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Evaluation of Redox Status, Energy Metabolites, and Immune-inflammatory Status in Dairy Cows at the Close-up Stage

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Abstract

The aim of this study was to elucidate current perspectives of redox status in dairy cows and their effects on energy metabolites and immune-inflammatory status during the close-up period. The study was conducted on dairy cows at various stages of lactation, between November 2019 and January 2020. An observational study was conducted on 36 cows, at ~ 4 weeks (28 day±2 day; means±SD) before the expected time of calving. Cows were proved healthy on both clinical and laboratory examinations. A blood sample was drawn from each cow on the 28th day before the expected time of calving to quantify selected biochemical variables. The studied cows were allocated into two groups based on serum oxidative stress index (OSi) concentrations, the first group included twenty-seven cows with high values of OSi, while the second group included nine cows with low values of OSi and served as negative controls. All cows were clinically healthy and showed no signs of illness throughout the study period. Cows with high OSi had higher serum levels of advanced oxidation protein products, reactive oxygen species (ROS), malondialdehyde, superoxide dismutase, non-esterified fatty acids, β-hydroxy butyric acid, glucose, serum amyloid A, haptoglobin, and immunoglobulin G than negative controls; while had low levels of serum antioxidant capacity (SAC), glutathione peroxidase, vitamin E, and insulin. The results herein confirmed that cows during the close-up period were likely under oxidative stress, and the latter makes cows vulnerable to the development of negative energy balance and significant immune-metabolic alterations. The results of OSi, ROS, and SAC could be used as reference variables to assess the redox status of transition dairy cattle.

KEYWORDS
Oxidative stress, Dairy cattle, Transition period, Advanced oxidation protein products

INTRODUCTION

The transition period, which lasts three weeks surrounding calving and is seen as a crucial period for the entire lactation cycle (Balamurugan *et al*., 2019; Szczubiał, 2020; Tsuchiya *et al*., 2020), is regarded as a challenging stage in the life of dairy cows. It is linked to an increased prevalence of a number of diseases as milk fever, mastitis, metritis, ketosis, and retained placenta due to abrupt changes in metabolic and immunological functions (Contreras and Sordillo, 2011). A number of coordinated metabolic and endocrine changes are also present as a result of the increased dietary requirements to promote milk production (Abuelo *et al*., 2015; Elischer *et al*., 2015).

The great majority of illness occurs during the first month of lactation. These ailments can last for a long time and cause large output losses for dairy farms (Mavangira and Sordillo, 2018). Oxidative stress (OS), which refers to the effects of oxidative stressors, is undoubtedly one of the less well-defined and misused terms used in biochemistry and cellular physiology (Valacchi *et al*., 2018). In bovine medicine, OS is quickly rising to the top of the list of essential research areas (Celi, 2011). Oxidative stress can occur as results of failure of maintaining the physiological redox steady state, which is a self-correcting physiological response to various stimuli (Ursini *et al*., 2016). As a result of this activating catabolic pathways, reactive oxygen species (ROS) production can increase at the cellular level (Surai *et al*., 2019).

Recently, there has been growing evidence that OS may play an important role in the initiation, progression, and maintenance of a variety of conditions, particularly in dairy cows during transitional periods (Abuelo *et al*., 2013; 2015). The energy status of perinatal dairy cows is linked to oxidative stress. This is due to the fact that the perinatal negative energy balance (NEB) affects various white blood cells and proteins that are important in fighting infections and disease (Elischer *et al*., 2015).

Cows adapt well to NEB when the release of non-esterified fatty acids (NEFAs) is limited and fully metabolized for energy needs, so lipid mobilization is a common feature of the condition (Sordillo and Raphael, 2013). Free radicals are normally produced

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in mitochondria during metabolic processes as a byproduct of cellular respiration in the electron transfer chain reaction. When NEFA formation exceeds hepatocyte processing capacity as a result of excessive lipid mobilization, liver function is compromised, resulting in fatty liver and ketone body overproduction (Sordillo and Raphael, 2013; Abuelo *et al*., 2015). Oxidative stress occurs when free radicals exceed the balancing effects of antioxidants (Abuelo *et al*., 2015).

