Original Article

Trace elements, oxidative stress biomarkers and hemato-biochemical indices of goat kids affected with nutritional dependent locomotor disturbances

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Abstract

This study aimed to investigate the biomedical significant of trace elements, oxidative stress and hemato-biochemical indices in the diagnosis of locomotor disturbances of affected goat kids. Blood samples were collected from 22 goat kids affected with locomotor disturbances. An equal number of healthy goat kids was used as a control. Clinical examination showed anemia and an incoordination in affected animals compared to the control. Malondialdehyde (MDA), creatine kinase (CK) and aspartate aminotransferase (AST) values were significantly higher in affected animals than in the control, while superoxide dismutase (SOD) and reduced glutathione (GSH) values were significantly lower. Concentrations of copper (Cu), iron (Fe) and ceruloplasmin (Cp) were significantly lower in affected goat kids than in the control. Concentrations of zinc (Zn), selenium (Se) and molybdenum (Mo) in affected animals were maintained significantly unchanged compared to the control. AST and CK activities and the concentration of MDA, were significantly lower in successfully treated goat kids (n =10) than in goat kids that failed to respond to treatment (n =12), while SOD activity was significantly higher. The treatment outcomes (success or failure) were found negatively correlate with AST and CK activities and positively correlate with SOD activity. Conclusively, lower copper concentrations may be the cause of goat kids' locomotor disturbances. Iron deficiency anemia was observed in animals affected with locomotor disturbances. As a result, administration of copper and iron elements is suggested in such cases. AST, MDA, CK, and SOD can also be used as diagnostic and prognostic biomarkers for nutritional related locomotor disturbances in goat kids.

Keywords: Minerals, copper, stress, antioxidants, biomarkers, goat kids

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Introduction

Goats represent a considerable proportion of the total animal population in Saudi Arabia. The total number of goats in Saudi Arabia is estimated to be about three million (AOCD, 2016). Trace element levels as well as the activity of certain enzymes, such as aspartate aminotransferase (AST), creatine kinase (CK), lactate dehydrogenase (LDH) have been critical in the diagnosis of clinical and subclinical cases of nutritional muscular dystrophy in lambs (Sobbiech and Kuleta, 2002). In Veterinary Medicine, oxidative stress biomarkers have recently been proposed as potential prognostic and diagnostic biomarkers for a variety of diseases (Al-Qudah, 2011; El-Bahr and El-Deeb, 2017). However, the use of these biomarkers in goats is restricted. The levels of malondialdehyde (MDA), a marker of lipid peroxidation, CK and AST have been significantly higher whereas, levels of antioxidant enzyme, superoxide dismutase (SOD), and reduced glutathione (GSH) have been significantly lower in lambs affected with enzootic ataxia than those in the control (El-Bahr and El-Deeb, 2017). A previous report (El-deeb, 2010) indicated that, oxidative stress biomarkers were significantly higher whereas, antioxidant levels were significantly lower in lambs affected with nutritional muscular dystrophy than in healthy control animals. In cashmere goats fed a basal diet supplemented with copper (Cu) and molybdenum (Mo) compared to controls, SOD and glutathione peroxidase (GPX) activities were significantly higher and MDA concentrations were much lower (Zhang *et al*., 2012). Concentration of copper (Cu), manganese (Mn), selenium (Se), zinc (Zn) and activity of antioxidant enzymes (SOD, GPX) have been significantly different in the plasma of Barki ewes fed silage of salt tolerant plants under South Sinai conditions (Amer *et al*., 2014). However, the investigation of the profile of trace elements and oxidative stress biomarkers in the serum of goat kids suffering from nutritional based locomotor disturbances has not been completely elucidated. Creatine kinase and superoxide dismutase (SOD) have been suggested as diagnostic and prognostic biomarkers in lambs affected with enzootic ataxia (El-Bahr and El-Deeb, 2017). However, data regarding diagnostic and prognostic biomarkers in goat kids affected with nutritional based locomotor disturbance is not available. Therefore, the current study aimed to evaluate selected trace elements concentrations, oxidative stress biomarkers levels and hemato-biochemical indices in the serum of goat kids affected with nutritional based locomotor disturbances. Some of these biomarkers have also been studied for their prognostic and diagnostic potential.

