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Biomarkers of Oxidative stress and the activities of antioxidant defense system during late pregnancy and onset of lactation and their modulation with certain micronutrients in Holstein–Friesian cows.

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Abstract

Oxidative stress and the antioxidant status in dairy cows during late pregnancy and onset of lactation revealed dramatic changes in the activities of antioxidant defense system. This study was carried out to assess the concentration of lipid peroxidation, antioxidants concentration, antioxidant enzymes activities and the inflammatory markers. One hundred of clinically healthy pregnant Holstein–Friesian cows approximately with an average age 42 months old were divided into five equal groups of 20 cows. The experiment was planned four weeks before and after parturition according to the farm records. Group I: (Control). Group II: cows received daily dose of 35mg/kg b.w. of zinc oxide food grade. Group III: cows received daily dose of 45mg/kg b.w. of manganese sulphate Group IV: cows received daily dose of 6 mg/kg b.w. of copper sulphate. Group V: cows received daily dose of 6,000IU/kg b.w. of α tocopherol. Blood samples for serum separation of serum were obtained and used directly for haptoglobin (HP) and C-reactive protein (CRP) estimation. Heparinized blood samples were taken for malondialdehyde (MDA), reduced glutathione (GSH), hydrogen peroxide (H2O2), nitric oxide (NO) concentrations and total antioxidant capacity (TAC), determination of glutathione peroxidase (GPx), glutathione reeducates (GR), superoxide dismutase (SOD), and Catalase (CAT) enzymes activities. Tht results revealed a higher increase in L-malondialdhyde (MDA), hydrogen peroxide (H2O2), Nitric oxide, C-reactive protein (CRP) and Haptoglobin (HP) concentration in control group. Meanwhile, a significant decrease was in L-malondialdhyde (MDA), hydrogen peroxide (H2O2), Nitric oxide, C-reactive protein (CRP) and Haptoglobin (HP) concentration observed after supplementation of zinc oxide, manganese sulphate, copper sulphate and α-tocopherol to the ration of cows moreover, a significant decrease was observed in reduced glutathione, and total antioxidant capacity concentrations in control group. Mainwhile, a significant increase were observed in reduced glutathione, and total antioxidant capacity concentrations, in addation, a marked increase was observed in glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase activities after supplementation of zinc oxide, manganese sulphate, copper sulphate and α tocopherol in the ration of Holstein–Friesian cows**.**

Keywords: *Oxidative stress, MDA, antioxidants, CRP, haptoglobin.*

Introduction

Oxidative stress is generally described as an imbalance between oxidant and antioxidant levels **(Lykkesfeldt and Svendsen, 2007).** When the production of oxidants exceeds the capacity of antioxidant defense, a condition of oxidative stress is produced resulting in oxidative damage to macromolecules such as lipids, DNA and proteins **(Sordillo *et al*., 2009).** The per parturient period of dairy cows involves major metabolic changes and release of reactive oxygen species (ROS) **(Halliwell and Gutteridge, 2007).** An increase in free radicals production or deficiencies of antioxidants may lead to oxidative stress **(Zhao and Lacasse 2008)**. Antioxidant vitamins and minerals such

as α-tocopherol, Zinc (Zn), copper (Cu) and manganese protect the body from free radicals either by directly scavenging free radicals or by inhibiting the activity of oxidizing enzymes **(Feng and Xiao 2015)**.

Lipid peroxidation may cause decrease in levels of some antioxidant molecules leading to an increase in oxidative stress **(Weiss *et al*., 2004).** Malondialdehyde (MDA) considered as indicator of oxidative stress (**Halliwell and Chirico, 1993).** Also reactive oxygen molecules (ROM) levels were shown to continue to increase even after parturition which suggests that the antioxidative capacity in dairy cows around calving seems to be insufficient to counteract the increase in ROM. **(Celi *et al*., 2012).** Reactive oxygen species (ROS) and antioxidants may be involved in some relevant physiological functions such as milk yield therefore it might be beneficial to supplement cows with antioxidants **(Pedernera *et al*., 2009)**. The possibility that oxidative stress during the transition period may be a major underlying cause of inflammatory and immune dysfunction in dairy cows **(Bouwstra *et al*., 2010)**.

SOD activity increases H2O2 production, protection from reactive oxygen species would controlled by a simultaneous increase in catalase and GSH-Px activities and availability of glutathione. **(Frei, 1994)**. Studies showed that blood GSH-Px activity is decreased during the postpartum period, suggesting some degrees of oxidative stress and lipid peroxidation**. (Celi *et al*., 2010).** SOD activity is decreased during the postpartum period probably as a consequence of lower peroxide generation as testified by the decrease in ROM concentrations. **Celi *et al*., 2010).** Dairy cows can experience oxidative stress which may be associated with metabolic diseases during the peripartum period **(Miller *et al*., 1993)**.

Antioxidant nutrient supplementation especially vitamin E and zinc can be used to attenuate the oxidative stress in ruminants **(Garg and Bansal, 2000).** In addition, **Kinal *et al*., (2005)** reported that replacing of the inorganic copper (Cu), manganese (Mn), and zinc(Zn) precalving until during early lactation could reduce lipid peroxidation in lactating cows. αtocopherol has antioxidant beside anti-inflammatory effects. Pregnancy and lactation had significant effects on blood haptoglobin concentrations. The oxidative stress during the transition period may be a major underlying cause of inflammatory and immune dysfunction in dairy cows **Sevanian and Ursini, (2000).** CRP concentrations tend to be higher in late pregnancy **(Young *et al*., 1991).** C-reactive protein (CRP) is a biomarker for inflammation status, Its serum level increases during inflammatory conditions or any stresses **Saboori *et al*., (2015).** C-reactive protein as inflammatory marker that interpreting biomarkers of micronutrient status. α-tocopherol supplement can significantly decrease the serum level of CRP (**Saboori *et al*, 2015).** Inflammation may affect antioxidant status by decreasing their concentrations in the serum and in this way it can mask the possible protective effects of antioxidants on CRP level (**Floegel *et al*., 2011**). α-Tocopherol supplementation may be a good strategy for decreasing inflammatory conditions (**Saboori *et al*, 2015).**

Therefore, the aim of this study was the evaluation of antioxidant inflammatory status in blood during late gestation and onset of lactation. it was planned with the view of the possible protective effect during supplementation of Zn, Cu, Mn or α- tocopherole during times of oxidative stress which may reduce oxidative damage during transition period it was decided to estimate the levels of MDA, GSH, GPx, Gr, SOD, catalase, H2O2, Nitric oxide, TAC, Haptoglobin and CRP and as anindex of oxidative stress, antioxidant status and inflammatory marker respectively and to see whether any difference was existed in these parameters during advanced pregnancy and early lactation.

