

The effects of betaine supplementation in diets containing different levels of crude protein and methionine on the growth performance, blood components, total tract nutrient digestibility, excreta noxious gas emission, and meat quality of the broiler chickens

J. H. Park and I. H. Kim¹

Department of Animal Resource and Science, Dankook University, Cheonan 31116, Republic of Korea

ABSTRACT The effects of betaine supplementation on growth performance, blood components, nutrient digestibility, excreta noxious gas emission, and meat quality of broiler chickens were examined using different dietary crude protein (**CP**) and methionine (**Met**) levels. A total of 768 Ross 308 broiler chickens were allotted to four treatments, with 12 replications of each treatment conducted over 6 wk. Treatments were factorially designed, with 2 levels of CP [Starter: CP 21% (low Met) and 23% (high Met); Finisher: CP 18% (low Met) and 20% (high Met)] and 2 levels of betaine supplementation (0 and 0.12%). Body weight gain and feed conversion improved significantly as dietary levels of protein increased ($P < 0.05$), but the results for betaine supplementation differed. The concentrations of serum total protein, albumin, and glutathione peroxidase (**GPx**) were elevated by either the supplementary betaine or the CP ($P < 0.05$). In addition, serum

albumin concentration significantly increased in groups fed low CP amounts and betaine 0.12% compared with groups fed low CP only ($P < 0.05$). Total tract digestibility of nitrogen in broilers fed high CP amounts or 0.12% betaine, was observed to be greater than that in groups fed low CP amounts or no betaine treatment ($P < 0.05$). Supplemental betaine affected excreta ammonia gas emission, and hydrogen sulfide concentrations decreased significantly in low CP-fed groups ($P < 0.05$). Breast meat quality and relative organ weights were not influenced by CP levels or dietary betaine supplementation. These results suggest that betaine does not increase productivity, but may affect serum total protein, albumin, GPx, excreta ammonia emission, and nitrogen digestibility in broiler chickens. In addition, betaine supplementation is more effective in increasing serum albumin concentration when it was added in low CP (low Met) diets.

Key words: antioxidant enzyme, excreta ammonia gas, productivity, trimethylglycine

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INTRODUCTION

Betaine (N, N, N-trimethylglycine) is a non-toxic amino acid derivative that is present in relatively large amounts in many foods, including sugar beets, wheat, spinach, and aquatic invertebrates. However, it is not present in significant quantities in most feed ingredients (Fernández et al., 2000). In the body, betaine is generated in the liver and kidney from choline, through the sequential action of choline oxidase and betaine-aldehyde dehydrogenase (Dilger et al., 2007). It serves as a methyl donor in the conversion of homocysteine to methionine, catalyzed by the betaine-homocysteine methyltransferase enzyme (Ueland et al., 2005). Due to its ability to donate a methyl group, betaine can be used to spare methionine and improve intestinal function against osmotic stress,

which occurs during diarrhea or coccidiosis in animals (Saunderson and MacKinlay, 1990; Amerah and Ravindran, 2015).

Many studies have shown responses to betaine supplementation in the diets of chickens. The positive effects of betaine on methionine sparing in chicks have been previously indicated (Kidd et al., 1997; Simon, 1999; Mahmoudi et al., 2018). In contrast, Esteve-Garcia and Mack (2000) used a wheat-soybean meal-based diet, supplemented with choline to provide an adequate supply of methyl groups, and found relatively small and non-significant responses to betaine supplementation. However, the effects of methionine were significant in improving body weight gain (**BWG**) and feed conversion ratio (**FCR**), and in increasing the breast yield of broiler chicks. Schutte et al. (1997) and Kermanshahi (2001) also observed that there was no evidence of betaine sparing DL-methionine as an essential amino acid supplement in broiler diets. Although betaine has been thought to function as osmolyte, play a role in fat distribution, and act as a methyl donor,

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¹Corresponding author: inhokim@dankook.ac.kr

the effects of betaine in the donation of methyl groups has long been controversial.

