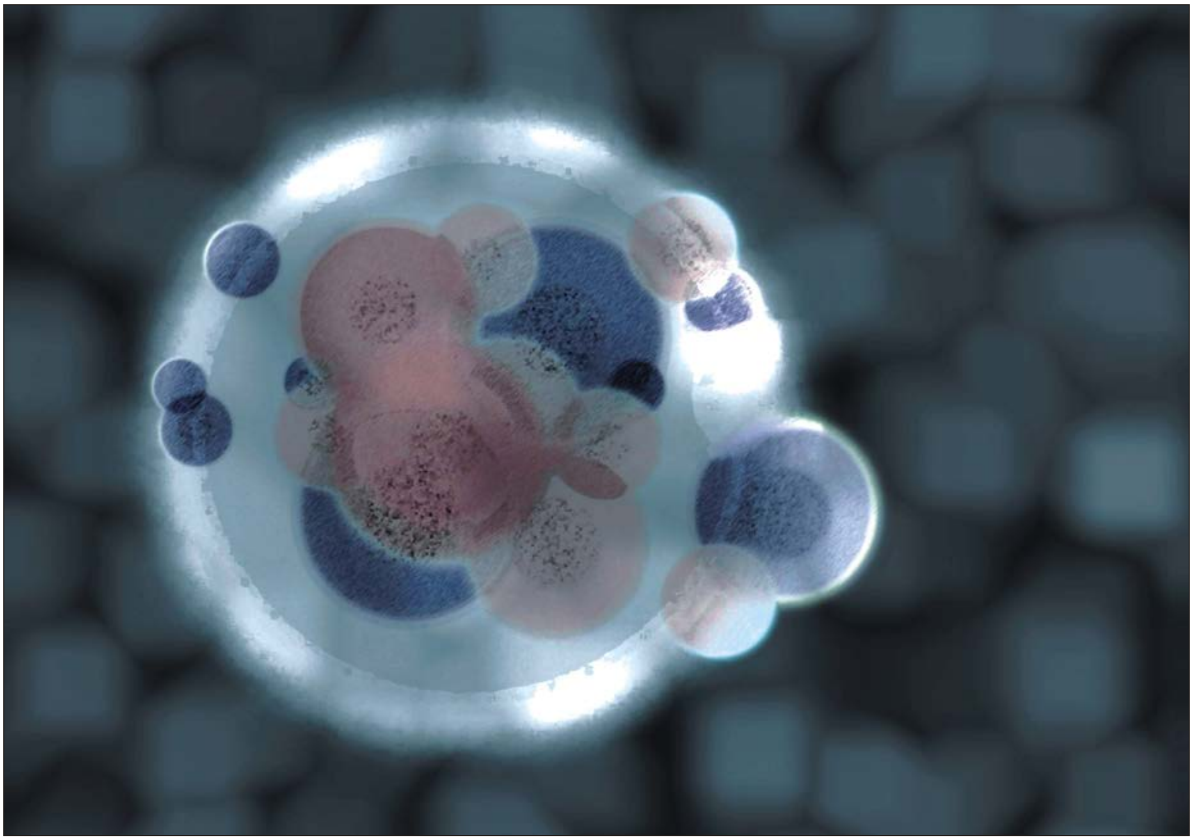

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Interactive Effects of Dietary Betaine and Saline Water on Carcass Traits of Broiler Chicks

Shirin Honarbakhsh, Mojtaba Zaghari and Mahmood Shivazad
Department of Animal Science, Faculty of Agriculture, University of Tehran, Iran

Abstract: The effect of saline water and dietary betaine on growth performance of 576 male broiler chicks from first to 42 days and on carcass composition (include: percentages of carcass, sartorial, breast, liver, abdominal fat and heart) of a sub sample of 96 birds was studied in a CRD experiment. Three levels of total dissolved solids (375, 1375 and 2375 mg L^{-1}) and 4 levels of dietary betaine supplementation (0.000, 0.075, 0.150 and 0.225%) were used in this trial. Feed was provided *ad libitum* and water was in free access. Data showed that betaine supplementation increased BW and improved FCR. Water salinity promoted BW at whole period, increased feed intake (11 to 21 and 29 to 42 days) and also improved FCR in grower and finisher periods ($p<0.01$). Breast weight was increased by consuming higher levels of total dissolved solids in water ($p<0.01$). The data imply that betaine supplementation maybe improved digestion and absorption conditions of the gastrointestinal tract and amended the usage of nutrients. These observations corresponded with results of FCR. It shows that betaine maybe involved in protection of intestinal epithelium against osmotic disturbance (saline water).