Materials and Methods

One hundred of clinically healthy pregnant Holstein–Friesian cows could be approximately an average of 42 months old. All the animals were clinically healthy dewormed and free from internal, external and blood parasites during the period of the study. The selected cows were in a good health, nutritional condition and approximately have the same body scores, were selected from private dairy farm at

sharkeia province during the late pregnancy, four and two weeks prior to calving,Day of parturition and early two and four weeks after parturition according to reproductive farm records. Animals were fed on total mixed ration TMR (concentrated ration and corn silage) according to **NRC (2001)** fresh and clean drinking water was supplied ad-libitum. The selected cows were in good health, nutritional condition and approximately have the same body scores

Animals Groups:
Cows under experiment were randomly divided into five equal groups of 20 cows each as follows:
Group I: (Control): cows fed on total mixed ration (TMR) four weeks before and after parturition.
Group II: (Zinc Oxide treated group): cows received daily dose of 35 mg/kg B. w. of zinc oxide food grade added to TMR four weeks before and after parturition.
Group III: (manganese sulphate treated group): cows received daily dose of 45mg/kg B. w. of manganese sulphate added to TMR four weeks before and after parturition.
Group IV: (copper sulphate treated group): cows received daily dose of 6 mg/kg B. w. of copper sulphate added to TMR all over the experimental periods.
Group V: (α tocopherol treated group): cows received daily dose of 6,000 IU/kg B. w. of α tocopherol added to TMR all over the experimental periods.

All cow groups fed on total mixed ration all over the experimental periods

Sampling:
Individual blood samples were collected from the jugular vein five times. at (4 weeks, and 2 weeks before parturition, a day of parturition and at 2 and 4 weeks postpartum).

Blood samples:
Approximately 10 ml of blood samples were obtained in clean, dry screw capped tubes and serum was separated by centrifugation at 3000 r.p.m for 10 minutes. The clear serum was obtained and received in dry sterile sample tube using sterilized pipettes, processed directly for haptoglobin and CRP. Also heparinized blood samples were taken in clean, dry screw capped tubes for malondialdehyde, reduced glutathione, hydrogen peroxide, nitric oxide concentrations and total antioxidant capacity. In addition, determination of glutathione peroxidase, glutathione reductase, superoxide dismutase, and Catalase activities were performed in whole blood.

Biochemical analysis:
L-Malondialdehyde (L-MDA), **Esterbauer *et al*., (1982).** GSH **Moron *et al*., (1979),** Gpx **Gross *et al*., (1967),** GR **Beutler (1975).** SOD, **Paoletti and Mocali, (1990).**C-reactive protein (CRP) **Tietz (1995)**. CAT, **Xu *et al*., (1997). H2O2, Sinha, (1972).** Nitric oxide, **Moshage *et al*., (1995).** Haemoglobin **Wintrobe, (1965).** TAC. **oracevic, *et al*., (2001).** Hptoglobin, **Eckersal, (2000).**
All biochemical analyses were performed in AHRI zagazig branch Using spectrophotometer turner 960,

Statistical analysis: The obtained data were statistically analyzed using analysis of variance (ANOVA) test and comparative of means were performed according to **Duncan** Multiple Range test for comparison of Means according tousing **SPSS14 (2006).**

Results

Table (1). Effect of oxidative stress during late pregnancy and onset of lactation on MDA (nmol/L) , GSH (mg/dL) Concentrations, GPx and Gr U/mL activeies and their modulation with Zinc oxide, Manganese sulphate, Copper sulphate and α- Tocopherol supplementation Holstein–Friesian cows (n=5).

		Before parturition		Day of parturition	After parturition	
	Animals groups	4 weeks	2 weeks		2 weeks	4 weeks
MDA (nmol/ L)	Control	80.57 ± 1.66^{a}	82.1 ± 4.95^{a}	88.2 ± 5.82^{a}	89.35 ± 3.59^{a}	89.19 ± 3.79^{a}
	Zinc oxide treated group	69.97± 2.34^{b}	66.83 ± 4.84^{b}	71.52 ± 5.97^{a}	69.65 ± 3.96^{b}	71.15 ± 6.67^{b}
	Manganese sulphate treated group	67.30 ± 4.04^{b}	61.70 ± 2.87^{b}	76.94 ± 2.25^{a}	63.70 ± 3.55^{b}	63.54 ± 2.30^{b}
	Copper sulphate treated group	63.53 ± 3.76^{b}	62.91 ± 2.43^{b}	82.81 ± 4.82^{a}	66.81 ± 3.06^{b}	65.81 ± 3.78^{b}
	α Tocopherol supplemented group	68.14 ± 4.38^{b}	68.82 ± 2.61^{b}	75.26 ± 6.50^{a}	67.28 ± 2.24^{b}	61.48 ± 2.11^{b}
GSH (mg/dL).	Control group	49.60 ± 1.91^{c}	53.81 ± 3.52^{c}	69.27 ± 2.59^{a}	59.46 ± 2.55^{b}	53.15 ± 2.34^{b}
	Zinc oxide treated group	69.60 ± 1.21^{b}	69.85 ± 2.01^{b}	75.11 ± 3.82^{a}	69.07 ± 2.21^{a}	77.77 ± 3.20^{a}
	Manganese sulphate treated group	69.40 ± 1.63^{b}	77.20 ± 4.04^{a}	77.00 ± 3.34^{a}	71.2 ± 2.18^{a}	72.8 ± 2.18^{a}
	Copper sulphate treated group	72.20 ± 1.82^{b}	75.10 ± 1.12^{a}	75.70 ± 2.72^{a}	72.00 ± 1.70^{a}	75.70± 4.17^{a}
	α Tocopherol supplemented group	79.66 ± 3.19^{a}	77.40 ± 3.34^{a}	73.20 ± 1.93^{a}	74.50 ± 1.69^{a}	76.8 ± 3.02^{a}
Gpx U/mL	Control group	27.25 ± 2.02^{b}	32.52 ± 3.92^{b}	52.45 ± 3.78^{a}	36.44 ± 3.33^{b}	30.31 ± 2.21^{c}
	Zinc oxide treated group	55.16 ± 8.30^{a}	44.63 ± 5.38^{a}	65.49 ± 5.65^{a}	50.48 ± 3.41^{a}	44.70 ± 5.43^{b}
	Manganese sulphate treated group	58.22 ± 5.21^{a}	53.21 ± 1.40^{a}	58.82 ± 5.36^{a}	51.94 ± 1.29^{a}	52.81 ± 1.67^{bc}
	Copper sulphate treated group	59.95 ± 11.65^{a}	51.76 ± 2.03^{a}	62.21± 4.41^{a}	50.14 ± 2.21^{a}	61.08 ± 4.08^{c}
	α- Tocopherol supplemented group	67.85 ± 4.22^{a}	54.65 ± 4.26^{a}	67.95 ± 4.51^{a}	56.01 ± 2.52^{a}	59.26 ± 4.93^{c}
GR U/mL	Control group	26.54 ± 0.52^{c}	28.46 ± 2.20^{b}	36.46 ± 5.03^{a}	25.68 ± 1.12^{b}	31.74 ± 1.68^{b}
	Zinc oxide treated group	35.50 ± 2.53^{b}	37.20 ± 3.74^{a}	41.69 ± 3.70^{a}	35.65 ± 2.29^{a}	37.77 ± 0.10a^{b}
	Manganese sulphate treated group	37.75 ± 0.10^{b}	43.08 ± 3.32^{a}	39.77 ± 1.94^{a}	37.68± 0.1^{a}	39.80 ± 1.94^{ab}
	Copper sulphate treated group	38.08 ± 0.23^{b}	41.53 ± 2.18^{a}	43.77 ± 3.39^{a}	40.35 ± 1.61^{a}	41.77 ± 3.95^{a}
	α Tocopherol supplemented group	46.82 ± 4.86^{a}	40.15 ± 1.33^{a}	45.75 ± 3.81^{a}	41.95 ± 3.9^{a}	43.81 ± 4.01^{a}

Mean with different superscript letters in the same column are significantly different at ($P \leq 0.05$).

