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Can Exogenous Betaine Be an Effective Osmolyte in Broiler Chicks under Water Salinity Stress?

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ABSTRACT : A CRD experiment was conducted to evaluate the effects of different exogenous betaine levels (0.000, 0.075, 0.150 and 0.225 percent) on 576 one-day-old male broiler chicks (Ross) under water salinity stress. Different levels of water salinity were made by adding 3 levels of NaCl (0, 1,000 and 2,000 mg/L) to drinking water. Feed and water were available *ad libitum*. Betaine increased body weight, improved feed conversion ratio, and decreased packed cell volume ($p<0.05$). Water salinity promoted body weight over the whole period, increased feed intake (11 to 21 and 29 to 42-d) and also improved feed conversion ratio in grower and finisher periods ($p<0.01$). Breast weight, water consumption (28-d and 42-d) and excreta moisture (28-d) were increased by elevating the level of water salinity ($p<0.01$). Interaction between dietary betaine and water salinity was significant on plasma osmolarity as well as epithelial osmolarity of the duodenum at 28-d. Epithelial osmolarity was decreased from duodenum to ileum. The data imply that betaine is involved in the protection of intestinal epithelia against osmotic disturbance which can be caused by saline water, but further research is needed to investigate the effects of betaine with higher levels of water salinity. (**Key Words** : Broiler, Exogenous Betaine, Saline Water, Osmolytic Effect, Packed Cell Volume, Performance)

INTRODUCTION

High levels of sodium chloride (NaCl) in drinking water result in increased blood pressure. In order to expel salt, water consumption increases, total amount of blood potassium concentration decreases and an increment occurs in litter moisture (Kalimuthu et al., 1987; Balnave and Gordon, 1993). Consequently, performance decreases, anion-cation ratio becomes imbalanced and diseases such as ascites and coccidiosis occur (Kalimuthu et al., 1987; Julian, 1993). Change in anion-cation balance affects a lot of physiological and metabolic functions of the body, and thereby can reduce performance and also increase feed conversion ratio (FCR) (Kalimuthu et al., 1987; Julian, 1993). Nowadays, poultry producers in regions with high levels of total dissolved solids (TDS), especially NaCl, in water try to solve the problem by reducing dietary NaCl content, purifying water etc. The current study examined betaine supplementation as an alternative way of solving this problem. Betaine is a naturally-occurring product present in relatively large quantities in sugar beet and aquatic invertebrates, but is not present in most animal feedstuffs (Wang et al., 2004). As a by-product of sugar beet processing, betaine is commercially available as a feed additive. Due to its chemical structure (Eklund et al., 2005) it has two primary metabolic roles: it is a methyl group donor and an osmolyte that assists in cellular water homeostasis (Klasing et al., 2002). Tissues that rely on zwitterionic betaine as an osmolyte include the leukocytes, kidney, liver, brain and intestines (Klasing et al., 2002). For example, the effect of betaine as an osmotically active substance may be more pronounced in animals exposed to osmotic disorders, such as coccidiosis in poultry (Eklund et al., 2005). Augustine et al. (1997) suggested that betaine may contribute to improved performance of coccidia-infected chicks directly, by partial inhibition of coccidial invasion and development, and indirectly by an improvement of intestinal structure and function in the presence of a coccidial infection. This improvement of the intestinal structure might occur in both infected and healthy animals. Betaine accumulation results in an increased water-binding capacity of the intestinal cell structure of the gut epithelium. Enhanced tensile structure has been reported in chicks (Remus and Quarles, 2000) and strength was improved in pigs (Siljander-Rasi et al., 2003).

