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Effects of Betaine on Performance, Carcass, Bone and Blood Characteristics of Broilers During Natural Summer Temperatures

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Abstract: This experiment was conducted to determine the dietary supplementations of betaine on performance carcass, bone and blood traits of broilers in natural summer condition. A total of 180 broilers were divided into three experimental groups. The experimental diets were; Control, without any dietary addition, dietary supplemental betaine (Betafin®) 1 g kg^{-1} and (3) 2 g kg^{-1}. The experimental diets were offered to respective broiler chicks for 6 weeks. Additional betaine decreased body weight at 6 weeks and body weight gain 3-6 and 0 to weeks ($p<0.01$). Feed intake and feed conversion ratio were not affected by treatments ($p<0.05$). Dietary betaine supplementation did not affect carcass weight and composition, breast and thigh meat pigmentation and pH value of thigh the broilers. Tibia width was decreased by 1 g kg^{-1} betaine ($p<0.05$), but shank and tibia measurements were not affected by betaine. Serum alanin aminotransferase levels were reduced by both 1 and 2 g kg^{-1} supplementation of betaine ($p<0.05$), but other blood constituents were not affected.

Key words: Broiler, betaine, performance, carcass, bone, blood traits

INTRODUCTION

Betaine (trimethyl-glicine) is the trimethyl derivative of the amino acid glycine. Betaine plays several roles such as source of methyl groups, osmoregulator (Kidd *et al*., 1997), anti-stress agent and fat redistribution in the body (Saunderson and Mackinlay, 1990) of animals. Methyl groups are necessary for the synthesis of numerous substances such as creatine, carnitine, phosphatidylcholine, adrenaline, methyl purines as well as methylated amino acids (Eklund *et al*., 2005). Betaine is a natural compound and it is the primary nitrogenous component of sugar beets and is commercially extracted from molasses. It can alleviate the growth depression caused by illnesses (Augustine *et al*., 1997). Also, it has an important role for human nutrient obtained from a variety of foods. It is rapidly absorbed and utilized and helps to maintain heart, liver and kidney health (Craig, 2004).

Seasonal heat stress may negatively influence the performance of poultry. The detrimental effects of high environmental temperature on broiler production have been well documented again and again. Strategies to minimize heat stress tend to concentrate on management practices or introducing dietary supplements. High environmental temperature may cause water imbalances and osmotic changes in the cell via dehydration. It is known that changes in cell water volume can affect cell activity. Intracellular betaine has been shown to preserve osmotic equilibrium and protect macromolecules against denaturation and allow the cell to maintain its metabolic activities (Tucker and Remus, 2001; Anonymous, 2006). The several responses to betaine addition across studies is likely due to the varying mode of action of betaine tested and the difference in animals' health and stress status between studies (Schrama *et al*., 2003). Osmotic function of betaine is useful in maintaining gut wall integrity and hydration of cells under stressful conditions such as heat stress or disease (Tucker and Remus, 2001). Researches have demonstrated that adding betaine in the feed or water may decrease dehydration by facilitating water retention in the body and also it may contribute to maintain both the bird's energy balance and feed intake (Eklund *et al*., 2005). There is some evidence that betaine reduces energy expenditure for ion pumping in cells exposed to hyperosmotic media (Moeckel *et al*., 2002). The spared energy may promote cell proliferation (Eklund *et al*., 2005). Therefore, with betaine in the feed, the bird is able to retain water allowing more energy for growth.

In some studies, betaine replacing of methionine (Rostagno and Pack, 1996) or choline and their sparing effect had been investigated (Waldroup and Fritts, 2005) and diets often designed to be limiting in methionine,

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choline or protein. Besides, betaine have been tested in broilers with coccidial challenge (Matthews and Southern 2000; Tucker and Remus, 2001; Klasing *et al*., 2002). Studies concerning effect of dietary betaine on performance of poultry show variable results. In some of experiments, betaine supplementation improved performance and carcass quality parameters (Esteve-Garcia and Mack, 2000; Wang *et al*., 2004; Zhan *et al*., 2006) whereas in the others revealed that betaine had no effect on these traits (Neto *et al*., 2000; Kermanshahi, 2001). Alterations in the distribution pattern of fat and protein in the body have been reported following betaine addition. Though the mode of action of betaine as a carcass modifier remains open, there is, however, growing evidence that betaine could have a positive impact both on animal performance and carcass quality (Eklund *et al*., 2005).