Table (2). Effect of oxidative stress during late pregnancy and onset of lactation on SOD (U/mL), CAT (U/mL)activeies and H2O2 (nmol/L) Concentrations, and their modulation with Zinc oxide, Manganese sulphate, Copper sulphate and α- Tocopherol supplementation Holstein–Friesian cows (n=5).

		Before parturition		Day of parturition	After parturition	
	Animals group	**4 weeks**	**2 weeks**	**0 Day**	**2 weeks**	**4 weeks**
SOD (U/mL)	**Control group**	43.65 ± 2.52^{b}	32.97 ± 3.37^{b}	49.45 ± 4.44^{a}	38.52 ± 2.27^{b}	34.66 ± 2.69^{b}
	Zinc oxide treated group	60.05 ± 5.47^{a}	54.25 ± 8.53^{a}	57.25 ± 3.78^{a}	57.66 ± 7.24^{a}	51.45 ± 3.04^{ab}
	Manganese sulphate treated group	54.25 ± 3.58^{a}	53.24 ± 4.57^{a}	59.05 ± 5.74^{a}	59.45 ± 5.07^{ab}	58.86 ± 6.03^{a}
	Copper sulphate treated group	64.71 ± 5.13^{a}	57.45 ± 3.99^{a}	61.26 ± 3.84^{a}	60..86 ± 3.42^{a}	55.45 ± 4.70^{a}
	α Tocopherol supplemented group	55.66 ± 3.48^{a}	61.85 ± 1.89^{a}	55.85 ± 3.04^{a}	61.04 ± 6.04^{ab}	59.06 ± 3.45^{a}
CAT (U/mL)	**Control group**	46.06 ± 3.82^{b}	38.72 ± 2.91^{b}	43.30 ± 3.80^{a}	41.85 ± 6.12^{b}	35.15 ± 1.55^{c}
	Zinc oxide treated group	60.46 ± 5.40^{a}	56.57 ± 6.63^{a}	49.82 ± 3.78^{a}	65.59 ± 3.26^{a}	50.21 ± 6.85^{b}
	Manganese sulphate treated group	63.25 ± 4.77^{a}	61.64 ± 5.50^{a}	57.04± 6.41^{a}	68.84± 2.31^{a}	53.81 ± 4.67^{ab}
	Copper sulphate treated group	62.64 ± 4.68^{a}	58.87 ± 4.14^{a}	54.34 ± 3.19^{a}	70.24 ± 4.86^{a}	48.22 ± 2.46^{b}
	α Tocopherol supplemented group	60.86 ± 3.42^{a}	67.99 ± 3.63^{a}	54.67 ± 3.19	77.65 ± 4.86^{a}	63.65 ± 3.80^{a}
H2O2 (nmol/L)	**Control group**	99.09 ±2.61^{a}	134.51 ± 2.59^{a}	122.99 ± 4.56^{a}	131.95 ± 2.49^{a}	130.80 ± 3.02^{a}
	Zinc oxide treated group	88.44±2.54^{b}	95.99 ± 8.53^{b}	111.45 ± 6.07^{a}	90.65 ± 4.13^{b}	111.40 ± 7.81^{b}
	Manganese sulphate treated group	84.65 ± 4.09^{c}	98.71 ± 3.57^{b}	118.75 ± 7.54^{a}	98.00 ± 3.39^{b}	93.00 ± 2.55^{c}
	Copper sulphate treated group	82.55 ± 3.58^{c}	92.61 ± 5.59^{b}	109.24 ± 5.94^{a}	95.75 ± 4.49^{b}	103.80 ± 2.24^{bc}
	α Tocopherol supplemented group	82.19 ± 3.41^{c}	97.44 ± 4.17^{b}	110.55 ±8.07^{a}	89.00 ± 1.87^{b}	95.25 ± 5.31^{c}

Mean with different superscript letters in the same column are significantly different at ($P \leq 0.05$).

Table (3). Effect of oxidative stress during late pregnancy and onset of lactation on Nitric oxide (mmol/L), TAC (u/gmHb), and Haptoglobin (mg/dL) Concentrations, and their modulation with Zinc oxide, Manganese sulphate, Copper sulphate and α- Tocopherol supplementation Holstein–Friesian cows (n=5).

		Before parturition		Day of parturition	After parturition	
	Animals group	**4 weeks**	**2 weeks**	**0Day**	**2 weeks**	**4 weeks**
Nitric oxide (mmol/L)	**Control group**	18.12 ± 0.89^{a}	19 .14 ± 1.07^{a}	19.34 ± 0.74^{a}	18.35 ± 0.71^{a}	18.53 ±0.75^{a}
	Zinc oxide treated group	13.62 ± 1.76^{b}	15.49 ± 1.29^{b}	16.59 ± 2.06^{a}	13.25 ± 1.63^{b}	11.28 ± 1.03
	Manganese sulphate treated group	11.10 ± 0.41^{b}	11.04 ± 041^{c}	16.19 ± 1.93^{a}	11.89 ± 0.87^{b}	14.02 ± 1.56^{b}
	Copper sulphate treated group	10.66 ± 0.63^{b}	13.14 ± 1.97^{bc}	16.35 ± 1.81^{a}	12.12 ± 0.10^{b}	14.12 ± 1.55^{b}
	α Tocopherol supplemented group	11.40 ± 0.88^{b}	10.36 ± 0.72^{c}	18.69 ± 1.61^{a}	11.74 ± 0.84^{b}	11.06 ± 0.33^{b}
TAC (u/ gmHb)	**Control group**	3.09 ± 0.23^{b}	2.71 ± 0.18^{b}	3.59 ± 2.61^{a}	2.99 ± 0.05^{c}	2.99 ± 0.05^{b}
	Zinc oxide treated group	4.10 ± 0.29^{a}	3.76 ± 0.39^{a}	3.95 ± 0.31^{a}	3.91 ± 0.21^{ab}	3.95 ± 0.30^{a}
	Manganese sulphate treated group	4.35 ± 0.22^{a}	4.21 ± 0.10^{a}	3.97 ± 0.19^{a}	3.78 ±0.23^{ab}	3.83 ± 0.25^{a}
	Copper sulphate treated group	3.61 ± 0.19^{ab}	3.58 ± 0.22^{a}	3.78 ± 0.23^{a}	3.85± 0.41^{bc}	3.92 ± 0.18^{a}
	α Tocopherol supplemented group	3.81 ± 0.23^{ab}	4.05 ± 0.13^{a}	3.95 ± 0.19^{a}	4.08 ± 0.14^{a}	4.03 ± 0.13^{a}
CRP (mg/ml)	**Control group**	2.84 ±0.28^{a}	2.56 ± 0.21^{a}	2.50 ± 0.15^{a}	2.57 ± 0.14^{a}	2.59 ± 0.13^{a}
	Zinc oxide treated group	1.75 ± 0.22^{b}	1.76 ± 0.24^{b}	2.16 ± 0.34^{a}	2.01 ± 0.33^{ab}	1.78 ± 0.34^{b}
	Manganese sulphate treated group	1.42 ± 0.11^{b}	1.82 ± 0.18^{b}	2.19 ± 0.15^{a}	1.55 ± 0.14^{b}	1. 87 ± 0.10^{b}
	Copper sulphate treated group	1.87 ± 0.21^{b}	1.75 ± 0.21^{b}	2.09 ± 0.10^{a}	1.98 ± 0.11^{ab}	1.93 ± 0.11^{b}
	α Tocopherol supplemented group	1.71 ± 0.15^{b}	1.77 ± 0.21^{b}	2.19 ± 0.14^{a}	1.76 ± 0.21^{b}	1.87 ± 0.21^{b}
Haptoglobin (mg/dL)	**Control group**	41.80 ± 3.60^{a}	43.80 ± 3.6^{a}	48.40± 5.06^{a}	42.00 ± 2.89^{a}	39.00 ± 2.53^{a}
	Zinc oxide treated group	29.20 ± 5.95^{b}	29.40 ± 4.01^{b}	43.50 ± 5.56^{a}	33.80 ± 1.74^{bc}	33.80 ± 1.74^{ab}
	Manganese sulphate treated group	28.20 ± 3.53^{b}	28.80 ± 2.33^{b}	38.80 ± 4.78^{a}	34.60 ± 2.69^{bc}	34.60 ± 2.69^{ab}
	Copper sulphate treated group	31.40 ± 2.44^{ab}	31.20 ± 2.92^{b}	45.60 ± 5.27^{a}	30.00 ± 1.58^{b}	30.00 ± 1.58^{b}
	α Tocopherol supplemented group	26.60 ± 1.07^{b}	25.00 ± 2.43^{b}	41.60 ± 3.29^{a}	27.80 ± 4.34^{c}	27.80 ± 4.39^{b}