Methionine is the first limiting amino acid in corn-soybean diets for broiler chickens. Dietary methionine treatment significantly affects the growth performance, serum protein metabolism, meat quality, and oxidative status of muscle in broiler chickens and pigs (Tian et al., 2016; Wen et al., 2017). In addition, Ratriyanto et al. (2017) demonstrated that the changes in bird productivity and nutrient digestibility depend on the amount of dietary crude protein (CP). In a review, Eklund et al. (2005) reported that the methionine sparing effect of betaine could enhance the efficient use of dietary protein. We speculated that betaine supplementation to low protein and low methionine diets may improve the efficiency of protein utilization and enhance the performance of broiler chickens, thereby reducing nitrogen excretion to the environment as well as providing the opportunity to formulate low cost ration compared with high protein diets with high methionine levels. Therefore, the objectives of this study were i) to evaluate whether betaine supplementation to low or high protein diets improve the production performance, blood components, nutrient digestibility, excreta noxious gas emission, and meat quality of broiler chickens ii) to see if there is interaction between dietary levels of CP and betaine.

MATERIALS AND METHODS

The research was carried out as per guidelines of the Animal Care and Use Committee of Dankook University, Republic of Korea.

Animals and Experimental Design

A total of 768, Ross 308 (male, 1-day-old) broiler chickens, weighing between 40 and 42 g, were selected for 6 wk of feeding trials. The broilers were sorted into 1 of 4 dietary treatments. Each treatment involved 12 replications and 16 broilers were used per replication. There were 2 phases of differing CP levels—the starter (1 to 21 d) and finisher periods (22 to 42 d). Treatments were arranged in a 2×2 factorial, with 2 dietary CP levels [high CP or low CP (high CP – 2% less)], and 2 levels of betaine [betafin[®] (CTC BIO Inc. Seoul, Korea), natural betaine anhydrous 96%] supplementation (0 or 0.12%). The diets (in mash form) were formulated according to recommendations from the Nutrient Requirements of Poultry (NRC, 1994), based on corn-soybean diets containing CP 23% (21%) and methionine 0.5% (0.45%) for 1 to 21 d, and CP 20% (18%) and methionine 0.38% (0.34%) for 22 to 42 d (Table 1).

Experimental Management

Broilers were housed in a temperature-controlled room with 3 floors of stainless steel battery cages (64 × 124 × 40 cm), allowing free access to feed and

water during the experiment. Consistent light was provided for 22 h, and temperature and humidity were adjusted according to the Ross 308 manual management guide.

Growth Performance

Body weight of broilers was measured on days 1, 21, and 42 of the experimentation period. After measuring the remaining feed on days 21 and 42, BWG, feed intake (FI), and FCR were calculated. Dead birds were recorded to determine the mortality (%) during the experiment.

Blood Profiles

Serum samples were collected from broiler chickens in each group at the end of the experiment, for analyses. Total serum protein, albumin, blood urea nitrogen (BUN), creatinine and uric acid concentrations were determined by standard laboratory methods, using an automatic analyzer (ADVIA, Bayer, Tokyo, Japan). The activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in the serum, were measured using a commercial kit from Enzo Life Sciences Company (Ann Arbor, MI, USA) as per instructions from the manufacturer.

Apparent Total Tract Digestibility

To determine the nutrient digestibility, 0.2% chromium oxide (Cr₂O₃), as an indigestible marker, was added to the diets, 5 d prior to the collection period. Clean stainless steel collection trays were placed under each cage, and excreta (feces and urine) from the broilers was collected twice daily, in the last 3 d of the experimental period. After drying at 60°C for 72 h, it was pulverized and passed through a 1-mm screen; apparent total tract retention of dry matter, nitrogen, and energy were analyzed (AOAC, 2000).

Excreta Noxious Gas Emission

Excreta (feces and urine) samples were collected using stainless steel collection trays from each replication in the last 3 d of the experiment, for analyses of ammonia, hydrogen sulfide, total mercaptan and acetic acid. The samples were stored in 2.6-L sealed plastic containers for 5 d, at room temperature (25°C). Following the fermentation period, a Gastec (model GV-100) gas sampling pump was used to detect gas (Gastec Corp., Tokyo, Japan). For these measurements, the adhesive plaster of each container was punctured, and 100 ml of air was sampled at approximately 5 cm above the feces.