Recently, the term 'oxInflammation' has been proposed to describe the interaction of inflammatory markers and the oxidative stress index. The cause of the inflammatory state is not clinically obvious in this case, but it does not appear to be infection or tissue damage (Valacchi *et al*., 2018; Gabai *et al*., 2019). This controlled inflammation may serve several functions, including labor facilitation, homeostasis, and adaptation to lactation initiation (Abuelo and Hernández, 2019). On the other side, widespread and poorly controlled inflammation can predispose transitional cattle to underlying metabolic and infectious diseases (Sordillo and Raphael, 2013). Therefore, it is worth investigating the potential link between oxidative stress, inflammation, and metabolic disturbances in dairy cows during the close up period. We hypothesized that cows may experience metabolic and oxidative stress as well as fluctuations in immune defense mechanisms during the observation period.

MATERIALS AND METHODS

Animal population

The current study was conducted on a commercial dairy herd in Dakahlia governorate, Egypt, from November 2019 to January 2020, with a stock population of 150 Holstein-Friesian cows. Cows on the farm were seemingly healthy, with an average of 305 days of normalized milk production; weighed (350 Kg±50) and profitability played an important role in farm decisions. The animals were multiparous, artificially inseminated after synchronization, milked twice a day. Cows had also a range of body condition score of 3-3.5 according to Edmonson *et al*. (1989). All cows were kept under identical conditions throughout the study period and were dried off 60 days before the expected date of calving.

All procedures were followed in accordance with the Guidelines for the Care and Use of Livestock in Research and Education, 3rd Edition (http://www.fass.org) and from Mansoura University Animal Care, approved by the Ethics Committee (Code number: 28910261500886/2016). The farm owner was asked to sign consent for agreeing to the proposed testing and treatment protocol and was given a document containing information about the significance of the research study.

Criteria for animal selection and inclusion

On the farm, there were ninety cows in various stages of lactation. An observational study was conducted on 36 cows, at ~ 4 weeks (28 day±2 day; means±SD) before the expected time of calving. The cows were seemingly healthy and were clinically examined using standard procedures (Radostits *et al*., 2000). All animals were monitored for potential health disorders throughout the study period. The transition cows had free access to water and were fed a daily total mixed ration.

Cows that have high values of ROS, and oxidative stress index (OSi) and low values of SAC were considered to have OS status, while those having low ROS and low OSi but having high SAC were considered negative controls. Cows were included in the study in the following conditions (1) if they were clinically healthy with no current or previous metabolic disorders, (2) if they did not receive any medications before their first testing, and (3) if their previous gestation period was> 260 days.

Blood samples

Ten milliliters of venous blood were drained from each cow via coccygeal venipuncture on the day 28th before the expected time of calving and were added to plain tubes (no anticoagulants) and tubes containing EDTA to yield serum and whole blood, respectively. Blood was rapidly cooled on crushed ice and transported to the laboratory, where it was centrifuged at 1400 x g for 10 minutes to separate serum. Only clear, non-hemolyzed sera were collected and aliquoted for quantification of selected biochemical variables according to manufacturer's instructions.

