

Betaine counteracts the harmful effects of saline water induced to growing lambs

M.M. Abdelsattar ^a, A.M.A. Hussein ^{b,*}, M. Haridy ^c, M.N. Abd El-Ati ^b, A.M. Saleem ^a, and N. Zhang ^d,

^a Department of Animal and Poultry Production, Faculty of Agriculture, South Valley University, Qena 83523, Egypt.

^b Department of Animal Production Department, Faculty of Agriculture, Assiut University, Assiut 71515, Egypt.

^c Department of Pathology & Clinical Pathology, Faculty of Veterinary Medicine, South Valley University, Qena 83523, Egypt.

^d Feed Research Institute of Chinese Academy of Agricultural Sciences, Key Laboratory of Feed Biotechnology of the Ministry of Agriculture and Rural Affairs, Beijing 100193, China.

* Corresponding author.

E-mail address: ahmed.hussein1@age.au.edu.eg (Ahmed Hussein)

ABSTRACT

The current study aimed to evaluate the effects of betaine on the growth performance, blood parameters, and histopathology of growing lambs offered fresh or saline water. A total of 20 healthy Ossimi male lambs, aged 9 months, were randomly assigned to four treatments (n=5). Lambs received NaCl (0% or 1.5%) with drinking water and betaine (0 or 2500 mg/kg diet) with ration. The experiment lasted 135 days. Saline water significantly decreased ($p<0.01$) lamb body weight, blood urea, sodium and alanine amino transferase. Betaine significantly increased ($p<0.05$) lamb body weight, Hb concentration, RBCs count and hematocrit value. However, betaine decreased ($p<0.05$) blood neutrophils, band cells and eosinophils, urea, and both alanine and aspartate amino transferases. Saline water induced swelling and disarrangement of hepatocytes, focal medullary tubular necrosis and calcium calculi in renal tissue, and fasciculation and focal lysis of cardiac muscle fibers. In contrast, betaine ameliorated these harmful effects of saline water. In conclusion, dietary betaine could enhance the growth performance and ameliorate the harmful effects of drinking saline water for sheep.

Keywords: Salinity; Betaine; Lambs; Growth; Harmful; Ameliorative

INTRODUCTION

The Nile River is the major source of fresh water in Egypt, which accounts for 75.2% of Egypt's annual available water resources (Abouzeid, 1992). In Egypt, 81.1% of water consumption is consumed by the agricultural sector. Currently, Egypt needs to cover its water deficit by increasing groundwater consumption. The available amount of groundwater in Egypt is 11.3 billion m³ annually. But annual usage is just 5.2 billion m³ (Abouzeid, 1992). Especially in arid areas, where groundwater has high salinity and desalination is expensive, lack of fresh water has seriously affected agricultural production (El Tahlawi et al., 2008). The poor water quality can influence the consumption of water and feed, and the health and production states of ruminants (NRC, 2007). Sheep can tolerate 5000 ppm TDS (Total Dissolved Solids) to maintain healthy growth, however sheep can, for a limited time, tolerate 10,000-13,000 ppm TDS (Markwick, 2007). The feed consumption of sheep depress when the saline concentration is over 15000 ppm

TDS (Yousfi and Salem, 2017). Betaine (trimethylglycine, glycine betaine) supplemented to the diets of livestock as an osmoregulator as well as a methyl donor (Eklund et al., 2005). Betaine as a feed additive in animal diets ranged from 0.10% to 1.0% (Matthews et al., 2001). Betaine is a nontoxic glycine derivative originates from nutritional sources such as sugar beets juice (Craig, 2004). It is generally accepted that betaine accumulates in cellular organelles as osmolyte to replace inorganic ions, protect enzymes and enhance cell membrane integrity in stressed cells (Petronini et al., 1992). Dietary betaine has positive effects on the body weight, feed intake, meat quality, and meat content of unsaturated fatty acid and free amino acids of growing lambs (Dong et al., 2019). Therefore, this study is designed to investigate the application of betaine in saline water stressed sheep and how the betaine as an osmolyte could counteract the harmful effects of saline water. The objective of this study was to evaluate the ability of betaine to ameliorate the

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harmful effects of drinking saline water and to investigate its effect on body weight, blood parameters, and histopathological pictures in growing lambs.

MATERIALS AND METHODS

Animals and Experimental Design

The experiment was carried out at the Department of Animal Production, Faculty of Agriculture, South Valley University, Qena, Egypt, under the ethical committee of animal use and care, Faculty of Veterinary Medicine, South Valley University approval no. 2016-0001.

Twenty healthy male Ossimi sheep lambs (9-month-old, 31.5 kg initial mean body weight) were randomly assigned to four groups (5 replicates each) with a 2×2 factorial design. The first factor was saline water (lambs received 0 or 1.5% NaCl saline water), the second factor was betaine (lambs received 0 or 2500 mg betaine/kg of diet). Betaine (100%) was obtained from Danisco Animal Nutrition, Cairo, Egypt. Lambs

were weighed at the beginning and the end of experiment. The experiment lasted for 135 days.

