

Scrutinizing of Trace Elements and Antioxidant Enzymes Changes in Barki Ewes Fed Salt-Tolerant Plants under South Sinai Conditions

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Abstract: In attempt to monitor the pattern of trace elements and antioxidant changes in ewes as a result of feeding silage of salt tolerant plants during different physiological status under South Sinai conditions. Forty two Barki ewes were randomly divided into two equal groups (21 each). The first group (control, G1) was fed berseen hay while the second group (G2) was fed silage form of salt tolerant plants and concentrates feed mixtures. This experimental was carried out at South Sinai Station (Ras Sudr) belonging to Desert Research Center (DRC), Ministry of Agricultural and Land Reclamation, Egypt.

Blood samples were collected from the all animals during dry, gestation (early, mid, late) and early lactation periods. The profiles of trace elements (Cu, Se, Mn and Zn), malondialdehyde (MDA), lipid peroxidation and oxidative stress markers [total antioxidant capacity (TAC), antioxidant catalase (CAT) enzymes] were analyzed in plasma. On the other hand, antioxidant enzyme (SOD and GPX) activities were analyzed in erythrocyte.

The obtained results declared that there were significant differences in plasma levels of trace elements (Cu, Mn, Se and Zn) and consequently antioxidant enzymes (SOD and GPX) between treatment groups. On the other hand, pregnancy and lactation constituted the most oxidative stress facing the animals of the two groups since oxidative stress index (MDA) was increased and TAC was decreased and were significantly different in treatments and physiological status.

It could be concluded that feeding silage form of salt tolerant plants was not harmful for desert Barki ewes raised under semi- arid condition of South Sinai. Furthermore, pregnancy and lactation periods constituted oxidative stresses on animals even fed traditional or untraditional (salt tolerant plants) diets. So, it is recommended that supplementing trace elements diet in order to improve antioxidant status (defense system) which consequently enhances growth performance and animal productivity.

[Hanan, Z. Amer; Ibrahim, N. H.; Donia, G. R; Younis, F. E. and Shaker, Y. M. **Scrutinizing of Trace Elements and Antioxidant Enzymes Changes in Barki Ewes Fed Salt-Tolerant Plants under South Sinai Conditions.** *J. Am. Sci.*, 2014; 10(2): 241- 249]. (ISSN: 1545-1003). <http://www.americanscience.org>. 34

Keywords: Salt tolerant plants, Barki ewes, pregnancy, trace elements, lipid peroxidation, oxidative stress, antioxidant enzymes,

1. Introduction

Demands of agricultural products, particularly in developing countries, are increasing rapidly. Consequently, land and water resources are unable to sustain such demands. Maximizing the efficiency of resource utilization and identification of alternative resources to support agricultural production are becoming a priority in many developing countries. Many arid and semi-arid regions in the world have soils and water resources that are too saline for most of the common conventional crop systems (Pitman and Lauchli, 2002). Halophytes and other salt-tolerant plants may provide sensible alternatives for many developing countries (Squires and Ayoub, 1994). The less and unpalatable plant species represent approximately 70% of the total coverage. Several attempts have been made towards utilization of such less and unpalatable halophytic plants

through proper processing methods to improve their palatability and nutritional utilization. Ensiling halophytic plants with other feed ingredients appeared to be the most convenient processing method under the prevailing conditions of aridity in Egypt (Abou El Nasr *et al.*, 1996).

Salinity of salt tolerant plants, pregnancy and early lactation are known to be stressful on organisms which accelerates the production of reactive oxygen species (ROS) and oxidative stress (Górecka *et al.*, 2002). These reactive oxygen species are normally neutralized by enzymatic and non-enzymatic defense systems of living organisms. The imbalance between the rate of ROS production and their neutralization leads to the oxidative stress (Adela *et al.*, 2006). These species of oxygen are highly cytotoxic and can seriously react with vital biomolecules such as lipids, proteins, nucleic acid, etc..., causing lipid

peroxidation, protein denaturing and DNA mutation. Evidences suggest that membranes are the primary sites of oxidative stress because ROS can react with unsaturated fatty acids causing peroxidation of essential membrane lipids in plasma membrane or intracellular organelles (Esfandiari *et al.*, 2007). Polyunsaturated fatty acids present in membrane phospholipids are the main target substrates for oxygen radical activity which results in disorganization of cell framework and function (Nazifi *et al.*, 2010a). Peroxidation of plasma membrane leads to the leakage of cellular contents, rapid desiccation and cell death. Lipid peroxides derived from polyunsaturated fatty acids are unstable and are decomposed to form a series of compounds, including malondialdehyde (MDA). The quantization of MDA is widely used as an indicator of lipid peroxidation and oxidative stress in cells and tissues (Simsek *et al.*, 2006). The cells have evolved a number of counteracting antioxidant defenses. Antioxidant enzymatic activities are of utmost importance because they may indicate how tissue or organ might respond to oxidative stress in oxidizing environment. The natural defense mechanisms against free radicals consist of enzymatic antioxidants like glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT) and non-enzymatic antioxidants like glutathione (GSH), ascorbate, urate, vitamin E and beta carotene (Erisir *et al.*, 2009). SOD is the first line of defense against ROS and is active in catalyzing detoxification of superoxide radical. The hydrogen peroxide generated in this reaction is restored to water in the presence of CAT and GPX (Nazifi *et al.*, 2010a).

