Archiv Tierzucht 57 (2014) 4, 1-12

Original study

Effect of betaine as an osmolyte on broiler chickens exposed to different levels of water salinity

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Abstract

This trial was conducted to examine the effect of betaine on performance, humoral immunity, small intestinal morphology and blood osmotic pressure parameters of broiler chicks consumed different levels of water salinity. Total of 520 day-old broiler chicks (Ross 308) were used with 2×4 factorial arrangement in completely randomized design. Experimental treatments were consisting of 2 levels of betaine supplementation (0 and 1.5 g/kg) and 4 levels of total dissolved solids (TDS) (250, 1 500, 3 000, 4 500 ppm). In this trial daily feed intake (DFI), daily weight gain (DWG) and feed conversion ratio (FCR) were recorded on days 14, 28 and 48. Small intestinal morphology was evaluated at the age of 28. Humoral immunity was assessed by evaluation of antibody titre against sheep red blood cells (SRBC) and also Newcastle and influenza antigens. At the end of the experiment, blood was taken and concentration of plasma Na, K, Cl and haematocrit blood percentage was determined. Results showed that DWG significantly decreased and FCR increased in 4 500 ppm of water TDS in growing, finishing and whole period of broilers production ($P<0.05$). Furthermore increased plasma Na and blood hematocrit percentage observed in 4 500 ppm of water salinity ($P<0.05$). Betaine significantly increased DFI during growing phase ($P<0.05$). Betaine supplementation substantially increased antibody titre against influenza and also bursa of fabricius relative weight ($P<0.05$). Additionally, betaine inclusion increased villi height in ileum ($P<0.05$). In conclusion, increasing trend of water TDS affect chickens performance and betaine supplementation is able to improve antibody titre against influenza.

Keywords: broiler chickens, betaine, total dissolved solids, performance, immune system

Archiv Tierzucht 57 (2014) 4, 1-12
doi: 10.7482/0003-9438-57-004

Received: 11 July 2013
Accepted: 17 January 2014
Online: 11 March 2014

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Abbreviations: DFI: daily feed intake, DWG: daily weight gain, FCR: feed conversion ratio, SRBC: sheep red blood cells, TDS: total dissolved solids

Introduction

Some areas of poultry production in the world are involved with relatively high levels of drinking water total dissolved solids (TDS). Animals tolerance to various levels of dissolved salts such as Cl and Na are differing depend on their species, age, water requirement, physiological condition, season of the year and salt content of total diet (Kellems & Church 1998). The NRC (1974) reported that, the water TDS of less than 1 000 ppm should present no serious burden to any class of poultry. On the other hand, consumption of water with high dissolved salts may cause performance decrease due to the change in the osmotic regulation and its negative impact on optimal regulation of intracellular macromolecules (Kettunen *et al*. 2001). Base on the conducted studies, once the TDS in drinking water of birds rises above 1 500 ppm, osmotic stress ensues causing a transient polyuria, and when above 3 000 ppm osmoregulatory homeostasis is compromised (Bagley *et al*. 1997, Goldstein & Skadhauge 2000). Poultries given drinking water with TDS in excess of 3 000 ppm evacuate watery faeces which may contribute to wet litter problems, growth rate depression and eventually increased flock mortality (Barton 1996). Similarly, consumption of high sodium chloride in drinking water reported to increase the blood pressure, water consumption and litter moisture of broiler chickens (Balnave & Gorman 1993).

In order to solve this problem, nutritional solutions like betaine can be considered. Betaine is the trimethyl derivative of the amino acid glycine and is a compound which naturally occur in animal and plant tissues (Kidd *et al*. 1997, Lipiński *et al*. 2012). Sugar beets and its by-products such as molasses are among the usual sources of betaine. This compound is capable of donating the methyl group for transmethylation reaction (Kidd *et al*. 1997) and also can play the role of an osmolyte via apply an osmoprotective effect by accumulating in cell organelles and cells exposed to osmotic and ionic stress (Petronini *et al*. 1992).