This study investigated the effect of betaine as an organic-compatible osmolyte because betaine was named as

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Table 1. Composition of basal diet in starter, grower and finisher periods

Ingredient	Starter (1-10 d)	Grower (11-28 d)	Finisher (29-42 d)
	g/kg diet		
Corn	607.5	655.2	687.3
Soybean meal	345.7	301.2	273.8
Oyster shells	8.3	7.6	7.5
Dicalcium phosphate	22.2	19.6	18
Salt (sodium chloride)	3.3	2.3	2.3
Sodium bicarbonate	0.5	1.9	1.9
Vitamin premix[1]	2.5	2.5	2.5
Mineral premix[2]	2.5	2.5	2.5
DL-methionine	2.6	2.7	1.9
Lysine·HCl	3.8	3.7	1.8
Choline·HCl	1.1	0.8	0.5
Calculated contents			
AME_n (kcal/kg)	2,829.47	2,889.60	2,926.50
CP (%)	20.88	19.32	18.17
Calcium (%)	1	0.9	0.85
Phosphorus (available, %)	0.5	0.45	0.42
Sodium (%)	0.16	0.16	0.16
Anion-cation (mEq/kg)	200	200	200
Lysine (%)	1.22	1.12	0.92
Methionine (%)	0.54	0.54	0.45

[1] Provided the following per kilogram of broiler diet: Vitamin A, 9,000 IU; Cholecalciferol, 2,000 IU; Vitamin E, 18 IU; Vitamin k_3, 2 mg; Vitamin B_{12}, 0.015 mg; Biotin, 0.1 mg; Folacin, 1 mg; Niacin, 30 mg; Calcium pantothenat, 10 mg; Pyridoxine, 3 mg; Riboflavin, 6.6 mg; Thiamine 1.8 mg, choline 500 mg.

[2] Provided the following per kilogram of broiler diet: Copper (as cupric sulfate $5H_20$).10 mg; Iodine (as calciumiodate), 1 mg; Iron (as ferrous sulfate $7H_20$).50 mg; Manganese (as manganese oxide), 100 mg; Selenium (as sodium selenite), 0.2 mg; Zinc (as zinc oxide), 100 mg.

one of the most likely candidates for osmoregulation, according to the report of Kettunen et al. (2001). Those researchers suggested that the presence of betaine helped the duodenal, but not jejunal, epithelium to maintain water balance in hyperosmotic conditions.

To date, little has been known about the effects of betaine on water salinity tolerance in broiler chicks. The aim of the present study was to examine the effect of exogenous betaine as an osmoprotectant in response to saline water consumption in broiler chickens.

MATERIALS AND METHODS

General

The experiment comprised 576 Ross 308 male broiler chickens (*Gallus domesticus*) from day-old up to slaughter at 42-d of age. The chickens were housed on wood shaving litter in 48 floored pens (1.25×2.5 m), situated in an insulated broiler house with concrete floors. Twelve experimental treatments were studied, each with four replicates of 12 chicks. This experiment was designed as a 4×3 factorial arrangement of treatments with four levels of added dietary betaine (0.000, 0.075, 0.150 and 0.225 percent) and three levels of total dissolved solids (TDS) in drinking water (375, 1.375 and 2,375 mg/L) which were made by adding three levels of NaCl (0, 1.000 and 2.000 mg/L). The levels of betaine which were supplemented in feed were lower, higher and the same dosage as recommended by the manufacturer (Fin feeds, P.O.Box777, Marlborough, Wiltshire, SN8 1XN, UK). The composition of the basal diet, based on corn soybean meal, and the calculated nutrient content are presented in Table 1 and met the requirements for Ross 308 broiler chicks (2002). Methionine plus cystine levels exactly met the requirement of this strain. Sufficient amounts of methyl-donating compounds were added (Choline chloride: 60% choline chloride) to eliminate the methionine-sparing effect of betaine. In order to avoid the effect of betaine as a coccidiostat enhancer, clean conditions were used to reduce the coccidial challenge. The diet contained no antibiotics, coccidiostat or feed enzymes. Chicks were raised without betaine, and TDS of their water was 375 mg/L during the first 10-d post hatching. In the morning of 11-d post hatching, after 4 h of feed deprivation, chicks were weighed and assigned to pens in a manner that ensured minimal variation in initial body weight among pens. Thus, the experimental period was subdivided into grower (11 to 28-d) and finisher (29 to 42-d) periods. Prior to the start of trial, two birds of each replicate were chosen randomly and wing-banded for taking blood and intestinal samples during the experiment. Betaine (Betafin S_1, 96% Betaine anhydrous, feed grade) and sodium chloride were added to the basal diet and drinking water, respectively, from 11 days of age when the main experiment started. The ingredients used were from a single batch in order to minimize differences in composition among the mixing batches. The experimental diets were prepared by splitting up this batch into sub charges, to which the required amount of betaine was added and mixed. Feed and water were available *ad libitum*. Ionic composition in drinking water of the control group, as measured by atomic absorption is shown in Table 2. TDS of the basal drinking water was 375 mg/L as measured according to AOAC (2000). Chicks were housed in an environmentally controlled house with 24 h light during the experiment. Ambient temperature on the 1st day was set at 32±1°C and then gradually decreased until 22±1°C was reached by the fifth week. The relative