Animal housing and Feeding

The twenty lambs were housed in open shaded pens under the same managerial conditions. The pen's space was 72 m² (12×6), surrounded by a 1.5 m high concrete wall, and covered by a roof of sheet metal at 4 m height. The animals' requirements of diets were calculated according to NRC (2007). The commercial concentrate mixture was obtained from El-Kawthar Factory, Sohag, Egypt. The animals fed once a day at 8:00 am and the feed intake was recorded.

The daily rations consisted of 20% wheat straw as roughage and 80% concentrate mixture. The composition of concentrate mixture was 25% corn, 20% sunflower cake, 47% broad wheat bran, 1.5% limestone, 1.5% salt, and 5% molasses. The samples from wheat straw and concentrate mixture were prepared for the chemical analysis shown in Table 1.

Table 1: The chemical analysis of concentrate mixture and wheat straw (on dry matter basis).

Sample	Crude protein %	Ether extract%	Crude fiber %	Ash %
Concentrate	17.00	3.15	13.00	9.70
Wheat straw	3.26	1.42	36.01	13.89

Hematological Assays

Blood samples from each lamb were collected monthly starting at 0 day of the experiment using EDTA (Spectrum Diagnostics, Egypt) as an anticoagulant for hematological examinations i.e. red blood cells count (RBCs), Hemoglobin concentration (Hb), packed cell volume (PCV %), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and the differential white cell count.

Biochemical Assays

Clear serum samples were obtained by centrifugation at 3000 rpm for 20 min and then kept in the refrigerator at (-20 C°) until used for biochemical analysis, i.e. liver and kidney function tests. The aspartate aminotransferase, alanine aminotransferase (ALT), plasma urea, creatinine and sodium were measured using local

commercial colorimetric assay kits (Spectrum Diagnostics, Egypt).

Histopathology

At the end of the experiment (135 days), twelve animals (three lambs from each group) were slaughtered with traditional halal (Islamic way). Tissue specimens from liver, kidney, and heart were collected, then fixed in 10% neutral buffered formalin, dehydrated in graded alcohol series, cleared with xylene and embedded in paraffin wax. Samples were sectioned (3-5µm thickness) using a microtome device (model Leica RM 2125RTS) and stained using hematoxylin and Eosin (H&E). Thereafter, the prepared samples were microscopically observed. Scale bars, 200 µm and 50 µm.

Statistical Analysis.

Prior to analysis, data were tested for normality all data normally distributed. The data were analyzed using the GLM procedure of SAS. Saline water (0% and 1.5%) and betaine (0 and 2500 mg/kg concentrate mixture) as the main factors conducted in Randomized Design, and the statistical analysis model was as follow:

$$Y_{ijk} = \mu + S_i + B_j + (SB)_{ij} + \gamma_k + \epsilon_{ijk}$$

Where Y_{ijk} is the observation, μ is the general mean, S_i is the effect of i^{th} level of saline water, B_j is the effect of j^{th} level betaine, $(SB)_{ij}$ is the interaction between i^{th} level of saline water and j^{th} level of betaine, γ_k is the random effect of lambs, and ϵ_{ijk} is the random residual error.

When factors effects were significant ($p < 0.05$), differences between treatments (interactions) were tested using PIDFF procedure.

Table 2: Effects of saline water and betaine on the growth performance of lambs

Items	S0		S1		p value		
	B0	B1	B0	B1	S	B	S×B
Initial body weight, kg	31.44±2.15	31.93±1.43	31.64±1.45	31.38±1.43	0.6269	0.6549	0.5095
Final body weight, kg	41.20±2.59	48.80±3.82	33.83±3.50	30.93±2.21	<.0001	0.023	0.0710
Average daily gain, g/d	94.34±12.24	113.2±18.46	27.45±17.26	46.44±13.68	0.0004	0.1765	0.8891
Dry matter intake, g/d	1250±10.33	1246±10.83	1048.97±13.45	1090.11±14.37	<.0001	0.1349	0.0694

Factors were saline (0%, S0; 1.5%, S1) and betaine (0 mg/kg, B0; 2500 mg/kg, B1). S = saline effect; B = betaine effect; S × B = interaction effect of saline and betaine.

Hematological Findings

Likewise, there were also no interactions ($p > 0.05$) between saline water and betaine for the blood indices including Hb concentration, RBCs count, HCT, MCV, MCH, and MCHC values, and WBC, and platelets counts (Table 3). Dietary betaine significantly increased ($p < 0.05$) the Hb concentration, RBCs count, and HCT value. However, drinking saline water did not significantly affect blood indices of lambs throughout the experimental period. In addition, betaine did not significantly change the blood MCV, MCH, MCHC, platelets and WBC through the experimental period.

RESULTS

Growth Performance.

The effects of saline water and betaine on the growth performance of lambs are shown in Table 2. There were no interactions ($p > 0.05$) between saline water and betaine on the growth performance of lambs. Nevertheless, symptoms of suffering from salt stress obviously appeared in saline water groups. Lack of feed intake followed with a decrease in body weight in some lambs compared with their initial weight. Lambs drank saline water showed a significant decrease ($p < 0.01$) in body weight, average daily gain and dry matter intake when compared to those drank freshwater. By contrast, betaine supplementation increased significantly the final body weight ($p < 0.05$), although the average daily gain and dry matter intake of lambs were not changed significantly.

