



Partial Replacement of Betaine on Broiler Birds Performance

Shivani Katoch et al.

Effect of Betaine Partially Replaced with Methionine on Performance and Carcass Quality of Broiler Birds

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ABSTRACT

A feeding trial was conducted to evaluate the methionine-sparing effects of betaine and their effects on growth performance of broiler chickens fed methionine (Meth)-deficient diets. A total of 266, 1-day-old broiler chicks (Vancobb-400) were used in a completely randomized study with 6 replicate pens of 11 birds each. The treatments comprised a positive control (control-; 0.27% Meth. - recommended level), 0.15% betaine (control- plus 0.17% Low Meth.), 0.20% betaine (control- plus 0.17% Low Meth.) and 0.25% betaine (control- plus 0.17% Low Meth.). Results of the study indicate that supplementation of betaine up to the level of 0.25 % in a low Meth. diet has the potential to support growth performance making the cost of feeding more economical. Supplementation of betaine at 0.25 % with low Meth. also function as carcass fat and cholesterol modifier significantly reducing the carcass fat and breast cholesterol content. Betaine supplementation at 0.25% increased serum albumin level indicating its role as a methyl donor. Supplementing diets up to 0.25% betaine reduced serum uric acid concentration, which in turn may reduce its excretion, thereby reducing ammonia emission in poultry houses and nitrogen emission into the environment.

KEY WORDS: Betaine, Broiler Chicken, Carcass quality, Growth performance, Serum metabolites.

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INTRODUCTION

Methionine is the first limiting amino acid in poultry. Its main use is in protein synthesis and feather growth. It also reduces oxidative stress by increasing glutathione. Besides this it acts as primary methyl donor for plethora of metabolic reactions in bird body (Finkelstein 1990). Methionine is converted to S-adenosylmethionine which donates methyl group for various biochemical reactions. However, under methionine-deficient conditions or stress, there is a need to recycle methionine back into the protein synthesis pool. This happens via trans methylation cycle, where final step is conversion of homocysteine to methionine. Betaine is formed from choline in mitochondria. Thus, part of methyl group sparing can be done by choline and betaine. Unlike plant and animal species, poultry have a limited ability to produce betaine within the body. A variety of foodstuffs of plant and animal origin contains betaine which is a naturally occurring amino acid derivative.

Owing to low quantity of betaine in animal feedstuffs; dietary supplementation can improve productivity and relieve the adverse effects of stress (Wang et al., 2004). Betaine, both serves as a methyl group donor and is an osmolyte assisting in cellular water homeostasis (Petronine et al., 1992). Betaine may serve as an important alternative methylating agent when normal methylating pathways are impaired by ethanol ingestion, drugs, or nutritional imbalances (Barak and Tuma, 1983). Betaine has also been found helpful in modifying the carcass characteristics by reducing the carcass fat content and increasing the percent of lean carcass, which may be of interest to satisfy consumer needs. Betaine is involved in protein and energy metabolism due to its methyl group donor function (Eklund et al., 2005). A study was therefore done to evaluate the methionine replacement by betaine containing methyl group analogues in chicken broiler birds at different supplementation levels viz. 0.15, 0.20 and 0.25 percent.

MATERIAL AND METHODS

Experimental design and management

The research work was carried out in Experimental Poultry House of Department of Animal Nutrition, DGCN College of Veterinary & Animal Sciences, CSK HP Agriculture University, Palampur, HP, India. At the start of the experiment, broiler chicks of Vancobb-400 strain were weighed individually on 0-day, wing tagged and distributed randomly according to the experimental plan in battery brooder for the first seven days. Chicks were immediately offered *ad libitum* feed and lukewarm water upon arrival. The standard management practices were followed for rearing the chicks in litter prepared with rice husk at a depth of around 6 inches. The temperature and relative humidity of poultry house was maintained at 21 to 30°C and 40 to 60 percent respectively. Proper ventilation and light were made available to birds throughout the trial.