Mean with different superscript letters in the same column are significantly different at ($P \leq 0.05$).

Results

The obtained data in our research revealed a higher increase in L-malondialdhyde (table 1), H2O2 (table 2), Nitric oxide, CRP and Haptoglobin concentration (table 3) within 4 and 2 weeks before parturition, day of parturition, 2 and 4 weeks after parturition. This increase became decrease after supplementation of zinc oxide, manganese sulphate, copper sulphate and α tocopherol to the ration of Holstein–Friesian cows in comparison with the control group. But a significant decrease observed in reduced glutathione concentration (table 1) and total antioxidant capacity (TAC) concentrations (table 3). This decrease became a significant increase after supplementation of zinc oxide, manganese sulphate, copper sulphate and α tocopherol in the ration of Holstein–Friesian cows in reduced glutathione, and total anteoxidant capacity (TAC) concentrations, in addition; a marked significant increase showed observed in glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase activities (table 1 and 2) at 4 and 2 weeks before parturition, day of parturition, 2 and 4 weeks after parturition in compared with the control.

Discussion

Pregnancy in dairy cows induces oxidative stress that can be a significant underlying factor leading to dysfunctional host immune and inflammatory responses that can increase the incidence and severity of diseases **(Sordillo, 2013).** During advanced pregnancy and early lactation increased demand of micronutrients do not usually fullfill the resulting deficiencies occurring due to natural protective substances or excess exposure to stimulators of "reactive oxygen metabolites" (ROM), and this might result in high lipid peroxidation and decreased level of antioxidant enzymes **(Allison and Laven, 2000)**.

A higher increase in MDA concentration at late pregnancy, during parturition and early lactation in Holstein Frisian cows was considered the final product of lipid peroxidation and a marker of oxidative stress. As can be observed in the (Table 1), a similar pattern was observed in studies regarding MDA and TAS (total antioxidant status) in dairy cows **(Castillo *et al*., 2006).** The high level of MDA in the first week after parturition correlates with a low activity of antioxidant enzymes (SOD, GPx and catalase) **(Trevisan, 2001).** The recorded significant elevation in MDA agree with **(Sharma *et al*., 2011)** who concluded that the oxidative stress in cows were increased during late pregnancy, parturition and initiation of lactation would be expected to increase the production of reactive oxygen species (ROS) resulting oxidative stress. Lipid hydroperoxides as biomarkers of lipid peroxidation were increase from calving through early lactation **(Castillo *et al*., 2005).** A similar result was observed by **Castillo *et al*., (2006)** who found that the increase in Malondyaldehyde (MDA) is considered a marker of oxidative stress. The high level of MDA in the first week after parturition correlates with a low activity of antioxidant enzymes SOD, GPX and catalase **(Trevisan, 2001).** The concentration of MDA may simply reflect the total oxidative damage formed in the rest of the body. Thus, the increase in blood MDA before during parturition and after calving because high amounts of free radicals are conformedby **Sharma *et al*., (2011)** who also reported that α tocopherol supplementation reduced oxidative damage and reduce MDA concentrations. In addition, **Sordillo *et al*., (2007)** who reported that the Lipid hydroperoxides increased significantly from the pre-partum, calving and onset of lactation. On the other hand, supplementations of zinc oxide, manganese sulphate, copper sulphate and α tocopherol in ration causes a significant decrease in MDA concentration in comparison with the non-supplemented animal group. Our results coincide with **Vallee and Falchuk (1993)**, who stated that the supplementations of zinc oxide, manganese sulphate, copper sulphate and α-tocopherol in ration causes a significant decrease in MDA concentration. Moreover, **Ohta *et al*., (2006)** concluded that MDA concentration was decreased after supplementation of α tocopherole when compared with the control group. In addition, **Maurya, *et al*., (2014)** reported that MDA level was lowered in α-tocopherol and zinc oxide supplementations and they have an important role in protecting lipid membranes from attack of reactive oxygen species. The best understood the role of α-tocopherol is that it acts as a lipid soluble cel-

lular antioxidant, free radical scavenger and protects against lipid peroxidation**. Bouwstra *et al*., (2008)** concluded thatα-tocopherol supplementation reduces oxidative damage and MDA production, α-tocopherol considered the most effective lipid-soluble antioxidant present in cell membranes; it plays a major role in maintaining cell membrane integrity by limiting lipid peroxidation by reactive oxygen species (ROS) **(Lavrovsky *et al*., 2000).** Zn is required for the formation of Mn-Zn SOD enzyme, deficiency of Zn effects on the activity of SOD in blood and tissues, which results in increased superoxide radicals hence oxidative stress takes place **(Gaafar *et al*., 2010).** As confirmed by **Kinal *et al*., (2005)** who attributed the replacing of the inorganic Cu, manganese (Mn), and Zn pre-calving and during lactation in dairy cows could result in reduction in superoxide radical. The current data (table 1) showed a significant decrease in GSH concentration in Holstein–Friesian cows 4 and 2 weeks in group I before parturition, day of parturition, 2 and 4 weeks after parturition. Our data coincide with **Aitken *et al*., (2009)** who mentioned that**,** blood GSH concentration, SOD and GPx activity was significantly lowered in advanced pregnant cows and early lactating cows. Moreover, reduced availability of antioxidant defense near the time of parturition accompanied with an increase of the oxidative stress which may contributed to per parturient disorders in dairy cows **(Waller, 2000).**