Key words: Betaine, saline stress, male, growth performance, carcass characteristics

INTRODUCTION

High levels of sodium chloride (NaCl) in drinking water results in increase blood pressure. In order to expulsion salt, water consumption increases, total amount of blood potassium (K) concentration decreases and an increment occurs in litter moisture (Kalimuthu *et al*., 1987; Balnave and Gordon, 1993). Consequently performance decreases, anion-cation becomes imbalance and some diseases such as ascites and coccidiosis happen (Kalimuthu *et al*., 1987; Julian, 1993). Change in anion-cation balance affects a lot of physiologic and metabolic functions of body, so it can reduces performance and also increases Feed Conversion Ratio (FCR) (Kalimuthu *et al*., 1987; Julian, 1993). Nowadays, poultry producers in regions with high levels of water TDS, especially NaCl in water, try to solve the problem by reducing diet NaCl content, purifying water or etc. Current study examined betaine supplementation for searching another way to get rid of 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 (Eklund *et al*., 2005). Chemically, betaine is trimethylglycine and it has been implicated in methionine sparing, osmotic stress protection and fat distribution (Saunderson and Mackinlay, 1990). The methionine-sparing and fat-distribution effects of betaine have been the subject of many studies about broilers (Virtanen *et al*., 1993; Saunderson and Mackinlay, 1990; Esteve-Garcia and Mack, 2000), pigs (Emmert *et al*., 1998; Campbell *et al*., 1995; Matthews *et al*., 2001b) and ducks (Wang *et al*., 2004). To date, concerning to our knowledge no information has been published about the osmotic stress protection effect of exogenous betaine on carcass traits of broiler chicks under water salinity stress, so in the present trial this role was studied.

MATERIALS AND METHODS

Animal and feed: Five hundred and seventy six male broiler chickens (Gallus domesticus) of type Ross 308 were used in the experiment. The birds were raised from day of hatch, up to slaughter at 42 days of age in the experimental chicken house of Animal Science Department Research Center, Faculty of Agriculture, at Tehran University in winter of 2006. 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. This experiment was designed in a 4×3 factorial arrangement of treatments with four levels of added dietary betaine (0.000, 0.075, 0.150 and 0.225%) and three levels of total dissolved solids (375, 1375 and 2375 mg L^{-1}). Total Dissolved Solids (TDS) levels were made by adding three levels of sodium chloride (0, 1000, 2000 mg L^{-1}) to basal drinking water. The levels of betaine which supplemented in feed were lower, higher and the

Corresponding Author: Shirin Honarbakhsh, Department of Animal Science, Faculty of Agriculture, University of Tehran, Karaj, Zip Code 31587-11167, Iran Tel: +98 2612248082 Fax: +98 2612246752

Table 1: Composition of basal diet

Ingredients	Starter (1-10 days)	Grower (11-28 days)	Finisher (29-42 days)
	----------------------	($g\ kg^{-1}$ 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	18.0
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 ($kcal\ kg^{-1}$)	2829.47	2889.60	2926.50
CP (%)	20.88	19.32	18.17
Calcium (%)	1.00	0.90	0.85
Phosphorus (available) (%)	0.50	0.45	0.42
Sodium (%)	0.16	0.16	0.16
Anion-cation ($mEq\ kg^{-1}$)	200.00	200.00	200.00
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, 9000 IU; Cholecalciferol, 2000 IU; Vitamin E, 18 IU; Vitamin k3, 2 mg; Vitamin B12, 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 $5H_2O$), 10 mg; Iodine (as calciumiodate), 1 mg; Iron (as ferrous sulfate $7H_2O$), 50 mg; Manganese (as manganese oxide), 100 mg; Selenium (as sodium selenite), 0.2 mg; Zinc (as zinc oxide), 100 mg