Experimental Plan and feed formulation

Birds were distributed randomly into four groups T0, T1, T2 and T3. Each group was divided into six replicates with 11 chicks in each replicate, thus each treatment had 66 birds. Birds were shifted from brooder house to deep litter system on 7th day. T0 served as positive control group and was given standard corn-soy flake-based ration (Table-1) with added 2.7 Kg DL-Methionine/ ton, treatment T1 served as treatment group with added betaine provided by M/S Jubilant Pvt. Ltd. Noida by the trademark Anichol Ultra® @ 1.5kg/Ton (0.15%) +1.7kg DL-methionine + 0.625 Kg Choline chloride. Treatment T2 was given standard ration with added betaine (AniChol Ultra®) @ 2.0kg/Ton (0.20%) +1.7kg DL-methionine + 0.625 Kg Choline chloride. Further treatment T3 was given standard corn- soy flake-based ration with added betaine (AniChol Ultra®) @ 2.5kg/Ton (0.25%) +1.7kg DL-methionine + 0.625 Kg Choline chloride.

Partial Replacement of Betaine on Broiler Birds Performance

Table 1. Composition of Experiment Diets (dry matter basis)

Physical Composition	Starter 1-21 days				Finisher 22-42 days			
	T0 (%)	T1 (%)	T2 (%)	T3 (%)	T0(%)	T1 (%)	T2(%)	T3(%)
Maize Yellow (9%)	50.36	50.36	50.36	50.36	57.00	57.00	57.00	57.00
Soybean meal (45%)	44.00	44.00	44.00	44.00	36.36	36.36	36.36	36.36
Palm Oil	2.00	2.00	2.00	2.00	3.00	3.00	3.00	3.00
Di-calcium Phosphate (DCP)	3.00	3.00	3.00	3.00	1.50	1.50	1.50	1.50
Limestone Powder (LSP)	0.00	0.00	0.00	0.00	1.50	1.50	1.50	1.50
Common Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine (99%)	0.27	0.17	0.17	0.17	0.27	0.17	0.17	0.17
L-Lysine (99%)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
AniChol Ultra %	0.00	0.15	0.20	0.25	0.00	0.15	0.20	0.25
Premix#	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Choline Chloride-60% (gm)	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5
Nutrient Composition (Fed Basis)								
Crude Protein %	22.39	22.39	22.39	22.39	20.00	20.00	20.00	20.00
Ether Extract %	1.80	1.80	1.80	1.80	1.88	1.88	1.88	1.88
Crude Fibre %	3.90	3.90	3.90	3.90	3.30	3.30	3.30	3.30
Total Ash %	2.88	2.88	2.88	2.88	3.24	3.24	3.24	3.24
Calcium %	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total Phosphorus %	0.86	0.86	0.86	0.86	0.57	0.57	0.57	0.57
Analysis(Calculated)								
Nitrogen Free Extract %	58.86	58.86	58.86	58.86	60.6	60.6	60.6	60.6
ME (Kcal/Kg)	2867	2867	2867	2867	2987	2987	2987	2987
Lysine %	1.30	1.30	1.30	1.30	1.18	1.18	1.18	1.18
Methionine %	0.52	0.40	0.40	0.40	0.45	0.35	0.35	0.35
Cysteine %	0.36	0.36	0.36	0.36	0.32	0.32	0.32	0.32
Threonine %	0.97	0.97	0.97	0.97	0.85	0.85	0.85	0.85
Tryptophan %	0.92	0.92	0.92	0.92	0.95	0.95	0.95	0.95
Digestible Lysine %	1.22	1.22	1.22	1.22	1.09	1.09	1.09	1.09
Digestible Methionine %	0.44	0.33	0.33	0.33	0.38	0.29	0.29	0.29
Digestible Tryptophan%	0.64	0.64	0.64	0.64	0.66	0.66	0.66	0.66
Digestible Cysteine %	0.25	0.25	0.25	0.25	0.22	0.22	0.22	0.22
Digestible Threonine %	0.82	0.82	0.82	0.82	0.72	0.72	0.72	0.72