Materials and Methods

Animals and clinical examinations: Twenty-two Ardi goat kids (8 ± 4 months old; 15 ± 5 kg body weight) affected with locomotor disturbances were admitted to the Veterinary teaching hospital, King Faisal University, Al-Ahsa, Saudi Arabia for clinical examination. A full history of the admitted animals was taken and their clinical signs were recorded. A control group of healthy goat kids (n=22; 8-14 months old; 20 ± 5 kg body weight) was used as well. The control animals were from the Research and Training station farm, King Faisal University, Saudi Arabia. All animal procedures were performed according to the guidelines of the Animal Ethics Committee of College of Veterinary Medicine, King Faisal University, Saudi Arabia (DSR150056). All admitted goat kids had anemia and a normal body temperature compared to the control animals. In addition, there were no signs or evidence of infection (coughing, diarrhea, and depression). When compared to the control animals, the predominant indications and complaints were related to locomotor difficulties. These locomotors problems were in the form of cambial gait, in the coordination of pelvic limbs and difficulties in standing up. Following the diagnosis of locomotor disturbances, a specific therapeutic scheme was implemented. Goat kids were treated with Ballinskelligs Veterinary Products, B.V.P. copper + Vitamin B_{12} (Ballinskelligs Veterinary Products Ltd Ballinskelligs, Co. Kerry, Ireland) injectable suspension. Copper methionate and vitamin B_{12} were given intramuscularly once at a dose of 20mg/50kg body weight and 1mg/50kg body weight, respectively. This dose was given again three weeks after the first.

Sample collection and analysis: Blood samples were collected from the jugular vein of all animals into plain and Ethylenediaminetetraacetic acid (EDTA) vacutainers. EDTA blood was used for determination of hemoglobin (Hb) concentration, total erythrocyte count (TEC) and differential leukocyte counts using electronic cell counters (VetScan HM5 Hematology system, Abaxis, Inc., CA, USA). The obtained sera were used for determination of total proteins, albumin, AST, CK, calcium, phosphorus, magnesium, trace elements (Cu, Zn, Mo, iron (Fe), Se), oxidative stress biomarkers (MDA, GSH, SOD) and Cp. Commercial diagnostic kits were used for the determination of total protein (EP56-660), albumin (EP03-570), AST (EP15-500), blood urea nitrogen (BUN, EP20-420), calcium (EP22-660), phosphorus (EP46-660), magnesium (EP50-660) on full automated chemistry analyzer Ellipse model (AMS Alliance, Rome, Italy). Kits were supplied by United Diagnostic Industry, UDI, Dammam, Saudi Arabia. The determination of trace elements (Cu, Zn, Mo, Fe, Se) was carried out using Atomic Absorption Spectrophometer, AA-6800 model (Shimadzu, Kyoto, Japan) (16). Standard stock solutions were prepared with deionized water. Calibration curves were obtained for different concentrations of standard solutions prepared from 1000 mg/L commercial stock solution (Merck, Darmstadt, Germany). Commercial Enzyme linked Immunosorbent Assay (ELISA) kits (Cayman, USA) was used for determination of MDA (Cat # 700870) and GSH (Cat # 703002) concentrations and SOD (Cat # 706002) activities on ELISA reader (Absorbance Microplate Reader ELx 800TM BioTek®, USA) equipped with an automated washer (Microplate Strip Washer ELx 50TM BioTek®, USA). MDA was detected using TBARS assay.

Statistical analysis: Data presented in Tables (1 – 4) was normally distributed as indicated by Kolmogorov-Simirnov (K-S) test with Lilliefors correction.