Breast Meat Quality

At the end of the experimentation period, 12 broilers were randomly selected from each treatment,

Table 1. Formulation and chemical composition of experimental diets (as-fed basis).

Ingredient, %	Starter ¹		Finisher	
	Low CP	High CP	Low CP	High CP
Corn (7.9%)	59.83	55.84	65.66	61.59
Soybean meal (45%)	19.69	20.50	17.51	18.67
Corn gluten meal (61%)	11.69	14.83	7.27	10.45
Wheat bran	2.00	2.00	3.00	3.00
Soybean oil	3.00	3.00	3.00	3.00
Tricalcium phosphate	1.83	1.81	1.31	1.29
Limestone	0.93	0.94	1.12	1.13
Salt	0.46	0.46	0.41	0.41
DL-Methionine (99%)	0.10	0.19	0.06	0.09
L-Lysine (99%)	0.27	0.23	0.23	0.19
Vitamin premix ²	0.10	0.10	0.10	0.10
Mineral premix ³	0.10	0.10	0.10	0.10
Nutrient composition				
ME, kcal/kg	3200	3200	3200	3200
CP, %	21.00	23.00	18.00	20.00
Ether Extract, %	5.62	5.61	5.68	5.67
Crude Fibre, %	2.64	2.67	2.67	2.70
Ash, %	5.48	5.59	4.96	5.06
Ca, %	1.00	1.00	0.90	0.90
Available P, %	0.45	0.45	0.35	0.35
Sodium, %	0.20	0.20	0.18	0.18
Lysine, %	1.10	1.10	1.00	1.00
Methionine, %	0.45	0.50	0.34	0.38
Cysteine, %	0.37	0.41	0.33	0.36
Threonine, %	0.75	0.82	0.66	0.72
Tryptophan, %	0.23	0.24	0.20	0.22
Glycine, %	0.78	0.84	0.71	0.76
Serine, %	1.03	1.13	0.90	1.01
Choline, mg/kg	997	1007	981	992
Folacin, mg/kg	0.55	0.55	0.56	0.56
Total sulfur amino acid, %	0.82	0.91	0.67	0.74
Digestible lysine, %	0.94	0.93	0.84	0.84
Digestible methionine, %	0.42	0.47	0.31	0.35
Digestible cysteine, %	0.25	0.28	0.23	0.26
Digestible threonine, %	0.63	0.70	0.54	0.60
Digestible tryptophan, %	0.16	0.17	0.14	0.15
Digestible glycine, %	0.60	0.64	0.53	0.57
Digestible serine, %	0.95	1.05	0.80	0.90
Digestible sulfur amino acid, %	0.67	0.75	0.54	0.61

¹Starter diets provided during d 1 to 21; finisher diet provided during d 22 to 42.

²Provided per kg of complete diet: 15,000 IU of vitamin A, 3,750 IU of vitamin D₃, 37.5 mg of vitamin E, 2.55 mg of vitamin K₃, 3 mg of thiamin, 7.5 mg of riboflavin, 4.5 mg of vitamin B₆, 24 µg of vitamin B₁₂, 51 mg of niacin, 1.5 mg of folic acid, 0.2 mg of biotin and 13.5 mg of Ca-Pantothenate.

³Provided per kg of complete diet: 37.5 mg Zn (as ZnSO₄); 37.5 mg Mn (as MnO₂); 37.5 mg Fe (as FeSO₄ · 7H₂O); 3.75 mg Cu (as CuSO₄ · 5H₂O); 0.83 mg I (as KI); and 0.23 mg Se (as Na₂SeO₃ · 5H₂O).

individually weighed, and slaughtered by cervical dislocation. The pH of collected breast muscle samples was measured using a pH meter (Istek 77P, Seoul, Korea). Meat color values were measured using a Minolta colorimeter (CR-300, Tokyo, Japan). Meat samples were measured at three different locations across the breast and expressed as L* (lightness), a* (redness), and b* (yellowness) values. The pH value of raw breast meat was measured using a digital pH meter (NWKbinar pH, K-21, Landberg, Germany) after blending 10 g of finely homogenized samples with 90 ml of double-distilled water. The water holding capacity (WHC) was determined using a method by Kim et al. (2009). Ten grams of breast meat samples was ground using a grinder (PA-82, Mainca, Barcelona, Spain) equipped with 3-mm plate, and heated to 70°C in a water bath for 30 min. The samples were then cooled with ice and centrifuged at 4°C at 1,000 × g for 10 min. The WHC

