PRELIMINARY STUDY ON LIPID PROFILE WITH RELATION TO TOTAL ANTIOXIDANT CAPACITY AND SOME HEMATOLOGICAL AND BIOCHEMICAL CHANGES OF PRE-POST-PARTUM BUFFALO HEIFERS AT ASSIUT CITY

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ABSTRACT

The objectives of this study were to evaluate the relation between lipid profile and total antioxidant capacity (TAC) and to investigate the changes in some hematological and biochemical parameters in buffalo heifers during pre and post-partum period. The study was conducted on 30 buffalo heifers from a buffalo's farm that belongs to Assiut City, Egypt. These pregnant buffaloes were followed until delivery. Blood samples were taken at 3 weeks pre-partum (late pregnancy) and one month post-partum (early lactation). Two types of samples were collected, whole blood samples for hematological picture, and serum samples for biochemical analysis which included total proteins, albumin, globulins, AST, ALT, triglycerides, total cholesterol, HDL-C, LDL-C, and total antioxidant capacity (TAC). The hematological indices including mean values of RBC, Hb, PCV, MCV, and MCH, TLC, lymphocyte % and neutrophil % revealed significant decrease in the early lactation period if compared with late pregnancy mean values. There were significant increase in total proteins, albumin and globulins in the early lactating group compared with late pregnancy group. The mean values of AST, revealed significant increase in the early lactating group compared with late pregnancy group. A significant decrease in the mean values of triglycerides was observed in the early lactating period compared with late pregnancy results. The mean value of total cholesterol, HDL-C and total antioxidant capacity (TAC) revealed significant increase, in the early lactating group compared with late pregnancy group. There were a positive correlation between Total antioxidant capacity (TAC), albumin, total cholesterol and HDL-C.

INTRODUCTION

Buffaloes are the main source of good quality meat and milk in the valley of the River Nile in Egypt and some other developing countries despite this species is mostly reared under harsh socioeconomic conditions and shows low productive and reproductive potentials (GOVS, 2005 and El-Wishy, 2006).

During the transition period from late pregnancy to early lactation, energy requirement that is needed for fetal growth and milk synthesis increases dramatically exceeding the amount of energy the cow can obtain from dietary sources (Castillo *et al.,* 2005).

This dramatic increase in energy requirements makes dairy animal highly susceptible to negative energy balance (NEB). The metabolic adaptation to NEB requires interactions of metabolic fuels and its failure

may occur in various tissues such as liver and adipose tissue (Herdt, 2000).

During NEB, periparturient cows meet their energy requirements by lipomobilization syndrome, i.e. in the dry period and early lactation, is accompanied by typical changes in the serum concentration of lipid parameters, particularly changes in triglycerides, the total cholesterol and High and low density lipoprotein (HDL-C and LDL-C) concentrations (Pysera and Opalka, 2000; Turk *et al.,* 2004, 2005, 2008; and Wathes *et al.,* 2013).

Under physiological conditions, the body usually has sufficient antioxidant reserves to cope with the production of free radicals (Miller *et al.,* 1993; and Castillo *et al.,* 2001), which are produced continuously during metabolism and may increase as a result of pathological and other circumstances (Roth, 2000). However, when free radical generation

exceeds the body's antioxidant production capacity, oxidative stress develops. In dairy cows, the peripartum and early lactation periods are especially critical and present considerable physiological challenges to homeostasis by imposing significant metabolic stressors that may contribute to the onset of diverse disorders (Goff and Horst, 1997).

Oxidative stress can be monitored with several biomarkers and several methods have been developed to assess total antioxidant capacity in view of the difficulty of measuring each antioxidant component separately and their interactions in serum (Cao and Prior, 1998; Niki and Noguchi, 2000 Young, 2001; and Castillo *et al.,* 2006). As a single measure, total antioxidant status (TAS) provides more biologically relevant information that may more effectively describe the dynamic equilibrium between prooxidants and antioxidants in the plasma compartment (Ghiselli*et al.,* 2000) although it has received little attention in clinical oxidative stress profiling.