Therefore, data presented as mean ± standard error of mean (SEM) using parametric test (student *t*-test). P < 0.05 was considered significant. The data presented in Table 5 was not normally distributed due to outliers of the normality tests; lack of symmetry (skewness) and pointiness (kurtosis) was 1.6 and 1.5, respectively. Therefore, the differences in selected biomarkers in treatment success or failure were estimated using non-parametric analysis (Wilcoxon Mann–Whitney) at *P* < 0.05 and data was presented as medians ± interquartile range (IQR). Regarding data of Table 6, Spearmen's correlation coefficient was estimated between biochemical markers (AST, CK, Cu, Fe, Cp, MDA, GSH and SOD) and treatment outcomes (success or failure) in goat kids' affected locomotor disturbances. All tests were performed in IBM SPSS 15.0 statistical software (SPSS Inc., Chicago, IL, USA). The sample size was based on that used previously in other studies with similar objectives (12). The sample size calculation was performed using power and sample size program (www.power-analysis.com); the type 1 error was 0.05 and the power was 85%

Results

Clinical examination: All admitted goat kids had anemia and a normal body temperature compared to the healthy control animals. In addition, there were no signs or evidence of infection (coughing, diarrhea or depression) in affected animals. Compared to the control animals, the predominant symptoms and complaints were locomotor disturbances.

Analysis of hematological indices: Table 1 shows that affected animals had significantly (P ≤ 0.05) lower Hb concentration than the control. However, the total erythrocyte count (TEC; 10^{12}/L), total leucocytes count (TLC) (10^9/L), neutrophils (%), lymphocytes (%), eosinophils (%), basophils (%) and monocytes (%) in affected animals were similar to that of the control (*P* > 0.05).

Analysis of selected biochemical indicators: The current findings (Table 2) revealed significantly increased (P ≤ 0.05) AST and CK activity in the serum of affected animals when compared to the controls. Other biochemical markers in the serum of affected animals (total protein, albumin, BUN, calcium, phosphorus, and magnesium) remained significantly unchanged when compared to control animals (Table 2).

Analysis of Trace elements: Data summarized in Table 3 indicates that, Cu and Fe concentrations in affected animals were significantly (P ≤ 0.05) lower than that of the control. However, the concentrations of Zn, Se and Mo remained significantly unchanged in the serum of affected animals compared to control (Table 3).

Analysis of oxidative stress biomarkers and ceruloplasmin (Cp): Data summarized in Table 4 shows that MDA concentration was significantly (*P* < 0.05) higher in affected animals than that of the control. There was a significant decrease (*P* < 0.05) in SOD activity and GSH concentration in affected animals compared to the control (Table 4). In addition, Cp concentration was significantly lower (*P* < 0.05) in affected goat kids than that of the control (Table 4).

Relationship between biochemical parameters and treatment response: Table 5 shows the relationship between AST, CK, Cu, Se, Fe, Cp, MDA, GSH, and SOD levels and treatment response (success or failure) in goat kids with locomotor disturbances. The activities of ALT, CK and SOD and the concentration of MDA were changed significantly (*P*<0.05) after the treatment of goat kids affected with locomotor disturbances. The treatment success was observed in affected goat kids with lower levels of AST, CK and MDA and with higher values of SOD (Table 5) than goat kids of treatment failure. Spearman's correlation (*r*) analysis (Table 6) shows a high negative (r ≥ −0·70; *P* < 0·001) correlation between treatment outcomes (success or failure) and the activities of CK and AST. Activities of superoxide dismutase (SOD) highly and positively (r ≥ 0.77; *P* < 0.001) correlated with the treatment response. In addition, CK activity positively correlated with serum AST (*r* = 0.724; *P* < 0.01) activity and inversely correlated with the activity of SOD (*r* = −0.611; *P* < 0.01) (Table 6). Malondialdehyde (MDA) concentration positively correlated with serum AST (*r* = 0.701; *P* < 0.01) and CK (*r* = 0.780; *P* < 0.001) activities and negatively correlated with SOD activity (*r* = -0.719; *P* < 0.01) (Table 6).

