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Effects of Betaine Supplementation to Methionine Deficient Diet on Growth Performance and Carcass Characteristics of Broilers

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Abstract: Problem Statement: The efficacy of Methionine (Met) sparing effect of Betaine (BET) has been shown to be associated with dietary compositions, animal physiological stage and living conditions. This study was to determine the extent to which dietary Met could be replaced by BET in broiler chickens under the feeding conditions specific to Chinese poultry industry.**Approach:** A total of 900 day-old Arbor Acres broiler chicks were fed three corn-soybean meal-based starter rations (d 1-21) and grower rations (d 22-42) for a total of 42 days. Met levels in the diets were: Diet 1, Met content at the recommended level (Control); diet 2, Met level at 85% of the Control supplemented with BET at the level of 400 (starter) or 300 (grower) mg kg^{-1} DM; Diet 3, Met level at 75% of the Control supplemented with BET at the level of 600 (starter) or 500 (grower) mg kg^{-1} DM. The broilers were raised in a temperature controlled house with 3 pens (replicates) per dietary treatment. **Results:** In general, treatment had no effect on body weight, feed intake or feed efficiency. Concentrations of growth hormone and insulin-like growth factor-1 in the serum of broilers fed Diet 3 were higher ($p<0.05$) than that of broilers of other treatments. Supplementation of BET at the level of replacing 25% of total Met increased ($p<0.05$) breast meat yield and protein content of breast meat and liver, but decreased ($p<0.05$) abdominal fat yield and ether extract content of liver. **Conclusions/Recommendations:** Supplementation of BET to replace up to 25% of total dietary Met did not affect the growth performance but improved the carcass quality of the broilers. BET could be used to spare 25% of the total Met in broiler diet that was formulated based on the Feeding Standard of China.

Key words: Broiler, betaine, methionine, growth performance, carcass characteristics

INTRODUCTION

Dietary labile methyl groups were proposed to be nutrients by du Vigneaud *et al*.[1]. Methionine (Met), choline, Betaine (BET) and folic acid are all considered as methyl donors to body metabolic reactions and have been shown to compensate for the partial deficiency of labile methyl groups in corn-soybean-based diets[2-5]. Modern nutrition has revealed that Met is one of the most limiting amino acids that play a crucial role in body protein synthesis and therefore it would be beneficial to spare its function as an methyl donor. It has been shown that folic acid has to take methyl group before liberating methyl group and choline first has to be activated and then converted to betaine before methyl groups are liberated to fulfill methylation function[6]. In contrast, BET contains three methyl groups in its structure and donates these in several metabolic reactions. On a molecular weight basis, BET contains about 3.75 times the methyl groups of Met and therefore would be an effective compound to spare dietary Met as methyl donor.

However, the Met sparing effect of BET has been the subject of some controversy. Some studies have shown positive responses of animals to BET supplementation in met deficient diets, which included improved animal performance and carcass characteristics[7-11]. These responses were obtained when 1 part of BET was supplemented to replace 2 parts of dietary DL-Met. However, the Met sparing effect of BET was not observed in other studies[12,13]. It seems that the efficiency of Met sparing effect of BET is associated with dietary compositions, animal physiological stage and living conditions. The objective of this study was to

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determine the effect of BET supplementation to a Met deficient diet on growth performance and carcass characteristics of broiler chickens under the feeding conditions specific to Chinese poultry industry.

MATERIALS AND METHODS

Animals and experimental design: Nine hundreds of day-old Arbor Acres (AA; mixed sex) broiler chickens obtained from a local hatchery were randomly distributed among 9 pens with 100 chickens per pen. The chicks in 9 pens were then randomly allocated to 3 dietary treatments that were arranged as a complete randomized block design. The experiment was conducted in 2 phases: starter (1-21 d) and grower (22-42 d). All birds were fed corn-soybean meal based basal diets (Table 1) that were formulated to meet the nutrients requirement for chickens (NY/T 33-2004) of Feeding Standard of the People's Republic of China except for Met content. The 3 dietary treatments were: Diet 1: Met content at the recommended level (Control) with no BET supplementation; Diet 2: Met level at 85% of the Control supplemented with BET at the rate of 400 (starter) or 300 (grower) mg kg^{-1} DM; Diet 3: Met level at 75% of the Control supplemented with BET supplementation at the rate of 600 (starter) or 500 (grower) mg kg^{-1} DM. Methionine content in each diet was achieved by supplementation of a commercial available methionine product (DL-Methionine) to the basal diet. The amounts of BET supplemented to Diets 2 and 3 were calculated on the basis of 1 BET replacing 2 Met as methyl donors to compensate for the Met deficiency in these two diets. Betaine hydrochloride (Weifang Sunwin Chemicals Co., Ltd, China) was used as the source of BET. The treatments and contents of Met and BET of each diet were summarized in Table 2. Each diet was pelleted in one batch, stored in covered containers and was used for the entire experimental period.