The objectives of this study were to evaluate the relation between lipid profile and total antioxidant capacity and to study the changes in some hematological and biochemical parameters in buffalo heifers during pre and post-partum period.

MATERIALS and METHODS

1. Animals:
The study was conducted on 30 buffalo heifers pregnant for the first time from a buffalo's farm that belongs to Assiut City, Egypt. These pregnant buffaloes were followed until delivery. The samples were taken at 3 weeks prepartum (late pregnancy) and one month postpartum (early lactation).

2. Samples:
-**Whole blood samples**: 10 ml of blood was drawn from jugular vein of each calf in clean centrifuge tubes containing EDTA as an anticoagulant for complete blood picture evaluation.

- **Serum sample**: 10 ml of blood was drawn from the jugular vein of each calf in clean vacutainer tube without anticoagulant. Sera were separated by centrifugation and stored at -20 C until used (Coles, 1986). The serum was clear and free from haemolysis.

3. Hematological picture:
Total red blood cell count (TRBCs T/l), hemoglobin volume (Hb g/l), packed cell volume (PCV%), mean corpuscular volume (MCV/ fl) and mean corpuscular hemoglobin (MCH /pg), were determined using a fully automated blood cell counter analyser, Abbott cell- dyne 1700, in the department of animal medicine Faculty of Veterinary Medicine-Assiut university,

Assiut. Total leucocytic count (TLCx10³/mm³ blood), Lymphocyte %, Monocyte %, Neutrophil%, Eosinophil %, and Basophil %, were determined (Coles, 1986).

4. Biochemical analysis:
Measurements of total proteins (TP), albumin (ALB), aspartate amino transferees (AST) activity, alanine aminotransferase (ALT) activity, triglycerides, total cholesterol, high density lipoprotein (HDL-C) and low density lipoprotein (LDL-C) concentrations, were carried out using reagent test kits supplied commercially by Biodiognestic, Egypt. Spectrophotometeric assay was performed using spectrophotometer in the department of Animal Medicine- Faculty of Veterinary Medicine Assiut University, Assuit Egypt.

Total Antioxidant capacity (TAC):
TAC was measured by colorimetric method according to (Koracevic *et al.,* 2001). The determination of the antioxidative capacity is performed by the reaction of antioxidants in the sample with a defined amount of exogenously provide hydrogen peroxide (H2O2). The antioxidant in the sample eliminate a certain amount of the provided hydrogen peroxide. The residual (H2O2) is determined calorimetrically by an enzymatic reaction which involves the conversion of 3, 5, dichloro-2- hydroxyl benzensulphonate to a colored product.

Statistical analysis:
Data were analyzed using the packaged SPSS program for windows version 10.01 (SPSS, 2000 Inc., Chicago, IL). Data were presented as mean ±standard deviation (SD). Differences between groups were determined by the one way analysis of variance (ANOVA). Significance level was set at P≤ 0.01 and P≤ 0.05.

RESULTS

The hematological indices including RBC, Hb, PCV, MCV, and MCH are shown in table (1). Mean values revealed significant (p ≤ 0.05) decrease in RBC, in the early lactation group compared with late pregnancy group, while highly significant decrease (p≤0.01) at the Hb, results of total and differential leucocytic count were shown in table (1). Mean values revealed highly significant decrease at (p ≤0.01) in the TLC, lymphocyte % and neutrophil % in the early lactating group compared with late pregnancy group. Non-significant changes in monocyte %, Esoniphile % and Basophile % were reported in both groups. The results of total proteins albumin and globulins were shown in table (2). Mean values revealed significant increase at (p ≥0.05) in total proteins, albumin and globulins (which calculated mathematically), in the early lactating group compared with late pregnancy group.