same dosages as recommended by the manufacturer (Finfeeds, P.O. Box 777, Marlborough, Wiltshire, SN8 1XN, UK). Basal diet was based on corn soybean meal. Basal diet (Table 1) provided nutrient requirements for broiler chicks of Ross 308 (2002) and the methionine plus cystine levels were exactly met the requirement of this strain. Sufficient amounts of methyl-donating compounds were added (Choline chloride: 60% choline chloride) which eliminates the methionine-sparing effect of betaine. In order to avoid the effect of betaine as coccidiostat enhancer, clean conditions were used to reduce the coccidiosis challenge. Diet contained no antibiotics, coccidiostats or feed enzymes. Chicks were raised without betaine and without added NaCl in water during the first 10 days post hatching. In the morning of 11 days post hatching, after 4 h deprived of feed, chicks were weighed and assigned to pens in a manner that ensured minimal variation in initial body weight among pens. Betaine (Betafin S_1, 96% Betaine anhydrous, feed grade) and sodium chloride were added to basal diet and drinking water, respectively, from 11 days of age when the main experiment started. The ingredients used were of a single batch in order to minimize differences in composition among mixing batches. The experimental diets were prepared by splitting up this batch in sub charges, to which the required amount of betaine were added and mixed. Feed was provided *ad libitum* and water was in free access. The amount of sodium in drinking water of the control group was 0.0035%. It was measured by atomic absorption. 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 up to 22±1°C was reached by the fifth week. The relative humidity in the broiler unit was approximately between 60 to 70% 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 days. Birds were deprived of feed for 4 h, before weighed. Mortality was recorded daily. Feed conversion ratios were corrected for mortality. After termination of finisher period at 42 days, carcass, breast, abdominal fat, sartorial, liver and heart were weighed on 96 sub sample birds (2 birds/pen) with body weight near the mean of each pen. They were deprived of feed for 4 h and individually weighed just prior to slaughter.

Statistics: Data were analyzed by using the General Linear Models procedure of SAS (2001) software appropriate for completely randomized design. Significant difference among individual group means was determined with Duncan's multiple range test option of the GLM procedure of SAS software. The data on a pen basis were subjected to nonlinear asymptotic (Noll *et al*., 1984) and sigmoidal (Robbins *et al*., 1979) regression. The regression models were described by Potter *et al*. (1995).

RESULTS AND DISCUSSION

Effect of betaine: According to data presented in Table 2, effect of added betaine to basal diet was not significant on feed intake. Supplementation of basal diet with betaine decreased FCR significantly ($p<0.05$). Quadratic relationship was observed between percentage of dietary added betaine and FCR on 42 days:

$$FCR_{42} = 1.86 - 1.21\ Bet + 3.79\ Bet^2,\ R^2 = 0.8$$

Data from responses of chicks fed methyl donor-adequate diet (Table 2), 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 pig (Feng and Yu, 2001; Wang and Xu, 1999), though the results of several other studies reveal no effect of supplemental betaine on animal performance (Cera and Schinckel, 1995; Cromwell *et al*., 1999; Fernandez-Figares *et al*., 2002; Kitt *et al*., 1999; LeMieux *et al*., 1996; Matthews *et al*., 2001a, b, 1998; Schutte *et al*., 1997;

Table 2: Body weight, feed intake and FCR of broiler chicks fed with different levels of dietary betaine and TDS in water†,‡,§

Main and interaction effects	Body weight 21 days (g)	Body weight 28 days (g)	Body weight 42 days (g)	Feed intake 11-21 days (g: bird: day)	Feed intake 22-28 days (g: bird: day)	Feed intake 29-42 days (g: bird: day)	FCR 21 days (g : g)	FCR 28 days (g : g)	FCR 42 days (g : g)
TDS(mg L^{-1})									
375	548.05^{b}	892.58^{b}	2117.27^{b}	62.89^{b}	105.01	162.06^{b}	1.68^{a}	1.86^{a}	1.86^{a}
1375	687.00^{a}	1140.57^{a}	2363.19^{a}	70.66^{a}	110.21	170.20^{a}	1.46^{b}	1.56^{b}	1.76^{b}
2375	684.79^{a}	1135.91^{a}	2404.46^{a}	70.42^{a}	113.97	173.50^{a}	1.46^{b}	1.58^{b}	1.76^{b}
SEM	5.21	11.15	24.37	0.74	2.71	1.69	0.01	0.03	0.01
Betaine (%)									
0.000	623.79^{b}	1023.96^{y}	2226.96^{y}	68.56	112.65	166.84	1.59^{a}	1.75^{x}	1.86^{a}
0.075	645.19^{a}	1060.31^{xy}	2292.57^{xy}	67.38	109.77	167.26	1.51^{b}	1.65^{y}	1.78^{b}
0.150	642.52^{a}	1066.00^{x}	2320.28^{x}	67.40	107.81	169.26	1.51^{b}	1.62^{y}	1.76^{b}
0.225	648.30^{a}	1074.92^{x}	2340.09^{x}	68.62	108.70	170.99	1.52^{b}	1.64^{y}	1.77^{b}
SEM	6.01	12.87	28.14	0.85	3.13	1.95	0.02	0.03	0.02
Betaine×TDS									
0.000×375	508.85^{z}	814.06	1941.38	62.17	103.72	153.91	1.79^{a}	2.01	1.97
0.075×375	546.35^{y}	888.02	2128.12	61.27	102.68	162.91	1.65^{b}	1.82	1.83
0.150×375	570.31^{y}	938.54	2221.45	63.87	101.34	166.92	1.63^{b}	1.75	1.79
0.225×375	566.67^{y}	929.69	2178.13	64.25	112.31	164.51	1.65^{b}	1.86	1.85
0.000×1375	677.60^{x}	1124.48	2337.48	71.12	113.24	171.08	1.49^{c}	1.60	1.80
0.075×1375	703.65^{x}	1162.50	2372.25	71.88	112.90	169.55	1.44^{c}	1.55	1.77
0.150×1375	682.29^{x}	1125.00	2321.88	69.13	105.21	164.96	1.45^{c}	1.53	1.74
0.225×1375	684.47^{x}	1150.28	2420.83	70.50	109.48	175.22	1.46^{c}	1.54	1.75
0.000×2375	684.90^{x}	1133.33	2401.70	72.40	120.98	175.52	1.49^{c}	1.65	1.80
0.075×2375	685.56^{x}	1130.40	2377.32	68.99	113.72	169.34	1.44^{c}	1.58	1.75
0.150×2375	674.95^{x}	1135.13	2417.52	69.19	116.88	175.91	1.46^{c}	1.59	1.76
0.225×2375	693.75^{x}	1144.79	2421.31	71.12	104.31	173.25	1.45^{c}	1.52	1.72
SEM	10.42	22.30	48.74	1.47	5.42	3.37	0.03	0.06	0.03