In a research, Honarbakhsh *et al*. (2007) reported the increased water consumption and litter moisture of chickens with increasing of water salinity. They also found the decreasing of packed cell volume by betaine supplementation. Levels of Na and Cl in drinking water also considerably affected live performance of broilers in the study of Watkins *et al*. (2005).

Although some trials have been carried out in this area but, it seems researches considering the effect of betaine on broiler chickens exposed to water TDS till 4 500 ppm is limited. Therefore, current study was conducted to evaluate the effect of dietary betaine, water salinity and their interaction on performance, immunity, intestinal morphology and biochemical blood components of broiler chickens.

Material and methods

Diets, birds and experimental design

Total of 520 day-old broiler chickens (Ross 308) were weighed individually and assigned to 8 treatments and 5 replicates of 13 birds with 2×4 factorial arrangement in completely randomized design. From day one, chickens were fed with basal diet supplemented with

2 levels of dietary betaine (0.00 and 1.5 g/kg), four levels of TDS in drinking water (250, 1 500, 3 000 and 4 500 ppm) and interaction between them. Water and feed were ad libitum. Chickens were grown on wood shavings as bedding material. Different levels of water TDS were provided via adding sodium chloride to the water and 96 % betaine anhydrous (Betafin S1) was applied as betaine form in this experiment. The composition of the basal diet and the calculated contents of nutrients are presented in Table 1.

Table 1
Ingredients and composition of the diet

	Starter (0-14)	Grower (14-28)	Finisher (28-42)
Diet composition			
Corn (7.1 % crude protein)	54.98	54.88	54.61
Soybean meal (42 % crude protein)	40.07	39.11	38.20
Soybean oil	0.68	2.52	3.88
Dicalcium phosphate	1.85	1.51	1.43
Calcium carbonate	1.10	0.98	0.94
DL-Methionine	0.29	0.20	0.14
L-lysine	0.18	0.00	0.00
Vitamin premix[a]	0.25	0.25	0.25
Mineral premix[b]	0.25	0.25	0.25
Salt	0.35	0.30	0.30
Calculated composition			
Metabolizable energy, kcal/kg	2 800	2 950	3 050
Crude protein, %	20.73	20.32	20.01
Lysine, %	1.32	1.16	1.14
Met + Cys, %	0.99	0.89	0.82
Calcium, %	0.97	0.84	0.80
Available phosphorous, %	0.48	0.42	0.40

[a]Vitamin premix per kg of diet: vitamin A (retinol), 2.7 mg; vitamin D3 (Cholecalciferol), 0.05 mg; vitamin E (tocopheryl acetate), 18 mg; vitamin K3, 2 mg; thiamine 1.8 mg; riboflavin, 6.6 mg; panthothenic acid, 10 mg; pyridoxine, 3 mg; cyanocobalamin, 0.015 mg; niacin, 30 mg; biotin, 0.1 mg; folic acid, 1 mg; choline chloride, 250 mg; antioxidant 100 mg, [b]Mineral premix per kg of diet: Fe ($FeSO_{4\cdot7}.H_2O$, 20.09 % Fe), 50 mg; Mn ($MnSO_4.H_2O$, 32.49 % Mn), 100 mg; Zn (ZnO, 80.35 % Zn), 100 mg; Cu ($CuSO_{4\cdot5}H_2O$), 10 mg; I (KI, 58 % I), 1 mg; Se ($NaSeO_3$, 45.56 % Se), 0.2 mg

Performance measurements, carcass traits and litter moisture

Daily weight gain (DWG) and daily feed intake (DFI) of each pen were recorded at 14, 28 and 48 days of age. Feed conversion ratio (FCR) (feed intake/weight gain) was also calculated. Carcass, abdominal fat, heart, bursa of fabricius, spleen, intestine, and liver were weighed and calculated as a percentage of live body weight. At the end of the experiment, litter samples were collected separately from each pen and subsequently dried in an oven at 65 °C for 72 h (Maiorka *et al*. 2004).

