Egyptian J. Nutrition and Feeds (2022), 25 (3):283-290

BIOLOGICAL AMELIORATION OF BLOOD METABOLITES INDICES OF BARKI EWES UNDER SALINE WATER STRESS DURING PREGNANCY

A.S. El-Hawy[1]; E.B. Abdalla[2]; H.A. Gawish[1]; Effat, M. Madany[2] and H.K. Zaghloul[3]

[1]*Animal and Poultry Division, Desert Research Center, Cairo, Egypt*

[2]*Animal Production Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt*

[3]*High Institute for Agricultural Co-Operation, Shoubra, Cairo, Egypt*

(Received 10/07/2022, accepted 20/09/2022)

SUMMARY

The present study aimed to evaluate the effect of zeolite addition to rations on liver and kidney functions, minerals profile and aldosterone hormone of Barki ewes affected by saline well water throughout gestation period in South Sinai, Egypt. Thirty Barki ewes (2.0-3.0 years old and 31.8 ± 1.4 kg average body weight) were randomly assigned into three equal groups (10 ewes / group). The 1st group (G1); ewes drank fresh (TW) tap water (274 ppm TDS) and fed the basal diet (control group). The 2nd group (G2) ewes drank saline (SW) well water (5980 ppm TDS) and fed the basal diet withot zeolite addition. The 3rd group (G3) ewes drank SW (5980 ppm TDS) and fed the basal diet supplemented with 60 g zeolite /kg diet (6 %). Rations were adjusted monthly to cover their nutritional requirements during the experimental period. Fresh and saline water were available freely all the day time. Results indicated that Alanine amino transferase (ALT) and aspartate amino transferase (AST) concentrations were significantly higher in saline group (G2) then G3 and control group (24.41, 19.63 and 16.29 IU/dl, respectively) and (34.19, 29.42 and 25.26 IU/dl) respectively. Urea and creatinine concentrations tended to increased significantly in saline group (G2) followed by zeolite group (G3), while control group recorded the lowest value. P and K concentrations were significantly ($P<0.05$) higher in control group than others, while Ca significantly decreased in control as compared to other groups. Cl and Na were insignificantly higher in G2 than other groups. Aldosterone concentrations were affected by drinking saline water. Both G2 and G3 recorded 24.1 and 17.5% reduction in aldosterone concentration at 140 day of pregnancy compared to control group. In conclusion, addition of zeolite at a level of 6 % to the diets of Barki ewes could be an attempt to reduce the negative effect of drinking saline water.

Key words: *Barki ewes, Zeolite, Saline water, Liver function, Kidney function, Minerals, Aldosterone.*

INTRODUCTION

Salinity, sodality, and aridity, in various combinations, impact around a third of the Earth's land surface (Vosooghi *et al.*, 2018). For sheep, the issue of water quality has long been highlighted. Salt is one component that impacts water intake. El-Gharbi *et al.* (2015) found that water with less than 13% NaCl is suitable for sheep. Water is an essential nutrient, and its amount and quality have a big influence on animal performance and health. The concentration of total dissolved salt in drinking water is one of the most effective parameters in determining the appropriateness of water for animals (Vosooghi *et al.*, 2018). Because powder-form zeolites are inert in the digestive system, comparable to many silicates (Ivkovic *et al.*, 2004), they have no chemical interactions with nutrients or body fluids, and hence may be utilised in animal feeding without causing harm. Natural zeolites have been shown to have beneficial benefits on the body's detoxification, immunological system, mineral metabolism, blood circulation, neurological system, and digestion, according to Hecht (2010). Some nations have embraced good agricultural techniques that are ecologically friendly, contribute considerably to human and animal health, do not allow the use of chemical and artificial fertilisers, and provide safe and healthy meals as a consequence of growing consumer awareness in recent years (Toprak *et al.*, 2016). For these reasons, scientists have been looking for safe and helpful chemicals that do not leave hazardous residues in the production of vegetables and animals. This category of additives includes zeolites. As a result, the goal of this study was to see how adding zeolite to rations affected the liver and renal functions, mineral profile, and aldosterone concentration of Barki ewes that drink saline water in the desert of South Sinai, Egypt.