The mean values of AST, revealed significant increase at (p ≥0.05), in the early lactating group compared with late pregnancy group table (2). The results of triglycerides, total cholesterol, high density lipoprotein (HDL-C), low density lipo protein (LDL-C) concentrations, and total antioxidant capacity (TAC) were shown in table (2). Mean values revealed significant decrease at (p ≤0.05) in the triglycerides in the early lactating group compared with late pregnancy group. The mean value of total cholesterol revealed highly significant (p ≥0.01) increase, also, HDL-C showed significant increase at (p ≥0.05) in the early lactating group compared with late pregnancy group. Non-significant change in LDL-C in both groups was reported. Mean values of total antioxidant capacity (TAC) revealed significant increase at (p ≥0.01) in the early lactating group compared with late pregnancy group.

There were a positive correlation between total antioxidant capacity (TAC) and albumin (r=0.420, p=<0.001), total cholesterol (r=0.342, p=<0.001) and HDL-C (r=0.321, p=<0.001).

Table 1: Mean values (±SD) of hematological indices in late pregnancy and early lactation period.

parameter	Late pregnancy	Early lactation
RBCs (T/l)	5.9±0.29	5.33±0.69*
Hb (gm/dl)	11.19±0.70	9.69±0.63**
PCV (%)	40.66±1.3	31.58±1.9**
MCV(fl)	52.28±2.1	47.73±3.4 **
MCH(pg)	17.93±0.82	15.62±0. 52**
TLCx 10³/mm³	12.45±0.91	10.22±1.51 **
Lymphocyte %	67.20 ±2.4	60.40±4.5**
Monocyte %	13.90±1.9	14.00±2.6 ns
Neutrophil %	22.60±0.83	14.10±0.85**
Eosinophil%	5.40±0.67	2.20±1.22 ns
Basophil %	0.66±0.51	0.33±0.51 ns

*significant difference at 0.05, * *significant difference at 0.01, ns non-significant

Table 2: Mean values (±SD) of measured biochemical parameters and lipid profile in late pregnancy and early lactation period.

parameter	Late pregnancy	Early lactation
Total proteins (gm/dl)	6.79±0.40	7.82±0.51 *
Albumin (gm/dl)	3.81±0.06	4.32±0.08 *
Globulins (gm/dl)	2.97±0.07	3.5 ±0.06 *
AST(IU/l)	67.90±15.98	80.02±18.8l*
ALT (IU/l)	44.37±12.78	49.79±13.09 ns
TAC (mmol/l)	0.57±0.05	0.66±0.12**
Triglyceriedes (mmol/l)	0.58±0.17	0.56 ±0.13*
Total cholesterol (mmol/l)	2.94±0.31	3.51±0.13 **
HDL-C (mmol/l)	2.14±0.01	2.82±0.06*
LDL-C (mmol/l)	2.06±0.04	2.42±o.o5 ns

significant difference at 0.05, *significant difference at 0.01, ns non-significant

DISCUSSION

During the periparturient periods the energy requirement significantly increases, essentially to support milk production, and the physiological adaptation to this condition is represented by the implementation of metabolic activities and by an extensive mobilization of body fat reserves, particularly in early lactation (Castillo et al., 2005, 2006; Kowalska and Jankowiak, 2009; and Roth 2000).

The hematological parameters were significantly altered in cows during the peri-parturient periods. RBCs count showed a significant decrease at (p ≤0.05) in the early lactating period when compared with the late pregnant one. The Hb, PCV, MCV, and MCH, showed a significant decrease at (p≤0.01) in the early lactating group in comparing with the late pregnant group. This result is similar to what observed by Găvan, et al. (2010) who attributed the reduction in RBCS count, MCV, and MCH to deficiency of iron during peri-parturient periods, that resulted in a decreased MCV (microcytosis) because cells undergo an extra division due to inadequate hemoglobin concentration (Roy et al., 2010). PCV% and Hb (gm/dl) showed a significant decrease during peri-parturient periods, that was attributed to anemia due to stress of pregnancy and parturation (Daramola et al., 2004).