Immune system measurements

At 9 days of age, Newcastle and influenza antigens were injected to chickens with dual vaccine of Newcastle-influenza. Two male chickens per pen were selected randomly for intraperitonal injection with a 1.0 ml of sheep red blood cells (SRBC) suspension diluted with phosphate buffer saline (pbs) on day 25. Five days later, the same wing-banded birds were bled to determine antibody titre against SRBC and also against influenza and Newcastle. Subsequently antibody titre against SRBC was measured by haemagglutination assay method and also antibody titre against influenza and Newcastle separately was measured by haemagglutination inhibition method. Haemagglutination inhibition antibodies were then converted to log2. Antibody titres against SRBC were measured by the microtitre procedure described by Wegmann & Smithies (1966). At the end of the experiment, birds were slaughtered after taking blood samples then spleen and bursa of fabricius weighed to determine the immune system development.

Small intestinal morphology

At day 28 of age, two male birds of each pen were slaughtered and intestinal samples were taken immediately from the duodenum; intestine from the gizzard to pancreatic and bile ducts, jejunum; midway between the point of entry of the bile ducts and Meckel's diverticulum, ileum; 10 cm proximal to the ileo-cecal junction were taken to evaluate the villus height, crypt depth and villus height: crypt depth ratio. Segments which were 1.5 cm in length were flushed with saline and fixed in 100gl-1 buffered formalin (pH=7.0). The fixed intestinal samples embedded in paraffin then sectioned (5 µm) and stained with hematoxylin-eosin and examined by light microscope. Villus height (µm) was measured from the tip of the villus to the villus crypt junction and crypt depth was measured from the base upward to the region of transition between the crypt and villus.

Biochemical blood components

At the end of the experiment, 2 ml of blood was taken from the branchial vein of 2 male birds and centrifuged immediately at 2 500 rpm for 10 min (Mirsalimi *et al*. 1993). Plasma was separated and used for determination of plasma Na, K and Cl concentration. Plasma Na and K concentration were measured by flame photometer (Jenway PFP 7, Bibby Scientific Ltd, Staffordshire, UK) and plasma Cl concentration was measured by spectrophotometer (UV 2100, Shimadzu, Kyoto, Japan). Haematocrit also was determined by using a micro haematocrit centrifuge.

Statistical analysis

Data were subjected to ANOVA using the general linear model procedure of SAS software 2008 (SAS Institute Inc., Cary, NC, USA) with the main effects of betaine supplementation, water TDS and the interaction between them. Means were compared using Tukey's test and were considered to be significant different at $P<0.05$.

Results

Performance measurements, carcass traits and litter moisture

Results presented in Table 2 show the significant decrease of DWG and increase of FCR using 4 500 ppm of water TDS compared to other levels in growing, finishing and also whole period ($P<0.05$). Heart weight was markedly increased using water TDS of exceed 250 ppm while other carcass traits included of carcass, abdominal fat and liver weights were not affected by water TDS augmentation (Table 6). Litter moisture trend insignificantly elevated with increasing of water TDS and the greatest litter moisture was observed in 4 500 ppm level of water TDS. Although Supplementation of betaine considerably increased DFI and FCR in growing period ($P<0.05$), no such increase was observed during whole period of production. Interaction of water TDS and betaine addition had no impact on performance and litter moisture of chickens.

Table 2
Effect of water TDS and betaine on different phase's performance and litter moisture of broiler chickens