MATERIALS AND METHODS

Animals and management:

Thirty adult Barki ewes (2.0-3.0 years old and averaged 31.8 ± 1.4 kg live body weight) were randomly assigned into three equal groups (10 ewes/ group). The 1st group (G1); ewes drank fresh tap water (Tw 274 ppm TDS) and fed the basal diet (control group). The 2nd group (G2); ewes drank saline well water (Sw 5980 ppm TDS) and fed the basal diet. The 3rd group (G3); ewes drank saline well water and fed the basal diet blues 60 g zeolite /kg diet (6 %). Chemical analysis of SW and TW are illustrated in (Table 1). Rations were adjusted monthly according to Kearl (1982) to cover their requirements. Fresh water (TW) and saline water (SW) were available all the day time to all groups.

Table (1): Chemical analysis of drinking saline well water and fresh tap water.

Parameters	SW	TW	SW/TW ratio
Total dissolved solids (mg/l)	5980	274	21.82
Electric conductivity (µs/cm)	9.96	0.53	18.79
Sodium (mg/l)	86.00	2.40	35.83
Chloride (mg/l)	61.34	2.47	24.83
Calcium (mg/l)	15.00	1.75	8.57
Magnesium (mg/l)	19.00	2.25	8.44
Potassium (mg/l)	0.36	0.15	2.40
Hardness* (mg/l)	34.00	4.00	8.50
Carbonate (mg/l)	0.20	0.40	0.50
Bicarbonate (mg/l)	3.00	2.60	1.15
pH	7.23	7.63	0.95

** Hardness is a measure of the amount of calcium and magnesium salts in water.*

Blood sampling and analysis:

For five months, blood samples (10 ml) were taken from ten ewes in each group in the morning before feeding through vein puncture (using a clinical needle) at biweekly internals. For serum separation, blood samples were centrifuged at 3000 rpm for 20 minutes and stored at -20 ºC until further analysis. Test kits provided by Diamond Diagnostic Company for Laboratory Services were used to determine the concentrations of both alanine (ALT) and aspartate (AST) amino transferases, while commercial test kits provided by Biodiagnostic Company for Laboratory Services were used to determine urea and creatinine concentrations as indicators of kidney function. Commercial kits provided by Biostc Company for Laboratory Services were used to assess the amounts of minerals in blood serum (Na, K, Ca, Cl, P, and Mg). ELISA kits provided by Immunospec Corporation were used to quantify aldosterone hormone (7018 OweNSMounth Ave. Suite 103 Canoga Park, CA 91303, USA).

Statistical analysis:

Data of blood parameters were analyzed by the least square analysis of variance using the General Linear Model Procedure (SAS, 2004). The model was one-way analysis as follows:

$$Yij = \mu + Gi + eij$$

Where:
Yij = any observations of ith animal within jth group.
µ = overall mean
Gi = effect of group, (i: 1-3)
eij = experimental error.
Means were compared using Duncan Multiple Range Test (Duncan, 1955).

RESULTS AND DISSCUTION

Liver and kidney functions:

The ALT and AST enzymes in the liver are used to evaluate liver damage (Mahgoub *et al.,* 2008). Alanine aminotransferase (ALT) is a blood enzyme that is elevated when cellular degeneration or destruction occurs and is particularly effective in evaluating hepatic necrosis (Nicoll *et al.,* 2004). In this study, G2 had substantially higher liver enzymes (ALT and AST) than the other groups (P0.05) (Table 2).Alanine amino transferase (ALT) concentration was (P<0.05) higher in G2 followed by G3, while the control group recorded the lowest value (24.41, 19.63 and 16.29 IU/dl, respectively). Similarly, aspartate amino transferase (AST) concentration showed a significantly (P<0.05) higher level in G2 then G3, while the control group recorded lowest value (34.19, 29.42 and 25.26 IU/dl, respectively).

Table (2): Effect of Treatments on blood biochemical parameters.