†: Standard Error of Mean (SEM) = $\sqrt{\text{MSE (mean square)}} \div \sqrt{\text{N (number of replicate)}}$ ‡ ^{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 ($p<0.05$)

Smith *et al*., 1994; Urbanczyk *et al*., 2000, 1999, 1997; Van Lunen and Simmins, 2000; Webel *et al*., 1995). 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 *et al*., 2005). Eklund *et al*. (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, carotenoid, intestinal lactic acid 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 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 current study about weight gain corresponded with results of feed conversion ratio. FCR at 21, 28 and 42 days in chicks fed highest levels of dietary betaine was 4.4, 6.3 and 4.8% lower than control group (Table 2). 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 correlated with the protein turnover rate. N retention is maximized when urea-N is minimized. The energy

Table 3: Effect of dietary betaine and TDS of water on carcass yield characteristics of broiler chicks †, ‡

Main and interaction effects	Heart	Liver	Abdominal fat	Breast	Sartorial	Carcass	Total mortality (%)
	-------------	-------------	-------------	(%BW)	-------------	-------------	
TDS (mg L⁻¹)							
375	0.44	1.98	1.63	23.99[b]	22.33	73.30	1.04
1375	0.45	2.01	1.57	25.45[a]	21.88	72.89	2.08
2375	0.43	1.95	1.56	25.60[a]	21.51	74.10	2.08
SEM	0.01	0.05	0.08	0.29	0.39	0.39	0.92
Betaine (%)							
0.000	0.45	1.90	1.51	24.61	22.03	73.28	2.08
0.075	0.43	1.98	1.58	24.97	21.43	73.71	1.39
0.150	0.44	1.99	1.61	25.04	22.20	72.85	1.39
0.225	0.44	2.05	1.65	25.43	21.97	73.89	2.08
SEM	0.01	0.05	0.09	0.34	0.45	0.46	1.06
Betaine×TDS							
0.000×375	0.45	1.83	1.85	22.76	22.20	72.58	2.08
0.075×375	0.44	1.93	1.57	23.90	21.41	73.90	0.00
0.150×375	0.43	2.07	1.69	24.14	22.66	72.85	2.08
0.225×375	0.42	2.10	1.47	25.16	23.03	73.87	0.00
0.000×1375	0.47	1.95	1.51	25.31	21.83	72.96	2.08
0.075×1375	0.45	2.07	1.61	25.19	21.53	73.29	2.08
0.150×1375	0.45	2.00	1.39	25.55	21.92	71.83	0.00
0.225×1375	0.45	2.00	1.76	25.74	22.24	73.47	4.17
0.000×2375	0.43	1.92	1.25	25.76	22.04	74.29	2.08
0.075×2375	0.40	1.95	1.56	25.84	21.34	73.93	2.08
0.150×2375	0.45	1.88	1.75	25.43	22.01	73.87	2.08
0.225×2375	0.46	2.06	1.73	25.39	20.62	74.33	2.08
SEM	0.02	0.09	0.15	0.59	0.77	0.79	1.84