However, the blood GSH concentration in table (1) was significantly decreased with the increased lipid peroxidation, therefore, an imbalance between increased production of ROS and reduced availability of antioxidant defense near the time of parturition might increase oxidative stress in dairy cows **(Gitto *et al*., 2002).** Our data confirmed by **(Circu and Aw, 2010)** who observed a significant decrease in glutathione reductase activities which indicates a decrease in the conversion of oxidized glutathione back to its reduced form GSH, which leads to further reduction of ROS during oxidative stress**.**

GPxcatalyse the reduction of inorganic and organic hydroperoxides, with glutathione as a reducing equivalent, via GPx action. GR acts to restore glutathione to its reduced form **(Dandekar *et al*., 2002).** The decreased Gpx activity contributes to the oxidative defense of animal tissues by catalyzing the reduction of hydrogen and lipid peroxides **(Halliwell and Chirico, 1993). Tuzun *et al*., (2002)** suggested that the reactive oxygen species increased, while blood SOD and GPx activities started to decrease as an indicator of oxidative stress immediately after birth. Moreover, our results coincided with **Sharma *et al*., (2011)** who concluded that significant decreases in GPX activity during advanced pregnancy and early lactation have been observed a decrease blood GPx in dairy cows during late pregnancy and early lactation might be due to as a loss of homeostatic control in the periparturant period **(Adela *et al*., 2006).** Gpx catalyzes the conversion of H2O2 to H2O produced in the course of normal cellular events and it is also catalyses the reduction of fatty acid hydroperoxides. moreover, another GPx in RBCs termed phospholipid hydroperoxides glutathione peroxidase participate in reduction of more complex phospholipid hydroperoxides using GSH **(Festila *et al*., 2012)**. Our results agree with **Celi *et al*., (2010)** who recorded a decrease of blood GSH-Px activity in dairy cows during day of calving and after parturition as a loss of homeostatic control in the postnatal period, who also reported a reduction in GSH-Px activity could be supported as increasing postnatal oxidative stress. SOD is known to be an important factor in protection against harmful free radical activity and is considered as the first defense mechanism against pro-oxidants**.** Essential trace minerals such as zinc, copper and manganese play a wide role in the antioxidant defense, development of the tissue, and immune function **(Elhashmi *et al*., 2016**). The obtained results demonstrated in (Tables 2 and 3) revealed that, the reduction in Cu and zn levels could represent contributing factors for reduction of total antioxidant capacity (TAC) since they are members on Cu-zn superoxide dismutase system **Spears and Weiss (2008)** observed that the decreased activity of SOD may be contributed to low levels of Cu which has been detected during pregnancy in dairy cows ration**. (Bernabucci *et al*., 2005)** mentioned that the higher erythrocyte SOD activity on the day of parturition indicates higher oxidative stress and

lower antioxidant status**.**

The obtained results were in agreement with those recorded by **(Górecka *et al*., 2002)** who reported that the oxidative stress during pregnancy showed an increase of MDA value and decrease of TAC level, CAT, and SOD activities. In addition, **Öztabak *et al*., (2005)** reported that plasma CAT activity was lowered during late pregnancy. Furthermore, **Sharma *et al*., (2011)** showed that the value of GPx and SOD activities were decreased in early lactation and during advanced pregnancy. Moreover, **Festila *et al*., (2012)** recorded that the decreased levels of blood SOD and GPx activities in cows in advanced gestation and early lactation. Accordingly, the low blood GPx level at postpartum was considered an indicator of oxidative stress that occurs when GPx reduces plasma lipid peroxidation. Immediately after birth, the reactive oxygen species increased, while blood GPx value started to decrease. GPX and SOD activity decreased after parturition, the antioxidant defense mechanisms protection decreased. **Celi *et al*., (2010)** also established a relationship between physiological changes associated with pregnancy and lactation periods and a decrease in total antioxidant capacity (TAC). Reduction in total antioxidant capacity approaching parturition has been recorded by **Liu *et al*., (2013)** who stated that the value of antioxidant decreases during gestation period and lactation. who also reported that the antioxidant supplementation in the diet has been shown to increase antioxidant capacity in plasma. Moreover, **Turk *et al*., (2013)** found that the antioxidant capacity in dairy cows decreases one week after calving. The obtained results were in agreement with that recorded by **(Gitto *et al*., 2002).** A relationship between the physiological changes associated with parturition and a loss in overall antioxidant potential was established in dairy cows **(Sordillo *et al*., 2007).**

Gaafar *et al*., (2010) recorded that the supplementation of zinc oxide, manganese sulphate, copper sulphate and α-tocopherol plays an essential role in Superoxide dismutase activties. SOD catalyzes the dismutation of superoxide radicals into hydrogen peroxide which degrades further to water by GPx and CAT. The possibility of oxidative stress during the transition period particularly during parturition may be a major underlying cause of inflammatory and immune dysfunction in dairy cattle **(Sordillo and Aitken, 2009).** GPx catalyzes the reduction of organic hydroperoxides, lipid peroxides, and hydrogen peroxide, using glutathione GSH as the reducing agent, thereby also protecting cells from oxidative damage resulting from normal oxidative metabolism **(Gaafar *et al*., 2010).** Moreover, (**LeBlanc *et al*. 2002)** concluded that, Dietary vitamin E is important for their ability to contribute ROS neutralization, thereby blocking the progression of inflammation and neutralizes free radicals **s**upplementing α-tocopherol in the prepartum period improves the antioxidant status and decreases inflammatory cytokine production of dairy cows during the peri and postpartum periods.

Stress disease and induction of the immune response increases nutrient requirements. The supplementation of antioxidant vitamins as α-tocopherol and antioxidant minerals such as Zinc and copper in dairy cow's ration is very important to help the animal recover the oxidative stress in dairy cows **(Feng and Xiao, 2015).**

SOD activity increases H_2O_2 production, protection from reactive oxygen would only be confered by a coordinated increase of catalase and glutathione peroxidase activities **(Sharma *et al*., 2011).** In support of this conjecture, catalase activity was found to be increased in cows near parturition and also lower catalase activity **(Maurya, *et al*., 2014).** Who also concluded that α- tocopherol supplementation improves the activities of both SOD and GPx**.** Our results coincided with **Beckman (1997)** who reported that the prevention of free radical induced damage to tissues for the maintenance of health and production superoxide radicals are reduced to hydrogen peroxide (H2O2) by superoxide dismutase in the presence of copper and zinc cofactors. An adequate level of copper in food is indispensable for optimization of immune system, since copper reduces the occurrence of development of metabolic and oxidative stress in dairy cows **(Cortinhas *et al*., 2012).**