There is no enough comprehensive study in terms of betaine usage in broiler diet in seasonal heat stress condition. The first aim of this study was to measure whether betaine in the broiler diet at seasonal high environmental temperature medium could reduce the water loss from the body via osmoregulation. The second objective was to examine whether betaine and sufficient methyl sources (methionine and choline) would provide a synergetic response; and finally we interested in whether adding betaine to basal diet would improve the carcass, bone and blood traits in broilers.

MATERIALS AND METHODS

Animals and diets: A total of 180 male day-old Ross-308 broiler chicks were individually weighed, wing banded and assigned into 18 pens with 10 chicks per pen (each treatment was consisted with 6 replicate groups, 10 birds m^{-2}). Floor was covered with wood shaving litter. The house was artificially heated to provide standard brooding temperatures (28-34°C) from hatch to 7 weeks and growing period temperature remained 23 °C. The lighting schedule was 24L: 0D from 0-7 days and 22L: 2D (darkness from 11.00 pm to 1.00 am) from 7-42 days. Floor pen was furnished with fresh wood shavings litter and population density of 10 chicks per square meter. Temperature and humidity of house was recorded daily at 8am, 3pm and 8pm, between 8-42 days. Average temperature and humidity was 28.14±0.47, 50.31±1.03; 33.13±0.27, 38.6±0.81 and 31.70±0.25, 37.03±0.69, respectively.

Three experimental diets based on maize-wheat-soybean were formulated using linear programming to be isoenergetic, isonitrogenic and to contain equal level of dry matter, crude fiber, crude ash, calcium, total phosphorus, sulphur amino acids and lysine. Therefore, the treatments were: Control, without any addition (C). betaine (Betafin® S1, Finnfeeds Danisco Cultor, Marlborough, United Kingdom) was added at the rate of 1 g per kg of diet (B1) and 2 g per kg of diet (B2). Small amounts of the basal diet were first mixed with the respective amounts of betaine as a small batch and then with a larger amount of the basal diet until the total amount of the respective diets were homogeneously mixed. Chicks were fed a starter diet throughout 0-21 days of age and fed a grower diet throughout 21-42 days of age. The diets used in this study were prepared according to NRC recommendation (NRC, 1994). The experimental diets contents are given in Table 1.

Table 1: The composition of starter diet at 0 to 3 weeks and grower diets at 3-6 weeks (g kg^{-1})

Ingredient	Starter diet (0-3 week)	Grower diet (3-6 week)
Yellow corn	433.18	400
Wheat	90.27	185.95
Soybean cake	207.71	195.36
Soybean meal	150.0	150.0
Fish meal	50.0	-
Vegetable oil	40.0	36.76
Ground limestone	12.24	14.74
Dicalcium phosphate	10.06	10.89
Salt	2.0	2.0
DL-Methionine	1.04	0.80
Anti-coccidiostat (clinacox)	1	1
Vitamin and trace mineral mixture[1]	2.5	2.5
Nutrient composition (g kg^{-1})*		
Dry matter	911.8	909.8
Crude protein	22.3	193.6
Crude fiber	55.2	36.5
Crude ash	56.8	53.3
Total calcium	9.9	9.3
Available phosphorus	4.0	3.5
Lysine	12.2	10.4
Methionine	4.5	3.8
Metabolizable energy, (ME, MJ kg^{-1})	13.39	13.34

[1]Supplied mg kg^{-1} of diet: Vitamin A, 15000 I.U; vitamin D3, 2000 I.U; vitamin E, 40.0 mg; vitamin K, 5.0 mg; vitamin B1 (thiamin), 3.0 mg; vitamin B2 (riboflavin) 6.0 mg; vitamin B6, 5.0 mg; vitamin B12, 0.03 mg; niacin, 30.0 mg; biotin, 0.1 mg; calcium D-pantothenate, 12 mg; folic acid, 1.0 mg; choline, 375 mg; manganese, 80.0 mg; iron, 35.0 mg; zinc, 50.0 mg; copper, 5.0 mg; iodine, 2.0 mg; cobalt, 0.4 mg; selenium, 0.15 mg, * Dry matter, crude protein, fiber and ash content of feed were analyzed; calcium, phosphorus, lysine, methionine and ME were calculated