†a, b: Means in a column with different superscripts differ significantly ($p<0.01$). ‡: Body weight minus blood, feather, head and feet

requirement for breakdown and re-synthesis of body protein as well as for N excretion contributes to a great extent to the animal's energy requirement for maintenance. The maintenance requirement for energy is reduced in pigs receiving betaine-supplemented diets. Provided that adequate dietary protein is available, a more efficient protein synthesis may be assumed. Present observations (Table 3) 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% of exogenous betaine was 4.64, 6.62 and 9.27% higher than control group. Obtained result about this trait isn't in agreement with many studies (Fernandez-Figares *et al.*, 2002; Matthews *et al.*, 2001b; Urbanczyk, 1997; Wang *et al.*, 2000a, b, 1999; Yu *et al.*, 2001; Zou and Lu, 2002), 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 control group showed that it were increased 1.46, 1.75 and 3.33% ($p>0.05$). It is the same as the reports of Schutte *et al.* (1997), McDevitt *et al.* (2000) and Wang (2000).

Effect of TDS: As shown in Table 2, high TDS of saline water increased BW, FI (at 11 to 21 and 29 to 42 days) and 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 ($p>0.05$). Linear relationship was observed between FCR and TDS content of drinking water:

$$FCR_{42} = 1.74 + (46.68/TDS),\ R^2 = 0.7$$

Figure 1 shows the quadratic relationship which obtained between breast percentage and TDS levels (mg L^{-1}) of drinking water. The correlation between BW and TDS levels was obtained 0.7 at whole investigated ages ($p<0.01$).

Interaction between dietary added betaine and drinking water TDS on BW (21 days) and FCR (21 days) were significant ($p<0.05$). According to Table 2, feed intake trend from 11 to 21 and 29 to 42 days of age, are similar to those reported by Maiorka *et al.* (2004). Results obtained from present study suggested that 0.16% Na in diet did not met the Na requirement of broiler chicks in starter period. Na as well as Cl and K, are essential elements in order to maintain the 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% of Na for 0-3 and 3-6 weeks of age, whereas the recommended value by Ross management manual (2002) is 0.16% for 0 to 42 days. By comparing these two suggestions, it is clear that value recommended by management manual, for

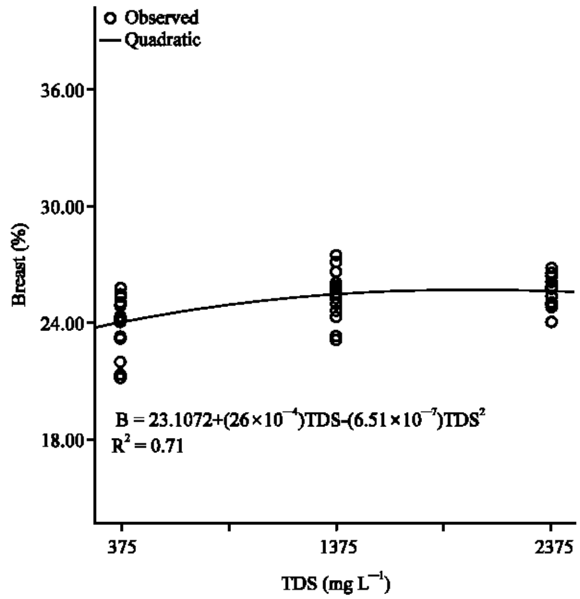

Fig. 1: Relationship between TDS (mg L^{-1}) and breast percent

the first three weeks of age, was 25% lower than actual requirement. Maiorka *et al.* (2004) showed that Na levels which promoted the best results during the first week of age were higher than those recommended by NRC (1984, 1994) and it is similar to the level suggested by Britton (1992). It seems that the obtained results could be related to the Na requirement. Feed intake of 29 to 42 days also correlated significantly with body weight of 42 days (R = 0.89). This correlation was evaluated about -0.64 for FCR. Usage high levels of Na affected feed intake and increased it because the stimulating role of this element on appetite is determined and it also affects 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. The adverse effect of TDS on mortality showed that young chicks are more susceptible to salt than older birds. A number of our mortalities 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. 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).

CONCLUSION

The data imply that betaine supplementation may be improved digestion and absorption conditions of the gastrointestinal tract and amended the usage of nutrients in broiler chicks under water salinity stress.

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