Table 1 Hematological parameters in the control (n=22) and in goat kids' affected with locomotor disturbances (n=22).

Variables	Unit	Control	diseased
TEC	(10^{12}/L)	11.9 ± 1.2	12.2 ± 2.0
TLC	(10^9/L)	11.5 ± 0.4	11.8 ± 0.7
Neutrophils	(%)	65.2 ± 5.2	67.7 ± 3.0
Lymphocytes	(%)	31.2 ± 1.2	29.1 ± 1.0
Esinophils	(%)	0.0 ± 0.0	0.0 ± 0.0
Basophils	(%)	0.0 ± 0.0	0.0 ± 0.0
Monocytes	(%)	0.5 ± 0.0	0.5 ± 0.0
Hb	(g/L)	130.0 ± 3.0	80.2 ± 4.0*

Data expressed as mean ± standard error of means (SEM). *Means are significantly different at the level (p ≤ 0.05) in the same row compared to the control.
TEC: total erythrocytes count; TLC: total leucocytes count; Hb: hemoglobin

Table 2 Biochemical parameters in the control (n= 22), and in goat kids' affected with locomotor disturbances (n= 22).

Variables	Unit	Control	diseased
Total Protein	(g/L)	65 ± 1.6	64 ± 2.0
Albumin	(g/L)	36 ± 2.2	37 ± 2.1
AST	(U/L)	90.0 ± 9.2	132.0 ± 8.6*
CK	(U/L)	83.8 ± 9.1	204.0 ± 9.7*
BUN	(mmol/L)	7.2 ± 1.4	7.5 ± 1.2
Calcium	(mmol/L)	2.4 ± 0.1	2.9 ± 0.1
Phosphorus	(mmol/L)	1.3 ± 0.2	0.7 ± 0.2
Magnesium	(mmol/L)	0.8 ± 0.1	0.9 ± 0.1

Data expressed as mean ± standard error of means (SEM). *Means are significantly different at the level ($p \leq 0.05$) in the same row compared to the control.
AST: aspartate aminotransferase; CK: creatine kinase; BUN: blood urea nitrogen.

Table 3 Trace element concentrations in the control (n=22), and in goat kids' affected with locomotor disturbances (n= 22).

Variables	Unit	Control	diseased
Cu	(µmol/L)	10.4 ± 1.2	3.1 ± 2.1*
Zn	(µmol/L)	3.6 ± 0.2	3.7 ± 0.1
Se	(µmol/L)	0.8 ± 0.1	0.7 ± 0.1
Fe	(µmol/L)	23.0 ± 3.1	15.9 ± 2.2*
Mo	(µmol/L)	0.3 ± 0.1	0.4 ± 0.1

Data expressed as mean ± standard error of means (SEM). *Means are significantly different at the level ($p \leq 0.05$) in the same row compared to the control.
Cu: copper; Zn: zinc; Se: selenium; Fe: Iron; Mo: Molybdenum

Table 4 Oxidative stress biomarkers and ceruloplasmin values in the control (n=22), and in goat kids' affected with locomotor disturbances (n= 22).

Variables	Unit	Control	diseased
MDA	(µmol/L)	4.90 ± 0.20	9.60 ± 0.10*
GSH	(mmol/L)	1.20 ± 0.20	0.70 ± 0.10*
SOD	(U/L)	156.00 ± 0.01	102.00 ± 0.01*
Cp	(µmol/L)	0.45 ± 0.01	0.08 ± 0.01*

Data expressed as mean ± standard error of means (SEM). *Means are significantly different at the level ($p \leq 0.05$) in the same row compared to the control.
MDA: malondialdehyde; GSH: reduced glutathione; SOD: superoxide dismutase; Cp: ceruloplasmin

Table 5 Biochemical parameters in relation to treatment response (success or failure) in goat kids affected with locomotor disturbances.