Commercial kits were used to measure ROS (Flurometeric assay kits, Elabscience, China, Cat. No: E-BC-K138-F), SAC (Colormeteric, Biodiagnostic, Egypt, Cat. No: TA 2513), Beta-hydroxybutyric acid (BHBA) (Pointe Scientific, INC, USA, Cat. No: H758758), NEFA (DIA Lab, ACOD-PAP, Australia, Cat. No: D07940), glucose (Biodiagnostic, Egypt, Cat. No: GP2524), insulin (Enzo, insulin ELISA Kits, New York, USA, Cat. No: ENZ-KIT141-0001), haptoglobin (Hp) (Antibodies, ELISA Kits, Germany, Cat. No: ABIN6574215), serum amyloid A (SAA) (Antibodies, ELISA Kits, Germany, Cat. No: ABIN5658746), aspartate amino transferase (AST) (ELI-Tech group solution, France, Cat. No: 19-0125), γ-glutamyl transferase (GGT) (ELI-Tech group solution, France, Cat. No: 6003-200); malondialdehyde (MDA) (Colormeteric, Biodiagnostic, Egypt, Cat. No: MD 2529), advanced oxidation protein products (AOPP) (Bioassay technology laboratory ELISA test, Shanghai Korain, Cat. No: E1266Hu), glutathione peroxidase (GPx) (Colormeteric, Biodiagnostic, Egypt, Cat. No: GP2524), superoxide dismutase (SOD) (Colormeteric, Biodiagnostic, Egypt, Cat. No: SD2520), Vitamin E (Bioassay technology laboratory ELISA test, Shanghai Korain, Cat. No: E1547Hu), and immunoglobulin G (IgG) (Ray Biotech, USA, ELISA, Cat. No: ELH-IGG-1). The oxidative stress index (OSi) was calculated by dividing ROS by the serum antioxidant capacity (SAC) (Abuelo *et al*., 2016).

Feed analysis

The ingredients of the total mixed ration were presented in Table 1 and were chemically analyzed using a commercial method described by the northeast DHI cooperative forage testing laboratory (Ithaca, NY, USA) (Table 2). Ration was adjusted accordingly to maintain the consistent nutrient levels (Erickson *et al*., 1992).

Table 1. The ingredients of the total mixed ration used in the farm.

Items	Per ton
Yellow corn	315 Kg
Soya	125 Kg
Wheat bran	80 Kg
Calcium bicarbonate	8 Kg
SodiumChloride	4 Kg
CalciumChloride	6 Kg
Salts	3 Kg
Multivitamins	1 Kg
Yeast	1 Kg
Biological antitoxic	300 g
Silica antitoxic	1 Kg

Table 2. Feed analysis including the protein (%), fat (%), energy (%) and the values of calcium and phosphorus.

Ingredients	Percentage
Protein	14.6
Fat	2.3
Ration energy	70.5
Calcium	0.58
Phosphorus	0.39

Statistical analysis

Data were statistically analysed using Statistical Software Program (SPSS, version 17, USA). Data were tested for normality by using Shapiro–Wilk test. Since the data were normally distributed, student t test was used. Means and standard deviation for each variable were calculated. At $p < 0.05$, results were considered statistically significant. Pearson correlation was also applied to emphasize the potential correlation among the tested variables. Correlation coefficient (r) and p value were calculated.

RESULTS

Clinically, all investigated cows showed no detectable clinical findings during the initial screening and appeared clinically sound throughout the study period. Biochemically, serum ROS concentrations and calculated OSi were higher in the positive group compared to the negative controls but did not differ significantly ($p = 0.055$, and $p = 0.104$, respectively), whereas SAC levels moved in the opposite direction with no significant difference between the two groups ($p = 0.181$) (Table 3).

Table 3. Reactive oxygen species (ROS) (U carr), serum antioxidant capacity (SAC) (µmolHClO/mL) and oxidative stress index (OSi) in dairy cattle with oxidative stress compared with negative controls during the close-up period.

Variables / Groups	Negative control (n=9)	Positive group (n=27)	P- value
ROS (U carr)	55.11±12.91	104.51±29.67	0.06
SAC (µmolHClO/mL)	356.71±124.37	247.95±78.85	0.18
OSi	0.168±0.064	0.46±0.20	0.10

Data are presented as Mean values±SD.
ROS: reactive oxygen capacity; SAC: serum antioxidant capacity; OSi: oxidative stress index

Serum MDA levels were significantly (p-value= 0.000) higher in the positive group compared to the negative controls. However, serum AOPP, SOD, GPx, and vitamin E concentrations did not differ significantly between the two groups (p-value= 0.565, 0.108, 0.070, and 0.971, respectively) (Table 4).

Table 4. Serum malondialdehyde (nmol/ml); advanced oxidation protein products (ng/ml); superoxide dismutase (U/ml), glutathione peroxidase (mu/ml) and vitamin E (nmol/ml) in dairy cattle with oxidative stress compared with negative control during the close-up period.