Moreover, saline water had no significant effect on differential leukocytic count including neutrophils (segmented neutrophils and band cells), eosinophils, basophils, monocytes, and lymphocytes through the experimental period (table 4). However, betaine significantly decreased the numbers of neutrophils ($p < 0.05$), band cell ($p < 0.01$) and eosinophil ($p < 0.01$). On the other hand, betaine did not show significant effects on the numbers of segmented neutrophils, basophils, monocytes and lymphocytes throughout the experiment. No interaction was found either with all treatments ($p > 0.05$).

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Table 3 : Effects of saline water and betaine on hematological picture of growing lambs

Items	S0		S1		p value		
	B0	B1	B0	B1	S	B	S×B
HB Conc.	9.22 ±0.23	9.97 ±0.32	9.54 ±0.36	10.17±0.45	0.3885	0.0271	0.8403
RBCs count	3.07 ±0.08	3.32 ±0.11	3.18±0.12	3.40±0.16	0.3652	0.0241	0.9102
HCT value	27.65 ±0.69	29.70 ±0.96	28.62 ±1.08	30.68 ±1.4	0.2834	0.0268	0.9985
MCV	89.88 ±0.19	90.47 ±0.28	90.10 ±0.04	90.17 ±0.1	0.8171	0.0807	0.1670
MCH	29.92±0.07	30.03±0.03	30.00±0.00	29.98±0.08	0.8487	0.4974	0.2949
MCHC	33.27±0.02	33.27±0.02	33.21±0.03	33.24±0.03	0.0763	0.6105	0.4904
WBCs count	12.43±0.93	12.31±0.56	13.26±0.68	12.85±0.62	0.3358	0.7085	0.8378
Platelets count	209.50±11.66	211.25±8.08	202.50±7.02	201.00±8.84	0.3660	0.9895	0.8642

Factors were saline (0%, S0; 1.5%, S1) and betaine (0 mg/kg, B0; 2500 mg/kg, B1). S = saline effect; B = betaine effect; S × B = interaction effect of saline and betaine.

Table 4: Effects of saline water and betaine on blood leukocyte picture of growing lambs.

Items	S0		S1		p value		
	B0	B1	B0	B1	S	B	S×B
Segmented Neutrophils	41.40±0.61	40.95±0.88	41.75±1.13	39.60±0.74	0.5617	0.1352	0.3254
Eosinophils	1.65±0.44	0.80±0.21	1.15 ±0.23	0.90 ±0.24	0.2423	0.0021	0.0821
Basophils	0.60±0.19	0.65±0.14	0.60±0.11	0.65±0.14	1.0000	0.7079	1.0000
Monocytes	6.40 ±0.32	6.90 ±0.29	5.95 ±0.35	6.35 ±0.27	0.0982	0.1357	0.8668
Lymphocytes	46.50 ±0.88	47.85 ±0.98	46.95 ±1.13	49.40 ±0.8	0.3092	0.0567	0.5745
Neutrophils	44.85±0.69	43.80±0.82	45.35±1.18	42.70±0.78	0.7406	0.0455	0.3791
Band cell	3.45 ±0.2	2.85±0.24	3.60±0.2	3.10 ±0.21	0.2981	0.0057	0.7937

Factors were saline (0%, S0; 1.5%, S1) and betaine (0 mg/kg, B0; 2500 mg/kg, B1). S = saline effect; B = betaine effect; S × B = interaction effect of saline and betaine.

Biochemical Parameters

No interaction was observed between saline water and betaine for the plasma biochemical parameters of growing lambs, except the plasma urea concentrations. With the saline water, supplementation of betaine in diet lowered ($p<0.01$) the plasma urea of lambs. However, with the fresh water, the plasma urea of lambs was not affected by the supplementation of

betaine (Table 5). In addition, Saline water group had significant decrease ($p<0.01$) in the plasma urea, ALT, and sodium concentrations compared with the freshwater group. Betaine significantly decreased ($p<0.01$) the plasma urea, ALT, and AST concentrations, but did not affected ($p>0.05$) the plasma sodium. In contrast, neither saline nor betaine affected ($p>0.05$) the plasma creatine level in sheep throughout the experiment.

Table 5 :Effects of saline water and betaine on plasma biochemical parameters of growing lambs.

Items	S0		S1		<i>p</i> value		
	B0	B1	B0	B1	S	B	S×B
Urea (mg/dl)	52.57 ^{ab} ±0.58	53.66 ^a ±0.73	51.55 ^b ±0.52	46.79 ^c ±0.60	<.0001	0.0002	<.0001
Creatinine (mg/dl)	1.32±0.066	1.27±0.031	1.25±0.057	1.25±0.038	0.4379	0.674	0.6551
Alanine aminotransferase (U/L)	16.17 ±0.82	14.06±0.64	15.32 ±0.71	12.14 ±0.45	0.0012	<.0001	0.1983
Aspartate aminotransferase (U/L)	22.58±0.93	20.27±0.65	21.73±0.82	19.63±0.43	0.1435	<.0001	0.8298
Sodium (mEq/L)	138.17±1.85	137.02±1.58	132.69±1.92	133.74±1.79	0.006	0.9753	0.4777

Means within the same row with different superscripted letters are significantly different ($p < 0.05$). Factors were saline (0%, S0; 1.5%, S1) and betaine (0 mg/kg, B0; 2500 mg/kg, B1). S = saline effect; B = betaine effect; S × B = interaction effect of saline and betaine.