Parameters	Treatment failure (n=12)				Treatment success (n=10)				α value	Confidence interval (%)	*P - value
	Median	25th quartile	75th quartile	IQR	Median	25th quartile	75th quartile	IQR			
AST (U/L)	134.70	129.10	140.30	11.20	98.90	90.80	107.00	20.16	0.05	95	0.021
CK (U/L)	203.10	195.70	210.50	14.80	131.10	124.40	137.80	40.13	0.05	95	0.011
Cu (µmol/L)	3.40	3.10	3.70	0.60	3.70	3.30	4.10	0.80	0.10	90	0.632
Se (µmol/L)	0.70	0.60	0.80	0.20	0.70	0.60	0.80	0.20	0.10	90	0.699
Fe (µmol/L)	4.90	2.30	17.50	15.40	16.20	13.90	18.50	4.60	0.10	90	0.533
Cp (µmol/L)	0.09	0.07	0.11	0.04	0.12	0.11	0.13	0.02	0.10	90	0.642
MDA (µmol/L)	9.40	9.30	9.50	0.20	5.60	5.40	5.80	40.0	0.05	95	0.697
GSH (mmol/L)	0.80	0.60	1.00	0.40	1.00	0.80	1.20	40.0	0.10	90	0.781
SOD (U/L)	105.80	98.20	113.40	15.20	132.80	123.70	141.90	20.18	0.05	95	0.031

*Data expressed as Medians ± interquartile range (IQR). **P*-value resulting from non-parametric Wilcoxon Mann-Whitney test. AST: aspartate aminotransferase; CK: creatine kinase; Cu: copper; Se: selenium; Fe: iron; Cp: ceruloplasmin; MDA: malondialdehyde; GSH: reduced glutathione; SOD: superoxide dismutase.

Table 6 Spearmen's correlation coefficient between each of the biochemical markers (AST, CK, Cu, Fe, Cp, MDA, GSH and SOD) and treatment outcomes (success or failure) in goat kids' affected locomotor disturbances.

Variables	AST	CK	Cu	Fe	Cp	MDA	GSH	SOD
CK	**0.724							
Cu	**-0.731	**-0.611						
Fe	**-0.598	**-0.672	***0.927					
Cp	**-0.599	***-0.749	***0.936	**0.688				
MDA	**0.701	***0.780	***-0.689	***-0.788	***-0.799			
GSH	**-0.481	**-0.501	**0.686	**0.687	***0.756	**-0.703		
SOD	**-0.524	**-0.611	**0.697	**0.677	***0.733	**-0.719	**0.698	
Treatment outcomes	**-0.701	***-0.791	**0.399	*0.254	*0.170	*-0.153	*0.067	***0.770

*Significant correlations at $P<0.05$; **Significant correlations at $P<0.01$ and *** Significant correlations at $P<0.001$.
AST: aspartate aminotransferase; CK: creatine kinase; Cu: copper; Fe: Iron; Cp: ceruloplasmin; MDA: malondialdehyde; GSH: reduced glutathione; SOD: superoxide dismutase.