Measurements: Feed and water were consumed *ad libitum*. Birds were weighed individually at 21 and 42 days. Body weight gain was calculated from 0-21 and 21-42 days. Total feed intake was measured per cage at 21 and 42 days. Mortality was recorded daily. Feed intake and feed conversion ratio were adjusted for mortality. Twelve males were selected from each dietary treatment group and weighted individually before killed by cervical dislocation at 42 days. In all, 60 males' feathers were plucked mechanically, eviscerated by hand. Carcass, abdominal fat pat (excluding gizzard fat), empty gizzard,

liver, heart, kidney weights were immediately recorded individually. Then breast and thigh portions were separated from carcass and weighted. Percentages of carcass yield, (carcass weight: live weight), breast, thigh, neck and wing were calculated as part weight: carcass weight ratio.

Chemical analyses: Six breast and thigh meat samples from each group (totally 30 samples) were collected in plastic trays, weighed and stored in an air thigh plastic bag in a freezer until samples were required for analysis, when they were homogenized using a blender and analyzed for dry matter, nitrogen, ether extract and crude ash. Dry matter content of feed, breast and thigh samples was determined by oven-drying at 105°C for 18 h. Ether extract content of breast and thigh samples were obtained by the Soxhlet extraction using anhydrous diethyl ether. Kjeldahl method was used for the analysis of total nitrogen content of feed, breast and thigh samples and crude protein was expressed as nitrogen×6.25 (AOAC, 1980). Crude ash content was determined after heating in a muffle furnace at 550°C for 16 h. The crude fibre content of feed was determined by using 12.5% H_2SO_4 and 12.5% NaOH solutions (Nauman and Bassler, 1993). The pH value of the samples were determined with a pH meter (Hanna Instruments-8413), using a direct probe by thrusting the probe into the breast and thigh. The colors of breast and thigh were measured using a Minolta colorimeter (CM508d) to measure CIE Lab values (L* measures relative lightness, a* relative redness and b* relative yellowness). The left tibia bones were removed. Bones were cleaned of adheral tissues and then weighed; length and width (at midpoint) were measured with a digital caliper. Bones were frozen at -20°C until analyses. After thawing to room temperature bone breaking strength was determined by Instron Testing Machine and the bones were subjected to test at the midshaft of each bone until they fractured (Norgaard-Nielsen, 1990). The centre of each bone was aligned with the breaking probe (10 mm diameter) which approached at 30 mm min^{-1}. The supports for each bone were 30 mm apart. The breaking strength was determined from the failure point (peak) of each loading curve. The ash content of tibia bone was determined after heating in a muffle furnace at 550°C for 16 h. Left shank length and width were measured with a caliper rule at 42 days.

Blood biochemical evaluation: Twelve blood samples per group (totally 60 birds) were obtained by venepuncture of left wing vein at 42 days for blood biochemical analysis. Then blood samples were kept on ice and transferred to the laboratory where they were centrifuged at 1500 g for 10 min and serums were removed and then stored at -20°C until analyzed. The serum total protein, albumin, glucose, triglyceride, total cholesterol, High Density Lipoprotein (HDL), á-amylase, uric acid, aspartate aminotransferase (SGOT, AST), alanine aminotransferase (SGPT, ALT) Lactate Dehydrogenase (LDH) and iron levels were measured using kit from the same manufacturer with an auto analyzer (model BT 3000 plus, Biotechnica, Rome, Italy). Also serum Low Density Lipoprotein (LDL) concentration was calculated as the difference between total cholesterol and HDL.

Statistical analysis: Data were subjected to ANOVA using General Linear Models (SAS, 1996). The model included levels of dietary betaine content are presented. Pen means served as the experimental unit for statistical analysis. Means were separated using Duncan's multiple range tests. The results of statistical analysis were shown as mean values and standard error of means (pooled SEM) in the tables.