Performance and litter moisture	Total dissolved solids (TDS)					Betaine (BT)			Source of variation (Significance)		
	250	1 500	3 000	4 500	SEM	0	1.5	SEM	TDS	BT	BT×TDS
DWG, g/d											
0-14	21.84	21.38	20.95	20.92	0.31	21.12	21.43	0.22	ns	ns	ns
14-28	54.98^{a}	51.08^{ab}	54.24^{a}	47.25^{b}	1.95	52.63	51.48	1.31	*	ns	ns
28-48	60.80^{a}	62.79^{a}	58.30^{a}	46.03^{b}	1.95	56.40	56.68	2.05	**	ns	ns
0-48	59.00^{a}	61.02^{a}	58.29^{a}	46.02^{b}	2.11	55.87	55.88	1.97	**	ns	ns
DFI, g/d											
0-14	35.71	34.88	34.32	34.93	0.55	34.91	35.01	0.40	ns	ns	ns
14-28	107.76	101.46	101.68	101.92	2.80	99.53^{b}	106.87^{a}	1.37	ns	**	ns
28-48	136.30	137.46	135.45	125.98	3.43	135.44	132.16	2.67	ns	ns	ns
0-48	112.65	111.13	111.43	105.16	2.21	109.38	110.75	1.68	ns	ns	ns
FCR											
0-14	1.63	1.63	1.63	1.66	0.002	1.65	1.63	0.02	ns	ns	ns
14-28	1.95^{b}	1.98^{b}	1.87^{b}	2.15^{a}	0.02	1.89^{b}	2.07^{a}	0.04	*	*	ns
28-48	2.24^{b}	2.18^{b}	2.32^{b}	2.73^{a}	0.07	2.40	2.33	0.08	**	ns	ns
0-48	1.90^{b}	1.82^{b}	1.91^{b}	2.28^{a}	0.09	1.95	1.98	0.07	*	ns	ns
Litter moisture Day 48, %	27.20	27.30	27.30	29.20	0.10	27.70	25.70	0.09	*	ns	ns

Values in the same row not sharing a common superscript differ significantly ($P<0.05$). ns: not significant; *$P<0.05$, **$P<0.01$

Table 6
Carcass traits on day 48

Parameters	Total dissolved solids (TDS)					Betaine (BT)			Source of variation (Significance)		
	250	1 500	3 000	4 500	SEM	0	1.5	SEM	TDS	BT	BT×TDS
Carcass	71.96	72.29	72.20	71.5	0.48	65.16	65.77	0.34	NS	NS	NS
Abdominal fat	0.54	0.78	0.58	0.52	0.1	0.65	0.57	0.07	NS	NS	NS
Heart	0.57^{b}	0.69^{a}	0.67^{a}	0.69^{a}	0.3	0.69	0.64	0.02	**	NS	NS
Liver	2.92	2.96	3.01	3.14	0.1	3.10	2.91	0.07	NS	NS	NS

Values in the same row not sharing a common superscript differ significantly ($P<0.05$). ns: not significant, $^{**}P<0.01$

Immune System measurements

The effect of water TDS, betaine and their interaction on the immune system summarized in Table 3. Results show that antibody titre against SRBC and influenza was not influenced by increasing trend of water TDS and the greatest antibody titre against Newcastle observed in 1 500 ppm ($P<0.05$). Spleen weight significantly decreased with increasing of water TDS ($P<0.05$). Betaine supplementation substantially increased antibody titre against influenza and also bursa of fabricius weight ($P<0.05$). Interaction of water TDS and betaine failed to show any significant effect on humoral immunity and lymphoid organs.

Table 3
Effect of water TDS and betaine on humoral immunity and lymphoid organs of broiler chickens

Variable	Total dissolved solids (TDS)					Betaine (BT)			Source of variation (Significance)		
	250	1 500	3 000	4 500	SEM	0	1.5	SEM	TDS	BT	BT×TDS
Influenza (log2)	3.35	4.53	4.46	3.50	0.60	3.36^{b}	4.59^{a}	0.38	ns	*	ns
New castle (log2)	7.35^{b}	8.38^{a}	7.61^{ab}	7.51^{ab}	0.55	7.68	7.74	0.23	*	ns	ns
SRBC (log2)	5.71	4.26	5.60	4.43	0.80	5.03	4.93	0.34	ns	ns	ns
Lymphoid organs, % bodyweight											
Bursa of fabricius	0.12	0.11	0.10	0.10	0.01	0.09^{a}	0.12^{b}	0.007	ns	**	ns
Spleen	0.20^{a}	0.14^{b}	0.12^{b}	0.14^{b}	0.01	0.16	0.15	0.009	***	ns	ns

Values in the same row not sharing a common superscript differ significantly ($P<0.05$). ns: not significant; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$

Small intestinal morphology

Results presented in Table 4 show that increasing of water TDS had no significant effect on villi height and also villi height to crypt depth ratio. On the other hand betaine inclusion increased villi height in ileum significantly ($P<0.05$). No interaction of water TDS and betaine was revealed.