Therefore, this study was carried out so as to monitor the changes in some trace elements (Cu, Zn, Mn and Se), malondialdehyde (MDA), lipid peroxidation and oxidative stress markers (total

antioxidant capacity (TAC), antioxidant catalase (CAT) enzymes) and antioxidant enzyme (SOD and GPX) activities which may occur during different physiological stages in Barki ewes fed salt tolerant plants raised under semi-arid conditions of South Sinai, Egypt.

2. Materials and Methods

This investigation was undertaken at South Sinai Station (Ras Sudr) which belongs to Desert Researcher Center, Ministry of Agriculture and Land Reclamation, Egypt, in order to designate the effect of feeding salt tolerant plants on changes in antioxidant enzymes and its relation with plasma profiles of trace elements (Cu, Zn, Mn and Se) during different physiological status.

Forty two Barki ewes aging 2.5- 3 years old and averaging 41.50 ± 4.85 kg body weight were divided into two equal groups. The first group was fed berseen hay (*Trifolium alexandrinum*, 4th cut) and served as control while the second one was fed silage form of salt tolerant plants (*Atriplex halimus*, 50%; fodder beet, 25%; *Pearl millet*, 15% and *Carthamus tinctorius* hay, 10%). Experimental animal were fed their nutrient requirements according to Kearn (1982).

The trace elements in terms of copper (Cu), manganese (Mn), selenium (Se) and zinc (Zn) were analyzed in the both rations of the two groups using microwave digestion technique (1.5 ml of sample + 8 ml of HNO₃ 65%, 2 ml of H₂O₂ 30% in a closed Teflon vessel under high temperature and pressure control) as reported by Littlejohn *et al.*, (1991). The metals were determined by spectroscopic methods, Flame photometer and Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) in the central Lab of Desert Research Center (Water and Soil Analysis Unit).

Table (1): Trace elements concentration (ppm) in the experimental rations

	Copper	Manganese	Selenium	Zinc
Control group	9.40	32.33	450	720.10
Silage group	13.26	76.30	350	68.61

Blood samples were collected into heparinized tube (10 ml) from all experimental animals. Heparinized blood was centrifuged at 3000 rpm for 15 min. at 4°C. Pipette off plasma without disturbing the white buffy layer. Plasma was kept and stored at -80°C pending analysis of trace elements, lipid peroxide (MDA), total antioxidant capacity (TAC) and catalase (CAT) enzymes.

Trace elements (Cu, Zn, Mn and Se) levels analysis in plasma were determined by flame atomic absorption spectrophotometer (Pye-Unicam SP9).

Lipid peroxidation was assayed by measuring the level of malondialdehyde (MDA) by the method of Ruiz-Larrea *et al.* (1994) using thiobarbituric acid (TBA). The acid reacts with MDA to form a stable pink color with maximum absorption at 532 nm and expressed as nmol/ml.

TAC (mU /l) and catalase (U/l) were measured by colorimetric techniques using a commercially available kit (Bio-diagnostic., Egypt) according to the method of Koracevic *et al.* (2001) and Aebi (1984), respectively.

For determination of superoxide dismutase enzyme (SOD), 0.5 ml of heparinized whole blood was centrifuged for 10 min. at 4000 rpm at 4°C and then plasma aspirated off. Then red blood cells washed four times with 3 ml of 0.9 % saline solution (NaCl), centrifuged for 10 min. at 4000 rpm after each wash. The washed centrifuged erythrocytes should then be made up to 2.0 ml with cold redistilled water. Mixed and left to stand at +4°C for 15 min. and stored at -80 °C until analysis. The lysate is diluted with distilled water (50 fold), so the % inhibition falls between 30% and 60%. SOD measured calorimetrically using a commercially available kit (Bio-diagnostic., Egypt) according to the method of Nishikimi *et al.*, (1972) and expressed as U/L.

For determination of glutathione peroxidase enzyme (GPX), the red blood cells collected by centrifugation of heparinized whole blood (4000 rpm x 10 min. at 4°C) then plasma drawn off. Erythrocytes washed once (one time) with 10 volumes of cold saline. The red blood cell pellets lysed by adding 4 volumes of cold deionized water to the estimated pellet volume, then the red cell stroma was removed by centrifuging (4000 rpm x 10 min. at 4°C). The resulting clarified supernatant was collected and stored at -80 °C until assay. GPX measured calorimetrically using a commercially available kit (Bio-diagnostic., Egypt) according to the method of Paglia and Valentine (1967) and expressed as mU/mL.

Experimental data were analyzed using General Linear Model Procedure (SAS, 2004).

3. Results and Discussion

The obtained results demonstrated that feeding salt tolerant plants silage lowered ($P < 0.01$) the mean values of plasma and manganese than control group (Table 2) although salt tolerant plants silage contained higher levels of manganese (Mn) (Table 1). Unfortunately, concentrations of Mn in plasma are not good indicators of Mn intake. Concentrations of Mn in the red blood cells are higher than in plasma and have been used to assess status (Hidiroglou *et al.*, 1978). Dietary Mn affects the concentration of Mn in bones and other tissues (Kincaid, 1999). Exhibiting the same trend, selenium (Se) levels in the blood serum was lower ($P < 0.01$) in ewes fed salt tolerant plants silage than in their counterparts fed the traditional ration (Table 2). These results might be due to the low content of (Se) in salt tolerant plants silage ration. Concentrations of Se in whole blood are responsive to Se intake (Levander, 1986).