#Premix was prepared by mixing the following in 200 g maize flour: VETRIX 10g (vitamin A-82,500 IU, B2-50 mg, D3-12,000 IU, K-10 mg/g); VENTRIBEE PLUS-20g (vitamin B1-25 mg, B6-35 mg, B12-250µg, E-225mg, Pantothenate- 225 mg, Niacinamide-300mg, Folic acid -20 mg/5g); E-care Se forte -25 mg (Vitamin E- 0.20g ,Se-0.04mg/g); Super DOT (Dinitro-o-TOULAMIDE-250 mg ,Ethopabate- 16 mg/g); Trace minerals- 100g (Ferrous oxide -2 g, Dicalcium phosphate -54 g, Copper sulphate -2g, Manganese sulphate- 3 g, Zinc sulphate -0.6g, Zinc oxide-1 g, Ferrous sulphate-10 g, Potassium iodide-2.5 g, Magnesium sulphate-25 g).

Proximate analysis

The feed samples were analyzed for proximate principles in the laboratory of the Department of Animal Nutrition following the procedure as recommended in (AOAC, 2005). The calcium in feed samples was analysed in Atomic Absorption Spectrometer (AAS) (LABINDIA AA8000 Atomic Absorption Spectrophotometer), while phosphorus content was estimated spectrophotometrically (Spectronic 200 spectrophotometer) using the method proposed by Parks & Dunn (1963). The metabolizable energy (ME) was calculated using the equation as proposed by Lodhi et al. (1976).

Feeding Management

Pre-weighed formulated feed was offered twice daily as per the experimental plan to the broiler birds from 0 day till 42 days of age. The diets (in mash form) were formulated according to recommendations from the ICAR-New Delhi (ICAR, 2013), based on corn-soybean diets containing CP 22% and methionine 0.52% (0.44%) in positive control T₀, CP 22% and methionine 0.40% (0.33%) in T1, T2 and T3 for 1 to 21 d, and CP 20% and methionine 0.45% (0.38%) in positive control T0, CP 20% and methionine 0.35% (0.29%) in T1, T2 and T3 for 22 to 42 d (Table 1).

Growth performance

Various parameters like body weight gain (BWG), Feed intake (FI) and Feed conversion ratio (FCR) were recorded. The body weight of broilers was measured on days 1, 7, 14, 21, 28, 35 and 42 of the experimentation periods. After measuring the remaining feed on days 1, 7, 14, 21, 28, 35 and 42, BWG, FI and FCR were calculated. Dead birds were recorded to determine the mortality (%) during the experiment. Viability, economic factors including the European efficiency factor (EEF), was calculated using the following formulae (Bera et al., 2010; Lup et al., 2012):

$$\text{Viability} = \{1 - (\text{n of dead birds} / \text{total birds})\} \times 100$$

$$\text{EEF} = \{\text{BW} \times \text{viability} \times 100 / (\text{FCR} \times \text{age})\}$$

Where EEF is the European efficiency factor and BW is the average body weight (kg). Age was measured in days.

Carcass parameters

At the end of feeding trial, three birds from each replicate were slaughtered by cervical dislocation. The birds were fasted over night to drain the intestinal content and sacrificed to assess the effect of various dietary treatments on live weight, eviscerated weight, dressing percentage, weight of adipose tissue, breast, skin, heart, liver, spleen and bursa.

Meat Cholesterol estimation

Muscle lipid extract was made based on the Folch et al. (1956) method. Lipid extract was prepared by taking one gram of meat sample and adding 10 mL of freshly prepared 2:1 chloroform:methanol solution and homogenized. Homogenate was filtered using Whatman No. 42 filter paper. An equal quantity of distilled water was added to 5 mL of filtrate, mixed and centrifuged at 3,000 rpm for 7 minutes. The top layer (methanol) was removed by suction. The volume of the bottom (chloroform) layer having cholesterol was recorded. Total cholesterol was determined as per Zaltkis et al. (1953) with some modification. The test sample was prepared by mixing 0.1 ml of lipid extract and 9.9 ml of ferric chloride acetic acid reagent and centrifuging at 2000 rpm for 10 minutes. 5 mL of this supernatant was used as a test sample. For standard, 4.9mL of ferric chloride reagent was mixed with 0.1mL of cholesterol solution of known concentration. 5 mL of ferric chloride reagent was used as blank. 3 mL of sulphuric acid was added to all tubes. Tubes were rested for 20-30 minutes. The O.D. of standard and sample against blank was taken at 560 nm. Total cholesterol mg percent was recorded as follows:

$$\text{Cholesterol (mg/100gm)} = \text{O.D. of sample} \times \text{Vol. of cholesterol (mL)} \times \text{Conc. Of std.}$$

$$\text{O.D. of standard} \times \text{weight of sample taken (gm)}$$

Blood profile

Approximately 3 to 5 mL of blood from randomly chosen birds of each replicate was collected from ulnar vein with the help of 24gauge (24G) needle and collected in 10 percent heparin coated centrifuge tubes. After collection of the whole blood, the blood was centrifuged at 2000 × g for 15 minutes. The resulting supernatant, designated plasma was collected and transferred into a clean polypropylene tube using a Pasteur pipette and were analyzed for blood biochemical profiles. The samples were then stored at - 20°C and later analyzed in the lab by using Erba Mannheim CHEM 5 ÷ plasma analyzer, with the help of the kits of Agape diagnostics

in the Department of Animal Nutrition, COVAS, Palampur.

Statistical Analysis

All the recorded and calculated data were subjected to analysis of variance (ANOVA) by methods of Snedecor and Cochran (1968) using complete randomized block design (CRBD). Results were evaluated at 5 percent level of significance.

RESULTS AND DISCUSSION

Average daily and weekly parameters of feed intake, weight gain and FCR/feed efficiency Mortality%, litter moisture% and EPEF are presented in Table 2.

Table 2. Effect of betaine supplementation at different levels on overall growth performance.

Parameter	T0	T1	T2	T3
	Meth-270g/100 kg	Betaine 0.15% + Meth-170g/100 kg	Betaine-0.20% + Meth-170g/100 kg	Betaine-0.25% + Meth-170g/100 kg
ADFI, g				
1-21d	1057±12.8	1076±27.4	1079±20.8	1022±7.12
21-42d	3041±34.0	3071±65.9	3030±39.5	3012±4.32
1-42d	4098±18.5	4147±138	4109±111	4034±48.7
ADWG, g				
1-21d	591±10.8	634±14.9	605±15.2	611±11.9
21-42d	1543±34.6	1579±34.2	1564±40.9	1513±36.1
1-42d	2135±36.8	2214±40.3	2169±42.6	2124±27.9
FCR g/g				
1-21d	1.79±0.039	1.70±0.045	1.79±0.050	1.67±0.037
21-42d	1.97±0.010	1.95±0.077	1.94±0.096	1.99±0.090
1-42d	1.91±0.119	1.87±0.062	1.89±0.099	1.89±0.096
Mortality %				
1-21d	1.51±0.012	3.03±0.01	3.03±0.02	Nil
21-42d	Nil	1.51±0.01	3.03±0.01	Nil
1-42d	1.51±.012	4.54±.012	6.06±.022	Nil
Litter Moisture %	66.95±3.54	70.09±3.90	72.09±2.71	69.33±3.06
EPEF	262	268	256	267

Average Daily Feed Intake (ADFI); Average Daily Weight Gain (ADWG); FCR (Feed Conversion Ratio); EPEF (European Poultry Efficiency Factor)

Results of the experiment revealed no significant (P>0.05) difference on growth, feed intake and weekly body weight gain. During the period from 1-42 d, mortality percent was within the permissible limits and did not show any significant difference. However, litter moisture percent was recorded to be comparatively higher in treatments T1, T2, T3 and exhibited higher EPEF of 268, 256 and 268 compared to 262 in positive control T0. The highest value

of EPEF was exhibited by treatment T1 and T3 offered low Meth. based diet.

Biochemical parameters

Results for blood biochemical parameters namely total protein, albumin, globulin, uric acid, creatinine, triglycerides, cholesterol, HDL-Cholesterol, LDL-Cholesterol, SGPT and SGOT are presented in Table 3.

Table 3. Effect of betaine supplementation at different levels on blood biochemical parameters.