Histopathological Examination

Liver of S0B0 group consisted of hepatic lobules, each lobule has a central vein and hepatic cords were arranged radially around it. The portal triad was situated at the periphery of hepatic lobules (Figure 1a). The hepatic sinusoids were lined by endothelial and Kupffer's cells. Portal triad revealed the bile ductule and portal vein. The liver of S1B0 group revealed dilated hepatic sinusoids. Hepatic tissue revealed disarrangement and swelling of hepatocytes with rounded borders, condensed nuclei, and increased cytoplasmic acidophilia. The nuclei were pyknotic and cytoplasm was granular (Figure 1b). The central veins were severely dilated and hepatocytes were swollen and cytoplasm was vacuolated (Figure 1c). Liver of sheep in S0B1 group revealed severely swollen hepatocytes with obliteration of hepatic sinusoids (Figures 1d). The hepatocytes showed granular cytoplasm (Figures 1e). The hepatocytes around the portal triad were healthy, however those of the centrilobular zone were swollen, sinusoids and obliterated (Figure 1f). Liver of sheep in S1B1 group revealed cellular debris in the central vein and the hepatocytes around central vein were degenerated and necrotic (Figure 1g). A healthy zone of hepatocytes was observed around the portal area (Figure 1h).

The renal cortex of S0B0 group revealed renal glomeruli and proximal and distal convoluted tubules (Figure 2a). Renal medulla consisted of collecting tubules supported by interstitial fibrous connective tissue and blood vessels. Kidney of the S1B0 group revealed focal areas of medullary tubular necrosis that regenerated and replaced by fibrous connective tissue (Figure 2b). Calcium calculi in the medulla surrounded by destroyed renal tubules, fibroblast proliferation and mononuclear cells infiltration (Figure 2c). Homogeneous eosinophilic proteinaceous material were observed in the Bowman's spaces. The surrounding convoluted tubules were degenerated (Figure 2d). A focal area of cystically-dilated tubules was surrounded by fibroblast hyperactivity. Regenerated renal tubules were observed. Kidney of S0B1 group revealed hypercellularity in the renal glomeruli

and tubular nephrosis (Figure 2e). Renal medulla has no significant change. In kidneys of sheep in the S1B1 group, the renal medulla revealed regenerated renal tubules almost similar to normal renal tubules (Figure 2f). Renal cortex has no prominent histological changes.

The cardiac muscles From sheep in the (S0B0) composed of cardiac muscle bundles. The bundles composed of striated fibers lined by cigar-shape nuclei at the periphery of the fiber

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beneath the sarcolemma (Figure 3a). Cardiac muscles of the S1B0 group revealed fasciculated fibers, and some fibers revealed myocardiolysis (Figure 3b). There was crowdedness in the nuclei of the bundles and atrophy of the muscle fibers (Figure 3c). Muscle bundles of the S0B1 group were mostly normal with slight increased basophilia (Figure 3d). The muscle bundles of the S1B1 group were more or less normal except for few muscle fibers were lysed (Figures 3e).

DISCUSSION

Growth performance of Lambs

The current study was carried out to evaluate the effect of betaine on growing fattening lambs drinking fresh or saline water. Saline water decreases dry matter intake and the body weight, average daily gain of growing lambs and betaine normalizes this effect. Similarly in Barbarine sheep, the administration of NaCl in water at rates 11 and 15 g/l decreased feed intake compared with tap water group (Yousfi and Salem, 2017). In addition, Mdletshe et al. (2017) indicated significant reduction in average daily feed intake and average daily gain of goats drinking saline water (5.5 - 11 g TDS/l) compared with fresh water groups. Moreover, the average daily gain was decreased by 19.3% in Murrah buffalo calves offered water with 8789 mg TDS/l compared with freshwater group (Sharma et al., 2017). Drinking NaCl saline water increases sodium and chlorides in the rumen, thereby increasing the ruminal osmotic pressure, causing gastric distension and thus decreasing feed intake (Phillip et al., 1981). In addition, saline water induces higher water consumption which could help the kidney to remove the exceeded salt from their body (Kii and Dryden, 2005). Consequently, the animals face digestive and metabolic disorders, which could decrease the body weight gain (Masters et al., 2007).

However, our results showed that betaine increased the body weight of the growing lambs. Similar results were observed on heifers (Lakhani et al., 2019), where betaine supplementation increased the body weight gain. In addition, dietary betaine (600 mg/kg of diets) decreased the body weight in growing piglets (Dong et al., 2012). The enhancement effect of betaine for the lambs performance is due to the role of betaine as an osmolytic and methyl donor (Eklund et al., 2005). Betaine as an osmolytic increases the cellular free water content and maintain the cell volume and water balance at the high osmolarity of salinity stress (Eklund et al., 2005; Gudev et al., 2011). In addition, improving water retention in muscle tissues associated with dietary betaine supplementation may be contributed to the body weight gain (Esteve-Garcia and Mack, 2000). Betaine also donates methyl groups for the transmethylation reactions of homocysteine to methionine which are necessary for protein synthesis (McDevitt et al., 2000). Accordingly, Li et al. (2019) described that hyperosmolarity formed by NaCl *in vitro* could inhibit digestive enzyme activities such as amylase and trypsin, while betaine improved digestive enzymes activities, increased digestion of nutrients and counteracted the inhibitory effect of hyperosmolarity. The effect of betaine and saline water on digestion and rumen microbes needs further research.

