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Research Article

Effect of Betaine Supplementation on Performance Parameters, Betaine-homocysteine S-methyltransferase Gene Expression in Broiler Chickens Consume Drinking Water with Different Total Dissolved Solids

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Abstract

The objective of the current work was to evaluate the effect of drinking water with various levels of total dissolved solids (TDS) and betaine supplementation on the performance and gene expression of betaine-homocysteine S-methyltransferase (BHMT) in broiler chickens. In a completely randomized design with a 3×2 factorial arrangement, chicks were assigned to six treatments with four replicates and 15 chicks per each. The treatments were included of three levels of total dissolved solids (400, 2.000 and 3.500 ppm) and two levels of betaine supplementation (0 and 0.2% of diet). Weight gain decreased and feed conversion ratio and water intake increased as TDS of water increased. Betaine supplementation had no effect on weight gain and water intake during the grower period, but had a significant effect on gain and feed conversion ratio in the finisher period. Mortality rate and excreta moisture content increased as TDS of water increased. Excreta moisture content decreased with betaine supplementation. Gene expression of BHMT decreased significantly with increases in TDS level and betaine supplementation increased its expression (28 folds) as compared with the non-additive group. It was concluded that consumption of drinking water with higher than 2.000 ppm TDS adversely and betaine supplementation positively affect the performance of broiler chickens. The lowest feed conversion ratio was seen in chicks fed 400 and 2.000 ppm TDS with betaine supplementation.

Keywords: *Betaine, Blood parameters, Broiler chick, Gene expression, Performance, Total dissolved solids*

Betain İlavesinin Değişik Miktarlarda Toplam Çözünmüş Katı Madde Tüketen Broiler Tavuklarda Performans Parametrelerine ve Betain-hemosistein S-metiltransferaz Gen Ekspresyonuna Etkisi

Özet

Bu çalışmanın amacı, içme suyu içerisinde değişik oranlarda toplam çözünmüş katı madde (TDS) ve betain ilavesinin broiler tavuklarda performans ve Betain-hemosistein S-metiltransferaz (BHMT) gen ekspresyonu üzerine etkisini değerlendirmektir. Tamamen rastgele olarak 3x2 faktöriyel dizayn kullanılarak, civcivler her birinde 15 hayvan ve 4 tekrar olmak üzere altı uygulama grubuna ayrıldı. Deneysel uygulamalar üç farklı dozda toplam çözünmüş katı madde (400, 2.000 ve 3.500 ppm) ve iki farklı dozda betain (diyetin %0 ve %2'si) takviyesinden oluştu. Suyun TDS'sinin artışıyla orantılı olarak kilo artışı azalırken yem dönüşüm oranı ve su tüketimi artış gösterdi. Betain ilavesi büyüme döneminde kilo artışı ve su tüketiminde etki etmezken bitirme döneminde kazanım ve yem konversiyon oranında anlamlı derece etki gösterdi. Suyun TDS'i arttıkça mortalite ve dışkı nem oranında artış tespit edildi. Dışkı nem oranı betain takviyesi ile azalma gösterdi. BHMT gen ekspresyonu TDS seviyesindeki artışla ilişkili olarak anlamlı oranda azalırken betain ilavesi betain uygulanmayan grupla karşılaştırıldığında 28 kat artış gösterdi. Sonuç olarak içme suyu içerisinde 2.000 ppm'den daha yüksek oranda TDS'in tüketilmesi broiler civcivlerde performansı olumsuz yönde etkilerken betain takviyesi pozitif yönde etki gösterdi. En düşük yem konversiyon oranı betain takviyesi ile birlikte 400 ve 2.000 ppm TDS'li civcivlerde gözlemlendi.