Contrariwise, animals salt tolerant silage group achieved higher ($P < 0.01$) copper (Cu) and zinc (Zn) mean values than control group (Table 2). The higher

values of copper (Cu) in salt tolerant plants silage group could be attributed to the higher Cu intake in the diet (Table 1). Ashton (1970) reported that copper levels in tissues and body fluids depend on diet, state of health, age and sex. Copper is a mineral element that activates several enzyme systems, and though in less numbers than Zn, it is considered an essential nutrient (Minatel and Carfagnini, 2000). The physiological role of Cu in the organism is related to several functions, which include cellular respiration, bone formation, connective tissue development, and essential catalytic cofactor of some metallo-enzymes, among other (McDowell, 2003 and Underwood and Suttle, 2003).

Unexpectedly, zinc concentrations in plasma of salt tolerant plants animals exceeded ($P < 0.01$) their counterparts of control group (Table 2) although the low levels of Zn intake (Table 1). This might be that animals fed salt tolerant plants were more efficient to utilize the low Zn intake. Elnageeb and Abdelatif (2010) suggested that a combination of low nutritional status and pregnancy in non-supplemented ewes may increase the efficiency of utilization of ingested Zn. The major part of the total body Zn is in the bones and competes with Cu for absorption from the intestinal tract (Kargin *et al.*, 2004). The need for Zn in most animals is based on its influence on enzymes and proteins and their activities, that are linked to vitamin A synthesis, carbon dioxide (CO₂) transport, collagen fiber degradation, free radical destruction, membrane stability of red blood cells, metabolism of essential fatty acids, carbohydrate metabolism, protein synthesis and metabolism of nucleic acids, among others (Powell, 2000; McCall *et al.*, 2000; Stefanidou *et al.*, 2006 and Rubio *et al.*, 2007).

Concerning the effect of physiological status, the present results showed that pregnancy and lactation influenced the levels of serum micro-minerals of ewes fed silage of salt tolerant plants (Table 2). Pregnancy and lactation constituted metabolic stress, associated with alterations in the minerals profile dependent on the reproductive status of small ruminants. Metabolism of mineral elements plays a significant role in the regulation of physiological functions of pregnancy and lactation. Moreover, substantial losses of body minerals occur during pregnancy and lactation (Ceylan *et al.*, 2009; Elnageeb and Adelatif, 2010). The concentration of minerals varies in blood as a result of interactions between those nutrients, transfer of nutrients to the fetus and initiation of milk synthesis (Kincaid, 2008). Pregnancy presents a considerable stress to trace mineral homeostasis in mammals (Mills and Davies 1979).

Table (2): Means of some trace elements concentrations of the experimental groups as affected by feeding silage of salt tolerant plants during different physiological status under South Sinai conditions

Minerals	Treat	Dry	Pregnancy				Lactatio n	Overall	±SE		
			Early	Mid	Late	T			S	TxS	
Cu, ppm	T1	1.96 ^{Aa}	1.54 ^{Aa}	1.07 ^{Ab}	0.94 ^{Ab}	1.67 ^{Aa}	1.44 ^A	0.07**	0.11**	0.15**	
	T2	2.00 ^{Aa}	2.36 ^{Ba}	2.36 ^{Ba}	2.10 ^{Ba}	0.94 ^{Bb}	1.95 ^B				
	Overall	1.98 ^a	1.95 ^a	1.71 ^{ab}	1.52 ^{bc}	1.30 ^c					
Mn, ppm	T1	0.137 ^{Aa}	0.499 ^{Ab}	0.855 ^{Ac}	0.675 ^{Ad}	0.163 ^{Aa}	0.47 ^A	0.02**	0.03**	0.04**	
	T2	0.487 ^{Ba}	0.481 ^{Aa}	0.233 ^{Bb}	0.199 ^{Bb}	0.154 ^{Ab}	0.31 ^B				
	Overall	0.319 ^a	0.490 ^b	0.543 ^c	0.437 ^b	0.158 ^d					
Zn, ppm	T1	7.05 ^{Aa}	6.73 ^{Aa}	10.22 ^{Ab}	8.25 ^{Aab}	7.20 ^{Aa}	7.89 ^A	0.29**	0.46**	0.65*	
	T2	6.34 ^{Aa}	6.38 ^{Aa}	12.59 ^{Bb}	11.22 ^{Bb}	8.57 ^{Aa}	9.02 ^B				
	Overall	6.69 ^c	6.56 ^c	11.40 ^a	9.73 ^b	7.88 ^c					
Se, ppm	T1	0.035 ^{Aa}	0.076 ^{Aa}	0.116 ^{Aa}	0.101 ^{Aa}	0.225 ^{Ab}	0.11 ^A	0.01*	0.02**	0.02**	
	T2	0.104 ^{Aa}	0.075 ^{Aab}	0.052 ^{Aab}	0.055 ^{Aab}	0.033 ^{Bab}	0.063 ^B				
	Overall	0.069 ^b	0.075 ^b	0.084 ^{ab}	0.078 ^b	0.129 ^a					

T1: animals fed berseem hay

T2: animals fed salt tolerant plants silage

In the same column, means in a certain item having the same capital letter do not differ significantly.

In the same row, means in a certain item having the same small letter do not differ significantly.

Serum Cu level was lower during lactation compared to the respective values obtained pre and during pregnancy (Table 2). This could be related to the response of the ewes to the needs of the foetus by increasing mobilization of stored Cu for the development of the nervous system (Elnageeb and Adelatif, 2010).

