم تظهر الدراسة فروق معنوية ($P > 0.05$) بين المرحلتين لتركيزات المالنوالدهيد ونشاط الكتاليز. أظهرت معاملات ارتباط بيرسون بين مؤشرات التمثيل الغذائي وهرمونات الغدة الدرقية ومؤشرات الأوكسدة في المراحل المبكرة والمتوسطة من الحليب ارتباطات إيجابية وايضا سلبية مختلفة. يمكن أن نستنتج أن هذه الدراسة تعطي دليلاً على أن الأبقار الحلابة تتعرض لإجهاد تأكسدي كبير يتعلق بالتكيف الأيضي والفسولوجي خلال فترة الحليب المبكرة إلى المتوسطة تحت تأثير ظروف درجات الحرارة المرتفعة في صعيد مصر.