Hematological findings

Dietary betaine supplementation increased Hb concentration, RBCs count, and HCT value in this study. Similarly, dietary betaine (1.5 g/kg of diet) increased RBCs count and Hb concentration in ducks offered saline or fresh water (El-Badry et al., 2015). The correction of Hb concentration, RBCs count, and HCT value by the dietary betaine in lambs might be due to the better nutrient

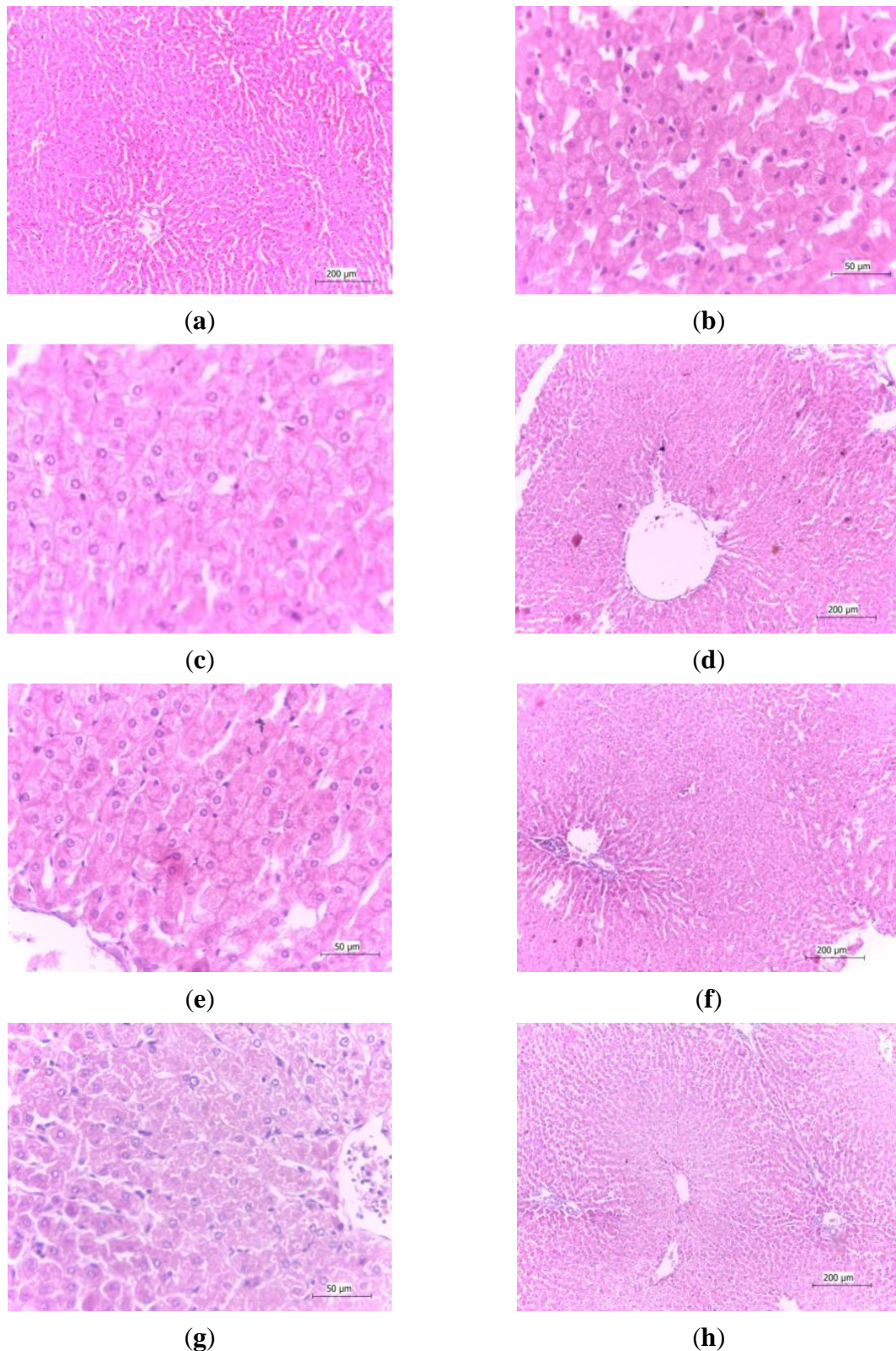


Figure 1. Liver of sheep revealed normal histological structure in control group (Fig. 1a), diffuse Hepatocellular swelling in saline water group (Fig. 1b-c), centrilobular swollen hepatocytes with granular cytoplasm in betaine group (Fig. 1d,e,f) and centrilobular degeneration and necrosis in saline water with betaine group (Fig. 1g-h) (H&E, Bar = 200 or 50 μm).