RESULTS AND DISCUSSION

Average livability value was 94.2±1.60 (%) for experiment and there were no treatment differences among groups. Esteve-Garcia and Mack (2005) and Waldroup *et al.* (2006) noted that betaine supplementation did not affect on livability (not shown in the tables). Body weight, gain, feed intake and feed conversion ratio of broilers are presented in Table 2. Betaine supplementation results vary in terms of performance characteristics. Body weight at 21 days and body weight gains at 0-21 days were not affected by dietary betaine (B1 and B2). However, the body weight at 42 days and body weight gains of B1 and B2 groups at 0-42 days were significantly lower than that of control ($p<0.01$). Feed intake and feed conversion ratio of broilers were not influenced by betaine supplementation. Performance traits of broilers were not influenced by the betaine levels.

Currently, literature related to the effects of supplemental betaine on broiler chickens under environmental heat stress conditions is limited. Our expectations betaine can improve chicks' performance and carcass traits in heat stress but results showed that betaine failed. During heat stress, the bodies cells of birds are subjected to osmotic stress and betaine have an osmoregulatory effect (Kidd *et al.*, 1997) and anti-stress agent (Saunderson and Mackinlay, 1990). Thus, betaine may help to chicks in ameliorate detrimental effects of

Table 2: Effects of betaine and levels on body weight, body weight gain, feed intake and feed conversion ratio

		Additional betaine level (g kg^{-1})				
Item	Weeks of age	0	1	2	SEM	Probability
Body weights (g)	0	43.0	43.5	43.3	0.46	NS
	3	752.2	731.7	736.1	10.4	NS
	6	2138^{a}	1994^{b}	2017^{b}	32.3	**
Body weight gain (g)	0-3	33.8	32.8	33.0	0.49	NS
	3-6	66.0^{a}	60.2^{b}	60.8^{b}	1.30	**
	0-6	49.9^{a}	46.4^{b}	47.0^{b}	0.76	**
Feed intake (g)	0-3	45.6	44.6	44.2	0.92	NS
	3-6	133.9	127.4	125.0	3.02	NS
	0-6	112.6	108.3	106.7	1.88	NS
Feed conversion ratio (feed/gain)	0-3	1.36	1.39	1.34	0.02	NS
	3-6	2.04	2.12	2.06	0.06	NS
	0-6	1.80	1.86	1.80	0.04	NS

^{a,b}: Means within a line in each variable with no common superscript differ significantly; **:($p<0.01$), NS: No significant differences were found ($p>0.05$); SEM: Standard Error of Means (pooled)

heat stress. Zulkifli *et al.* (2004) reported that betaine supplementation may alleviate effect of heat stress in broilers. They showed that betaine led to decrease in heterophile/lymphocyte ratio (H/L) and body temperature. However, they noted that betaine supplementation to diet or water had no any significant effect on final body weight, feed intake and feed conversion ratio. On the other hand the present results of the experiment are similar with Matthews *et al.* (1997) and Matthews and Southern (2000). They reported that average daily gain was decreased in coccidiosis uninfected chicks fed with betaine (0.1 and 0.075%). Also, presented results are in agree with those of Rostagno and Pack (1996), Schutte *et al.* (1997), Esteve-Garcia and Mack (2000) and Zulkifli *et al.*, (2004), Waldroup and Fritts (2005), they determined that supplemental betaine does not effect feed efficiency. On the other hand, Zhan *et al.* (2006) reported that betaine increased weight gain and feed efficiency but not feed intake. In contrast to these observations the data there are some reports related to betaine improved live weight and feed efficiency in boilers (Waldensted *et al.*, 1999; Neto *et al.*, 2000) and in ducks (Wang *et al.*, 2004) when fed methionine adequate diets.