Anahtar sözcükler: *Betain, Kan parametreleri, Broiler civciv, Gen ekspresyonu, Performans, Toplam çözünmüş katı madde*

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INTRODUCTION

Water, as an important nutrient, is supplied for broiler chickens in sufficient access and amount, but the quality of water and its importance on the health and production has been considered less or neglected. Total dissolved solids (TDS), especially calcium, magnesium, and sodium salts, in the drinking water considered as the important factors that affect water quality. The presence of these salts in drinking water could change the osmotic status of cells and body [1] and have negative impacts on optimal regulation of intracellular macromolecules [2]. Hence, high levels of salts in water cause negative effects on water intake [3] and on feed intake [4,5] and consequently health [6,7] and growth performance [8]. In an interesting study, feed efficiency and body weight gain were affected negatively at TDS level of 3448 ppm and the highest weight gain was observed at the level of 2610 ppm [9].

To solve this problem, several management and nutritional procedures were suggested. Of which, reducing dietary salt content, purifying the drinking water and etc., that are less effective or cost consuming. Nowadays, alternative procedures that increase the animals' tolerance to high levels of TDS recommended. Tolerance of livestock animals to high levels of TDS differs and depends on species, classes of animals in each species, physiological status, dietary salt content, feed additives and season [4,8]. Therefore, feed additives like betaine that reduce the cells osmolality and rearing of livestock strains with tolerance to high levels of TDS are two executable alternative procedures to overcome the problem.

Betaine is a tri-methyl derivative of glycine and a naturally occurring compound in animal body and plant tissues [10]. Petronini et al.[11] speculated that betaine can accumulate in the cells exposed to osmotic and ionic stress and increase the water-binding capacity of cells and as an osmolyte can assist the water homeostasis in the cells, especially in the intestinal epithelium cells [12]. Supplementation with natural betaine reduced the impact of coccidia challenge on intestinal lesion scores and positively affected nutrient digestibility and feed efficiency in broilers [13]. Betaine content of body tissues in rats depends on the level of BHMT gene expression [14]. Increase in osmolality due to high concentration of salts in drinking water caused a decrease in the level of BHMT gene expression and consequently increased the level of tissue betaine level and tolerance to this condition in rat [15].

The effect of betaine on health and performance of broiler chicks exposed to salinity water (mainly sodium salt) was evaluated [16], but the effect of drinking water with high TDS (from calcium, magnesium and sodium salts) on performance and gene expression of BHMT in broilers was not defined. In addition, in the literature the effect of strain in this subject remained unclear. Therefore, the main objective of the current work was to evaluate the effect of drinking water with various levels of TDS and betaine supplementation on the performance and gene expression of BHMT in broiler chickens.

MATERIAL and METHODS

Chickens and Experimental Design

Chicks used in this study received human care and the experimental protocol was approved by the Research Committee of Islamic Azad University, Science and Research Branch (approval date: 20.06.2014; no: 27999).

A total of 360 one-day-old Ross 308 male broiler chicks (43±1.2 g) was obtained from a local hatchery and allocated to 24 wire-floored pens covered with weed shaving. In a completely randomized design with a 3×2 factorial arrangement, chicks were assigned to 6 experimental treatments with four replicates and 15 chicks per each. The treatments were included of three levels of total dissolved solids (400, 2.000 and 3.500 ppm) and two levels of betaine supplementation (0 and 0.2% of diet). Water samples was taken from natural wells in the Qazvin Province of Iran and analyzed for different quality parameters. Then, water with different total dissolved solids was provided *(Table 1)* and kept in the large plastic tanks and given to chicks based on the treatments using automatic Plasson drinker. In Qazvin province, the lowest level of TDS in drinking water of natural source is 400 ppm and the highest level is 3.500 ppm. As TDS level of 3448 reduced and around 2.000 increased the performance, these ranges of TDS were selected. Drinking of water during the first week was the same for all chicks and thereafter drinking water with different TDS was initiated. The diets of chicks were formulated based on the strain guides for nutrition. All chickens were fed the same starter, grower and finisher diet based on Ross recommendation [17]. Feed and water

Table 1. *Characteristics and elements in waters supplied for broilers*

Treatments	Parameters						
TDS mg/L	EC (µ mhos/cm)	pH	Na mEq L^{-1}	Ca mEq L^{-1}	CL mEq L^{-1}	Mg mEq L^{-1}	HCO_3 mEq L^{-1}
400	626	7.31	63.2	47.5	198	34.6	152
2000	3250	7.35	350	128	475	48.06	121
3500	5360	8.17	560	171	590	53.01	143

were provided for *ad libitum* intake. Chicks were kept in the clean and sanitary house building and under temperature and humidity controls. The house temperature was raised to 32°C in the first week and declined gradually to 21°C at the end of the experiment (days 42 of age).