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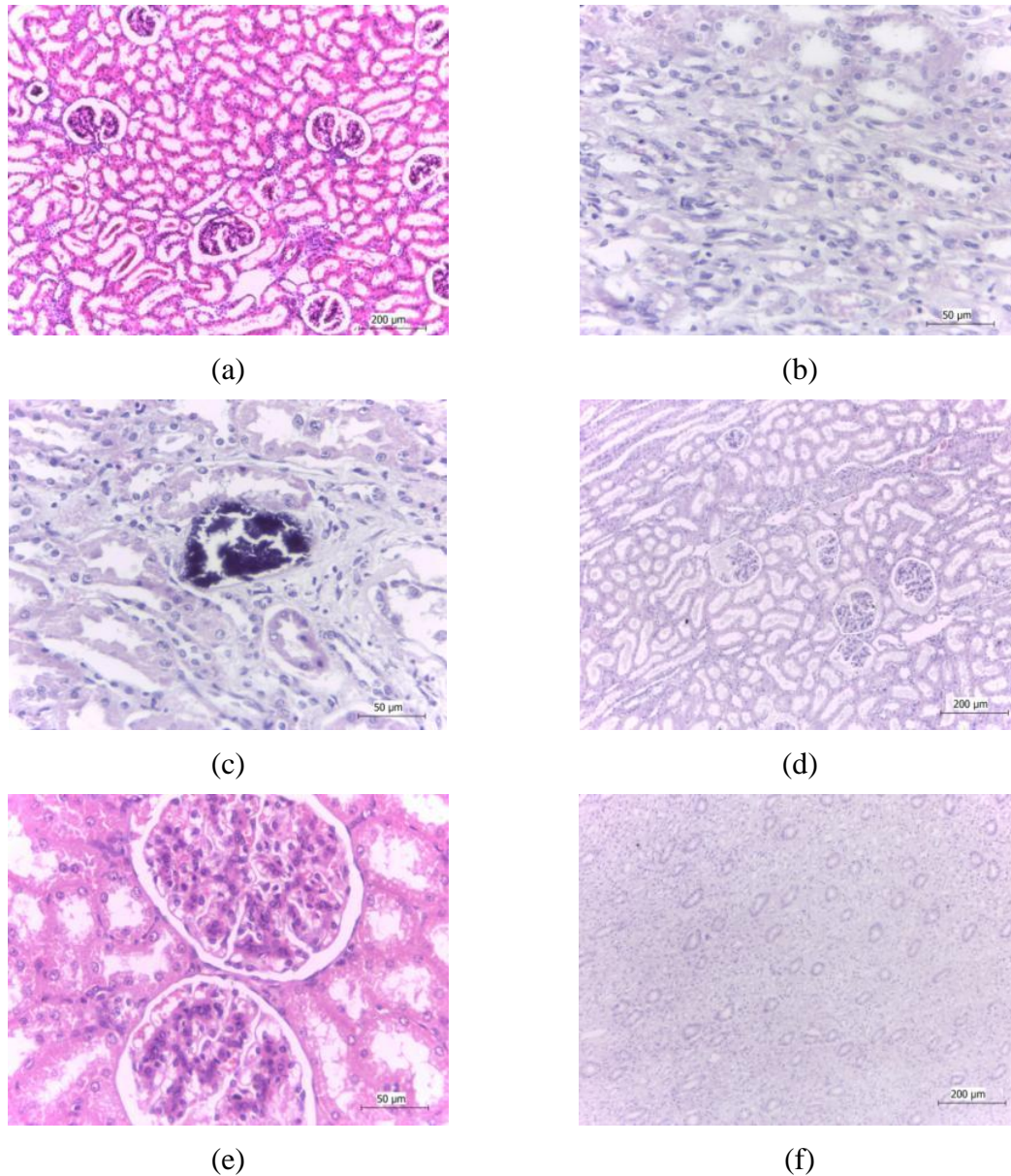


Figure 2. Kidney of sheep revealed normal histological structure in control group (Fig. 2a), focal medullary tubular necrosis in saline water group (Fig. 2b,c,d), glomerular hypercellularity and tubular nephrosis in betaine group (Fig. 2e) and more or less normal tubules in renal medulla in saline water with betaine group (Fig. 2f) (H&E, Bar = 200 or 50 μm).