The present results demonstrated that the differences in Zn levels among the periods of dry, pregnancy and lactation were significant ($P < 0.01$). However, the levels of Zn were higher in mid, late pregnancy and lactation period as compared to the dry period (Table 2). These results could be attributed to the increase in the rate of accumulation of Zn in the foetus. Williams *et al.* (1972) reported that the developing foetus accumulates 1 to 2 mg of Zn/ day and the pregnant ewe increases the demands for Zn towards the end of pregnancy. However, Elnageeb and Abdelatif (2010) reported that no significant changes were observed in Zn level during the experimental periods (dry, pregnancy and lactation periods). The serum Zn level was slightly higher during pregnancy compared to the value obtained during early flushing period. There is also evidence in sheep and cattle that the Zn status and intake affect Zn absorption from the gut (Kirchgessner, 1976 and Suttle, 1988).

Concerning the effect of physiological status on the manganese level, the obtained results demonstrated that pregnancy increased ($P < 0.01$) the Mn level from the dry period to reached its peak at mid- pregnancy then it decreased gradually till the

lactation period which had the lowest Mn values (Table 2). Similarly, El-Tohamy *et al.* (1986) reported lower plasma manganese in non-pregnant camels. However, according to the authors, no variation owing to pregnancy was observed, contrary to other trace elements.

During pregnancy and lactation, the concentration of Se decreased in ewes fed silage of salt tolerant plants as compared with control ewes that fed berseem hay (Table 2) which might be attributed to the low Se concentration in salt tolerant plants (Table 1). This may be explained by the concentration of selenium in plants varies widely and depends on the selenium content and characteristics of the soil (Pechová *et al.*, 2008). The selenium concentration in soil is low in many parts of the world including South Sinai Research Station where it is poorly available and incapable of providing the required amount to animals because of the presence of the antagonistic relationship between water irrigation salinity and Se available in the soil (Sadek, 1995 and Fahmy *et al.*, 2009).

The detection of free radical damage and the protection against it has become very important in the studies related to ruminant production and reproduction as the level of lipid peroxidation and antioxidant status give complementary information about the metabolic status of the animal rather than metabolic parameters alone (Castillo *et al.*, 2003).

The obtained results, as shown in Table (3), revealed that malondialdehyde (MDA) level was found to be significantly ($P < 0.01$) increased in the

two experimental groups along the advanced of pregnancy. This gradual increase with the progression of pregnancy was associated with decreases antioxidant enzyme levels; total antioxidant capacity (TAC), antioxidant catalase (CAT) enzyme, superoxide dismutase (SOD) and glutathione peroxidase (GPX). The maximum level of lipid peroxidation was observed in late pregnancy and early lactation in control ewes that fed berseem hay comparing with the level of MDA in ewes fed silage of salt tolerant plants in late pregnancy. This finding is in agreement with the findings of Toescu *et al.*, (2002), Upadhyaya *et al.*, (2005) and Patil *et al.*, 2006 and 2007) who reported that markers of lipid peroxidation (MDA) to be increased during the progression of normal pregnancy. MDA is considered the final product of lipid peroxidation and a marker

of oxidative stress. In the same time, trace elements showed the same trend of decrease in ewes fed silage of salt tolerant plants especially Se in all experimental periods, Cu in early lactation and Mn in late pregnancy and early lactation (Table 2). It is known that various kinds of stress such as salinity, pregnancy and lactation accelerate the production of reactive oxygen species and oxidative stress (Górecka *et al.*, 2002). These species of oxygen are highly cytotoxic and can seriously react with vital biomolecules such as lipids, proteins, nucleic acid, etc., causing lipid peroxidation, protein denaturing and DNA mutation (Esfandiari *et al.*, 2007). In health, reactive oxygen species (ROS) and antioxidants remain in balance but this balance is disrupted in cases of oxidative stress (Aurousseau *et al.*, 2006).

Table (3): Means of malondialdehyde and antioxidant enzymes concentrations of the experimental groups as affected by feeding silage of salt tolerant plants during different physiological status under South Sinai conditions

Minerals	Treat	Dry	Pregnancy			Lactation	Overall	±SE		
			Early	Mid	Late			T	S	TxS
MDA (nmol/ml)	T1	0.78 ^{Aa}	1.26 ^{Ab}	1.30 ^{Ab}	1.33 ^{Ab}	1.33 ^{Ab}	1.20 ^A	0.02**	0.02**	0.03 ^{NS}
	T2	0.68 ^{Ba}	1.14 ^{Bb}	1.12 ^{Bb}	1.23 ^{Aab}	1.13 ^{Bb}	1.06 ^B			
	Overall	0.73 ^c	1.20 ^b	1.21 ^{ab}	1.28 ^a	1.23 ^{ab}				
TAC (mU/L)	T1	1.32 ^{Aa}	1.06 ^{Ab}	0.70 ^{Ab}	1.04 ^{Ab}	1.14 ^{Ab}	1.13 ^A	0.02**	0.02**	0.03*
	T2	1.35 ^{Aa}	1.02 ^{Ab}	0.83 ^{Bc}	0.95 ^{Ab}	1.04 ^{Ab}	1.04 ^B			
	Overall	1.33 ^a	1.04 ^{bc}	0.95 ^d	1.00 ^{cd}	1.09 ^b				
Catalase (U/L)	T1	203.02	153.20	148.53	70.21	61.80	127.35	2.30 ^{NS}	3.64**	5.14 ^{NS}
	T2	204.46	170.65	139.05	78.04	57.14	129.87			
	Overall	203.74 ^a	161.93 ^b	143.79 ^c	74.12 ^d	59.47 ^e				
SOD (U/L)	T1	235.12 ^{Aa}	244.34 ^{Aa}	222.78 ^{Aa}	197.40 ^{Ab}	187.58 ^{Ab}	217.44 ^A	3.16*	4.99**	7.06**
	T2	246.36 ^{Aa}	210.36 ^{Bb}	171.25 ^{Bc}	189.59 ^{Ab}	215.92 ^{Bb}	206.70 ^B			
	Overall	240.74 ^a	227.35 ^a	197.02 ^b	193.49 ^b	201.75 ^b				
CPX (mU/L)	T1	453.91 ^{Aa}	402.04 ^{Ab}	252.89 ^{Ac}	376.10 ^{Ab}	233.44 ^{Ac}	343.67 ^A	5.62**	8.88**	12.56**
	T2	434.46 ^{Aa}	337.19 ^{Bb}	149.14 ^{Bc}	226.96 ^{Bd}	207.50 ^{Ad}	271.05 ^B			
	Overall	444.19 ^a	369.61 ^b	201.02 ^d	301.53 ^c	220.47 ^d				