Parameter	T0	T1	T2	T3
	Meth-270g/100 kg	Betaine-0.15% + Meth-170g/100 kg	Betaine-0.20% + Meth-170g/100 kg	Betaine-0.25% + Meth-170g/100 kg
Total protein g/dL	4.30±.54	4.28±.29	3.12±.25	4.10±.95
Albumin g/dL	1.80±.17	2.19±.21	2.25±.25	2.17±.08
Globulins g/dL	2.84±.37	2.09±.08	0.88±.24	1.93±.87
Uric Acid mg/dL	10.40±1.57	6.48±1.25	6.59±1.76	4.79±.33
Creatinine mg/dL	0.11±.10	0.51±.11	0.40±.32	0.39±.15
Triglyceride mg/dL	66.47±11.9	95.76±16.7	53.45±15.6	67.57±15.9
Cholesterol mg/dL	83.80±9.87	148.14±39.8	114.13±15.1	121.45±9.90
HDL -Cholesterol mg/dL	41.82±4.11	37.94±11.0	43.91±3.30	41.09±8.27
LDL-Cholesterol mg/dL	26.77±1.30	28.57±6.96	44.70±4.47	50.69±9.59
ALT IU/L	15.38±10.4	43.96±12.6	33.89±3.36	25.99±6.18
AST IU/L	178.76±16.1	228.16±26.6	246.88±37.7	311.06±31.28

Treatments T1, T2 and T3 exhibited higher serum albumin, but low globulin level whereas total protein level comparatively did not show any significant ($P>0.05$) difference. Similarly, uric acid level tended to be higher in control diet compared to all betaine supplemented diets which recorded lower uric acid level. The serum creatinine level tended to be on higher side in all betaine supplemented diets whereas low serum creatinine level was recorded in T0. Treatment diets T1, T2, T3 exhibited higher serum cholesterol and LDL-Cholesterol with highest value recorded in T3 at 0.25 percent betaine addition. The triglyceride and HDL- cholesterol level was almost

similar in all the treatments. AST and ALT level in all the treatment groups T1, T2, T3 were within a range of 15.38 to 43.96 IU/L and 178.76 to 311.06 IU/L and did not exhibit any significant ($P>0.05$) difference.

Carcass characteristics

Results of the experiment demonstrated no significant ($P>0.05$) effect on dressing, skin, liver heart, spleen and bursa percent (Table 4) but numerically, lower weight of adipose tissue was recorded in betaine supplemented treatments.

Partial Replacement of Betaine on Broiler Birds Performance

Table 4. Effect of betaine supplementation at different levels on carcass characteristics.

Parameter	T0	T1	T2	T3
	Meth-270g/100 kg	Betaine-0.15% + Meth-170g/100 kg	Betaine-0.20% + Meth-170g/100 kg	Betaine-0.25% + Meth-170g/100 kg
Live Weight- gm	2468±33.5	2556±48.0	2580±119.94	2406±40.96
Dressing %	75.58±1.80	79.06±.89	75.63±1.58	77.03±1.20
Breast%	24.06±0.90	23.75±0.83	24.11±0.68	25.02±0.38
Adipose tissue %	1.96±0.18	1.58±0.20	1.67±0.32	1.23±0.09
Skin%	7.68±0.45	7.93±0.53	6.97±0.39	7.07±0.30
Liver %	2.00±0.02	2.05±0.05	2.35±0.08	1.81±0.18
Heart %	0.68±0.01	0.61±0.02	0.65±0.00	0.54±0.02
Spleen %	0.07±0.01	0.09±0.01	0.10±0.01	0.11±0.01
Bursa%	0.20±0.00	0.24±0.02	0.26±0.03	0.26±0.01
Dry Matter%	24.25±0.65	24.70±0.31	24.81±0.95	26.19±0.27
Crude Fat%	23.83±1.51	25.61±2.37	26.45±3.02	22.59±1.60
Crude protein%	68.60±2.47	66.61±2.51	67.66±1.67	68.24±0.76
Cholesterol (Leg) mg/100g	46.94±11	61.15±11.82	53.21±5.54	68.55±4.70
Cholesterol (Breast) mg/100g	48.28 ^a ±3.08	15.30 ^b ±3.48	13.15 ^b ±2.89	14.20 ^b ±1.46

Fig. with different superscripts in a row are statistically ($P < 0.05$) significant from each other.