Variables / Groups	Negative control (n=9)	Positive group (n=27)	P- value
MDA (nmol/ml)	29.03±1.22	44.5±9.43	0.000**
AOPP (ng/ml)	15.90±5.58	17.15±8.1	0.57
SOD (U/ml)	243.29±48	392.68±89.92	0.11
GPx (mu/ml)	29.97±2.45	20.74±4.5	0.07
Vitamin E (nmol/ml)	44.17±12.13	42.37±11.42	0.97

Data are presented as Mean values±SD.
MDA: malondialdehyde; AOPP: advanced oxidation protein products; SOD: superoxide dismutase; GPx: glutathione peroxidase, **: p-value is significant at the level ≤ 0.010

Serum NEFA and SAA concentrations were significantly higher in cattle with OS compared to controls (p-value =0.006; p-value = 0.016). Nonetheless, there was no significant difference in blood glucose, insulin, BHBA, Hp or IgG levels between the two groups (p-values = 0.584, 0.492, 0.138, 0.532, 0.116, respectively). On the other side, serum levels of AST, and GGT were significantly elevated in the positive cows compared with negative controls (p-value= 0.017, 0.008, respectively) (Tables 5, 6).

Table 5. Glucose (mmol/l), insulin (µlU/ml), non-esterified fatty acid (mmol/l), β-hydroxy butyric acid in dairy cattle with oxidative stress compared with negative control during the close-up period.

Variables / Groups	Negative control (n=9)	Positive group (n=27)	P- value
Glucose (mmol/l)	3.35±0.41	4.33±0.39	0.58
Insulin (µlU/ml)	3.23±0.47	2.40±0.58	0.49
NEFA (mmol/l)	0.32±0.08	0.69±0.23	0.006**
BHBA (mmol/l)	0.75±0.19	1.12±0.27	0.14

Data are presented as Mean values±SD.
NEFA: Non-esterified fatty acid; BHBA: β-hydroxy butyric acid; **: p-value is significant at the level ≤ 0.010

Table 6. Serum amyloid A (SAA) (µg/ml); haptoglobin (µg/ml), immunoglobulin G (mg/ml)AST (U/L) and GGT (U/L)in dairy cattle with oxidative stress compared with those of negative control during the close-up period

Variables / Groups	Negative control (n=9)	Positive group (n=27)	*P*- value
SAA (µg/ml)	3.13±4.05	15.96±12.95	0.016*
Hp (µg/ml)	23.11±3.88	19.51±4.79	0.53
IgG (mg/ml)	3.07±0.67	5.31±1.55	0.12
AST (U/L)	50.55±8.08	90.18±21.81	0.017*
GGT (U/L)	14.77±4.02	30.92±9.62	0.008**

Data are presented as Mean values±SD.
SAA: Serum amyloid A, Hp: Haptoglobin; IgG: Immunoglobulin G; AST: aspartate aminotransferase; GGT: γ-glutamyl transferase. *: p-value is significant at the level ≤ 0.050

OSi was positively correlated with ROS (r = 0.824; p-value < 0.01), MDA (r=0.498; p-value < 0.01), SOD (r=0.480, p-value < 0.01), SAA (r= 0.493; p-value < 0.01), NEFA (r= 0.433; p-value < 0.01), BHBA (r=0.535; p-value < 0.01), glucose (r= 0.514; p-value < 0.01), IgG (r= 0.480; p-value < 0.01), AST (r= 0.432; p-value < 0.01), GGT (r= 0.493; p-value < 0.01), and negatively correlated with SAC (r = -0.79; p-value < 0.01) and GPx (r =-0.408, p-value < 0.01).

DISCUSSION

To date, only a few studies have reported the reference intervals for OSi, ROS, and SAC in Egyptian dairy farms during the transition period, while most studies have used traditional variables for evaluating OS indices such as MDA, SOD, CAT, GPx, and SAC (MOSTAFA *et al*., 2007; Ahmed *et al*., 2009; Ghanem and Abdel-Hamid, 2010; Hady *et al*., 2018; El-Sharawy *et al*., 2019; El-sayed *et al*., 2019).