T1: animals fed berseem hay

T2: animals fed salt tolerant plants silage

In the same column, means in a certain item having the same capital letter do not differ significantly.

In the same row, means in a certain item having the same small letter do not differ significantly.

The rise of oxidative stress markers could be due to pregnancy and early lactation which are considered as stressful stages accompanied by a high metabolic demand and elevates the requirements for tissue oxygen (Patil *et al.*, 2007 and Idonije *et al.*, 2011) and causes an increase of reactive oxygen species production. This could be explained by the fact that 80% of foetus growth occurs in the last 2 months of pregnancy, so ewes exhibit a dramatic increase in metabolism during this period (Cristian and Jauhianinen, 2001). The rapid growth of foetus during the last 6 weeks of pregnancy, causes an increase in fatty acid consumption from the mother's

fat reserve and production of hydrogen peroxide that has been enhanced by intense lipolysis and mobilization of fatty acids from the body deposits during pregnancy (Öztabak *et al.*, 2005 and Rezapour and Roudbaneh, 2011) and during lactation in order to sustain the lactogenesis (Adela *et al.*, 2006). Moreover, the placenta is a major source of oxidative stress because of its enrichment with polyunsaturated fatty acids (PUFA) (Gitto *et al.*, 2002).

In addition to pregnancy, salinity of salt tolerant plants is another stressor on ewes. The soils where halophytes normally grow becomes more saline due to rapid evaporation of water particularly during

summer, therefore, surface of the soil tend to have higher soil salinity and higher water potential (Khan and Gul, 2002). The adverse effects of the salt on cell membranes are results of the accumulating toxic ions and ROS (Cicerali, 2004) and evidence suggests that membranes are the primary sites of salinity injury to cells and organelles because ROS can react with PUFA and results in disorganization of cell framework and function and cause peroxidation of essential membrane lipids in plasma membrane or intracellular organelles. Peroxidation of plasma membrane leads to the leakage of cellular contents, rapid desiccation and cell death. Lipid peroxidation is an indicator of oxidative stress in cells and tissues (Esfandiari *et al.*, 2007).

The result of the present study indicated that there was a negative relationship between antioxidant enzyme activities and lipid peroxidation or MDA content (Table 3). Enzymes with important antioxidant functions include: i) superoxide dismutase (SOD), which catalyses the dismutation of superoxide radical to hydrogen peroxide and water, ii) catalase (CAT), which catalyses the breakdown of hydrogen peroxide to oxygen and water, and iii) glutathione peroxidase (GPX), which facilitates the destruction of both hydrogen peroxide and organic peroxides. SOD is the first line of defense against ROS and is active in catalyzing detoxification of superoxide radical (Nazifi *et al.*, 2010a). The hydrogen peroxide generated in this reaction is restored to water in the presence of CAT and GPX.

Several studies have indicated that antioxidative defense system is modified during normal pregnancy. Late-pregnant ruminants tend to have raised lipoperoxidative processes as a consequence of increased production of free oxygen radicals and therefore have a low antioxidative status (Dimri *et al.* 2010). The susceptibility of cells to oxidative stress is a function of the overall balance between the degree of oxidative stress and the antioxidant defense capability. Decreased concentrations of catalase, SOD and GPX activities may reflect oxidative stress in pregnant ewes (Erisir *et al.*, 2009).

It is worthwhile to mention that the decrease of antioxidant enzymes level during late pregnancy and early lactation in our study is dependent on trace elements profile where trace elements showed the same trend of decrease in both experimental groups; Se in all experimental periods, Cu in early lactation and Mn in late pregnancy and early lactation (Table 2 and 3).

Animals fed plants grown in selenium-deficient areas and not supplemented with minerals are vulnerable to oxidant stress (Steen *et al.*, 2008). Many authors confirmed a positive correlation between GSH-Px activity and selenium concentration

in whole blood where about 11.8% of total Se in the organism is bound in GSH-Px. (Awadeh *et al.*, 1998) and Trávníček *et al.*, (2008) proved a high correlation between Se content in the whole blood and GSH-Px activity in the blood of ewes. Our findings demonstrates that the correlation between GSH-Px activity and selenium concentration in the whole blood of ewes is very close and that GSH-Px activity could be considered as a good indicator and diagnostic tool for the determination of selenium status in sheep (Pavlata *et al.*, 2012) and consequently in the evaluation of antioxidant status (Adela *et al.*, 2006).