Table 4
Villi height, crypt depth and villi height to crypt depth ratio (v/c) of broiler chickens fed different levels of water TDS and betaine

Parameters, µm	Total dissolved solids (TDS)					Betaine (BT)			Source of variation (Significance)		
	250	1 500	3 000	4 500	SEM	0	1.5	SEM	TDS	BT	BT×TDS
Duodenum											
Villi height	746	770	721	595	89.21	700	665	36.62	ns	ns	ns
Crypt depth	193	145	262	125	68.94	186	179	3.77	ns	ns	ns
V/C	4	5.3	3.6	4.9	0.91	4.22	4.57	0.44	ns	ns	ns
Jejunum											
Villi height	601	692	606	671	46.16	686	600	44.43	ns	ns	ns
Crypt depth	130	111	146	126	19.6	129	128	1.17	ns	ns	ns
V/C	4.6	4.8	4.7	5.2	0.49	9.4	5	2.43	ns	ns	ns
Ileum											
Villi height	487	522	404	438	59.40	403^{b}	518^{a}	56.5	ns	*	ns
Crypt depth	112	125	131	113	9.46	108	132	12.96	ns	ns	ns
V/C	4.8	4.3	3.2	3.4	0.83	3.6	4.3	0.45	ns	ns	ns

Values in the same row not sharing a common superscript differ significantly ($P<0.05$). ns: not significant, *$P<0.05$

Biochemical blood components

Table 5 indicates the increased blood haematocrit percentage in 3 000 and 4 500 ppm of water TDS compared to 250 ppm ($P<0.05$). Similar trend was also observed for plasma Na only at the level of 4 500 ppm ($P<0.05$). On the other hand plasma K was significantly reduced by water consumption of more than 250 ppm TDS ($P<0.05$). In comparison to 250 ppm, the minimum concentration of plasma Cl was observed in 3 000 ppm water salinity ($P<0.05$). Interaction of dietary betaine and drinking water TDS showed the decreasing trend in Na, K and Cl concentration in different levels of water TDS ($P<0.05$). Interaction of water TDS and betaine supplementation significantly influenced plasma Na, K and Cl ($P<0.05$).

Table 5
Blood osmotic pressure parameters and haematocrit (HCT) percent of broiler chickens fed with different levels of water TDS and dietary betaine

Parameters	Total dissolved solids (TDS)					Betaine (BT)			Source of variation (Significance)		
	250	1 500	3 000	4 500	SEM	0	1.5	SEM	TDS	BT	BT×TDS
Na, mEq L^{-1}	142.44^{b}	144.03^{b}	147.03^{b}	152.25^{a}	2.29	150.23	147.63	1.42	**	ns	*
K, mEq L^{-1}	7.38^{a}	6.68^{b}	6.54^{b}	6.36^{b}	0.26	6.78	6.69	0.17	*	ns	*
Cl, mEq L^{-1}	104.80^{a}	100.22^{ab}	96.59^{b}	97.24^{ab}	3.90	100.92	98.76	1.98	*	ns	*
HCT, %	33.46^{b}	35.12^{ab}	37.06^{a}	37.12^{a}	1.05	36.09	35.35	0.79	*	ns	ns

Values in the same row not sharing a common superscript differ significantly ($P<0.05$). ns: not significant, *$P<0.05$, **$P<0.01$