Groups / Items	G1	G2	G3	± SE	Pvalue
AST (IU/l)	25.26^{a}	34.19^{a}	29.42^{b}	0.39	0.04
ALT (IU/l)	16.29^{C}	24.41^{a}	19.63^{b}	0.31	0.01
Urea (mg/dl)	21.69^{c}	29.56^{a}	26.06^{b}	0.44	0.05
Creat (mg/dl)	1.18^{b}	1.70^{a}	1.37^{b}	0.07	0.01

a-c means within rows with different superscripts differ significantly (P<0.05).
G1=ewes drank fresh tap water (control), G2=ewes drank saline well water, G3=ewes dank saline well water + zeolite (6 %).

These results are in harmony with those reported by Preeti *et al.* (2018) who observed an increase in the level of plasma AST with values being 78.2, 78.5 79.7, 82.4 and 83.4 UI/dl in animals drank SW at levels 500, 2000, 4000, 6000 and 8000 ppm, respectively. They reported that ALT levels also increased to 33.0, 32.8, 34.1 and 34.3 in 2000, 4000, 6000 and 8000 ppm as compared to control (500 ppm) where the value was 32.9. The same results were reported by Assad and El-Sherif (2002) who found that AST in sheep was increased (P<0.05) by drinking SW for a long period from 44.13 to 53.00 IU/L, also the level of ALT increased (P<0.01) from 10.4 to 15.87 UI/L, indicating liver hyperfunction in sheep due to the increase in salinity of drinking water. Moreover, Gawish *et al.* (2013) reported that ALT and AST were significantly increased in Shamy goats which drank SW as compared to goats drank FW. Generally, both ALT and AST are liver marker enzymes and depict the function of the liver. Although, the values increased by providing SW but their values were still within the normal physiological range.

Water balance, electrolyte balance, acid/base balance, osmotic pressures of bodily fluids, and the elimination of metabolic waste products and other hazardous compounds are all critical functions of the kidneys (Sherwood, 1997). Serum blood urea and creatinine concentrations are known to indicate glomerular filtration rate and kidney function (Kaneko, 1989).

In the present study, urea and creatinine concentrations tended to increased significantly in saline group (G2) followed by zeolite group (G3), while the control group recorded the lowest value Table (2). These results indicated that urea and creatinine were influenced by SW but this effect was reduced by given the zeolite in the diet. These findings are consistent with those of Eltayeb (2006), who found that when NaCl levels in drinking SW rose, urea concentration in Nubian goats increased much more than in the control group.

Preeti *et al*. (2018), on the other hand, discovered that SW had no effect on urea and creatinine levels. According to Robert *et al.* (1992), there were no significant changes in blood urea concentrations between Holstein steers that drank high SW or FW, indicating no deleterious short-term impacts on renal function. This might be due to a rise in the glomerular filtration rate (GFR). GFR was considerably greater in goats consuming SW with high NaCl concentrations compared to those getting FW with low NaCl concentrations, according to Godwin and Williams (1986). With high concentrations of NaCl in the drinking water, this had the effect of making more urea accessible to the nephron tubule than with low concentrations of NaCl in the drinking water. It might also be explained by an increase in urea supply to the rumen. The kidney adjusts the ratio of urea to sodium in the medullary interstitium in favour of the former, according to Meintjes and Engelbrecht (2004), and this may have an effect on urea concentrations, or possibly under conditions of excess salt intake, the kidney adjusts the ratio of urea to sodium in the medullary interstitium in favour of the former. The current findings in sheep are consistent with Meintjes and Engelbrecht's (2004) findings.

Blood serum electrolytes:

Minerals are essential for bodily fluid management, acid-base balance, and metabolic functions (Milne, 1996). Data in Table (3) presented the effects of SW and zeolite supplementation on serum electrolytes of Barki ewes.