According to Sattar *et al.* (2007), pregnant animals are more susceptible to selenium deficiency than non-pregnant animals and maternal selenium concentrations and glutathione peroxidase activity fall during pregnancy (Mistry and Williams, 2011). This decrease in selenium status which is progressive as gestation proceeds may be partly attributed to hemodilution from the blood volume increase associated with pregnancy (Boskabadi *et al.*, 2010). Moeini *et al.*, (2011) reported that Se concentration decreased during the final 60 days of gestation, emphasizing the importance of se supplementation during late gestation

Humphries *et al.* (1983) revealed that in experimental copper deficiency in calf, plasma concentration of copper and SOD activity of erythrocytes severely decreased. Similarly, Nazifi *et al.* (2010b) found a positive correlation between plasma concentration of copper and SOD activity of erythrocytes because copper, zinc and magnesium are the main components of SOD that plays a vital role as an antioxidant and protects from oxidative stress (Nazifi *et al.*, 2010b).

From the above results, it could be concluded that feeding desert animals; Barki ewes, silage form of salt tolerant plants was not hazardous under semi-arid conditions of South Sinai. Furthermore, the pregnancy and lactation periods constitute oxidative stresses on animals even fed traditional or untraditional (salt tolerant plants) diets. So, we recommended supplementing trace elements to the diets so as to improve the antioxidants capacity (defense system) which consequently enhance growth performance and animal productivity.

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Acknowledgment

The authors would thank Prof. Dr. El-Shaer, the coordinator and Prof. Dr. Badawy, the key person of

the regional project titled “Adaptation to climate changes in WANA marginal environments through sustainable crop and livestock diversification” which is funded by International Center for Biosaline Agricultural (ICBA), UAE for their financial support to achieve this work.

References

- Abou El- Nasr, H. M., Kandil, H. M., El-Kerdawy, D. A., Khamis, H. S. and El-Shaer, H. M., (1996). Value of processed under arid condition of Egypt. *Small Ruminant Res.* 24: 15-20.
- Adela, P.; Zinveliu, D.; Pop, R. A.; Andrei, S. and Kiss, E. (2006). Antioxidant status in dairy cows during lactation. *Buletin USAMV-CN.*, 130 – 135.
- Aebi, H. (1984). Catalase *in vitro*. *Methods Enzymol.*, 105: 121 – 126.
- Ashton, W. M. (1970). Trace elements in enzyme systems with special reference to deficiency of copper and cobalt in some animal diseases. *Outlook Agricul.*, 6: 95- 101.
- Aurousseau, B.; Dominique, G. and Durand, D. (2006). Gestation linked radical ROS fluxes and vitamins and trace element deficiencies in the rudiment. *Reproduction Nutrition Development*, 46: 601- 620.
- Awadeh, F. T.; Kincaid, R. L. and Johnson, K. A. (1998). Effect of level and source of dietary selenium on concentrations of thyroid hormones and immunoglobulins in beef cows and calves. *J. Anim. Sci.*, 76: 1204 – 1215.
- Boskabadi, H.; Omran, F. R.; Tara, F.; Rayman, M. P.; Ghayour-Mobarhan, M.; Sahebkar, A.; Tavallaie, S.; Shakeri, M. T.; Alamdari, D. H.; Kiani, M.; Razavi, B. S.; Oladi, M. and Ferns, G. (2010). The effect of maternal selenium supplementation on pregnancy outcome and the level of oxidative stress in neonates. *Iranian Red Crescent Medical Journal*, 12(3): 254 – 259.
- Castillo, C.; Hernández, J.; López-Alonso, M.; Miranda, M. and Benedito, J. L. (2003). Values of plasma lipid hydroperoxides and total antioxidant status in healthy dairy cows: preliminary observations. *Arch. Tierz.*, 46: 227 – 233.
- Ceylan, E.; Tarritanir, P. and Dede, D. (2009). Changes in some macro-minerals and biochemical parameters in female healthy Siirt Hair goats before and after parturition. *J. Animal and Veterinary Advances*, 8 (3): 530 – 533.
- Cicerali, I. N. (2004). Effect of salt stress on antioxidant defence systems of sensitive and resistant cultivars of lentil (*Lens culinaris M.*). M.Sc. thesis, submitted to the Graduate School of Natural and Applied Science of Middle East Technical University, Turkey.
- Cristian, R. S. and Jauhianinen, L. (2001): Comparison of hay and silage for pregnant and lactating finish langrage ewes. *Small Rum. Res.*, 39: 47 – 57.
- Dimri, U.; Ranjan, R.; Sharma, M. C. and Varshney, V. P. (2010). Effect of vitamin E and selenium supplementation on oxidative stress indices and cortisol level in blood in water buffaloes during pregnancy and early postpartum period. *Trop. Anim. Health Prod.*, 42: 405 – 410.
- Elnageeb, M. E. and Adelatif, A. M. (2010). The minerals profile in desert ewes (*Ovis aries*): Effects of pregnancy, lactation and dietary supplementation. *American-Eurasian J. Agric. Environ. Sci.*, 7 (1): 18 – 30.
- El-Tohamy, M. M.; Salama, A. and Youssef, A. E. M. (1986). Blood constituents in relation to the reproductive state in she-camel (*Camelus dromedarius*) *Beitrage fur Trop. Landwirtschaft und Vet. Med.*, 24, 425-430.
- Erisir, M.; Benzer, F. and Kandemir, F. M. (2009). Changes in the rate of lipid peroxidation in plasma and selected blood antioxidants before and during pregnancy in ewes. *Acta Vet. Brno.*, 78: 237 – 242.
- Esfandiari, E.; Shekari, F.; Shekari, F. and Esfandiari, M. (2007). The effect of salt stress on antioxidant enzymes’ activity and lipid peroxidation on the wheat seedling. *Not. Bot. Hort. Agrobot. Cluj.*, 35 (1): 49 – 56.
- Fahmy, A. A.; Howaida, A. M. and Mahmoud, H. S. (2009). Growth performance of Barki lambs fed alfalfa forage treated with selenium. *Egypt. J. of Appl. Sci.*, 24 (3 B): 406 – 420.
- Gitto, G.; Reiter, R. J.; Karbownik, M.; Tan, D. X.; Gitto, P.; Barberi, S. and Barberi, I. (2002). Causes of oxidative stress in the pre 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.
- Hidiroglou, M.; Ho, S. K. and Standish, J. F. (1978). Effects of dietary manganese levels on reproductive performance of ewes and on tissue mineral composition of ewes and day-old lambs. *Can. J. Anim. Sci.*, 58: 35- 41.
- Humphries, W. R.; Philippo, M.; Young, B. W. and Bremner, I. (1983). The influence of dietary iron and molybdenum on copper metabolism in calves. *British J Nutr.*, 49: 77 – 86.
- Idonije, O. B.; Festus, O.; Okhiai, O. and Akpamu, U. (2011): A comparative study of the status of oxidative stress in pregnant Nigerian women.