The content of adipose tissue deposition numerically decreased with increased supplementation of betaine at 0.25 percent compared to 0.20 and 0.15 percent. Similarly, higher betaine supplementation at 0.25 percent also recorded non-significant ($P > 0.05$) but numerically higher (25.02) breast and dressing percent (77.03). Results of the experiment demonstrated no significant ($P > 0.05$) effect of low methionine in treatments on analysis of chicken meat for dry matter, crude fat and crude protein (Table 4). Similarly, percent crude fat was lowest in 0.25 percent betaine supplemented treatment T3 signifying leaner meat. Treatment diets T1, T2 and T3 tend to deposit higher cholesterol content in leg meat but lower content in breast meat.

Results of the experiment for growth studies revealed no significant difference for growth performance and feed conversion in treatment birds offered lower methionine than recommended levels

when supplemented with betaine. Many of the authors have reported that under normal conditions (no coccidiosis or heat stress), dietary supplementation of betaine (0.05 to 0.15%) increased ADG, gain: feed (Hassan et al., 2005), breast yield (Waldroup and Fritts, 2005). On the other hand, others have reported no effect on growth (Waldroup and Fritts, 2005) or carcass quality (Schutte et al., 1997). Results of the experiment signify that supplementation of betaine at graded levels of supplementation viz. 0.15, 0.20 and 0.25 percent in low meth-based treatment diets displayed comparable growth and feed conversion to positive control treatment offered diet with higher methionine. In this regard Rostagno and Pack (1995) and Turker et al. (2004) reported that betaine supplementation to corn soybean meal-based diets marginally deficient in methionine and choline improved growth performance and resulted in similar

percentage of carcass as well as meat protein and ether extract than those of positive control. Moreover, betaine can replace up to 25% of meth. without adversely affecting the production performance of the broilers, indicating that up to 25% of dietary meth. is involved in methylation reactions in broilers (Sun et al., 2008). Betaine supplemented treatments at 0.15, 0.20 and 0.25 percent exhibited higher EPEF of 273, 265 and 260 compared to 258 in positive control. The mortality percent was similar and did not exhibit any significant ($P>0.05$) difference and was well within the acceptable standard management practices. Results of the experiment also emphasized the potential of betaine as a methyl group donor replacing methionine and support growth and feed conversion in broilers offered low methionine-based diet. Previous studies have also reported better results under sub optimal conditions (Spreeuwenberg et al., 2007), though Wang et al., (2004) did not show any effect of betaine supplementation on low methionine-based diets on carcass yield in meat duck.

Betaine has also been reported to function as an osmolyte. It is reported that betaine may stabilize osmotic stress by minimizing water loss of the cells lining the gastrointestinal tract (Kettunen et al., 2001; Klasing et al., 2002). Betaine supplementation in the water (2.5 g/l) has been reported to alleviate diarrhea and reduce litter moisture in turkey production systems (Ferket, 1995) however, in this experiment no such effect was observed probably because there was no heat stress which generally triggers betaine to function as an osmolyte, whereas in our experiment there was no such trigger. The results of the experiment thus signify, that betaine can support optimal BWG, FI and FCR to the extent of 0.25 percent supplementation in low methionine-based diets in broiler chicken without any adverse effects.

Betaine has been reported to influence the characteristics and quality of chicken meat. Many of the previous studies reported enhanced breast and carcass yield without affecting body weight of unsexed broilers (Ratriyanto et al., 2014). Similarly,