Discussion

The deteriorated performance of chickens due to using water salinity of 4 500 ppm in this study might be related to the anion-cation imbalance and disturbed amino acids absorption and its negative impact on growth of broiler chickens. Since water prepare the vehicle for solute movement across epithelial membranes, it is crucial to the process of both nutrient absorption and waste excretion. Further, water flux across the epithelium is strongly influenced by solute content (Collet 2012). These findings of the current study are in consistent with those of Pourreza *et al*. (2000) and Ahmed (2013) who reported the decreased body weight of broilers by increasing of water TDS exceed 3 000 ppm. Inversely, Honarbakhsh *et al*. (2007) indicated chickens given drinking water with 375, 1 375 and 2 375 ppm water salinity suggested improved body weight, DFI and FCR. They concluded that Na deficiency in the diet may counteracted by Na levels in drinking water. The significant interaction of dietary and water levels of Na and Cl have been previously shown by Watkins *et al*. (2005). Accordingly, it is likely that if the diet Na meet the chickens demand, water salinity exceed the 4 000 ppm will exert the compromising impact on the performance of the birds (NRC 1974).

Anion-cation imbalance has direct effects on controller centre of the food intake in hypothalamus, Change the ionic composition of the hypothalamus tissue and subsequently affect feed intake (Barton 1996). Therefore, impact of betaine on DFI in this research might be attributed to osmoprotectant and methyl group donor properties of betaine. These results are matched with the reports of Wang (2000) who found the increased DFI of meat ducks by betain supplementation. It seems that DFI increase versus constant DWG of chickens fed with betaine supplemented diet is the reason of FCR increase during growing period. Unlike this study, Honarbakhsh *et al*. (2007) found the elevated body weight after betaine inclusion whereas several other researchers revealed no effect of supplemental betaine on animal performance (Matthews *et al*. 2001, Fernández-Fígares *et al*. 2002, Feng *et al*. 2006).

Increase in cardio-pulmonary blood flow accompany with increase in resistance to blood flow forces the right ventricle of heart to increase the pulmonary arterial pressure in order to propel the cardiac output through the lungs as usually measures by the right to total ventricle ratio (Julian *et al*. 1989, Wideman 2007). Furthermore, Increases in blood mean cell volume and blood viscosity due to the excess sodium utilization are the predisposing factors to increase the cardiac output (Xiang *et al*. 2004). Although the right to total ventricle ratio was not measured in the current experiment, the higher relative heart weight might be the indicator of cardiac output elevation. In a research Xiang *et al*. (2004) reported the increased right to total ventricle ratio after the administration of excess sodium from sodium chloride in drinking water. The symptoms of right ventricle failure resulting from sodium overdose also have been previously reported by Mirsalimi *et al*. (1992).

The litter moisture insignificantly elevated with increasing of water TDS. When sodium consumption exceeds the normal level, renin secretion decrease and leads to angiotensin II formation. This hormone is natural stimulant of water consumption which causes reduction in water reabsorption, elevation of water and Na excretion to litter and consequently increase the litter moisture (Kalimuthu *et al*. 1987). Honarbakhsh *et al*. (2007) observed the increased litter moisture using increasing levels of water TDS. They also exhibited that betaine addition could reverse the compromising effect of using high water TDS on litter moisture.

Additionally, Watkins *et al*. (2005) reported that faecal moisture content is directly correlated with the level of salt in the diet. The greater excreta moisture or litter wetness scores with elevated dietary Na also has been found by several researchers (Smith *et al*. 2000, Ahmad *et al*. 2009, Jankowski *et al*. 2011).