was calculated as the ratio (%) of liquid weight after centrifugation, to that of the original liquid. For cooking loss estimation, raw meat samples (3 × 5 × 8 cm) were placed in plastic bags, after being weighed, and were cooked in a water bath at 80°C for 1 h. The samples were then cooled to 4°C for 2 h and weighed again. Cooking losses were calculated based on the differences in weight between the raw and cooked samples. Drip loss (%) was determined by weight loss. Samples were removed from broiler breast meat, weighed, and suspended in a zipper bag for 7 d, at 4°C. The initial and final weight (at 1, 3, 5, and 7 d) of each sample was used to calculate drip loss.

Relative Organ Weight

Following blood collection, the same broilers were weighed individually and killed by cervical dislocation.

Table 2. Effect of betaine (Bet) supplementation on growth performance in broilers.¹

Items	Low CP		High CP		SEM ²	CP		Bet		P-value		
	Bet 0	Bet 0.12%	Bet 0	Bet 0.12%		Low	High	0%	0.12%	CP	Bet	CP × Bet
1–21 d												
BWG ³ , g	682	680	695	704	9.25	681	688	689	692	0.2059	0.2059	0.1960
FI ⁴ , g	1098	1093	1101	1102	8.42	1096	1097	1100	1098	0.2342	0.3533	0.7899
FCR ⁵	1.61	1.61	1.58	1.57	0.02	1.61	1.60	1.60	1.59	0.5268	0.4411	0.1055
22–42 d												
BWG, g	1913	1941	2203	2109	18.15	1927	2156	2058	2025	0.0187	0.7128	0.2666
FI, g	3789	3851	3849	3726	16.75	3820	3788	3869	3789	0.4096	0.4875	0.6261
FCR	1.98	1.98	1.75	1.77	0.02	1.98	1.76	1.88	1.87	0.0048	0.3753	0.5353
1–42 d												
BWG, g	2595	2621	2898	2813	20.48	2608	2856	2747	2717	0.0062	0.8540	0.1094
FI, g	4887	4944	4950	4828	21.76	4916	4889	4919	4886	0.3126	0.4080	0.8011
FCR	1.88	1.89	1.71	1.72	0.02	1.89	1.71	1.79	1.80	0.0002	0.1583	0.1539
Mortality, %	6.3	6.8	7.4	6.7	1.26	6.6	7.1	6.9	6.8	0.4595	0.7725	0.6258

¹Low crude protein (CP) diet, 21 and 18% CP, 0.45 and 0.34% methionine for starter and finisher periods; high CP diet, 23 and 20% CP, 0.50 and 0.38% methionine.

²Standard error of means.

³Body weight gain.

⁴Feed intake.

⁵Feed conversion ratio.

The liver, spleen, bursa of Fabricius, breast meat, abdominal fat, and gizzard were removed weighed. Organs, breast muscle and abdominal fat were expressed as a percentage of live body weight.

Statistical Analysis

The cage was used as the experimental unit for performance. Individual birds were used as experimental units for digestibility, blood profile, excreta noxious gas, and meat quality measurements. To investigate the effects of different dietary CP levels and betaine, data were analyzed in a 2 × 2 factorial design. Two-way ANOVA (using SAS software) was used analyze the interactions between the 2 factors. When interactions ($P < 0.05$) existed between the dietary CP levels and betaine, multiple comparisons were conducted by the Tukey's method to compare significant differences between treatments, using a significance level of $P < 0.05$.

RESULTS

Growth Performance

Broiler chickens fed a 0.12% betaine supplemented diet during the periods of 1 to 21 d, 22 to 42 d, and 1 to 42 d showed no significant differences in BWG, FI, and FCR. During the period of 22 to 42 d and 1 to 42 d BWG and FCR improved under high CP treatments compared with low CP treatments ($P < 0.05$). No significant differences were observed in mortality during the experiment. There was no significant interactive effect of betaine supplementation and dietary CP on performance of broilers in the entire experiment period (Table 2).