Sample Collection and Measurement

In the initial day 28 and 42 of age, body weight (BW) and also total feed intake (FI) were recorded, and then feed conversion ratio (FCR) was calculated by dividing corrected weight gain on feed intake. Correction for hen day was done by daily collection of dead chicks in each pen. Carcass weights of dead chicks were included in the calculations of feed conversion ratio.

At day 28 and 42 of age, excreta moisture contents were determined in each pen by collecting total excreta samples immediately after excretion. The samples were mixed together in each pen and one homogeneous sub-sample was weighed, oven dried at 105°C for 24 h, and reweighed to determine moisture content.

At days 28 and 48 of age, the blood samples of eight chicks per treatment (two chicks per pen) from wing vein were obtained in heparin-gel containing vacuum tubes. Plasma was separated after centrifugation at 2.500 × g for 15 min and stored at -20°C for further analysis. Calcium, potassium and sodium concentration in the plasma of chicks were measured by atomic absorption spectrophotometry [18].

At day 42 of age, eight chicks per treatment (two chicks per pen) were sacrificed by cervical dislocation, then liver was removed. Liver sample was taken, then frozen quickly in liquid nitrogen and stored at -80°C until analysis for gene expression of BHMT [19].

BHMT Gene Expression

The mRNA relative abundance of BHMT was determined by q-PCR [19]. The frozen liver was crushed in a sterile mortar, and the powder was applied for total RNA extraction using a suitable kit (Fermentas Gene JET RNA purification). cDNA for BHMT gene was synthesized based on reverse transcription technique using kit (Revert Aid TM first stand cDNA). Generation analysis and Melting Curve was performed by using Real time PCR Step One™ Real-Time PCR Systems (Applied Bio systems, Foster City, CA). Quantitative PCR was performed with a specific primer pairs (forward: 5′-GCCTGAAACAGGGCAAAAGG-3′; reverse: 5′-TCCCTGTGAAGCTGACGAAC-3′) using Quanti Fast SYBER Green PCR kit (QIAGEN). B-actin (forward: 5'-CCACCGCAAATGCTTCTAAAC-3', reverse: 5'-AAGACTGCT GCTGACACCTTC-3') was chosen as a reference gene. Amplification of liver BHMT gene was performed for 35 cycles, which consisted of an initial activations step (95°C, 5 min), denaturation cycle (95°C, 30s) and combined annealing (58°C, 30s) and extension (72°C, 45s). The relative expression ratio of BHMT as a target gene was normalized to B-actin gene using method as previously described by Livak and Schmittgen [20]. Quantification for each treatment group was performed in triplicates.

Statistical Analysis

The statistical analysis was performed with ANOVA of SAS for Windows version 9.1 (SAS Institute Inc., Cary, NC) [21]. At first, the normality of data distribution was checked using the Kolmogorov–Smirnov test. ANOVA GLM (general linear model) procedure and Tukey test was used to compare the means. Statistical significance was accepted when $P<0.05$.

RESULTS

The effect of treatments on feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR) and water intake at period 7 to 28 and period of 29 to 42 of age are presented in *Table 2*. There were no main effects of TDS and betaine on feed intake at two periods and main effect of strain was significant. There were differences among main and interactions effect of TDS on body weight gain, feed conversion ratio and water intake. Weight gain decreased and feed conversion ratio and water intake increased ($P<0.05$) as TDS of water increased. Betaine supplementation had no effect ($P>0.05$) on weight gain and water intake during grower period ($P<0.05$), but had significant effect on gain and feed conversion ratio in finisher period ($P<0.05$). The lowest feed conversion ratio was seen in chicks fed 400 and 2.000 ppm TDS with and without betaine supplementation.