Reduction in zinc and copper availability in the early postpartum period of dairy cows might explain the reduction of SOD activity **(Muehlenbein *et al*., 2011).** Who also reported rapidly decline in SOD activity during the late pregnancy and after calving. **Chandra *et al*., (2013)** an increase in the SOD activity of vitamin E treated cows before calving to the day of calving. **(Maurya *et al*., 2014)** reported an increase in the SOD activity of vitamin E and zinc cows before calving to the day of calving. In addition, Cu act as a modulator of the inflammatory process, as an acute-phase protein which rise in the inflammatory events **(Gropper *et al*., 2005).** In our research the increased concentration CRP (table 3) confirmed by **Floegel *et al.*, (2011)** who stated that, the significant beneficial effects of vitamin E supplements on the serum level of CRP, with somewhat greater reductions achieved on the serum level of CRP. In addition, **Saboori *et al*, (2015)** found that vitamin E supplementation may be a good strategy for decreasing inflammatory conditions in susceptible people, although large well designed randomized controlled trials are needed. C-reactive protein (CRP) is an acute phase reactant protein and a biomarker for inflammation status. Its serum level increases during inflammatory conditions or any stresses. Furthermore, **Cooney *et al*., (2008)** reported a negative association between plasma levels of CRP and α-tocopherol, who also mentioned that α-tocopherol more effective in lowering serum level of CRP. NO has an important role in primary defense system that eliminate intracellular pathogens **(O'Flaherty *et al*., 2003).** Peroxynitrite, a reactive nitrogen metabolite, derived from oxidation of NO **(Beckman *et al*., 1990).** This means that excessive release of NO results in oxidative damage **(Atakisi *et al*., 2010). Nazifi1 *et al*., (2008)** reported an increase in serum Hp during pregnancy and especially near parturition due to cortisol and non-steroid fatty acids. Haptoglobine was a sensitive marker in various inflammatory conditions in cattle. **Saboori1 *et al*., (2015)** suggested that, supplementation with vitamin E could reduce serum CRP levels. A relationship between oxidative stress (lipid peroxidation) and antioxidant status was found significantly positive in advanced pregnant cows **(Sharma *et al*., 2011).**

Conclusion

The results obtained in this research showed dynamic changes in enzymatic antioxidant capacity of blood serum during transition period of dairy cows. A significant increase were observed in MDA, H_2O_2, NO, CRP and HP during stress. Moreover, the activities of antioxidant enzymes SOD, GPX, Gr, and CAT are significantly decreased in period of late pregnancy and early lactation. After addition of micronutrients Zn, Cu, Mn or α- tocopherole during times of oxidative stress revealed An increase in SOD, GPX, Gr, and CAT acteveties GSH concentration, and A significant decrease were observed in MDA, H_2O_2, NO, CRP and HP represents adaptive changes of cows in response to oxidative stress. Therefore, if there is an imbalance between increased production of ROS and reduced antioxidant capacity close to the time of parturition may increases oxidative stress and contribute to disorders in dairy postpartum cows.

References

Adela, P.; Zinveliu, D.; Pop, R.A.l.; Andrei, S. and Kiss, E. (2006). Antioxidant status in dairy cows during lactation. Bulgarian Journal of Agricultural Science 21: 655-661.

Aitken, S.L.; E.L. Karcher; P. Rezamand; J.C. Gandy; M.J. Vandehaar; A.V. Capuco and L.M. and Sordillo (2009). Evaluation of antioxidant and proinflammatory gene expression in bovine mammary tissue during the transition period. J. Dairy Sci. 92: 589-598.

Allison, R.D. and R.A. and Laven (2000). Effect of vitamin E supplementation on the health and fertility of dairy cows: a review. Vet. Rec. 147: 703-708.

Amir, H.; Asgari, S. and Nasroallah, M.K. (2013). Trace Mineral Requirements for Dairy Cattle. Int J Adv. Biol. Biom. Res.; 2 (2): 427-432.

Atakisi, O.; Oral, H.; Atakisi, E.; Merhan, O.; Pancarci, S.M.; Ozcana, A.; Marasli, S.; Polat, B.; Colak, A. and Kaya, S. (2010). Subclinical mastitis causes alterations in nitric oxide, total oxidant and antioxidant capacity in cow milk. Research in Veterinary Science, 89: 10-13.

Beatty, D.T.; Barnes, A.; Taylor, E.; Pethick, D.; McCarthy, M. and Maloney,

and S.K. (2006). Physiological response of *Bostaurus*and *Bosindicus* cattle to prolonged, continuous heat and humidity. J . Anim. Sci., 84: 972–985.

Beckman, J.S.; Beckman, T.W.; Chen, J.; Marshall, P.A. and Freeman, B.A. (1990). Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proceedings of the National Academy of Sciences of the United States of America, 87: 1620-1624.

Beckman, K.B. (1997). Ames BN. Oxidative decay of DNA. J Biol. Chem; 272: 19633–19636.

Bernabucci, U.; B. Ronchi; N. Lacetera and A. Nardone (2005). Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. J. Dairy Sci. 88: 2017-2026.

Bouwstra, R.J.; Goselink, R.M.A.; Dobbelaar, P.; Nielen, M.; Newbold, J.R. and van Werven, T. (2008). The Relationship Between Oxidative Damage and Vitamin E Concentration in Blood, Milk, and Liver Tissue from Vitamin E Supplemented and Non-supplemented Periparturient Heifers J. Dairy Sci. 91: 977–987.

Bouwstra, R.J.; Nielen, M.; Newbold, J.R.; Jansen, E.H.; Jelinekm, H.F. and van Werven, T. (2010). Vitamin E supplementation during the dry period in dairy cattle. Part II: Oxidative stress following vitamin E supplementation may increase clinical mastitis incidence postpartum. J Dairy Sci. 93: 5696-5706.

Castillo, C.; J. Hernandez; A. Bravo; M. Alonso-Lopez, V. and Pereira, J.L. Benedito, (2005). Oxidative status during late pregnancy and early lactation in dairy cows, The Veterinary Journal, 169, 286-292

Castillo, C.; J. Hernandez; I. Valverde; V. Pereira; J. Sotillo; M. Alonso-Lopez and J.L. Benedito (2006). Plasma malonaldehyde (MDA) and total antioxidant status (TAS) during lactation in dairy cows, Research in veterinary Science, 80, 133-139**.**

Celi, P.; Di Trana, A. and Claps, S. (2010). Effects of plane of nutrition on oxidative stress in goats during the peripartum period. Vet. J184, 95–99.

Celi, P.; M. Merlo; O. Barbato and G. Gabai (2012). Relationship between oxidative stress and the success of artificial insemination in dairy cows in a pasture-based system. The Veterinary Journal. 193: 498-502.

Chandra, G.; Aggarwal, A.; Singh, A.K.; Kumar, M. and Upadhyay, R.C. (2013). Effect of Vitamin E and Zinc Supplementation on Energy Metabolites, Lipid Peroxidation, and Milk Production in Peripartum Sahiwal Cows. Asian-Australasian Journal of Animal Sciences, 26: 1569-1576.