Blood Profiles

There were no significant differences in creatinine, uric acid, and GPx concentrations in broiler chickens fed different levels of CP or betaine in their diets. The BUN concentration tended to increase in broiler chickens fed a diet containing 0.12% betaine ($P = 0.0889$). Broiler chickens in the high CP groups showed higher total serum protein concentrations than the low CP groups ($P < 0.05$). Total protein, albumin, and GPx concentrations were elevated in broiler chickens fed a diet containing 0.12% betaine compared with those fed no betaine diet ($P < 0.05$). There was a significant interaction between CP levels and betaine supplementation for albumin concentrations ($P < 0.05$). Broiler chickens fed a low CP containing 0.12% betaine, showed increased serum albumin concentration compared with birds fed low CP diet alone (Table 3).

Total Tract Nutrient Digestibility

As shown in Table 4, nitrogen digestibility in broiler chickens fed high CP diets was greater than in those fed low CP treatments ($P < 0.05$). Moreover, betaine supplementation increased nitrogen digestibility ($P < 0.05$), and tended to increased dry matter ($P = 0.1020$) and energy digestibility ($P = 0.0598$). However, betaine and 2 CP levels did not have any significant effects on the digestibility of dry matter and energy of broilers. There was no significant interactive effect of betaine supplementation and dietary CP on nitrogen digestibility.

Excreta Noxious Gas Emission

Ammonia gas emission in broiler chickens fed betaine was lower than in those fed no betaine treatments

Table 3. Effect of betaine (Bet) supplementation on blood profiles of broilers.¹

Items	Low CP		High CP		SEM ²	CP		Bet		P-value		
	Bet 0	Bet 0.12%	Bet 0	Bet 0.12%		Low	High	0%	0.12%	CP	Bet	CP × Bet
Total protein, g/dL	3.0	3.1	3.4	3.7	0.1	3.1	3.6	3.2	3.4	<.0001	0.0050	0.1163
Albumin, g/dL	1.2 ^b	1.4 ^a	1.4 ^a	1.4 ^a	0.1	1.3	1.4	1.3	1.4	0.1159	0.0126	0.0408
BUN ³ , mg/dL	2	3	2	3	0.2	3.0	3.0	2.0	3.0	1.0000	0.0889	0.2515
Creatinine, mg/dL	0.15	0.16	0.15	0.15	0.01	0.16	0.15	0.15	0.16	0.6158	0.4526	0.6158
Uric acid, mg/dL	2.9	3.2	3.2	3.4	0.3	3.1	3.3	3.1	3.3	0.4920	0.5699	0.9046
SOD ⁴ , U/mg protein	171.7	178.1	171.2	183.3	2.0	174.9	177.3	171.5	180.7	0.9343	0.4011	0.8690
GPx ⁵ , U/mg protein	199.4	211.5	200.8	219.7	2.5	205.5	210.3	200.1	215.6	0.4265	0.0364	0.8167

¹Low crude protein (CP) diet, 21 and 18% CP, 0.45 and 0.34% methionine for starter and finisher periods; high CP diet, 23 and 20% CP, 0.50 and 0.38% methionine.

²Standard error of means.

³Blood urea nitrogen.

⁴Superoxide dismutase.

⁵Glutathione peroxidase.

Table 4. Effect of betaine (Bet) supplementation on nutrient digestibility in broilers.¹

Items, %	Low CP		High CP		SEM ²	CP		Bet		P-value		
	Bet 0	Bet 0.12%	Bet 0	Bet 0.12%		Low	High	0%	0.12%	CP	Bet	CP × Bet
Dry matter	72.08	72.75	72.64	73.53	0.46	72.42	73.09	72.36	73.14	0.1542	0.1020	0.8091
Nitrogen	66.34	67.31	67.22	68.11	0.34	66.83	67.67	66.78	67.71	0.0179	0.0097	0.9104
Energy	69.13	71.95	69.83	71.94	0.49	70.56	70.89	69.48	71.95	0.1964	0.0598	0.7573

¹Low crude protein (CP) diet, 21 and 18% CP, 0.45 and 0.34% methionine for starter and finisher periods; high CP diet, 23 and 20% CP, 0.50 and 0.38% methionine.