Table 2. Ionic composition in drinking water of control group

Ion type	Ca	Na	K	Mg	Zn	Fe
	%				ppm	
Ionic amount	0.0073	0.0035	0.0011	0.003	0.031	0.207

humidity in the broiler unit was approximately 60 to 70 percent during the trial period.

Body weight (BW) and feed intake (FI) were measured on a pen basis at the end of 21, 28 and 42-d. Birds were deprived of feed for 4 h before weighing. Mortality was recorded daily. Feed conversion ratios were corrected for mortality.

At 28 and 42-d, water consumption was measured over 24 h by manual distribution in a plastic bell shape drinker with 1 L capacity (one drinker per pen). Four similar drinkers which were inaccessible to the birds were placed at various locations in the house for estimating evaporative water loss. Fecal samples were collected separately from each pen, immediately after excretion, at 28 and 42-d in order to measure excreta moisture. Litter parts attached to excreta were separated by forceps. Excreta were subsequently dried in an oven at 65°C for 72 h (Maiorka et al., 2004).

Two ml of blood was taken from the brachial vein of wing banded birds and centrifuged immediately at 2,500 rpm for 10 min (Mirsalimi et al., 1992), at 28 and 42-d. Plasma was separated and used for determination of plasma osmolarity and sodium (Na), potassium (K), chloride (Cl) and albumin concentration. Plasma Na and K concentrations were measured by flame photometer (Genway Clinical PFP7) and a colorimetric procedure was used to determine the amount of Cl and albumin in plasma.

At 42 days of age two wing-banded birds from each pen were slaughtered one by one and intestinal samples were taken immediately from these birds for evaluation of osmolarity along the intestine. Segments (5 cm) were obtained from the duodenum, jejunum at a position midway between Meckel's diverticulum and the entrance of the bile ducts and ileum at a position midway between Meckel's diverticulum and the ileocaecal junction. The contents of the lumen were flushed vigorously with saline to remove

Table 3. Effect of dietary betaine and TDS of water on packed cell volume (PCV), osmolarity of blood plasma and intestinal epithelia osmolarity of broiler chicks

Main and interaction effects	PCV	Plasma osmolarity	Intestinal epithelia osmolarity		
	42-d	42-d	Duodenum	Jejunum	Ileum
	%	mOsmol/L			
TDS (mg:L)					
375	29.5	331	705	687	540
1,375	30.4	329	727	711	584
2,375	32.3	331	849	799	656
SEM	0.96	2.14	46.19	46.64	50.13
Betaine (%)					
0.000	34.4^{a}	332	649	660	486
0.075	30.1^{b}	327	780	781	578
0.150	29.0^{b}	332	848	748	626
0.225	29.2^{b}	331	764	740	687
SEM	1.08	2.47	53.33	53.86	57.88
Betaine×TDS					
0.000×375	31.3	333	415^{z}	441	329
0.075×375	28.9	337	684^{xyz}	795	556
0.150×375	28.5	328	805^{xy}	691	588
0.225×375	29.7	328	915^{x}	820	689
0.000×1,375	33.9	333	583^{yz}	610	448
0.075×1,375	29.9	319	774^{xy}	758	628
0.150×1,375	28.6	333	904^{x}	793	626
0.225×1,375	29.2	332	648^{xyz}	685	634
0.000×2,375	37.1	329	949^{x}	929	683
0.075×2,375	31.6	325	883^{xy}	791	550
0.150×2,375	30.2	335	835^{xy}	761	651
0.225×2,375	28.8	333	730^{xy}	716	739
SEM	1.78	4.29	92.38	93.28	100.25
	Probabilities				
ANOVA					
Betaine	0.007	0.45	0.08	0.44	0.11
TDS	0.22	0.77	0.07	0.21	0.27
Betaine×TDS	0.81	0.12	0.01	0.07	0.56

[a,b] Means in a column with different superscripts differ significantly ($p<0.01$).