The main effects of TDS and betaine supplementation on mortality rate and excreta moisture content measured at days 28 and 42 of age are shown in *Table 3*. Mortality rate and excreta moisture content increased ($P<0.05$) as TDS of water increased. Main effect of betaine supplementation was not differ ($P>0.05$) for mortality rate. Excreta moisture content significantly decreased with betaine supplementation. The interactions were not significant and data not shown.

Table 4 shows the main effects of TDS and betaine supplementation on blood sodium, potassium and calcium concentration. There were no main effects ($P>0.05$) on these measured parameters in the blood, except for main effect of TDS on sodium concentration. There were no differences for interactions and data not shown

The main effects of TDS *(Fig. 1A)* and betaine supplementation *(Fig. 1B)* on the relative abundance of mRNA for BHMT gene were significant. Gene expression of BHMT decreased significantly with increases in TDS level and betaine supplementation increased its expression (28 folds) as compared with non-additive group.

Table 2. *Effects of treatments on performance parameters of broiler chickens at total period*

Treatments	Feed Intake (g)		Body Weight (g)		Feed Conversion Ratio		Water Intake (cm³)	
	days 8-28	days 29-42	days 8-28	days 29-42	days 8-28	days 29-42	days 8-28	days 29-42
TDS main effect								
400	1680	2240	1162^{a}	2210^{a}	1.55^{b}	1.80^{b}	150^{b}	279^{b}
2000	1722	2268	1169^{a}	2295^{a}	1.52^{b}	1.75^{b}	151^{b}	282^{b}
3500	1701	2212	1118^{b}	1910^{b}	1.67^{a}	2.05^{a}	160^{a}	298^{a}
SEM	35.2	48.3	61	74	0.35	0.42	4.28	6.41
P-value	0.885	0.648	0.030	0.015	0.027	0.038	0.019	0.034
Betaine main effect								
0	1711	2254	1122	2170^{b}	1.59	1.88^{a}	154	289
0.2	1693	2240	1180	2295^{a}	1.53	1.73^{b}	153	284
SEM	32.31	47.39	70.1	81.3	0.32	0.39	5.66	8.62
P-value	0.145	0.291	0.075	0.036	0.312	0.024	0.215	0.449
TDS × Betaine								
0×400	1764^{b}	2268^{b}	1218^{b}	2544^{a}	1.58^{c}	2.04^{b}	160^{b}	292^{a}
0.2×400	1701^{b}	2184^{b}	1290^{a}	2655^{a}	1.51^{c}	1.85^{c}	161^{b}	290^{a}
0×2000	1827^{a}	2324^{a}	1287^{a}	2768^{a}	1.51^{c}	1.78^{c}	168^{a}	291^{a}
0.2×2000	1869^{a}	2338^{a}	1304^{a}	2686^{a}	1.50^{c}	1.74^{c}	153^{b}	278^{b}
0×3500	1785^{b}	2366^{a}	1187^{b}	2426^{b}	1.75^{a}	2.20^{a}	165^{b}	306^{a}
0.2×3500	1701^{b}	2184^{b}	1312^{a}	2558^{a}	1.75^{a}	1.98^{b}	172^{a}	311^{a}
SEM	47.1	56.8	40.6	64.2	0.14	0.18	3.2	5.8

Within the same column, means with different superscripts are significantly differ (P<0.05)

Table 3. *The effect of treatments on mortality and excreta moisture content*

Treatments	Mortality (%)		Excreta Moisture Content (%)	
	d 28	d 42	d 28	d 42
TDS main effects				
400	5.15^{c}	2.18^{b}	80.3^{b}	81.8^{b}
2000	7.11^{b}	3.04^{a}	82.7^{ab}	82.3^{b}
3500	8.49^{a}	3.23^{a}	84.4^{a}	85.9^{a}
SEM	0.69	0.31	0.69	2.33
P-value	0.036	0.014	0.036	0.036
Betaine main effects				
0	6.23	2.24	84.5^{a}	84.2^{a}
0.2	5.71	2.11	81.9^{b}	82.2^{b}
SEM	0.31	0.28	2.88	2.41
P-value	0.936	0.864	0.031	0.039

Within the same column, means with different superscripts are significantly differ (P<0.05)

DISCUSSION

This study was carried out to evaluate the impact of drinking water with different TDS levels and betaine supplementation on performance, excreta moisture content, and gene expression of BHMT that affect health and welfare of broiler chicks.