²Standard error of means.

Table 5. Effect of betaine (Bet) supplementation on concentrations of bacterial metabolites in broiler excreta.¹

Items, ppm	Low CP		High CP		SEM ²	CP		Bet		P-value		
	Bet 0	Bet 0.12%	Bet 0	Bet 0.12%		Low	High	0%	0.12%	CP	Bet	CP × Bet
Ammonia	31.4	30.4	31.7	31.0	0.3	30.9	31.4	31.6	30.7	0.2155	0.0225	0.6441
Hydrogen sulfide	2.1	2.2	2.6	2.5	0.2	2.2	2.6	2.4	2.4	0.0371	0.8562	0.6233
Mercaptan	1.5	1.6	1.6	1.5	0.1	1.6	1.6	1.6	1.6	1.0000	0.9217	0.1746
Acetic acid	26.1	25.1	26.0	25.8	1.2	25.6	25.9	26.1	25.5	0.8218	0.5919	0.7487

¹Low crude protein (CP) diet, 21 and 18% CP, 0.45 and 0.34% methionine for starter and finisher periods; high CP diet, 23 and 20% CP, 0.50 and 0.38% methionine.

²Standard error of means.

($P < 0.05$). The decreased hydrogen sulfide emission in low CP treatments was comparable with that in high CP treatments ($P < 0.05$). However, changes in betaine and CP levels did not have any significant effects on the emission of total mercaptan or acetic acid. There was no significant interactive effect of betaine supplementation and dietary CP on excreta noxious gas emission (Table 5).

Meat Quality and Organ Weights

Dietary betaine supplementation and different CP levels did not have any significant effects on pH, meat color, WHC, cooking loss, or drip loss in broiler chickens. There was no significant effect of betaine supplementation and dietary CP on the relative organ weights of broiler chickens. There was no significant interactive effect of betaine supplementation and dietary CP on meat quality and organ weights (Table 6).

DISCUSSION

The present study aimed to determine the effects of dietary protein levels and betaine supplementation, and their relationships with growth performance, blood profiles, nutrient digestibility, noxious excreta gas emission, and meat quality of broiler chickens.

The application of betaine in poultry nutrition has been known to influence productivity. Addition of betaine in feed, has been the subject of studies on improvement of BWG and FCR (Virtanen and Rosi, 1995; Hassan et al., 2005; Chen et al., 2018). During periods of osmotic disturbance caused by heat stress in broiler chickens, betaine is involved in the protection and development of the morphological characteristics of the intestinal epithelia, resulting in improved growth and feed efficiency (Hamidi et al., 2010; Sakomura et al., 2013). However, other studies have revealed minimal or no effect of betaine supplementation on production

Table 6. Effect of betaine (Bet) supplementation on carcass quality and organ weight in broilers.¹

Items	Low CP		High CP		SEM ²	CP		Bet		P-value		
	Bet 0	Bet 0.12%	Bet 0	Bet 0.12%		Low	High	0%	0.12%	CP	Bet	CP × Bet
pH value	5.33	5.37	5.33	5.36	0.05	5.35	5.35	5.33	5.37	0.9842	0.4899	0.9684
Breast muscle color												
Lightness (L*)	54.76	54.28	55.14	54.41	0.53	54.52	54.78	54.95	54.35	0.6569	0.3048	0.8311
Redness (a*)	10.38	10.39	10.24	10.33	0.19	10.39	10.29	10.31	10.36	0.6307	0.7967	0.8496
Yellowness (b*)	8.45	8.59	8.55	8.37	0.19	8.52	8.46	8.50	8.48	0.7605	0.9163	0.4128
WHC ³ , %	55.36	55.13	56.61	55.48	1.48	55.25	56.05	55.99	55.31	0.8425	0.4405	0.5320
Cooking loss, %	18.43	18.45	18.45	18.46	0.55	18.44	18.46	18.44	18.46	0.9795	0.9738	0.9993
Drip loss, %												
d 1	4.14	3.91	3.97	3.85	0.19	4.03	3.91	4.06	3.88	0.5631	0.3892	0.7923
d 3	8.87	8.77	8.85	8.73	0.17	8.82	8.79	8.86	8.75	0.8597	0.4868	0.9564
d 5	11.43	11.41	11.41	11.32	0.15	11.42	11.37	11.42	11.37	0.7314	0.7455	0.8416
d 7	14.07	14.00	13.93	13.56	0.17	14.04	13.75	14.00	13.78	0.0817	0.1744	0.3650
Relative organ weight ⁴ , %												
Breast muscle	18.13	17.69	18.23	18.96	0.66	17.91	18.60	18.18	18.33	0.2857	0.8192	0.3652
Liver	2.87	2.87	2.90	2.93	0.07	2.87	2.92	2.89	2.90	0.5547	0.8128	0.7925
Bursa of Fabricius	0.16	0.18	0.17	0.16	0.01	0.17	0.17	0.17	0.17	0.8859	0.7379	0.2364
Abdominal fat	1.48	1.50	1.52	1.53	0.11	1.49	1.53	1.50	1.52	0.7266	0.8808	0.9964
Spleen	0.10	0.10	0.11	0.11	0.01	0.10	0.11	0.11	0.11	0.5833	0.9374	0.8138
Gizzard	1.08	1.12	1.13	1.17	0.05	1.10	1.15	1.11	1.15	0.2193	0.3637	1.0000