The principal anion, chloride (Cl), balances sodium, potassium, and other cations. Cl deficiency is rare and never accurse with salt in the diet or water (NRC, 2007). Cl concentration was insignificantly higher in control group than other to saline groups with values being 93.21, 98.53 and 94.08 mg/dl for G1, G2 and G3, respectively. Following ingestion of high salty water, Na and Cl ions can be excreted at a faster rate due to (i) an increase in glomerular filtration rate and filtration fraction without a significant change in renal plasma flow, (ii) a reduction in sodium chloride reabsorption in individual nephrons of the sheep kidney, and (iii) an increase in water retention (Potter, 1968).

P and K concentrations significantly decreased in G2 (3.70 and 2.89 mg/dl) than other groups. Zeolite group recorded the highest values of P and K (4.80 and 3.68 mg/dl, while control group recorded 4.45 and 3.62 mg/dl, respectively. The renin angiotensin system (RAS) regulates salt and water balance by releasing hormones such as renin, angiotensin I and II, aldosterone, which controls sodium retention, and arginine vasopressin (AVP), which regulates water reabsorption. When you eat too much salt, your plasma osmolality rises, which exerts a negative feedback on aldosterone, lowering concentrations and encouraging sodium excretion. Additionally, as plasma osmolality rises, so does water consumption and osmoreceptors signal the pituitary gland to produce AVP. On the other side, Ca recorded the highest value in G2 (13.3 mg/dl) followed by zeolite group (11.14 mg/dl), while the control group recorded the lowest value (9.8 mg/dl). The same trend was also observed in Na concentration, whereas saline group recorded the highest value (151.54 g/dl), then zeolite group (135.54 mg/dl), while the control group recorded the lowest value (122.62 g/mdl).

Table (3): Effect of Treatments on blood minerals profile (mg/dl) of experimental groups

Item \ Group	G1	G2	G3	±SE	P value
Cl	93.21	98.53	84.08	1.72	0.28
P	4.45^{a}	3.70^{b}	4.80^{a}	0.17	0.05
Ca	9.8^{b}	13.3^{a}	11.14^{a}	0.31	0.03
K	3.62^{a}	2.89^{b}	3.68^{a}	0.11	0.05
Na	122.62	151.54	135.54	4.75	0.12

a-b means within rows with different superscripts differ significantly (P<0.05).
G1=ewes drank fresh tap water (control), G2=ewes drank saline well water, G3=ewes dank saline well water + zeolite (6 %).

The above results are in harmony with that reported by El-Hawy (2013) who found that Cl, Ca insignificantly increased, Na significantly increased and P and K insignificantly decreased in Shami goats drank saline water than others drank tap water. Also, Eltaieb (2006) found that Na concentration significantly increased, while K concentration significantly decreased in Nubian goats with NaCl concentration increased in drinking water. Silicon, aluminium, or sodium content of zeolite may also have beneficial effects on calcium metabolism, therefore enhancing Ca and phosphorus (P) utilisation (Leach *et al.*, 1990 and Watkins and Southern, 1991).

On the other hand, Amer (1990) discovered that consuming saline water had no effect on Ca and Mg levels in goats. Furthermore, Jaster *et al.* (1978) found that Ca and P levels in cows consuming saline water were unaffected and remained generally steady (2500 ppm NaCl). According to zeolite supplementation, Topark *et al.* (2016) reported that Ca significantly increased in lambs fed diet supplemented with 2 and 3% Zeolite than control and 1% zeolite. They also found no differences in Na, K and Cl, while P concentration was significantly decreased in lambs fed diet supplemented with 2% zeolite. In dairy cows, Pconcentration did not affected by zeolite addition to ration, while Ca concentration was significantly increased in cows fed ration supplemented with 200 g zeolite per cow per day (Khachlouf *et al.,* 2019).

Increased sodium and chloride ions in plasma following consumption of high saline water can be excreted by (i) increased glomerular filtration rate and changes in renal plasma flow, (ii) reduced sodium chloride reabsorption in individual nephrons and (iii) increased water retention (Digby *et al.*, 2011). Another powerful adaptive mechanism is the Na K ATPase enzyme, induced in the ilium, liver and kidney after exposure to saline water (Mancarella *et al.*, 2016). In its function, it increases the pumping of sodium out of cells and in return the pumping of potassium into the intracellular space.