Up to date the most of researches have been done focused on to evaluate whether betaine has spared effect with methionine and choline (Matthews *et al.*, 1997; Wang *et al.*, 2004; Zhan *et al.*, 2006) and effectiveness of betaine in chicks infected with coccidiosis (Matthews *et al.*, 1997; Matthews and Southern, 2000). However the present diets contained methionine and choline in adequatly levels. Thus potential positive effects of betaine may shadowed by the presence of dietary methionine and choline. Also, in the present study, chicks received an anticoccidial in the diet and there was no apparent problem in terms of coccidial infection. Thus potential positive effects of betaine under a coccidiosis challenge did not play a role. The positive response might appear when chickens were fed a moderately methionine-deficient diet or in the existence of coccidial problem (Matthews *et al.*, 1997; Wang *et al.*, 2004; Zhan *et al.*, 2006). However, Matthews *et al.* (1997) and Matthews and Southern (2000) reported that average daily gain was decreased in coccidiosis uninfected chicks fed with betaine. Also it is reported that betaine supplementation failed improve weight gain in uninfected broilers (Matthews *et al.*, 1997; Schutte *et al.*, 1997). On the other hand, it is noted that the efficiency of additional betaine is reduced at dietary levels above 0.08% (Xu *et al.*, 1999). However, we used 0.1 and 0.2% supplemental betaine dosage in diet and these dosages higher than that of 0.08%. Because, betaine is an N-containing substance which requires energy to be excreted (Eklund *et al.*, 2005). Consequently, excessive betaine in the ration may cause energy loss due to their excretion and increasing the dietary betaine level may reduce its efficacy. This may be main cause of decrease body weight and gain.

Effects of betaine levels on slaughter weight, carcass weight and yield and carcass part weights and relative weights of parts of male broilers were presented in Table 3. The results of this study indicated that little or no positive benefits was obtained in carcass traits ($p>0.05$). However, there was a tendency concerning decrease abdominal and carcass fat in chicks fed with betaine. Nowadays, consumers prefer lean meat and higher breast meat yield. Betaine is so called carcass modifier due to fat redistribution (Eklund *et al.*, 2005). The present results and others indicated that betaine was effective to providing lean meat via increasing breast meat and decreasing abdominal fat (Wang *et al.*, 2004; Hassan *et al.*, 2005; Zhan *et al.*, 2006). However, Waldroup and Fritts (2005) reported that betaine in diet (1000 mg kg^{-1}) day of age significantly increased carcass dressing percentage at 42 days of age but did not at 49 days of age. McDevit *et al.* (2000) suggested that supplemental betaine caused relatively lighter breast

Table 3: Effects of betaine on slaughter weight (g), carcass weight (g) and yield (%) and carcass part weights (g) and relative weights[1] (%) of parts of male broilers

	Additional betaine level (g kg^{-1})				
Absolute weights (g)	0	1	2	SEM	Probability
Slaughter	2230.9	2045.8	2102.9	55.5	NS
Carcass	1670.3	1522.3	1576.4	44.8	NS
Thigh	680.8	629.8	646.2	18.8	NS
Breast	697.3	618.0	644.8	19.8	NS
Wing	179.8	164.9	174.6	4.48	NS
Liver	39.8	36.6	40.1	1.72	NS
Gizzard	28.0	29.3	28.8	1.42	NS
Heart	11.0	9.67	11.6	0.67	NS
Kidney	13.8	12.6	13.6	0.93	NS
Abdominal fat	38.1	34.3	31.1	2.28	NS
Relative weights (%)					
Carcass	74.6	75.3	74.9	0.58	NS
Thigh	40.7	41.4	40.9	0.40	NS
Breast	41.7	40.5	40.9	0.48	NS
Wing	10.8	10.9	11.1	0.25	NS
Liver	2.36	2.40	2.55	0.09	NS
Gizzard	1.67	1.95	1.85	0.09	NS
Heart	0.65	0.64	0.73	0.03	NS
Kidney	0.82	0.82	0.86	0.05	NS
Abdominal fat	2.27	2.26	1.97	0.15	NS

[1]As a proportion of carcass weight, NS: No significant differences were found ($p>0.05$); SEM : Standard error of means (pooled)

Table 4: Effect of betaine on thigh composition and pH values of male broilers

	Additional betaine level (g kg^{-1})				
Item	0	1	2	SEM	Probability
Dry matter (%)	25.82	25.52	25.25	1.00	NS
Crude protein (%)	21.68	21.33	21.83	0.63	NS
Ether extract (%)	3.48	3.15	3.07	0.66	NS
Crude ash (%)	0.97	1.08	1.12	0.05	
Thigh pH	6.44	6.48	6.48	0.03	
Breast pH	6.09	6.09	6.06	0.03	
Meat pigmentation					
Breast					
L*	50.6	50.8	48.2	1.03	NS
a*	2.45	3.84	3.37	0.39	NS
b*	10.39	10.88	10.34	0.76	NS
Thigh					
L*	52.72	53.89	54.78	2.57	NS
a*	2.72	3.89	2.98	0.60	NS
b*	10.73	10.85	11.18	0.95	NS