Discussion

In the current study, clinical examination revealed that all affected goat kids had anemia and a normal body temperature compared to the control. In addition, there were no signs or evidence of infection (coughing, diarrhea or depression) in affected animals. Compared to the control animals, the predominant symptoms and complaints were locomotor disturbances. In the current study, TEC, TLC, neutrophils %, lymphocytes %, Eosinophils %, Basophils % and Monocytes % of Ardi goat kids were all within the normal ranges of goats (Al-Bulushi *et al*., 2017; Mohammed *et al*., 2016). Low hemoglobin concentration verified the clinically reported anemia of the affected goat kids when compared to the controls. In the current study, low concentrations of Hb may be linked to Cu and Fe deficiencies (Sanders and Sander, 1983). In the current study, serum concentration of Cu was lower in affected goat kids than in the control. Copper is required for the absorption and transfer of iron. As a result, anemia was associated with Cu deficiency in affected goat kids when compared to the control. Parallel to the current findings, Rong *et al*. (2011), reported unchanged concentrations of selenium in the serum of Guizhou semi-fine wool sheep in pastures in the southwest China karst mountain area. The normal control values of trace elements (Cu, Zn, Mo, Fe, and Se) were similar to that detected previously in goats (Al-Sobaiyl, 2010; Marques *et al*., 2011). Similar studies (Al-Bulushi *et al*., 2017; Mohammed *et al*., 2016) found that the concentrations of total protein, albumin, BUN, calcium, phosphorus and magnesium in different breeds of goats were comparable. A state of muscular injury is indicated by significantly increased CK and AST activity in the serum of injured animals compared to the controls (Tunca *et al*., 2009; Ataollahi *et al*., 2013). The injury of muscle tissues may be attributed to copper deficiency (Kutil, 2010). Lipid peroxidation was produced in the current investigation, as shown by increased serum MDA concentrations in affected animals compared to the controls. Higher MDA concentrations in the serum of affected animals compared to the control could indicate that copper plays a role in oxidative stability (Cheng, 2011). The presence of greater levels of lipid peroxides in the serum of affected animals causes an imbalance between oxidative and antioxidant processes, favor oxidation and results in oxidative stress. The significant reduction in the level of serum enzymatic (SOD) and non-enzymatic (GSH) antioxidants of affected animals compared to the control confirms their antioxidant role against the existing state of oxidative stress. Serum values of SOD positively correlated with that of Cu in cattle, camels and yaks (Shen, 2009). Therefore, estimation of SOD activity is a sensitive marker for diagnosis of Cu deficiency in ruminants. In addition, SOD activity was significantly lower in Guizhou semi-fine wool sheep affected with copper deficiency than the control (Rong *et al*.,2011).

In successfully treated goat kids, activities of AST and CK and MDA concentration were significantly reduced compared to that of kids with treatment failure. In addition, the activity of SOD significantly increased in successfully treated goat kids compared to that of kids with treatment failure. In the current study, AST and CK activity, and MDA concentration were lower than 107 U/L, 137.8 U/L and 5.80 µmol/L, respectively and the activity of SOD was higher than 141.90 U/ml in successfully treated goat kids. In 12 of 18 lambs with treatment failure, AST and CK activity, as well as MDA concentrations, were more than 129.1 U/L, 195.7 U/L, and 9.3 mol/L, respectively. SOD activity was lower than 113.40 U/ml in 12 of the 18 lambs who failed to respond to the treatment. Higher AST and CK activities, as well as MDA concentrations than 129.1 U/L, 195.7 U/L and 9.3 µmol/L, respectively and lower serum activity of SOD than 113.40 U/ml may be used as prognostic biomarkers for treatment failure during the initial examination of goat kids affected with nutritionally based locomotors disturbances. Increased serum concentrations of CK over 400 U/L at initial examination was a bad prognostic indicator in lambs affected with enzootic ataxia (El-Bahr and El-Deeb, 2017). A similar study (El-Bahr and El-Deeb, 2017) reported negative correlation

between treatment outcomes (success or failure) and activities of AST and CK in lambs affected with enzootic ataxia. The same study (El-Bahr and El-Deeb, 2017) found a similar positive correlation between treatment outcomes (success or failure) and SOD activity in lambs affected with enzootic ataxia. Based on the obtained results, the current study concluded that, locomotor disturbances of the admitted goat kids could be attributed to copper deficiency. Lower copper concentrations may be the cause of goat kids' locomotor disturbances. Iron deficiency anemia was observed in animals affected with locomotor disturbances. As a result, administration of copper and iron elements is suggested in this situation. AST, MDA, CK, and SOD can also be used as diagnostic and prognostic biomarkers for nutritional related locomotor disturbances in goat kids.

Conflicts of interest: There were no conflicts of interest that may have biased the work reported in this study.

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Authors' contributions: I..E, T.F., A.A., and W.E. carried out the experimental design, clinical examination and hematological analysis. SME carried out the biochemical analysis, oxidative stress biomarkers estimation and wrote the article. All the authors drafted and approved the final version of the manuscript.

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