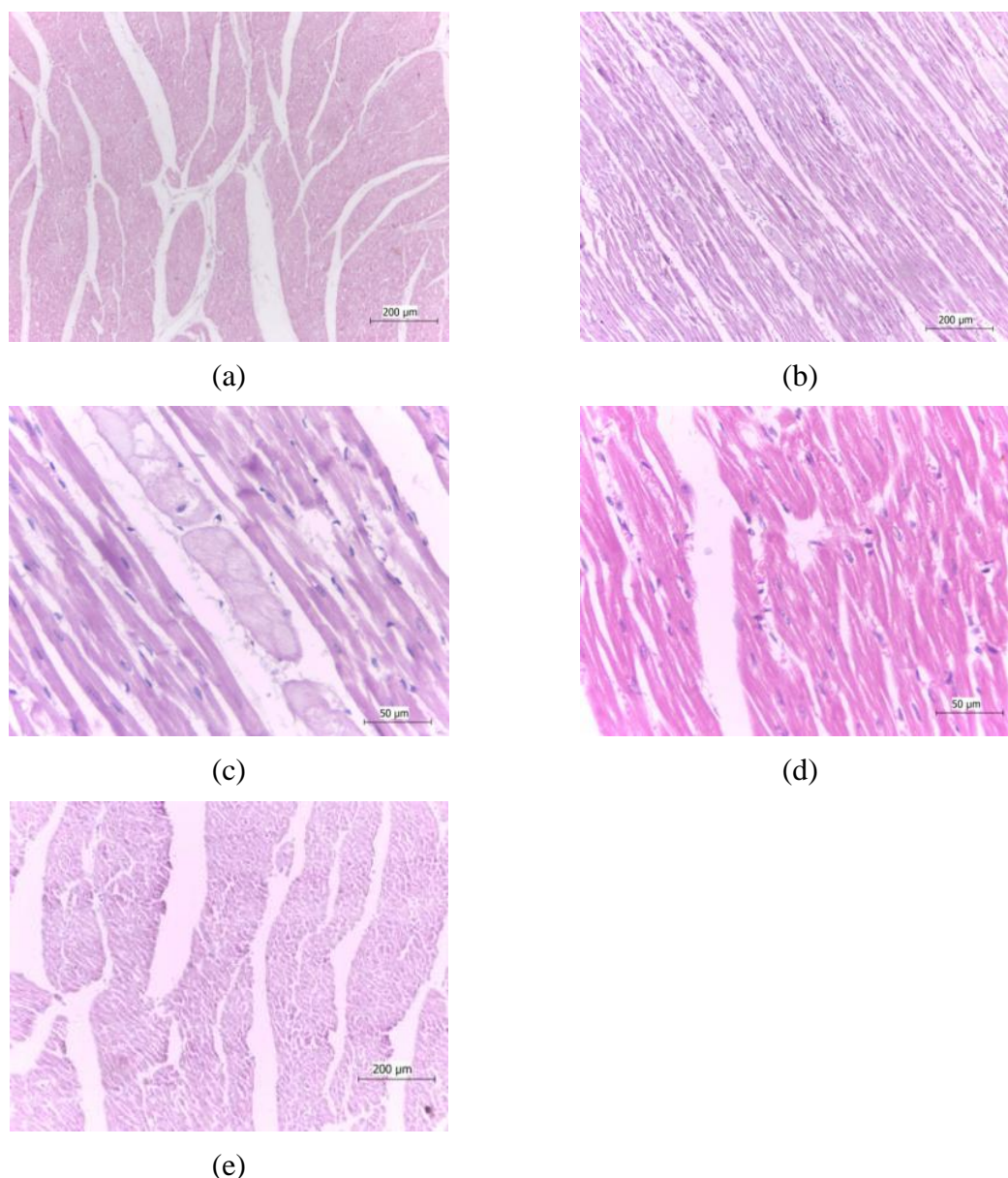


Figure 3. Cardiac muscles of sheep revealed normal histological structure in control group (Fig. 3a), fasciculate bundles and some fibers revealed cardiomyolysis in saline group (Fig. 3b-c), myocardial muscles appeared almost normal comparable to control animals in betaine group (Fig. 3d). few muscle fibers were lysed in saline/ betaine group (Fig. 3e) (H&E, Bar = 200 or 50 μm).

utilization especially of iron. The beneficial effects on nutrient digestibility of betaine are due to that betaine promotes intestinal microbes against the higher osmotic stress and maintain its growth and survival, also betaine improves microbial fermentation activity (Ratriyanto et al., 2009). Furthermore, betaine increased the water-binding capacity of the intestinal cells in broiler chicks (Kettunen et al., 2001). In addition, the increase in the production of

immature neutrophils in the blood circulation (band cell) is called the left shift or blood shift which indicates severe inflammatory responses (Kumar et al., 2014). Then, low blood eosinophil and band neutrophils percentages due to betaine indicate the healthy status of lambs dietary.

Biochemical parameters

Biochemically, saline water intake group decreased plasma urea and sodium compared

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with the freshwater intake groups in this study. Similarly, the serum urea concentrations reduced in Barbarine sheep drinking 10% NaCl water compared with 5% NaCl water (Arjomandfar et al., 2010). In the same line, saline water (5 or 10 g TDS/l) decreased the serum sodium in bulls (Visscher et al., 2013). Moreover, in sheep, Meintjes and Engelbrecht, (2004) reported that saline water containing 4.5 and 9 g/l NaCl decreased both plasma sodium and urea concentrations compared with the freshwater. They suggested that this effect was due to the increased glomerular filtration rate, which improve the excretion of urea through nephron tubule as well as the fractional excretion of sodium due to the rise of atrial natriuretic peptide, thus decreased sodium reabsorption from the tubule (Sonnenberg, 1990). In the present study, saline water lowered the levels of plasma ALT. Similarly, saline water decreased plasma ALT in growing Boer goat (Yirga et al., 2018). The low levels of ALT may be due to the blood haemodilution, which may result in low reference ranges for ALT and AST enzymes (Girling et al., 1997).