Chicks were housed in a temperature-controlled room with raised wire floor. Room temperature was maintained at 35°C for the first 3 d and then gradually reduced to 24°C by the rate of 2°C every 3 days. Feed were provided for ad libitum intake and the birds had free access to water throughout the entire experimental period. Light was provided 24 h continuously with overhead incandescent lighting by 10 lux during the first 2 wk period of the experiment and was then decreased gradually (2 h day^{-1}) to 20 h daily by wk 3 and maintained at this level till the end of the experiment.

Growth and carcass measurements: Body Weight (BW), feed intake (dry matter basis) and mortality of each pen were recorded weekly and Average Daily Gain (ADG), Average Daily Feed Intake (ADFI) and Feed: Gain (F:G) were calculated at the ages of 21 and 42 d. On d 21 and 42 of the feeding trial, nine birds per treatment were randomly picked out (3 per pen) after an overnight (12 h) fast, weighed and sacrificed to evaluate carcass characteristics according to the procedure described by Wang[14]. Prior to being slaughtered, birds were individually weighed and blood sample (5.0 mL) was taken from wing vein of each bird into eppendorf tubes containing coagulant (Haimen city, Jiangsu province, P. R. China). The blood samples were immediately centrifuged (1744×g, 10 min, 4 °C) with a low speed table centrifuge (80-2B, Shanghai, P. R. China) and the serum was stored at -20°C in sealed container till analyzed. The serum samples were analyzed for Growth Hormone (GH) and Insulin-like Growth Factor-1 (IGF-1) with radioimmunoassay method. All samples were analyzed in one batch to avoid inter-assay variations.

Table 1: Composition (%) and nutrient content of the experiment basal diets[z]

Ingredient (%)	Starter	Grower
Corn	48.94	55.52
Wheat-middlings	10.00	10.00
Peanut meal	12.00	11.00
Soybean meal, CP 43%	10.00	4.00
Cottonseed protein	4.00	4.00
Cottonseed meal	7.00	7.00
Corn protein	2.00	1.50
Animal oil	1.50	2.50
Lysine	0.40	0.51
Threonine	-	0.05
Dicalcium phosphate	1.00	0.80
Phytase	0.01	0.01
Limestone	1.70	1.70
Salt	0.25	0.25
1%Premix[Y]	1.00	1.00
Calculated composition		
Methionine (%)	0.28	0.24
Crude protein (%)	21.00	18.60
Metabolizable Energy (Kcal·kg^{-1})	2869	2979
Calcium (%)	0.98	0.87
Total phosphorus (%)	0.71	0.63

[z]Nutrient level of the diets was based on feeding standard of chicken of the People's Republic of China (NY/T 33-2004)

[Y]Premix supplied the following amounts of vitamin and minerals to per kg of diet for age of 1-21 d: vitamin A, 15300 IU; vitamin D_3, 3740 IU; vitamin E , 40.8 IU; vitamin K_3, 5.1 mg; thiamin, 3.4 mg; riboflavin, 10.2 mg; vitamin B_6, 5.1 mg; vitamin B_{12}, 0.0204 mg; choline chloride, 1,000 mg; pantothenic, 15.3 mg; niacin, 61.2 mg; biotin, 0.204 mg; folic acid, 1.7 mg; Mn,108 mg; Fe,100 mg; Zn, 88 mg; Cu, 9.6 mg; I, 0.374 mg and Se, 0.224 mg; and for age of 22-42 d: Vitamin A, 13500 IU; vitamin D_3, 3300 IU; vitamin E, 36 IU; vitamin K_3, 4.5 mg; thiamin, 3 mg; riboflavin, 9 mg; vitamin B_6,4.5 mg; vitamin B_{12}, 0.018 mg; choline chloride, 800 mg; pantothenic, 13.5 mg; niacin, 54 mg; biotin, 0.18 mg; folic acid, 1.5 mg; Mn, 108 mg; Fe, 100 mg; Zn, 88 mg; Cu, 9.6 mg; I, 0.374 mg and Se, 0.224 mg

Table 2: Arrangement of treatments and amounts (mg kg^{-1} DM) of Methionine (Met) and betaine supplemented to each diet

	Starter				Grower			
	Met Basal diet	Met Supplement	Total Met	Betaine Supplement	Met basal diet	Met Supplement	Total Met	Betaine Supplement
Diet 1[Z]	2800	2000	4800	0	2400	1600	4000	0
Diet 2	2800	1200	4000	400	2400	1000	3400	300
Diet 3	2800	800	3600	600	2400	600	3000	500

[Z] Diet 1: Total methionine content at the recommended level (Control); Diet 2: Total methionine content at 85% of the Control supplemented with betaine at the ratio of 1 (betaine): 2 (methionine) to compensate for the 15% reduction of total methionine; Diet 3: Total methionine content at 75% of the Control supplemented with betaine at the ratio of 1(betaine): 2 (methionine) to compensate for the 25% reduction of total methionine. Methionine content in each diet was achieved by supplementation of a commercial available methionine product (DL- methionine) in the amount (mg.kg^{-1} DM) as described in the table

Table 3: Body weigh (BW), average daily gain (ADG), average daily feed intake (ADFI), feed:gain ratio (F:G) and mortality of broilers chicken fed experimental diets during a 42-d period of feeding experiment