Circu, M.L. and Aw, T.Y. (2010). Reactive oxygen species, cellular redox systems, and apoptosis. Free RadicBiol Med, 2010; 48: 749-762.

Cooney, R.V.; Franke, A.A.; Wilkens, L.R.; Gill, J. and Kolonel, L.N. (2008). Elevated plasma γ-tocopherol and decreased α-tocopherol in men are associated with inflammatory markers and decreased plasma 25-OH vitamin D. Nutr Cancer; 60: 21–29.

Cortinhas, C.S.; Freitas junior, J.E.; Faves, J.R.; Porcionato, M.A.F., Silva, l.F.P.; Renno, F.P. and Santos, M.V. (2012). Organic and inorganic sources of zinc, copper and selenium in diets for dairy cows: intake, blood metabolic profile, milk yield and composition. Revistarasileira de Zootecnia, 41, 6, 1477-1483.

Dandekar, S.S.; Graham, E.M. and Plant, G.T. (2002). Lipid peroxidation and antioxidant enzymes in male infertility. J. Postgrad Med; 48: 186–90.

Eckersal, P.D. (2000). Recent advances and future prospects for the use of acute phase proteins and markers of disease in animals. Revue Med. Vet. 151(7): 577-584.

Elhashmi, Y.H.; Mohamed, A. and Ayman, O. (2016). A Review on Role of Zinc, Manganse and Copper in Rumen Metabolism and Immune Function: Article. Open Journal of Animal Sciences; 6304-6324.

Esterbauer, H.; Cheeseman, K.H.; Danzani, M.U.; Poli, G. and Slater, T.F. (1982). Separation and characterization of the aldehyde products of ADP/Fe^{2+}C stimulated lipid peroxidation in rate liver microsomes. Biochem J, 208: 129-40.

Feng, Li Yang and Xiao, Shan Li (2015). Role of antioxidant vitamins and trace elements in mastitis in dairy cows J. Adv. Vet. Anim. Res., 2(1): 1-9.

Festila, I.; Miresan, V.; Răducu, C.; Cocan, D.; Constantinescu, R. and Coroiane, A. (2012). Evaluation of oxidative stress in dairy cows through antioxidant enzymes glutathione peroxidase (GPX) and superoxide dismutase (SOD). Animal Science and Biotechnologies 69(1-2), pg. 107-110.

Floegel, A.; Chungm, S.J.; von Ruesten, A.; Yan, M.; Chung, C.E. and Song, W.O. (2011). Antioxidant intake from diet and supplements and elevated serum C-reactive protein and plasma homocysteine concentrations in US adults: a crosssectional study. Public Health Nutr 2011; 14: 2055–2064.

Frei, B. (1994). Natural Antioxidants in Human Health and Disease. San Diego, CA: Academic Press.

Gaafar H.M.A.; Basiuoni, M.I.; Ali, M.F.E.; Shitta, A.A. and Shamas, A.S.E. (2010). Effect of zinc methionine supplementation on somatic cell count in milk and mastitis in Friesian cows. Archiva Zootechnica, 13: 36-46.

Gabai, G.; Testoni, S.; Piccinini, R.; Marinelli, L. and Stradaioli, G. (2004). Oxidative stress in primiparous cows in relation to dietary starch and the progress of lactation. Anim Sci. 79, 99–108.

Garg, M.C. and Bansal, D.D. (2000). Protective antioxidant effect of vitamins in streptozotocin induced stress in diabetic rats. Indian J. Exp. Biol. 101-104.

Gitto, E.; R.J. Reiter; M. Karbownik; D.X. Tan; P. Gitto; S. Barberi and I. Barberi (2002). Causes of oxidative stress in thepre- and perinatal period. Biol. Neonate 81: 146-157.

Górecka, R.; Kleczkowski, M.; Kluciński, W.; Kasztelan, R. and Sitarska, E. (2002). Changes in antioxidant components in blood of mares during pregnancy and after foaling. *Bull. Vet. Inst. Pulawy*, 46: 301-305.

Gropper, S.S.; Smith, J. and Groff, J. (2005). Advanced Nutrition and Human Metabolism: Copper transport and uptake. 4thed. Wadsworth. Belmont, CA; pp 449-451.

Gross, R.T.; Bracci, R.; Rudolph, N.; Schroeder, E. and Kochen, J.A. (1967). Hydrogen peroxide toxicity and detoxification in the erythrocytes of newborn infants. Blood, 29: 481–493.

Halliwell, B. and Chirico, S. (1993). Lipid peroxidation: its mechanism, measurement, and significance. American Journal of Clinical Nutrition, 57 (1993), pg. 715-725.

Halliwell, B. and Gutteridge, J.M.C. (2007). Free Radicals in Biology and Medicine. Oxford University Press, Oxford, UK.

Kinal, S.; Korniewicz, A.; Jamroz, D.; Zieminski, R. and Slupczynska, M. (2005). Dietary effects of zinc, copper and manganese chelates and sulphates on dairy cows. Journal of Food, Agriculture and Environment, 3: 168-172.

Koracevic, D.; Koracevic, G.; Djordjevic, V.; Andrejevic, S. and Osic, V. (2001). Method for the measurement of antioxidant activity in human fluids. J. Clin. Pathol., 54: 356-361.

Lavrovsky, Y.; Chatterjee, B.; Clark, R.A. and Roy, A.K. (2000). Role of redox-regulated transcription factors in inflammation, ageing and age-related diseases. Exp. Gerontol.; 35: 521-32.

LeBlanc, S.J.; Duffield, T.F.; Leslie, K.E.; Bateman, K.G.; Ten Hag, J.; Walton, J.S. and Johnson W.H. (2002). The effect of prepartum injection of vitamin E on health in transition dairy cows. J. Dairy Sci. 85: 1416-1426.

Liu, H.W.; Zhou, D.W. and Lit, K. (2013). Effects of chestnut on performance and antioxidative status of transition dairy cows. J. Dairy Sci. 96: 5901-5907.

Lykkesfeldt, J. and Svendsen, O. (2007). Oxidants and antioxidants in disease: Oxidative stress in farm animals. Vet. J. 173, pg. 502-511.

Maurya, P.K.; Aggarwal, A.; Singh, S.V.; Chandra, G.; Singh, A.K. and Chaudhari, B.K. (2014). Effect of vitamin e and zinc on cellular antioxidant enzymes in karan fries cows during transition period. Indian J. Anim. Res., 48 (2): pg. 109-119.

Moron, M.S.; Depierre, J.W. and Mannervik, B. (1979). Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochimicaet Biophysica ACTA, 582: 67-78.

Moshage, H.; Kok, B.; Huizenga, J.R. and Jansen, P.L. (1995). Nitrite and nitrate determinations in plasma: A critical evaluation. ClinChem; 41: 892-96.

Muehlenbein, E.L.; Brink, D.R.; Deutscher, G.H.; Carlson, M.P. and Johnson, A.B. (2011). Effects of inorganic and organic copper supplemented to first-calf cows on cow reproduction and calf health and performance. 2001, 79: 1650-1659.J ANIM SCI.