Total leucocytic count (10³/mm), lymphocyte %and neutrophile% showed a significant decrease at (p ≤0.01) in the early lactating group compared with late pregnancy group. In fact, near parturition, usually the total WBCs, lymphocyte and neutrophils mean values were increased due to the antepartum rise in cortisol (Preisler et al., 2000 and Kim et al., 2005).

Mean values of serum total proteins, were significantly elevated at (p ≥0.05) in the early lactating group than the late pregnant one. This may be attributed to both elevation of serum albumin and globulins. Serum total proteins as parturition approach usually decreased may be due to the fact that the fetus synthesizes all its proteins from the amino acids derived from the dam, and growth of the fetus increases exponentially reaching a maximum level, especially in muscles, during late pregnancy (Jainudee, and Hafez, 1994), and also due to the haemoconcentration and water losses occurred following parturition (Ghanem et al., 2012).

Serum albumin showed a significant increase at (p ≥0.05) in the early lactating group. This result could be attributed to endogenous production of antioxidants, such as albumin, in an attempt to cope with the increase in lipoperoxide production, especially one week before calving and early post-partum (Mantovani et al., 2010).

Serum globulins showed a significant increase at (p ≥0.05) in the early lactating group. The reduction of globulins in the late pregnancy group and its increase in the early lactation group associated mainly with the immunological response to provide the newly born calf with sufficient globulins in colostrum (Janku et al., 2005).

AST activity was numerically higher during early post parturient periods in comparison with its value at calving (indicating that hepatic metabolism might be more stressed and tissue catabolism was more pronounced during this period Greenfield, (2000) However, because enzyme activities were within the normal range, integrity and functionality of liver tissue was obviously maintained during these periods. A similar trend was observed by Reist et al. (2003) for Holstein dairy cows, where AST activity was lowest pre - partum and reached its peak during the first week post parturient.

The observed peripartum increase in serum enzymes indicated increased hepatic effort during this period. It has previously been reported that reduced dry matter intake around parturition may lead to hepatic lipidosis that alters the normal function of the liver (Greenfield et al., 2000 and Drackley, 2002), although in our study the increase in ALT was small and certainly not significant.

Mean values of total antioxidant capacity (TAC) revealed significant increase at (p ≥0.01) in the early lactating group compared with late pregnancy group. TA capacity concentrations obtained in both late pregnancy and early lactating groups of buffaloes were in accordance with the values obtained in previous reports of peripartuent cows (Castillo et al., 2003; Bozukluhan et al., 2013 and Mousa and Galal 2013) were they found TAC concentration was significantly lower before calving and the highest concentration of TAC was observed 8 weeks post-partum (Castillo et al., 2006). The lowest mean value of TAC (3 weeks before calving) was coincident with the absence of vitamins and mineral supplementation, i.e., exogenous antioxidants, during the dry period. As regards antioxidant defense, the observed relationship between TAC and albumin confirms the role played by this protein as a component with antioxidant capacity (Castillo et al., 2001), and this role is especially important in the period when the animals did not receive vitamin/mineral supplementation Sordillo, and Raphael (2013).

Triglycerides Mean values revealed significant decrease at (p ≤0.05) in the early lactating group compared with late pregnancy group. As reported previously, Triglycerides concentration significantly decreased at calving and remained at the lowest concentration until the eighth week of lactation. The reasons for such disturbances probably are in

triglycerides accumulation in the liver (Bremmer *et al.,* 2000 and Turk *et al.,* 2004, 2005); and in triglycerides taking up by the mammary gland for milk fat synthesis and secretion (Bernard *et al.,* 2008 and Mantovani *et al.,* 2010).

The mean value of total cholesterol revealed significant increase at ($p \geq 0.01$) in the early lactating group compared with late pregnancy group. The descending trend in the serum total cholesterol concentration in late pregnant buffaloes compared to lactating buffaloes was observed as mentioned before by (Nath, 2005). The current study revealed lower levels of serum total cholesterol and HDL-C, in buffalo heifers when compared with those reported by Tajik and Nazifi (2001), which may be attributed to variation in the breed and age of the animals investigated.