¹Low crude protein (CP) diet, 21 and 18% CP, 0.45 and 0.34% methionine for starter and finisher periods; high CP diet, 23 and 20% CP, 0.50 and 0.38% methionine.

²Standard error of means.

³Water holding capacity.

⁴Based on live body weight (average 2604, 2625, 2890, and 2810 g) per each treatment.

performance in terms of BWG, feed efficiency, or egg production during growth in broilers or laying hens (Saunderson and Mckinlay, 1990; Harms and Russell, 2002; Park and Ryu, 2011). Through its methyl donating property, betaine may have spared the dietary methionine, which could be available for other vital functions like protein synthesis and immune modulation, resulting in increased growth performance in broiler chickens. However, as methionine was low in the diet in the present study, there was no indication that the addition of betaine had the potential to spare methionine. The results of the present study were consistent with those of previous studies by Saunderson and Mckinlay (1990) and Park and Ryu (2011), who also showed that dietary replacement of methionine with betaine did not improve productivity in broilers and laying hens. This finding suggests that betaine has no direct effect on the improvement of productivity performance in growing broiler chickens, in comparison with 2 levels of CP levels and methionine.

Total serum protein represents the sum of albumin and globulins, the measurements of which indicate the total amount of protein in blood. There is scientific evidence to confirm that some plasma constituents can be used as indicators of health and functional effectiveness of some organs and immune efficiency of animals. For instance, total protein is positively related to synthesis of tissue for the growth of pigs and broiler chicks (Kapelanski et al., 2004; Piotrowska et al., 2011; Law et al., 2017). Therefore, total protein can reflect protein synthesis and nutritional status. It can also reflect dehydration, kidney disease, liver disease, and many other conditions. If total protein is abnormal, further tests

must be performed to identify which protein fraction is abnormal, so that a specific diagnosis can be made. The mean values obtained in the present study are within the range cited by Harr (2002) that is 2.5 to 4.5 g/dl. In addition, BUN is the product of protein metabolism of poultry, which can be affected by dietary protein intake and protein catabolic rate. Reduced concentrations of serum BUN were confirmed in broilers fed low-protein diets (Yang et al. 2009). In the present study, total serum protein, albumin, BUN ($P = 0.0889$) concentrations were greater in broilers fed high levels of CP and 0.12% betaine diets, than in broilers fed low levels of CP and no betaine diets. In addition, increase in serum albumin was observed to be more effective by the supplementation of 0.12% betaine in low CP diets. This could indicate that protein status was improved in broilers fed betaine-supplemented diet. The increase in serum total protein, albumin, and BUN due to 0.12% betaine supplementation could be associated with its characteristic as a methyl group donor, which is fairly consistent in protein metabolism. Improvements in total protein, albumin, and BUN due to betaine supplementation, could be evidence of the role of methyl donor groups in protein metabolism, although there were no methyl donor effects on overall growth performance.