In the present study, the level of TDS had no effect on feed intake, although TDS level of 4.300 ppm trends to decrease feed intake. Drinking water with higher levels of TDS (6.000 ppm) caused the negative effect on feed intake significantly [4,5]. Decrease in feed intake may be related to higher water intake as shown in *Table 2*. The high level of TDS in water caused an increase in water output from

Table 4. *The effect of treatments on plasma level of sodium, potassium and calcium*

Treatments	Sodium mEq		Potassium mEq		Calcium mEq	
	d 28	d 42	d 28	d 42	d 28	d 42
TDS main effects						
400	149[b]	150	5.36	5.33	10.20	10.31
2000	155[ab]	155	5.81	5.47	10.45	10.69
3500	161[a]	156	6.40	5.68	9.86	10.01
SEM	2.71	2.89	0.13	0.2	0.25	0.31
P-value	0.025	0.13	0.186	0.150	0. 367	019
Betaine main effects						
0	156[a]	157	5.79	5.15	10.22	10.52
0.2	132[b]	151	7.04	6.38	10.08	10.17
SEM	3.16	2.75	0.24	0.19	0.31	0.33
P-value	0.011	0.12	0.064	0.066	0.493	0.16

Within the same column, means with different superscripts are significantly differ (P<0.05)

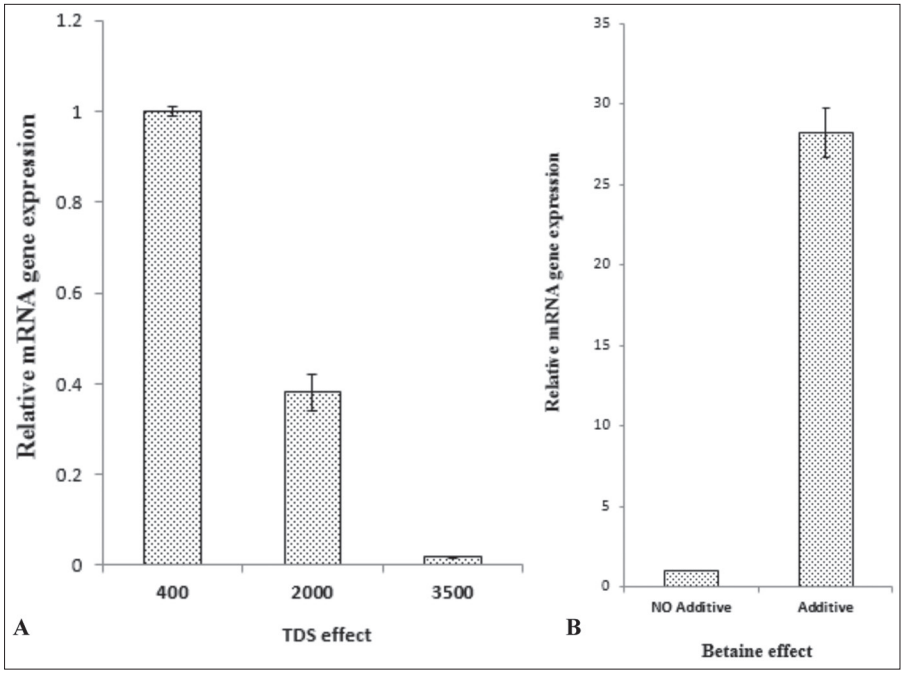

Fig 1. The effect of treatments on gene expression of BHMT

kidneys for excretion of anions and cations, hence chicks drink more water and again more salts, and consequently chicks increases its water intake. Increases in water intake impact negatively on feed intake.