Nazifi1, S.; Rezakhani, A.; Koohimoghadam, M.; Ansarilar, M. and Esmailnezhad, Z. (2008). Evaluation of serum haptoglobin in clinically Healthy cattle and cattle with inflammatory Diseases in shiraz, a tropical area in Southern iran Bulgarian Journal of Veterinary Medicine, 11, No. 2, 95−101.

NRC (2001). Nutrient Requirements of Dairy Cattle Seventh Revised Edition, 2001.

O'Flaherty, C.; Beorlegui, N. and Beconi, M.T. (2003). Participation of superoxide anion in the capacitation of cryopreserved bovine sperm. International Journal of Andrology, 26: 109-114.

Ohta, Y.; M. Kongo-Nishimura; Y. Imai; T. Matsura; A. Kitagawa and K. Yamada (2006). α-Tocopherol protects against alpha-naphthylisothiocyanate- induced hepatotoxicity in rats less effectively than melatonin. Chem. Biol. Interact. 161: 115–124.

Ohta, Y.; M. Kongo-Nishimura; Y. Imai; T. Matsura; A. Kitagawa and K. Yamada (2006). α-Tocopherol protects against alpha-naphthylisothiocyanate- induced hepatotoxicity in rats less effectively than melatonin. Chem. Biol. Interact. 161: 115–124.

Öztabak, K.; Civelek, S.; Özpinar, A.; Burçak, G. and Esen, F. (2005). The effects of energy restricted diet on the activities of plasma Cu-Zn SOD, GSH-Px, CAT and TBARS concentrations in late pregnant ewes. *Turk. J.* Vet.Anim. Sci., 29: 1067-1071.

Paoletti, F. and Macali, A. (1990). Determination of superoxide disumutase activity by purely chemical system based on NAD(P)H oxidation. Methods Enzymol 186: 209–220.

Pedernera, M.; Celi, P.; Garca, S.C.; Salvin, H.E.; Barchia, I. and Fulkerson, W.J. (2009). Effect of diet, energy balance and milk production on oxidative stress in early-lactating dairy cows grazing pasture. Vet. J. In Press.

Saboori, S.; Shab-Bidar, S.; Speakman, J.R.; Yousefi, Rad E. and Djafarian, K. (2015). Effect of vitamin E supplementation on serum C-reactive protein level: a meta-analysis of randomized controlled trials European Journal of Clinical Nutritionm69, 867 –873.

Saboori1, S.; Shab-Bidar, S.; Speakman, J.R.; Yousefi, Rad E. and Djafarian, K. (2015). European Journal of Clinical Nutrition 69, 867–873.

Saeed, S.A.; N. Ahmad and S. Ahmed (2007). Dual inhibition of cyclooxygenase and lipoxygenase by human haptoglobin: its polymorphism and relation to hemoglobin binding. Biochem Biophys Res Commun. 353: 915-920.

Saleh, M.; A. Salam and MEL Mileegy, I. M.H. (2007). Oxidative antioxidant status during transition from late pregnancy to early lactation in native and cross bred cows in the Egyptian oasis. Assiut. Vet. Med. J. 53: 113.

Sales, J.N.S.; Pereira, R.V.V.; Bicalho, R.C. and Baruselli, P.S. (2011). Effect of injectable copper, selenium, zinc and manganese on the pregnancy rate of crossbred heifers (Bosindicus × Bostaurus) synchronized for timed embryo transfer. Livestock Science. 142(1–3): 59–62.

Sevanian, A. and Ursini, F. (2000). Free Radic. Biol Med; 29: 306-311.

Sharma, N.; Singh, N.K.; Singh, O.P.; Pandey, V. and Verma, P.K. (2011). Oxidative stress and antioxidant status during transition period in dairy cows. Asian-Aust J. Anim-Sci., 24: 479-484.

Sharma, N.; Singh, N.K. and Bhadwal, M.S. (2011). Relationship of somatic cell count and mastitis: an overview. Asian-Australas J Anim. Sci., 24: pg. 429-438.

Sinha, A.K. (1972). Colorimetric assay of catalase. Analytical biochemistry, 47, 389.

Snitynskyj, V.V.; Antoniak, H.L. and Bershadskyi, V.I. (1996). The age-related changes in the antioxidant system enzymes of the erythroid cells of swine at the early stages of postnatal development. Fiziol. Zh. 42, 19–25.

Sordillo, L. (2013). Selenium-dependent regulation of oxidative stress and immunity in periparturient dairy cattle. Vet Med Int. 154045.

Sordillo, L.M. and Aitken, S.L. (2009). Impact of oxidative stresson the health and immune function of dairy cattle. Vet. Immunol.

Immunopathol., 128: 104-109.

Sordillo, L.M.; O'Boyle, N.; Gandy, J.C.; Corl, C.M. and Hamilton, E. (2007). Shifts in thioredoxinreductase activity and oxidant status in mononuclear cells obtained from transition dairy cattle. J. Dairy Sci. 90, pg. 1186-1192.

Spears, J.W. and Weiss, W.P. (2008). Role of antioxidants and trace elements in health and immunity of transition dairy cows. Vet. J., 176: 70-76.

SPSS. 14 (2006). Statistical Package for Social Science, SPSS for windows Release 14.0.0, Standard Version, Copyright SPSS Inc.

Tietz, N.W. (1995). Clinical Guide to Laboratory Tests, 3rd Ed, WB Saunders Company, Philadelphia, P. A.

Trevisan, M.; R. Browne; M. Ram; P. Muti; J. Freundenheim; A.N. Carosella and D. Armstrong (2001). Correlates of markers of oxidative status in the general populatin. Am. J. Epidemiol., 154, 348-356.

Turk, R.; Podpečan, O.; Mrkun, J.; Kosec, M.; Flegar-Mestrić, Z.; Perkov, S.; Starič, J.; Robić, M.; Belić, M. and Zrimsek, P. (2013). Lipid mobilization and oxidative stress as metabolic adaptation processes in dairy heifers during transition period. Anim. Reprod. Sci., 141: 109-115.

Vallee, B.L. and Falchuk, K.H. (1993). The biochemical basis of zinc hysiology. Physiol. Rev. 73: 79-118.

Waller, K.P. (2000). Mammary gland immunology around parturition. Influence of stress, nutrition and genetics. Adv. Exp. Med. Biol. 480: 231-245.

Weiss, W.P.; Hoganm, J.S. and Smithm, K.L. (2004). Changes in vitamin C concentrations in plasma and milk from dairy cows after an intramammary infusion of Escherichia coli. ournal of Dairy Science, 87: 32-37.

Wintrobe, M.M. (1965). Clinical hematology, 4----th. Ed. Iea&febiger, Philadelphia Biochm. 11: 189-200.

Xu, J.B.; Yuan, X.F. and Lang, P.Z. (1997). Determination of catalase activity and catalase inhibition by ultraviolet spectrophtometry. Chinese Environ. Chem. 16: pp. 73-76.

Young, B.; Gleeson, M. and Cripps, A.W. (1991). C-reactive protein: a critical review. Pathology.; 23: 118–24.

Zhao, X. and Lacasse, P. (2008). Mammary tissue damage during bovine mastitis: causes and control. J Anim Sci 86(13 Suppl): 57-65.