The antioxidant mechanism of betaine remains unclear. A previous study speculated that the antioxidant properties of betaine were related to higher free radical scavenging abilities (Alirezai et al., 2012). However, there are currently insufficient data to support this notion in poultry. There are several antioxidant defence mechanisms to preserve the integrity of biological membranes from detrimental oxidative stress caused by

free radicals and reactive oxygen species. Among them, enzymes such as SOD and GPx, are first line defense antioxidants (Ray and Husain, 2002). Alirezaei et al. (2012) reported that betaine enhanced SOD and GPx activities in the muscle of broiler chickens, experiencing methionine deficiency induced oxidative stress. The antioxidant activity of betaine may also be due to its ability to block the induction of mitochondrial lipid peroxidation (Ganesan et al., 2007). Erman et al. (2004) demonstrated that betaine exerted cellular and subcellular membrane stabilization in the liver by restoring both non-enzymatic and enzymatic antioxidants. It has been shown that betaine is involved in the synthesis of methionine, which serves as a major supplier of cellular cysteine, via trans-sulfuration pathways, for the synthesis of reduced glutathione, that protects the cell from reactive metabolites and reactive oxygen species (Craig, 2004). An increase in the GPx enzyme was observed in betaine treatments in the present study. Therefore, betaine has antioxidant properties, which could protect cells and organ systems against oxidative damage in broiler chickens.

In this study, broilers fed diets containing betaine revealed higher levels of nitrogen, dry matter ($P = 0.1020$), and energy ($P = 0.0598$) digestibility as compared with broilers that were fed a no betaine diet. This result confirms previous observations that betaine improves total tract digestibility of CP, amino acids, and dry matter (Remus et al., 1995; Augustine and Danforth, 1999; Eklund et al., 2006; El-Husseiny et al., 2007; Mosenthin et al., 2007) in pigs and poultry. Therefore, in this study, improvement in energy digestibility may be attributed to increased organic matter digestibility. Kettunen et al. (2001) identified that betaine decreased the ratio of villus height/crypt depth both in healthy and coccidian challenged broilers, which may partially explain the increased digestibility of nutrients when supplemented with betaine. The osmotic properties of betaine also support intestinal cell growth and survival, and enhance cell activity, thereby potentially influencing nutrient digestibility (Ratriyanto et al., 2009). The present study indicates that betaine decreases excreta ammonia gas emission in broilers. We found no studies regarding the influence of betaine on excreta gas emission in broilers. However, excreta gas emissions correlated with nutrient digestibility. Considering that betaine improves nitrogen digestibility, as shown in the present study, theoretically, the reduction in ammonia emission levels should be easily understood. Therefore, we expect that supplemental betaine can decrease odors in broiler housing due to reduced excreta ammonia gas emission in growing broilers.

Betaine has been reported to affect some carcass characteristics and meat qualities. It has been shown that betaine accumulates in the muscles of pigs (Matthews et al., 2001), thus possibly affecting meat quality. Alterations in the water-retention capacity of the muscle tissue following dietary betaine supplementation may increase total body weight and carcass weight (Esteve-Garcia and Mack, 2000). Some

studies suggest a close relationship between the pH of meat and supplemental dietary betaine. The initial pH of the meat was increased in pigs, in association with a decreased drip loss of the meat, when the diet was supplemented with betaine (Matthews et al., 2001). In contrast, other authors have reported that betaine supplementation was less effective in improving carcass characteristics of broiler chickens and pigs (Waldroup and Fritts, 2005; Fernandez-Figares et al., 2008). The results of the present study confirmed that dietary betaine has no effect on the meat quality of broiler chickens. Therefore, further study is needed to determine the effects of betaine on meat quality in broiler chickens, as there is a lack of such information.

In conclusion, the results of this study indicate little or no positive benefits, in terms of productivity and meat quality, from the supplementation of betaine to the different CP diets of broiler chickens. However, betaine supplementation, regardless of dietary CP levels, increased serum protein, albumin, and GPx concentrations, and nitrogen digestibility, whereas excreta ammonia gas emission decreased. In addition, increase in serum albumin was observed to be more effective by the supplementation of 0.12% betaine in low CP diets. Betaine supplementation seems to be related to the efficient use of protein for the methionine sparing effect, but it did not lead to improvement in the broiler performance.

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