Aldosterone hormone:

Data presented in Figure (1), showed that aldosterone concentrations were affected by drinking SW. Both G2 and G3 recorded 24.1 and 17.5% reduction in aldosterone concentration at 140 day of pregnancy compared to control ones. Consumption of a 13 % NaCL diet reduces aldosterone levels in pregnant sheep by nearly twofold (Digby *et al.*, 2008). Furthermore, high salt consumption has been shown to regulate energy partitioning in sheep (Blache *et al.*, 2007), including a direct effect on insulin concentrations. Digby *et al.* (2008) also mentioned that high salt feeding during pregnancy is associated with changes in circulating concentrations of insulin, leptin, and thyroid hormones, and that these changes may have effects on the offspring, and that changes in maternal thyroid hormone concentrations can affect birth weight.

The present results are in harmony with Digby *et al.* (2008), who found similar results in Merino ewes, whereas Shaker *et al.* (2008) reported similar results with Barki lambs. This increase in aldosterone concentration compared to 0 time might be related to the increased salt content in the water Shaker et al (2008). In addition, Vosooghi-Postindoz *et al.* (2018) looked at how drinking SW affected the aldosterone hormone in Baluchi lambs. They discovered that when lambs drank water with a high TDS concentration (8000 mg/L) compared to lambs drank water with a low TDS concentration (400 mg/L), aldosterone concentration reduced insignificantly. Furthermore, aldosterone concentrations were lower in the SW groups than in the tap water groups, according to El-Bassiouny (2013) on shami goats and Abou-hashim (2015) on lamb rams.

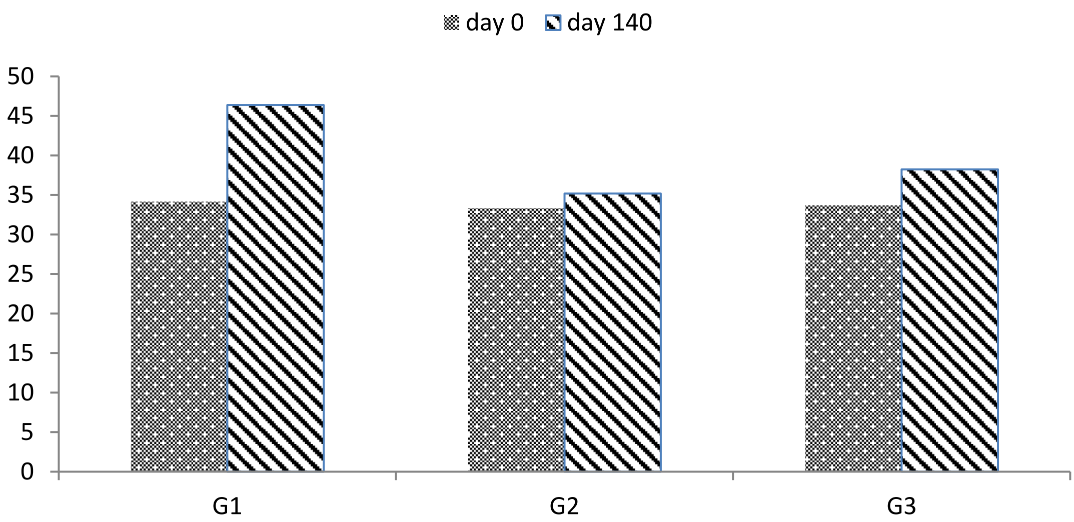

Figure (1): Aldosterone hormone (ng/dl) of different groups during experimental period.

G1=ewes drank fresh tap water (control), G2=ewes drank saline well water, G3=ewes dank saline well water + fed zeolite (6 %).

Ultimately, a high-salt diet causes a drop in aldosterone hormone levels, which reduces sodium reabsorption and increases sodium excretion. If the consumption of fresh water is adequate to maintain a salt and water balance, high salt intake did not cause a change in AVP concentration **(Cowley *et al.*, 1986)**.