[x,y,z] Means in a column with different superscripts differ significantly ($p<0.05$).

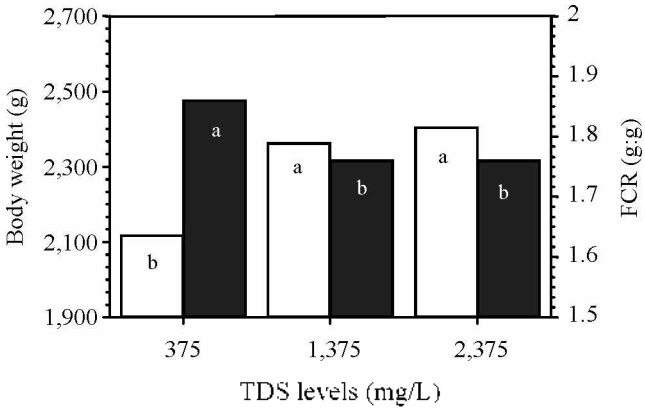

Figure 1. Effect of TDS levels on body weight (hatched bar) and FCR (solid bar) at 42-d in broiler chicks. Different letters above the columns denotes a significant difference between means ($p<0.01$).

the digesta. Washed segments were opened longitudinally and scraped with glass slides to obtain the mucosa. Collected mucosa was measured. Then 10 times the measured volume of scrapings were added as pure deionized water and the suspension was homogenized (Klasing et al., 2002). Osmolarity was determined by using a freezing point osmometer (Cryoscopic osmometer, osmomat 030, Genotec). Since the dilution was 10-fold the osmolarity measured was multiplied by a factor of 10. Care was taken to remove any external liquid such as water, blood, etc. on the surface where the sampling took place and the instruments were cleaned and dried regularly.

After termination of the finisher period at 42-d, carcass, breast, abdominal fat, sartorial, liver and heart were weighed in 96 sub-sample birds (2 birds/pen) with body weights near the mean of each pen. They were deprived of feed for 4 h, and individually weighed just prior to slaughter.

Statistical analysis

Data were analyzed by using the General Linear Models procedure of SAS software appropriate for a completely randomized design. Significant difference between individual group means was determined with Duncan's multiple range test option of the GLM procedure of SAS software. Relationships between independent variables (betaine and TDS) and dependent variables were studied by regression analysis and appropriate regression lines fitted to the data. Nonlinear asymptotic (Noll et al., 1984), sigmoidal (Robbins et al., 1979), cubic, quadratic and linear regression was performed to finally select the best-fit model according to predicted R^2. Detailed procedures for regression models were described by Potter et al. (1995). Correlation analyses between feed intake and body weight, feed intake and FCR, feed intake and water consumption, betaine and PCV, betaine and excreta moisture, TDS and body weight and TDS and water consumption were performed.

RESULTS

Effect of betaine

According to Figure 2, addition of betaine to the basal diet increased body weight at 21, 28 and 42-d ($p<0.05$), but there was no significant effect on feed intake. Figure 2 also shows that supplementation of the basal diet with betaine decreased FCR significantly ($p<0.05$). Betaine had no significant effect on carcass, sartorial, breast, abdominal fat, liver, heart and total mortality percentages. Observations indicated that between all blood osmotic pressure parameters which were measured in this study (Na, K, Cl and albumin), betaine supplementation only increased plasma Na concentration at 28-d ($p<0.05$). Table 3 implies that dietary added betaine resulted in decreased packed cell volume (PCV) at 42-d ($p<0.01$). PCV was significantly correlated to dietary levels of betaine ($R = -0.47$).