Some authors claimed that crossbred cows and buffaloes could exhibit OS due to heat stress during the summer season (Hady *et al*., 2018), but the authors evaluated OS in their study using conventional biomarkers such as MDA, GPx, SOD, and CAT. In comparison to our findings, those authors reported low MDA levels and high SOD activity four weeks post calving. However, the data reported by others, particularly for MDA and SOD, were consistent with our findings (Ghanem and Abdel-Hamid, 2010; El-Sharawy *et al*., 2019). In general, the actual data on Egyptian dairy farms in terms of ROS, SAC, and OSi are still limited, and

require additional research.

Our findings revealed a positive correlation between ROS and OSi and a negative relationship between OSi and SAC. This could be explained by an imbalance between ROS production and antioxidant capacity, or when ROS are produced faster than antioxidant defense can safely counteract them (Celi, 2011; Abd Ellah, 2016; Abuelo *et al*., 2016). It has previously been demonstrated that ROS and SAC did not differ significantly between control and OS cows during the pre-partum period (Abuelo *et al*., 2013). Another recent study found no difference in OS indices in healthy Holstein cows during the dry period (Putman *et al*., 2018). The increased oxygen requirements to meet high metabolic demands are likely to generate ROS, leading to the development of OS.

Oxidative stress index could be used to improve the accuracy of OS when compared with the use of ROS and SAC as individual variables (Abuelo *et al*., 2016). Nonetheless, Invernizzi *et al*. (2019) have found that the three measurable markers (ROS, SAC, and OSi), were capable of detecting OS in dairy cattle around the time of calving and should be used in tandem rather than separately to assess the redox status in transition dairy cattle (Invernizzi *et al*., 2019). Our measurable markers differed slightly from those of Italian, Greek, and Spanish farms (Invernizzi *et al*., 2019), as well as those of Spanish farms (Abuelo *et al*., 2013), particularly SAC values, which were lower in our study than those of the three farms, and ROS values, which were nearly similar to those of the Greek farms but lower than those of the Spanish and Italian farms. The OSi, on the other hand, were closely related to Italian farms and were higher than Greek farms but lower than Spanish farms.

Cows with OS had high serum levels of MDA, AOPP, NEFA, BHBA, glucose, and SAA and low insulin concentrations when compared to negative controls. The high metabolic demands of the pre-partum period (i.e. fetal development and colostrogenesis) may result in the generation of free radicals and, as a result, OS (Hatice Esra *et al*., 2017). High levels of NEFA can reduce glucose clearance by lowering insulin sensitivity during the dry period (De Koster and Opsomer, 2013; Salin *et al*., 2017). Furthermore, excessive NEFA liberation from adipose tissue may generate a large amount of oxygen radicals, such as ROS, which can initiate OS (Xu *et al*., 2014). Higher ROS production and/or decreased antioxidant capacity may result in low peripheral tissue insulin sensitivity and glucose tolerance capacity (Abuelo and Hernández, 2019), resulting in higher MDA concentrations during the transition period (Xu *et al*., 2014).

Oxidative stress may also be a factor in the dysfunction of host immune and inflammatory responses, which increases dairy cattle susceptibility to a variety of health disorders during the transition period because oxygen metabolism is accelerated and ROS production is increased due to significant metabolic and physiological adaptations during the transition period (Sordillo and Aitken, 2009; Yasui, 2013; Abuelo *et al*., 2016). The high concentration of serum AOPP may indicate protein damage caused by OS. It is known that AOPP is a synthetic marker of protein oxidation, and that it is a more appropriate marker of OS linked to phagocyte activity (Celi and Gabai, 2015). These findings were consistent with previous research (Celi, 2011; Celi *et al*., 2012; Abuelo *et al*., 2015).

CONCLUSION

Oxidative stress is occurring in hyper-metabolic dairy cattle during the transition period due to increase in ROS and/or impairment in antioxidant capacity. Accurate assessment of ROS is difficult due to their bewildering variety, low serum concentrations, high reactivity, and the extremely short half-life of ROS. The AOPPs are likely considered novels alternative marker of OS because they are stable and easy to detect.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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