In addition, betaine decreased the plasma urea concentrations in this study. Similarly, dietary betaine (600 mg/kg of diets) decreased the serum urea nitrogen in growing piglets (Dong et al., 2012). These results indicate increased efficiency of protein utilization due to the role of betaine as a methyl donor. Moreover, betaine decreased the plasma ALT and AST in this study. Similarly, dietary betaine decreased the plasma concentrations of ALT and AST in Pekin ducks and ducklings (Awad et al., 2014; El-Badry et al., 2015). The reduction in liver enzymes (plasma ALT and AST) might be due to the lipotropic effects of betaine, which in turn may decrease the liver fat content, indicating better liver function (Xu and Feng, 2002).

Histopathological examination

To the best of our knowledge, this study is the first to investigate the role of betaine to control the effects of water salinity in livestock. In the current study, the liver of S1B0 group showed hepatic swelling. The longtime of consumption of high saline water throughout the experiment period may be followed by increased intracellular inorganic molecules such as sodium

and chloride concentration. Intracellular sodium chloride acts to increase the water retention of tissue (Cheng and Sun, 2008). However, higher cellular NaCl concentration can lead to salt poisoning, thirst, increased urination, loss of appetite and death (Curran and Robson, 2007). Betaine, on the other hand, accumulates and replaces inorganic ions in cells exposed to hypertonic state to protect enzymes and cell membranes from inactivation by inorganic ions (Petronini et al., 1992; Wunz and Wright, 1993). In addition, betaine maintains water retention and prevent the water loss as well as cell dehydration (Kidd et al., 1997). Therefore, the livers of the S0B1 group revealed centrilobular hydropic degeneration. It is striking that the kidney of the S1B0 group revealed calcium calculi in the medulla. The high sodium chloride intake increases the risk of kidney stones formation by increasing the urinary calcium excretion and decreasing the citrate levels in urine (Sakhaee et al., 1993). Citrate is an inhibitor against the crystallization of calcium oxalate. However, the kidney of the S0B1 group showed increased cytoplasmic basophilia in renal glomeruli and tubule cells, suggesting higher protein content relied on betaine as a methyl donor. Some cardiac muscles fibers in S1B0 group revealed rhabdomyolysis due to systemic potassium deficiency (Larner, 1994). When animals drink high sodium saline water, the kidney flushing excess sodium by increasing glomerular filtration rate (Meintjes and Engelbrecht, 2004).

CONCLUSION

This study demonstrates that the supplementation with betaine had a protective effect against the harmful effects caused by saline water in sheep. Saline water decreased body weight, average daily gain and feed intake of lambs. However, betaine restored body weight of lambs but without effect on the average daily gain and feed intake. In addition, saline water decreased blood urea, sodium and alanine amino transferase concentration. Betaine also decreased blood urea, alanine and aspartate amino transferases concentration, but did not affect blood sodium. Saline water induced deleterious effects on the histological pictures of

kidney, liver and heart. Betaine minimized these effects.

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الملخص العربي

مقاومة البيتاين للأثار الضارة الناجمة عن شرب الماء المالح في الحملان النامية.

محمود محمد عبد الستار، احمد محمد عبدالله، محي هريدي، محمد نصرت محمود، عاطف سليم، نافين زانج

- هدفت الدراسة الحالية الى تحديد دو البيتاين على مكونات الدم والتشريح المرضي للحملان النامية التي تستهلك اما الماء العذب او المالح. تم استخدام ٢٠ من ذكور حملان الاوسيمي السليمة صحيا والتي في عمر ٩ شهور. تم توزيعها على اربع معاملات (٥ حملان لكل معاملة). تم معاملة الحملان بمستويين من كلوريد الصوديوم (٠ و ١,٥٪) المذاب في الماء ومستويين من البيتاين (٠ و ٢٥٠٠ ملجم/كجم عذاء) في العليقة. واستمرت التجربة لمدة ١٣٥ يوم. وقد أسفرت التجربة عن النتائج التالية: -
- أدى استهلاك الحملان النامية للماء المالح الى انخفاض معنوي ($P<0.01$) في وزن الجسم ومحتوي الدم من اليوريا ومستوي الصوديوم في الدم وكذلك مستوى انزيم Alanine amino transferase.
 - في حين أدت إضافة البيتاين الى غذاء الحملان النامية الى زيادة معنويه ($P<0.01$) في وزن الجسم وهيموجلوبين الدم وزيادة في عدد كرات الدم الحمراء ونسبة الهيماتوكريت (نسبة المكونات الخلوية) في الدم.
 - في حين عمل البيتاين على خفض مستوى بعض مكونات الدم من الخلايا البيضاء (وحيدة النواه والمتعادلة) ومستوى البلازما من اليوريا وانزيمي Alanine and Aspartate amino transferase.
 - أدى استهلاك الحملان النامية للماء المالح الى انتفاخ وتشتت الخلايا الكبدية عن ترتيبها الطبيعي. كما نتج عنه بؤر ميتة في النخاع الانبوبي للكلى وكذلك حصوات كلسية في الكلى وتحلل موضعي في عضلة القلب. على النقيض، فقد أدى استخدام البيتاين الى التعافي من هذه الأثار الضارة للماء المالح
 - وتوصي الدراسة الحالية بان إضافة البيتاين في علائق الحملان النامية من شأنه ان يحفز نمو وأداء الحملان. كما ان له القدرة على معادلة الأثار الضارة لشرب الماء المالح.

Betaine counteracts the harmful effects of saline water induced to growing lambs