No significant effect of supplemented betaine was found on water consumption and excreta moisture. There was a negative correlation between excreta moisture and dietary added betaine at 42-d ($p<0.05$). Betaine had no significant effect on plasma osmolarity and epithelial osmolarity of the small intestine (Table 3).

Effect of TDS

The high TDS of saline water increased BW (Figure 1) and FI (at 11 to 21 and 29 to 42-d) and also improved FCR ($p<0.01$). This trend was observed for breast yield ($p<0.01$). In contrast to breast yield, abdominal fat and sartorial percentages were decreased ($p>0.05$). The correlation between BW and TDS level was $R = 0.7$ over all investigated ages ($p<0.01$). Water consumption (28 and 42-d) and excreta moisture (28-d) were increased from the first to third level of water salinity ($p<0.01$); Figure 3 shows these results at 42-d of age. Water consumption was correlated significantly ($R = 0.7$) with TDS levels. The data implied that higher levels of TDS increased PCV, osmolarity of the duodenum, jejunum, ileum (42-d) and plasma osmolarity at 28-d, but these results were not significant ($p>0.05$).

Interactions between dietary added betaine and drinking water TDS on BW (21-d), FCR (21-d), plasma osmolarity (28-d) and epithelial osmolarity of the duodenum were significant ($p<0.05$).

DISCUSSION

Effect of betaine

The response (Figure 2) of chicks fed on a methyl donor-adequate diet shows that betaine was effective in promoting body weight in male broiler chicks ($p<0.05$). This is in agreement with the reports of many studies on poultry (Virtanen and Rosi, 1995; Wang, 2000) and pigs

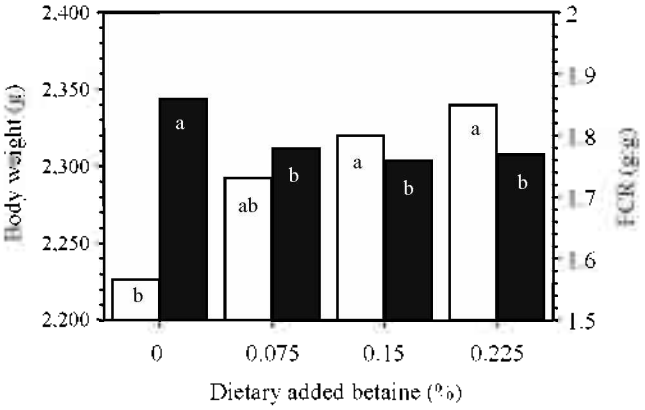

Figure 2. Effect of dietary added betaine on body weight (hatched bar) and FCR (solid bar) in broiler chicks.

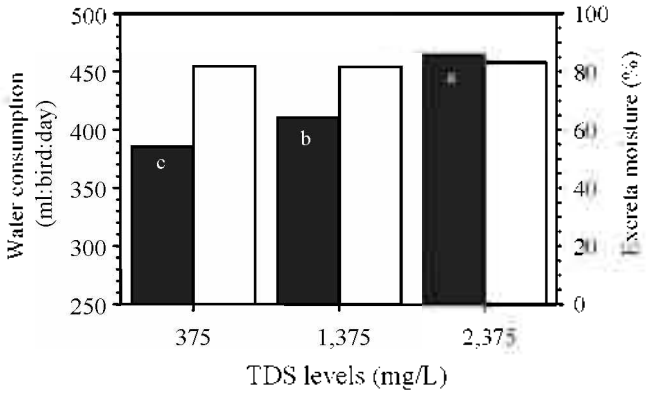

Figure 3. Effect of TDS levels on water consumption (solid bar) and excreta moisture (hatched bar) in broiler chicks. Different letters above columns denotes significant difference between means ($p<0.01$).