McDevitt et al., (2000) reported improved carcass composition including enhanced breast yield in broilers supplemented with betaine offered low methionine-based diets. In the present study, the potential of betaine as a carcass modifier was limited to the reduction of adipose tissue and meat fat content (0.25% betaine) and significantly ($P<0.05$) lowering the cholesterol content in the breast muscle. Further, numerically but non-significantly higher carcass yield and breast percent at 0.25% betaine supplementation was in line with the previous studies (Ratriyanto A et al., 2014). Many of the authors have reported no effect of betaine supplementation on carcass characteristics (Waldroup and Fritts, 2005; Fernandez-Figares et al., 2008). Effect of betaine supplementation is still inconsistent influenced by factors like methyl group donor level in the diet, betaine level and source, as well as age and species of animals (Ratriyanto A et al., 2009). Results of the experiment revealed that supplementation of betaine at 0.15 and 0.20 percent generated a similar response while 0.25 percent supplementation displayed higher efficiency in reducing abdominal fat deposition and meat fat content. The results are consistent with previous reports of the potential of betaine as a carcass modifier (Ratriyanto et al., 2014; 2009). Similarly, Zhan et al., (2006) reported decreased abdominal fat deposition in broilers fed diet deficient in methionine supplemented with betaine. Supplementation of betaine in diets of poultry (Hassan et al., 2005; Zhan et al., 2006; Xing et al. 2011) depressed overall fat deposition and increased lean deposition. Betaine as a carcass modifier in reducing the carcass fat content involves many of the mechanisms associated with lipotropic activity. Like methyl group donated by betaine increases the synthesis of lecithin, carnitine, creatinine and hormone sensitive lipase activity which functions to prevent fat deposition (Ratriyanto et al., 2019). Similarly, Huang et al. (2008) found that betaine supplementation decreased the activity of acetyl-CoA carboxylase, fatty acid synthase (FAS), and malic enzyme in subcutaneous adipose tissue of pigs by 18.0%, 18.8%, and 14.5%, respectively. Additionally, betaine supplementation also increased the activity

of hormone sensitive lipase and increased concentrations of NEFA were found in the plasma (Huang et al., 2006). This suggests that betaine can reduce lipogenesis and increase lipolysis via hormonal changes. Dietary betaine may influence lean deposition by stimulating the secretion of growth hormone (GH) and insulin-like growth factor (IGF)-1, enhancing the insulin receptor signaling pathway, increasing creatine synthesis, or by preventing muscle dehydration (protein denaturation) (Cholewa et al., 2014).

Treatment diets supplemented with betaine exhibited higher serum albumin but low globulin level whereas total protein level comparatively did not show any significant difference except for 0.20 percent betaine supplementation which recorded lowest level. Total serum protein is the sum of albumin and globulins. Total protein level in the blood is an indicator of the health and immune status of the animals and is positively correlated to the synthesis of the tissues for 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 (Park et al., 2019). It can also reflect dehydration, kidney disease, liver disease and many other conditions. The mean value obtained in this study is within the range quoted by Harr (2002) that is 2.5 to 4.5 g/dL. In the present study, the concentration of serum albumin was found to be more in all betaine supplemented treatment diets. The increase in serum albumin in all the betaine supplemented diets may be related to its characteristic as methyl group donor and indicates an improved protein status in broilers. Thus, supplementation of betaine at 0.15, 0.20 and 0.25 percent level seems to be role of betaine as a methyl group donor although it did not affect the overall growth performance. Serum uric acid level was well within the range in all betaine supplemented diets whereas uric acid level tended to be higher in control diet with high methionine and no betaine supplementation. Supplementing diets with 280 mg/kg of choline significantly reduced the serum concentration of uric acid (Mohammad Mahmoudi et al., 2018). The urea values determined in the

present study for betaine supplement are consistent with those found by Batina et al. (2005) for broilers, ranging between 6.5 and 7.76 mg/dL. Uric acid is the main product of protein nitrogen and purine metabolism in poultry. Approximately 90% of the uric acid is secreted by the proximal convoluted tubules in healthy broilers (Harr, 2002). Since serum uric acid levels are influenced by the diet, hydration status, and species (Lumeij, 2008) it seems that betaine supplementation up to 0.25 percent in the present study balanced the rate of urea excretion without putting any extra load on the kidneys. The serum creatinine level tended to be similar and within the range for all low methionine and betaine supplemented diets. Therefore, relatively low levels of creatinine and uric acid may be an indication of the renal protective effects of the feed supplements containing betaine.

CONCLUSION

Betaine up to the level of 0.25 percent in low methionine-based diets can support growth performance, reduced carcass fat and breast cholesterol content. Further Betaine supplementation at 0.25 percent increased serum albumin level, reduced serum uric acid and creatinine levels. Thus, it has potential to reduce ammonia emission in poultry houses and nitrogen emission into the environment. However, the latter needs further investigation.

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