Increase in TDS from 400 to 2.000 ppm increased body weight gain of broilers insignificantly. In consistent with our finding, a study [3] reported that increasing the water TDS level up to 2.375 ppm improved body weight and feed conversion ratio In this study, the decrease in body weight gain with increasing the level of TDS in drinking water exceed 2.000 ppm might be related to the increase water intake, the anion-cation imbalance [6], also lower nutrient digestion and absorption [22]. The finding of this study are in agreement with study of Pourreza et al.[23] and Ahmed [5] who speculated a decrease in body weight of broiler chicks as water TDS increased over 3.000 ppm.

In this study, betaine supplementation had no effect on feed intake of broilers and water consumption, in contrast, Wang et al.[24] found an increase in feed intake of meat ducks by betaine supplementation. Betaine supplementation had no effect on weight gain and feed conversion ratio at the grower period, but increased weight gain and decreased feed conversion ratio at the finisher period. In agreement to our finding, Honarbakhsh et al.[3] found improvements in growth performance of broilers with increases in dietary betaine levels. In contrast to our results, authors [25,26] reported no effect of dietary betaine supplementation on growth performance. The improvement in growth performance at the finisher period might be due to the osmolytic property of betaine [27] and its impact on energy expenditure for pumping ions in cells especially those exposed to hyperosmotic media [28]. Also, increase in the water retention capacity of tissues may be increased weight gain in broilers received dietary betaine as explained by Eklund et al.[29]. Moreover the osmolytic property of betaine impact positively on the growth, survival and contractile activity of intestinal cell,

thereby increase the pancreatic secretion and digesta mixing and consequently enhance nutrient digestibility [30].

In the present study, the negative effect of total dissolved solids on mortality percentage showed that young chicks (days 8-28 of age) are more susceptible to total dissolved solids in drinking water than older birds (days 29-42 of age). The most mortality in broilers of our study were caused by ascites. It has been revealed that ascites induced by salt stress in broilers was the result of osmotic difference between the plasma and tissue [31]. Betaine had no effect on mortality.

The results of this study showed an increase in water consumption and consequently excreta and excreta moisture percentage with increases in total dissolved solids. In line with our finding, Watkins et al.[7] and Honarbakhsh et al.[3] reported an increase in the excreta moisture as levels of water TDS increased. The greater litter moisture or litter wetness scores with elevated dietary Na also has been found by several researchers [5,32,33]. When salt consumption, especially sodium salts, exceeds the normal level, the secretion of renin decrease and formation of angiotensin II increase. Angiotensin II is a stimulant of water consumption which results in reduction in kidney water reabsorption [1]. Consequently it results in elevation of water excretion to excreta and increase the excreta moisture content. Betaine supplementation decreased the moisture content of excreta. In agreement, Honarbakhsh et al.[3] found that betaine supplementation could reverse the compromising effect of using high water TDS on excreta moisture.

There was no significant difference among different total dissolved solids levels, betaine supplementation and strains for the plasma levels of potassium and calcium, but differences exist for sodium level. In agreement to our finding, Balnave et al.[22] and Pourreza et al.[23] found a significant increase in blood levels of sodium by increasing TDS levels in drinking water. In another study, Talha et al.[34] showed an increase in sodium and calcium levels as TDS levels increased.

The gene expression of BHMT reduced as the level of TDS in drinking water increased. The reduction in BHMT gene expression may be caused to accumulate betaine in tissues and is a natural response to increase the tolerance of cells to hyper-osmosis induced by high levels of TDS. The enzyme BHMT activity causes a reduction in tissue betaine level as it catalyzes the transport of the methyl group from the betaine to homocysteine and syntheses methionine [29]. The result obtained in this study for the effect of TDS on BHMT gene expression are consistent with finding of Hoffman et al.[13]. Betaine supplementation increased BHMT gene expression, thereby reduce the excess betaine in the tissue for methionine synthesis. This finding was in line with report of Puchala et al.[35] in calves.

It was concluded that consumption of drinking water with higher than 2.000 ppm TDS adversely and betaine supplementation positively affect the performance of broiler chickens. Therefore, betaine supplementation can work as an osmolyte in diets of broiler which faced with high levels of TDS in drinking water. The lowest FCR was seen in chicks fed 400 and 2.000 ppm TDS with betaine supplementation.

Conflict of Interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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