(Wang and Xu, 1999; Feng and Yu, 2001; Yu et al., 2004), though the results of several other studies reveal no effect of supplemental betaine on animal performance (Smith et al., 1994; Cera and Schinckel, 1995; Webel et al., 1995; LeMieux et al., 1996; Schutte et al., 1997; Urbanczyk, 1997; Matthews et al., 1998; Cromwell et al., 1999; Kitt et al., 1999; Urbanczyk et al., 1999; Urbanczyk et al., 2000; Van Lunen and Simmins, 2000; Matthews et al., 2001a; Matthews et al., 2001b; Fernandez-Figares et al., 2002; Feng et al., 2006). It seems that alterations in the water-retention capacity of the muscle tissue following dietary betaine supplementation may increase total body weight and carcass weight as well. Eklund and co-workers (2005) explained that enhanced water-retention capacity can be due to different mechanisms. Increased water retention may be attributed to the osmolytic capacity of the accumulated betaine. Furthermore, increased mineral absorption and retention following dietary supplementation of betaine may also contribute to an increased water retention capacity of the muscle tissue (Eklund et al., 2005). Additionally, labeled betaine was detected in the water phase obtained from breast muscle tissue, suggesting the presence of free betaine or derivatives in muscle cells. Consequently, betaine may not interfere with the intramuscular fat depots. The osmolytic property of betaine supports intestinal cell growth and survival and enhances cell activity, thereby potentially influencing nutrient digestibility (Eklund et al., 2005). Results of many studies on broilers identified that digestibility of methionine, protein, lysine, fat and carotene and intestinal lactic and volatile fatty acid production were enhanced by dietary betaine. Transepithelial electrophysiological studies suggest that betaine changes the transport of ions in the intestinal epithelium of pigs (Eklund et al., 2005). Since betaine uptake is in part characterized as an Na^+-dependent co-transport, supplemental dietary betaine might result in higher absorption rates of Na^+ and Na^+-dependent ions (Eklund et al., 2005). The degree of fermentation in the digestive tract of single-stomached animals is affected by dietary betaine supplementation (Eklund et al., 2005). Improved nutrient digestibility could also be the result of a betaine-induced increase in the contractile activity of the duodenal smooth muscle cells. This increase is associated with enhanced pancreatic secretion and digesta mixing (Eklund et al., 2005). Observations of weight gain in the current study corresponded with results of feed conversion ratio. FCR at 21, 28 and 42-d in chicks fed the highest levels of dietary betaine were 4.4, 6.3 and 4.8 percent lower than in the control group. According to Eklund et al. (2005), improvements in feed conversion ratio ranging between 2.8% and 7.9% in laying hens and pigs were reported when betaine was added to the diet. This may be explained by a more efficient utilization of dietary protein for lean accretion, which is supported by reduced blood urea-N levels, increased N retention and reduced requirement for metabolisable energy. Dietary betaine supplementation has been shown to reduce serum or plasma urea-N content in pigs by up to 47%. Blood urea-N levels are correlated with protein turnover rate and N retention is maximized when urea-N is minimized. The energy requirement for breakdown and re-synthesis of body protein, as well as for N excretion, contributes to a great extent to the animal's maintenance energy requirement. The maintenance energy requirement is reduced in pigs receiving betaine-supplemented diets. Provided that adequate dietary protein is available, a more efficient protein synthesis may be assumed.

Our observations showed that betaine increased percentages of liver, breast and abdominal fat numerically ($p>0.05$). Abdominal fat in chicks fed 0.075, 0.150 and 0.225 percent of exogenous betaine was 4.64, 6.62 and 9.27% higher than in the control group. These results are not in agreement with many studies (Urbanczyk, 1997; Wang and Xu, 1999; Wang et al., 2000a; Wang et al., 2000b; Matthews et al., 2001b; Yu et al., 2001; Fernandez-

Figares et al., 2002; Zou and Lu, 2002; Yu et al., 2004; Feng et al., 2006), but in contrast with the aforementioned lipotropic properties of betaine, increases in energy retention may enhance fat accretion. Loest et al. (1998) showed that dietary betaine addition may result in higher carcass fatness in steers.