NS: No significant differences were found ($p>0.05$); SEM : Standard error of means (pooled), L*: relative lightness; a*: relative redness; b*: relative yellowness

muscles but relatively higher abdominal fat pad. On the other hand, Schutte *et al.* (1997) showed that supplemental betaine (0.04%) did not affect growth and tended to increase breast meat yield. Esteve-Garcia and Mack (2000) reported that betaine significantly improved carcass percentage but not carcass and breast weight, breast yield, abdominal fat weight and abdominal fat percentage. On the other hand some researchers reported that abdominal fat increased by betaine supplementation (Attia *et al.*, 2005). Rostagno and Pack (1996) noted that betaine did not have a consistent effect on carcass yield and abdominal fat pad. Neto *et al.* (2000) reported that abdominal fat and liver weight were not affected by betaine. Türker *et al.* (2004) reported that betaine did not improve carcass weight and yield of broilers when the diet contains adequate methionine.

The meat consistent of tight was presented in Table 4. Dry matter, crude protein, ether extract and crude ash content of the tight meat was not affected by betaine supplementation ($p>0.05$). Schutte *et al.* (1997) and Türker *et al.* (2004) reported that the effects of betaine were not significant in breast muscle protein and fat. In some researches betaine supplementation in diet had no affect on muscle protein (Neto *et al.*, 2000), dry matter, crude protein, ether extract and ash of breast muscle (Hassan *et al.*, 2005). However, others obtained an increment at meat protein and fat content (Attia *et al.*, 2005; Zhan *et al.*, 2006). Türker *et al.* (2004) reported that dressing percentage, crude protein and ether extract percentage of meat was not significant in broilers fed betaine in ration which adequate content of methionine. Meat Ph of broiler was not affected by betaine in current study. The pH values of tight and breast meats were not affected by betaine supplementation in the current study. Matthews *et al.* (2001) reported that betaine supplementation may maintain pH of meat within certain limitations via different action such as decreased drip loss of the meat, retarded lactic acid accumulation and promoted effect on muscle creatine content in pigs.

Lightness (L), redness (a) and yellowness (b) and pH values of the thigh and breast meats were not affected by betaine supplementation ($p>0.05$) (Table 4). During the hot summer season, birds' breast products are formed with poor water-holding capacity, poor texture and pale color (McKee and Sams, 1997). Betaine has an osmoregulatory effect on the body water (Anonymous, 2006; Tucker and Remus, 2001), therefore it may affect on meat quality. There is no literature related to effect of betaine on meat color in poultry. However, Matthews *et al.* (2001) reported that subjective meat color of pigs was not affected by fed with betaine (0.250%) while Matthews *et al.* (1998) reported that subjective color of the loin muscle in pigs was decreased fed with betaine (0.125%).

Shank length and width and tibia weight and length were not affected by dietary betaine (Table 5, $p>0.05$). Tibia width decreased chicks fed betaine 1 g kg^{-1} of feed as compared with control ($p<0.05$). Tibia breaking strength and ash was not affected by betaine supplementation ($p>0.05$) (Table 6). There is no available literature regarding the effect of betaine on bone properties. Neto *et al.* (2000) reported that bone ash was not affected by betaine in diet.