The serum total cholesterol concentration was minimum following calving and got build up as the lactation progresses Grummer (1993). The higher level of cholesterol with advancement of lactation was a physiological adjustment to meet the lactation requirements.

Serum HDL-C showed a significant increase at ($p \geq 0.05$) in the early lactating group compared with late pregnancy group. The decrease in HDL-C may be attributed to depressed lipoprotein lipase (LPL) as there is a positive association between LPL and HDL-C (Bernard *et al.,* 2008). Total cholesterol and HDL-C were significantly lower one week around parturition with the lowest values one week prior to calving and increased after parturition and reached significantly higher values at 4 and 8 weeks postpartum (Mantovani *et al.,* 2010).

These lipids change present typical patterns of lipomobilisation syndrome during transition period and were also demonstrated in previous investigations reported by Mazur *et al.* (1988), Grummer (1993) and Turk *et al.* (2008).

Total antioxidant capacity (TAC) showed significant positive correlation with albumin (r=0.420, p=<0.001), total cholesterol (r=0.342, p=<0.001) and HDL-C (0.321, p=<0.001). Our results suggest that lipid mobilization and oxidative stress are part of a complex metabolic adaptation to low energy balance which reaches equilibrium later in advanced lactation.

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دراسة اولية عن صورة الدهون وعلاقتها بمضادات الاكسدة الكلية وبعض التغيرات الدموية والبيوكيميائية فى عجلات الجاموس قبل وبعد الولادة فى مدينة اسيوط

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استهدفت الدراسه معرفه العلاقه بين صوره الدهون ومضادات الاكسده الكلية ودراسه بعض التغيرات الدمويه والبيوكيميائيه المصاحبه لذلك فى عجلات الجاموس قبل وبعد الولاده ولقد تم اختيار عدد ثلاثون من عجلات الجاموس وتم اخذ عينات من هذه الحيوانات قبل الولاده بثلاث اسابيع وبعد الولاده باربع اسابيع (شهر). تم تجميع عينات الدم احداها على ماده مانعه للتجلط وذلك لقياس التغيرات فى صورة الدم والتى اشتملت على العد الكلى لكرات الدم الحمراء والهيموجلوبين وكذلك كرات الدم المصممه وايضا معرفه التغيرات المصاحبه فى العد الكمى والنوعى لكرات الدم البيضاء. كما تم تجميع عينات دم للحصول على سيرم نقى لقياس بعض التغيرات البيوكيميائيه والتى اشتملت على البروتين الكلى والالبيومين والجلوبيولين وتقدير نشاط كلا من انزيمى الاسبرتيت امينو ترانسفيريز والالينين امينو ترانسفيريز وكذلك الدهون الثلاثيه والكوليستيرول الكلى والدهون عاليه ومنخفضة الكثافه وايضا قياس مدى نشاط مضادات الاكسده الكليه فى عجلات الجاموس قبل وبعد الولاده. واوضحت النتائج انخفاض معنوى ملحوظ فى كلا من العد الكلى لكرات الدم الحمراء والهيمجلوبين وكذلك فى نسبة كرات الدم المصممطه والعد الكمى والنوعى لكرات الدم البيضاء. كما اوضحت النتائج زياده معنويه فى كلا من البروتين الكلى والالبيومين والجلوبيولين وكذلك نشاط انزيم الاسبرتيت امينو ترانسفيريز بعد الولاده فى المرحله المبكره للحليب وايضا لوحظ انخفاض معنوى فى قيمة الدهون الثلاثيه بعد الولاده فى المرحله المبكره للحليب كما وجد زياده معنويه فى كلا من الكوليستيرول الكلى والدهون عاليه الكثافه وكذلك نشاط مضادات الاكسده الكليه فى عجلات الجاموس بعد الولاده فى المرحله المبكره للحليب. ووجد ايضا ارتباط معنوى بين نشاط مضادات الاكسده الكليه والالبيومين والكوليستيرول الكلى والدهون عاليه الكثافه.