Comparing breast percentage of male broiler chicks fed second, third and fourth levels of added dietary betaine with the control group showed that it was increased by 1.46, 1.75 and 3.33 percent ($p>0.05$). This is a similar result to that reported by Schutte et al. (1997), McDevitt et al. (2000) and Wang (2000). Excreta moisture of the groups which were supplemented with 0.075, 0.150 and 0.225 percent of betaine, was lower by 1.18, 0.85 and 0.89 percent compared with the control group at 28-d ($p>0.05$). Comparing the excreta moisture at 42-d for the same levels of betaine with the control group showed a 0.56, 2.07 and 1.85 percent decrease, respectively ($p>0.05$).

Interestingly, betaine decreased PCV (Table 3). To our knowledge, decreased PCV due to betaine has not been reported previously. It seems that the homeostatic regulation system of the body defends against changes in blood parameters and increased total blood volume. The present data imply that betaine helped minimize water loss despite a prevailing osmotic gradient (betaine×TDS: 0×2375, 0.075×2375, 0.150×2375 and 0.225×2375 in Table 3 for duodenum and jejunum). Eklund and co-workers (2005) noted that betaine exerts an osmoprotective effect by accumulation in cell organelles and cells exposed to osmotic and ionic stress, thereby replacing inorganic ions and protecting enzymes as well as cell membranes from inactivation by inorganic ions. Thus, water homeostasis is an important factor for cells exposed to different osmotic pressures. For example, intestinal cells always have to cope with variable osmotic media since the luminal content of the intestine is hyperosmotic in relation to blood plasma. Moreover, the process of nutrient digestion and absorption necessitates osmolytic protection mechanisms since intestinal cells mediate the exchange of water, small solutes such as ions, nutrients and macromolecules between plasma and intestinal fluid. Betaine is thought to be an important organic osmolyte for the control of the osmotic pressure inside the intestinal epithelial cells. Osmotic protection would allow for the maintenance of water balance and intestinal cell volume, thereby facilitating secretion of digestive enzymes (Eklund et al., 2005). According to Eklund et al. (2005) if betaine stimulates cell proliferation in the intestinal tissue, the enlarged gut wall epithelium would provide an increased surface for nutrient absorption. In the present study, osmolarity was measured by scraping the intestinal epithelium away from the underlying sub mucosa. Thus the values represent solute concentrations within the cells of the villi, as well as intestinal fluids and any luminal fluids trapped in the folds and not removed during washing. Overall means of duodenum, jejunum and ileum epithelial osmolarity (760, 732 and 593 mOsmol/L) showed that osmolarity decreased from duodenum to ileum. This result is in agreement with those reported by Klasing and co-workers (2002). Our observed value of ~760 mOsmol/L in the duodenum is very hyper-osmotic compared with normal plasma, which we found to be 330.5 mOsmol/L. The chicks consumed feed *ad libitum* and the intestines were full of digesta; thus, the high osmolarity could be the result of active absorption of nutrients (Klasing et al., 2002). The differences between the results obtained from the present study and those of Klasing and co-workers is due to different species, strain, individual variations, levels of added betaine and ages in which osmolarity were determined. Most of the betaine was absorbed in the duodenum and jejunum with little left for absorption in the ileum. Betaine is transported by the Na^+ dependent amino acid transport system A and by the Na^+ and Cl^- dependent betaine-γ-amino butyric acid (GABA) transporter (Klasing et al., 2002). Kettunen et al. (2001) reported the presence of a Na^+ dependent active transport system for betaine in the duodenum and jejunum of broiler chicks (Klasing et al., 2002). They also found that supplementation of betaine to the diet increased the Na^+ dependent component in betaine uptake as well as the total quantity taken up by the duodenum (Klasing et al., 2002). The duodenum also had the highest osmolarity because this tissue uses betaine for protection against a hyperosmotic environment (Klasing et al., 2002).