Table 5: Effect of betaine on shank and tibia measurements

	Additional betaine level (g kg^{-1})				
Item	0	1	2	SEM	Probability
Tibia					
Weight (g)	18.95	18.13	18.62	0.5	NS
Length (mm)	105.8	105.2	106.7	0.9	NS
Width (mm)	9.83^{a}	8.97^{b}	9.43^{ab}	0.2	*
Breaking strength (kg-force)	32.0	29.2	31.9	2.64	NS
Ash (%)	41.5	43.4	40.0	1.00	NS
Shank					
Length (mm)	65.8	65.8	65.8	0.35	NS
Width (mm)	12.9	12.6	12.8	0.12	NS

^{a,b}: Means within a line in each variable with no common superscript differ significantly; *:($p<0.05$), NS: No significant differences were found ($p>0.05$); SEM : Standard error of means (pooled)

Table 6: Effect of betaine on blood serum constitutes

	Additional betaine level (g kg^{-1})				
Item	0	1	2	p	SEM
Glucose (mg dL^{-1})	156.7	158.4	177.0	0.791	14.59
Triglyceride (mg dL^{-1})	37.14	47.0	39.57	0.546	4.55
Cholesterol (mg dL^{-1})	128.4	125.4	126.0	0.984	8.05
Direct HDL (mg dL^{-1})	53.86	56.57	61.00	0.170	2.72
Total Protein (g dL^{-1})	3.30	3.43	3.58	0.698	0.19
Albumin (g dL^{-1})	1.514	1.557	1.557	0.956	0.072
Urea (mg dL^{-1})	4.71	4.71	4.14	0.475	0.473
Uric Acid (mg dL^{-1})	8.27	8.36	7.01	0.207	1.02
LDH (U L^{-1})	759.4	975.4	1128.6	0.171	115.2
SGOT (AST, U L^{-1})	3.86	7.33	4.14	0.337	1.75
SGPT (ALT, U L^{-1})	14.86^{a}	9.57^{b}	8.57^{b}	0.04	2.74
Alfa-Amylase	278.3	322.6	245.7	0.253	36.09
Iron (ug dL^{-1})	145.5	177.4	156.6	0.962	35.41

^{a,b}: Means within a line in each variable with no common superscript differ significantly; *:($p<0.05$), NS: No significant differences were found ($p>0.05$); SEM : Standard error of means (pooled)

The blood consistent was showed in Table 6. The treatments did not affect blood components without serum glutamic-pyruvate transaminase (ALT). It was decreased by betaine supplementation ($p<0.05$). ALT catalyzes the transfer of an amino group from alanine to a-ketoglutarate, the products of this reversible transa-mination reaction being pyruvate and glutamate. It is used as a liver function test and elevated levels monitored liver malfunction (Yalçınkaya *et al*., 2008). These results agree with Attia *et al*. (2005), who indicated that betaine supplementation had no significant effect most of serum constituents except for ALT and a significant decrease in blood serum ALT was observed at 0.07% betaine compared to the control group. However, Hassan *et al*. (2006) revealed that betaine supple-mentation in diet (0.072-0.144%) caused an increase in serum total protein and globulin but cholesterol, AST and ALT levels were not affected by betaine supplementation. These results are in agreement with our results. Zhan *et al*. (2006) found that dietary betaine addition decreased serum uric acid but not affect on triglyceride content in broilers. Zou and Lu (2002) showed that betaine supplementation (600 mg kg^{-1}) increased serum glucose in laying hens.

In this experiment, betaine supplementation to broiler diets did not improve performance, carcass, bone and blood parameters during summer heat condition. However betaine decreased body weight and body weight gain in broilers. These effects of betaine on body weight and gain of broilers may refer to excess methyl groups originated from methionine, choline and supplemental betaine in the diets caused energy looses due to their excretion (Xu *et al*., 1999; Eklund *et al*., 2005). Because it is reported that betaine did not improve performance and carcass traits of poultry both uninfected coccidiosis (Matthews *et al*., 1997) and when diet contains sufficient methionine and choline (Harms and Russel, 2002). On the other hand, Schrama *et al*. (2003) reported that the several responses to betaine addition across studies is likely due to the varying mode of action of betaine tested and the difference in animals' health and stress status between studies. However, there have been limited studies in relation to effect of betaine supplementation on performance, carcass, meat, bone and blood properties in broiler diets during hot summer condition.

CONCLUSION

In conclusion, results of this experiment suggested that additional betaine in broiler diets had no beneficial effect on examined parameters of broilers during summer heat condition. However, there were a number of fundamental differences among reports, including the basal diets, genetic stocks used and environmental conditions of the experiments. Further experiments should need to be conducted to determine whether the effect of betaine at different condition in broilers.

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