The immune response against Newcastle disease in this experiment improved for 1 500 ppm water TDS which seemed to positively affect the immune response to Newcastle disease in broilers. Mechanism of immune responses activation is established through foreign antigen attack to host tissues by viral or bacterial infection. The immune response rate depend on numbers of exposure to antigen but intensity of the immune responses is related to immune adequacy which individually associated with factors such as immune system evolution, infection severity, attenuator factors of the immune system and Nutritional status (Svensson *et al*. 2001). In the current trial, the bursa of fabricius relative weight decreased. Bursa of fabricius and spleen have direct relations with immune system (Esmailzadeh *et al*. 2013) and are primary and secondary lymphatic organs, respectively which support cellular and humoral immunity. Decreasing relative weight of these organs lead to decrease immune response and consequently birds would not be able to resist against disease. Therefore it seems that water TDS beyond 1 500 ppm is weakening factor of immunity because of its effect on the relative weight of spleen. The higher levels of TDS in drinking water might partially suppress the immune response via probable increase in glucocorticoid level (Mashaly *et al*. 1993). Pourreza *et al*. (2000) expressed the decreased antibody titre against Newcastle with water consumption of more than 3 000 ppm TDS.On the other hand, Ahmed (2013) assumed that drinking water with TDS level of 2 610 ppm seemed to positively influence the immune response to ND in broilers.

Observations indicated that betaine increased antibody titre against influenza and also increased bursa of fabricius weight. These results are matched with the findings of Hamidi *et al*. (2010) and Klasing *et al*. (2002) who suggested that betaine promoted immune system of broiler chickens. It is postulated that reduction in the concentration of dietary methionine affect immunity in chickens therefore, sparing dietary methionine with betaine may influence the immune responses (Rao *et al*. 2011).

Betaine as an important organic osmolyte is capable of controlling the osmotic pressure inside the intestinal epithelial cells (Hochachka & Somero 1984). Dietary betaine regulates the concentration of betaine in the intestinal epithelium (Klasing *et al*. 2002). Whenever intestinal cells expose to osmotic and ionic stress, betaine apply its osmoprotective effect by accumulating in cell organelles and in cells exposed to stress via substituting inorganic ions, and protecting enzymes as well as cell membranes from inactivation (Petronini *et al*. 1992). Furthermore osmolytes such as betaine increase the cytoplasmic volume and free water content of the cells at high osmolarity, and thus permit cell proliferation under stress conditions (Csonka 1989). In this trial, betaine significantly increased villi height in ileum which may be attributed to the mentioned characteristics of betaine in the intestine which are in agreement with findings of Kettunen *et al*. (2001).

Increase of Water TDS increased plasma Na concentration and haematocrit percentage whereas decreased plasma Cl and K concentrations were seen in 3 000 ppm water TDS and salinity levels of more than 250 ppm respectively. Among essential elements; Na, Cl and K

are able to maintain the osmotic pressure and acid-base balance (Honarbakhsh *et al*. 2007). Aldosterone's activity is increased through stress occurrence like water salinity which increases the Na reabsorption and K excretion from kidneys. Increasing of Na consumption, increases bicarbonate concentration (Roberts & Balnave 1992) and consequently reduces plasma Cl due to the inverse relationship between plasma bicarbonate and Cl concentration. On the other hand, Na elevation in plasma leads to increased osmotic pressure and enhances water absorption which increases blood volume (Julian 1993). Additionally, stress may stimulate epinephrine secretion, leads to spleen constriction and entrance of red blood cells within blood which consequently increases haematocrit percentage. Haematocrit elevation leads to increased blood viscosity and decreased blood flow. Blood viscosity might be the reason of diseases such as ascites. Unlike the present study Ahmed (2013) have not found any significant difference among increasing levels of water TDS.

According to our observations, betaine decreased plasma Na concentration and haematocrit percentage which may be related to osmoprotective effect of betaine as accumulates inside the cell organelles and cells exposed to osmotic and ionic stress then protect enzymes as well as cell membrane from inactivation by organic ions (Eklund *et al*. 2005). These results are in agreement with findings of Honarbakhsh *et al*. (2007), who suggested that betaine decreased haematocrit percentage in broiler chickens under water salinity stress.

In conclusion, drinking water consumption with TDS of more than 3 000 ppm negatively affects the performance of broiler chickens. Therefore, drinking water should be analysed for TDS before bird's usage. According to beneficial effect of dietary betaine on immune system and small intestinal villi observed in this research, it seems that dietary betaine can work as an osmolyte in commercial farms which faced with water salinity problem.

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