Effect of TDS

Results of this research showed that TDS caused an increment in feed intake. Feed intake trends from 11 to 21 and from 29 to 42 days of age are similar to those reported by Maiorka and co-workers (2004). Results obtained from the present study suggested that 0.16% Na in the diet did not meet the Na requirement of broiler chicks in the starter period. Na as well as Cl and K are essential elements in maintaining osmotic pressure and acid-base balance within normal values. The last edition of the National Research Council increased Na level from 0.15% (NRC, 1984) to 0.20% (NRC, 1994) for the first three weeks of age. NRC (1994) suggested 0.2 and 0.15 percent of Na for 0-3 and 3-6 weeks of age, whereas the recommended value by the Ross management manual (2002) is 0.16% for 0 to 42-d. Comparison of these two suggestions shows that the value recommended by the management manual for the first three weeks of age was 25% lower than actual requirement. Maiorka and co-workers (2004) showed that the Na level which promoted the best results during the first week of age was higher than that recommended by NRC (1984 and 1994), and it was similar to the level suggested by Britton at

1992. It seems that the results obtained could be related to the Na requirement. Feed intake was significantly correlated to water consumption at 42-d (R = 0.63). Furthermore, feed intake for 29 to 42-d was also correlated significantly (R = 0.89) to body weight of 42-d. This correlation was -0.64 for FCR. Using high levels of Na increased feed intake, which may be due to the stimulating role of this element on appetite and its role on absorption. Sklan and Noy (2000) demonstrated that Na has a very important role on feed intake just after hatching and also in secretion and activity of some digestive enzymes. Results of the chicks response during the current study indicated a quadratic relationship between breast percentage and TDS levels (mg/L) of drinking water:

$$B = 23.1072+(26\times10^{-4})TDS-(6.51\times10^{-7})TDS^2$$

$$R^2 = 0.71$$

According to this relationship, by increasing level of TDS up to 1997 mg/L breast percentage was increased. It seems that this finding was related to promotion of BW and partly due to increasing water holding capacity of breast muscle.

The adverse effect of TDS on mortality showed that young chicks are more susceptible to salt than older birds. A number of our mortalities were caused by ascites. For many years it has been suggested that ascites caused by salt overdose in poultry was the result of osmotic difference between the plasma and tissue (Mirsalimi et al., 1992). However, modern studies indicate that pH is the main cause of the right ventricular failure that is induced by salt, hypoxia, or which occurs spontaneously (Mirsalimi et al., 1992).

The effect of TDS was not significant on PCV, although comparing PCV in chicks which consumed the second and third levels of TDS with the control group showed a 3.05 and 9.49% increment, respectively.

The results showed an increasing effect of TDS on water consumption (at 28 and 42-d) and excreta moisture at 28-d ($p<0.05$) and also an increment in plasma osmolarity at 28-d ($p>0.05$). Decrease in blood volume is a powerful stimulus for rennin secretion (Hazon and Flik, 2002). Thus, plasma angiotensin II (ANG II) concentration increased after consuming high levels of salinity. These results indicate that an increase in plasma osmolarity, a decrease in blood volume, and ANG II are involved in the regulation of water consumption. The osmotic, volaemic and ANG II stimuli are also dipsogenic in truly terrestrial species such as birds (Hazon and Flik, 2002). In these truly terrestrial animals, cellular dehydration of osmosensitive neurons in the hypothalamus caused by osmotic stimuli is the strongest stimulus to elicit water drinking (Hazon and Flik, 2002). The potent osmotic effect is assessed by the fact that vigorous drinking occurs after the stimulus, even though the other two dipsogenic stimuli, the volaemic stimulus and ANG II, are suppressed because of a simultaneous increase in blood volume (Hazon and Flik, 2002). By contrast, hypo volaemia is only a week dipsogenic stimulus in birds even though plasma concentration of ANG II is by itself a strong stimulus for drinking (Hazon and Flik, 2002).

The results indicated that consumption of saline water caused a hyperosmolarity condition (betaine×TDS: 0×375, 0×1375 and 0×2375 in Table 3 for the three parts of the intestine). The increasing effect of TDS and the interaction effect of TDS and betaine on blood osmolarity at 28-d, but not at 42 days, of age also imply that young chicks are more susceptible to salt than older birds. Furthermore, because provided levels of TDS were not marginally limited in this study, blood osmolarity was in the ranges reported by Freeman (1983), Kettunen and co-workers (2001), and Klasing et al. (2002) for plasma osmolarity in normal chicks.

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