- Research Journal of Obstetrics and Gynecology, 4 (1): 28 – 36.
- Kargin, F.; Seyrek, K.; Bulduk, A. and Aypak, S. (2004). Determination of the levels of zinc, copper, calcium, phosphorus and magnesium of Chios ewes in the Aydıń Region. *Turk. J. Vet. Anim. Sci.*, 28, pp. 609- 612.
- Kearl, I. C. (1982). Nutrients requirements in developing countries. *Utah Agric. Exp. Stat., Utah State University, Logan, USA.*
- Khan, M. A and Gul, B. (2002). Salt tolerant plants of coastal sabkhas of Pakistan. In H. Barth and B. Boer [eds.], *Sabkha ecosystems: the Arabian Peninsula and adjacent countries*, vol. 1, 123–140. Kluwer, Dordrecht, Netherlands.
- Kincaid, R. (2008). Changes in the concentration of minerals in blood of peripartum cows. *Mid-South Ruminant Nutrition Conference*. 1 – 8.
- Kincaid, R. L. (1999). Assessment of trace mineral status of ruminants. *Proceedings of the American Society of Animal Science.*
- Kirchgessner, M. (1976). Trace element deficiency and its diagnosis by biochemical criteria. In: *Nuclear Techniques in Animal Production and Health*. Nienna (IAEA), pp: 607.
- Koracevic, D.; Koracevic, G.; Djordjevic, V.; Andrejevic, S. and Cosic, V. (2001). Method for the measurement of antioxidant activity in human fluids. *J. Clin. Pathol.*, 54: 356 – 361.
- Littlejohn, D.; Egila, J. N.; Gosland, R. M.; Kunwar, U. K. and Smith, C. E. (1991). Graphite furnace analysis. *Analyt. Chim. Acta*, 250: 71 - 84.
- Levander, O. A. (1986). Selenium. In: W. Mertz (Ed.) *Trace Elements in Human and Animal Nutrition* vol. 2. pp 209-279. Academic Press, New York.
- McCall, K. A.; Huang, C. and Fierke, C. A. (2000). Function and mechanism of zinc metalloenzymes. *The Journal of Nutrition*. 130:1437-1446.
- McDowell, L. R. (2003). *Minerals in Animal and Human Nutrition*, Second Edition. Elsevier Science B. V., Amsterdam, The Netherlands.
- Mills, C. F. and Davies, N. T. (1979). Perinatal changes in the absorption of trace elements. *CIBA Foundation Series* 70: 247-265.
- Minatel, L. and Carfagnini, J. C. (2000). Copper deficiency and immune response in ruminants. *Nutrition Research*. 20:1519-1529.
- Mistry, H. D. and Williams, P. J. (2011). Review Article: The importance of antioxidant micronutrients in pregnancy. *Oxidative Medicine and Cellular Longevity*, 1 – 12.
- Moeini, M. M.; Kiani, A.; Karami, H. and Mikaeili, E. (2011). The effect of selenium administration on the selenium, copper, iron and zinc status of pregnant heifers and their newborn calves. *J. Agr. Sci. Tech.*, 13: 53 – 59.
- Nazifi, S.; Ghafari, N.; Farshneshani, F.; Rahsepar, M. and Razavi, S. M. (2010a). Reference values of oxidative stress parameters in adult Iranian fat-tailed sheep. *Pakistan Vet. J.*, 30(1): 13 – 16.
- Nazifi, S.; Shahriari, A. and Nazemian, N. (2010b). Relationships between thyroid hormones, serum trace elements and erythrocyte antioxidant enzymes in goats. *Pak. Vet. J.*, 30 (3): 135 – 138.
- Nishikimi, M.; Rao, N. A. and Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochemical and Biophysical Research Communications*, 46 (2):849 – 854.
- Öztabak, K.; Civelek, S.; Özpınar, 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.
- Paglia, D. E. and Valentine, W. N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.*, 70: 158 – 169.
- Patil, S. B.; Kodliwadmath, M. W. and Kodliwadmath, S. M. (2007). Study of oxidative stress and enzymatic antioxidants in normal pregnancy. *Indian J. Clin. Biochem.*, 22: 135 – 137.
- Patil, S. B.; Kodliwadmath, M. V. and Kodliwadmath, S. M. (2006). Lipid peroxidation and nonenzymatic antioxidants in normal pregnancy. *J. Obstet. Gynecol. India*, 56 (5): 399 – 401.
- Pavlata, L.; Misurova, L.; Pechova, A.; Husakova, T. and Dvorak, R. (2012). Direct and indirect assessment of selenium status in sheep – a comparison. *Veterinarni Medicina*, 57 (5): 219 – 223.
- Pechová, A.; Janštová, B.; Mišurová, L.; Dračková, M.; Vorlová, L. and Pavlata, L. (2008). Impact of supplementation of various selenium forms in goats on quality and composition of milk, cheese and yoghurt. *Acta Vet. Brno.*, 77: 407– 414
- Pitman, M. G. and Lauchli, A. (2002). Global impact of salinity and agricultural ecosystems. Lauchli, A. and Luttge, V. (Eds.), *Salinity: Environment-Plants Molecules*, Kluwer, The Netherlands, pp. 3– 20.
- Powell, S. R. (2000). The antioxidant properties of zinc. *The Journal of Nutrition*. 130:1447-1454.
- Rezapour, A. and Roudbaneh, M. T. (2011). Effect of food restriction on oxidative stress indices in Ghezel ewes. *Journal of Animal and Veterinary Advances*, 10 (8): 980 – 986