CONCLUSION

From our results, we can conclude that, the addition of zeolite at a level of 6 % to the diets of Barki ewes could be an attempt to reduce the negative effect of drinking SW on liver and kidney as well as minerals and aldosterone profiles.

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التحسين البيولوجى لمكونات الدم للنعاج البرقى المعرضة لإجهاد الملوحة فى ماء الشرب أثناء الحمل

أحمد صبحى الحاوى[1], عصمت بكرى عبدالله[2], حمدى عبدالعزيز جاويش[1], عفت مراد مدنى[1], حلمى زغلول[3]

[1]شعبة الانتاج الحيوانى والدواجن, مركز بحوص الصحراء , القاهرة, مصر

[2] قسم الإنتاج الحيوانى, كلية الزراعة, جامعة عين شمس, القاهره, مصر

[3] المعهد العالى للتعاون الزراعى, شبرا, القاهره, مصر

تهدف الدراسة إلى تحسين مكونات الدم فى النعاج البرقى المتأثرة بشرب الماء المالح بمحافظة جنوب سيناء. حيث استخدم فى هذه الدراسة 30 نعجة برقى (عمر 2-3 سنة) بمتوسط وزن (31,8±1,4 كجم) قسمت عشوائيا إلى 3 مجموعات متساوية (10 نعاج لكل مجموعة). المجموعه الاولي (الضابطة) غذيت علي العليقه الاساسية مع شرب الماء العذب (247 جزء فى المليون أملاح كلية ذائبة) بينما غذيت المجموعه الثانيه علي العليقة الأساسية مع شرب ماء البئر المالح (5890 جزء فى المليون أملاح كلية ذائبة) وغذيت المجموعه الثالثة علي العليقة الأساسية مضاف اليها الزيولايت بمعدل 60جم زيولايت / كجم علف (6 %) مع شرب ماء البئر المالح.

أظهرت النتائج زيادة تركيز انزيمات الكبد (ALT – AST) فى المجموعة الثانية نتيجة شرب الماء المالح بينما ادت إضافة الزيولايت للعلائق الى إنخفاض تركيز هذه الانزيمات فى المجموعة الثالثة وكذلك المجموعة الضابطة. كما تأثرت تركيزات اليوريا والكرياتينين معنويا بشرب الماء المالح حيث زادت التركيزات فى المجموعة الثانيه مقارنة بالمجموعات الثالثة والضابطة. وسجل تركيزالفسفور زيادة معنوية في المجموعة الضابطة والثالثة مقارنة بالمجموعة الثانية وكانت القيم 4.45 و 4.8 و 3.70 ملجم/ديسيلتر للمجموعات الضابطة والثالثة والثانية على الترتيب. كما زاد تركيز البوتاسيوم معنويا فى المجموعة الضابطة والثالثة (3.62 و 3.68 ملجم/ديسيلتر) على الترتيب فى حين سجلت المجموعة الثانية اقل تركيز (2.89 ملجم/ديسيلتر). وارتفع معنويا تركيز الكالسيوم فى المجموعة الثانية (13.3 ملجم/ديسيلتر) ثم المجموعة الثالثة (11.14 ملجم/ديسيلتر) بينما سجلت المجموعة الضابطة أقل تركيز (9.8 ملجم/ديسيلتر). وكانت الزيادة غير معنوية فى تركيزات الصوديوم والكلورايد. وسجل مستوى هرمون الالدوستيرون زياده غير معنوية فى المجاموعة الضابطة ثم مجموعة الزيولايت وسجلت المجموعة الثانية اقل مستوى.

من النتائج السابقة توصى الدراسة بإضافة الزيولايت إلى علائق الأغنام البرقى بنسبة 6% لتقليل الأثار الضارة لشرب الماء المالح على الكبد والكلى وكذلك على تركيز العناصر المعدنية وهرمون الألدوستيرون.

الكلمات الدالة: *الأغنام , الزيوليت, الماء المالح , انزيمات الكبد, وظائف الكلى.*