- Rubio, C.; González, D.; Martín-Izquierdo, R. E.; Revert, C.; Rodríguez, I. and Hardisson, A. (2007). El zinc: oligoelemento esencial. *Nutrición Hospitalaria*, 22:101- 107.
- Ruiz-Larrea, M. B.; Leal, A. M.; Liza, M.; Lacort, M. and de Groot, H. (1994). Antioxidant effects of estradiol and 2-hydroxyestradiol on iron induced lipid peroxidation of rat liver microsomes. *Steroids*, 59: 383 – 388.
- Sadek, Laila A. (1995). The effect of selenium, boron and salinity on biomass and mineral composition of *Medicago sativa* L. *Alex. J. Agric. Res.*, 40 (1): 293 – 304.
- SAS Institute (2004). Statistical Analysis System, STAT/ user's guide, Release 9.1, SAS Institute, Cary NC. USA.
- Sattar, A.; Mirza, R. H. and Hussain, S. M. (2007). Effect of prepartum treatment of vitamin E-selenium on postpartum reproductive and productive performance of exotic cows and their calves under subtropical conditions. *Pakistan Vet. J.*, 27(3): 105 – 108.
- Simsek, S.; Yuce, A. and Utuk, A. E. (2006). Determination of serum malondialdehyde levels in sheep naturally infected with *Dicrocoelium dendriticum*. *Firat. Univ. Saglik. Bil. Dergisi.*, 20: 217- 220.
- Squires, V. R. and Ayoub, A. T. (1994). Halophytes as a Resource for Livestock and for Rehabilitation of Degraded Lands. Kluwer Academic Publisher, Dordrecht Boston, London, 315 p.
- Steen, A.; Strøm, T. and Aksel Bernhoft, A. (2008). Organic selenium supplementation increased selenium concentrations in ewe and newborn lamb blood and in slaughter lamb meat compared to inorganic selenium supplementation. *Acta Veterinaria Scandinavica*, 50:1 – 7.
- Stefanidou, M.; Maravelias, C.; Dona, A. and Spiliopoulou, C. (2006). Zinc: a multipurpose trace element. *Archives of Toxicology*, 80: 1- 9.
- Suttle, N. E. (1988). Assessment of the mineral and trace element status of feeds. In: *Feed Information and Animal Production*. (Editors: Robards, G. E. and Packham, R. G.). The International Network of Feed Information Centres (INFIC), pp: 516.
- Toescu, V.; Nuttall, S. L.; Martin, U.; Kendall, M. J. and Dunne, F. (2002). Oxidative stress and normal pregnancy. *Clin. Endocrinol.*, 57: 609 – 613.
- Trávníček, J.; Racek, J.; Trefil, L.; Rodinová, H.; Kroupová, V.; Illek, J.; Doucha, J. and Písek, L. (2008). Activity of glutathione peroxidase (GSH-Px) in the blood of ewes and their lambs receiving the selenium-enriched unicellular alga *Chlorella*. *Czech J. Anim. Sci.*, 53, (7): 292 – 298.
- Underwood, E. J. and Suttle, N. F. (2003). *Los minerales en la nutrición del ganado*, Tercera Edición. Editorial Acribia, Zaragoza, España.
- Upadhyaya, C.; Mishra, S.; Singh, P. P. and Sharma, P. (2005). Antioxidant status and peroxidative stress in mother and newborn – a pilot study. *Indian J. Clin. Biochem.*, 20: 30 – 34.
- Williams, J. W.; Beutler, E.; Reselev, A. J. and Rundels, R. W. (1972). *Haematology*. McGrawhill. New York, London, pp: 100- 124.