	Control[z]	Diet 2	Diet 3	SEM[y]
BW (g)				
1 d	46	46	46	0.750
21 d	657[b]	695[a]	680[ab]	9.350
42 d	2414	2420	2425	39.680
ADFI (g d^{-1})				
1-21 d	46.5	47.8	46.6	0.530
1-42 d	103.7[a]	103.5[a]	99.5[b]	0.920
ADG (g d^{-1})				
1-21 d	29.1[b]	31.0[a]	30.2[ab]	0.460
1-42 d	57.5	57.6	57.7	0.950
F: G				
1-21 d	1.60	1.54	1.54	0.024
1-42 d	1.84	1.83	1.76	0.037
Mortality (%)				
1-21 d	1.30	1.30	1.00	0.270
1-42 d	2.30	2.00	1.70	0.540

[z] Please see Table 2 for the description of dietary treatment
[y] SEM, standard error of means
[a,b]: Within a row, means without a common superscript letter differ ($P<0.05$)

The birds were slaughtered after blood samples taken and the carcass plucked to determine Carcass Weight (CW). Intestines, windpipe, reproductive organ, gall bladder, spleen, oesophagus and content and cuticle of gizzard were then removed and semi-eviscerated carcass weight was obtained. The semi-eviscerated carcass was further processed to remove head, neck, legs, heart, liver, proventriculus, gizzard and abdominal fat to obtain the eviscerated weight. Abdominal fat including the fat that manually excised from the abdominal cavity (i.e., fat adhering to the gizzard, surrounding the bursa of fabricius, the cloaca and adjacent muscles) and breast meat was weighed after separation. Sub-samples were taken from liver and breast meat and stored at -20°C for subsequent analyses of Crude Protein (CP) and Ether Extract (EE) content[15,16]. Yield of Abdominal Fat (AFY), Carcass Yield (CY), Semi-Eviscerated Yield (SEY) and Eviscerated Yield (EY) were determined as percentage of live body weight at slaughtering, whereas Breast Meat Yield (BMY) was calculated as percentage of the eviscerated weight. This study was performed in accordance with local ethical guidelines.

Statistical analysis: Data were statistically analyzed by one-way ANOVA using GLM procedure of SAS[17] with individual pen as statistical unit. Differences among dietary treatment were compared using Duncan's multiple range tests. A significance level of $p<0.05$ was used.

RESULTS

Growth performance: Broiler chickens consumed Diet 2 had higher ($p<0.05$) BW and ADG compared to the broilers consumed Control diet, whereas this difference was not observed between birds ate Control diet and Diet 3 during the first 21-d experiment (Table 3). All broiler chickens had similar ($p>0.05$) BW at the end of 42-d experiment and similar ($p>0.05$) ADG over the entire experiment. On the contrary, all birds had similar ADFI during the starter phase (1-21 d) and F: G in two phases regardless of the dietary treatments. However, broiler chickens consumed Diet 3 had lower ($p<0.05$) ADFI compared to the broilers consumed Control diet and Diet 2. Furthermore, no significant difference was observed in mortality among treatments for birds during the entire experimental period.

GH and IGF-1: Birds consumed Diet 3 had highest levels of serum GH and IGF-1 on both 21 and 42 d of ages (Table 4). Supplementation of BET to diet with 25% Met deficiency increased ($p<0.05$) serum concentrations of GH at 42 d and IGF-1 at 21 and 42 d of ages. However, birds consumed Diet 2 (BET supplement to 15% Met deficient diet) had similar serum concentrations of GH and IGF-1 ($p>0.05$) to the Control birds regardless of the age.

Carcass characteristics: Carcass characteristics of broiler chickens as affected by dietary treatments were

Table 4: Concentration of Growth Hormone (GH) and insuline-like growth factor-1 (IGF-1) in the serum of broiler chickens at 21 or 42-d of age

	Diet 1(Control)[z]	Diet 2	Diet 3	SEM[y]
GH (ng mL^{-1})				
21 d	1.11	1.17	1.37	0.174
42 d	1.14[b]	1.16[b]	1.61[a]	0.212
IGF-1 (ng mL^{-1})				
21 d	10.83[b]	14.17[b]	19.39[a]	2.543
42 d	19.96[b]	19.38[b]	26.20[a]	0.870

[z] Please see Table 2 for the description of dietary treatment
[y] SEM, standard error of means
[a-c]: Within a row, means without a common superscript letter differ (P<0.05)

Table 5: Carcass Yield (CY), Semi-Eviscerated Yield (SEY), Eviscerated Yield (EY), Breast Meat Yield (BMY) and Yield of Abdominal Fat (AFY) of broilers chicken slaughtered at 21 or 42-d of age

	Diet 1(Control)[z]	Diet 2	Diet 3	SEM[y]
CY (%)				
21 d	92.83	92.87	93.62	0.511
42 d	92.85[ab]	92.50[b]	93.53[a]	0.274
SEY (%)				
21 d	82.89	83.89	83.86	0.384
42 d	86.79	86.23	86.91	0.345
EY (%)				
21 d	68.00	68.67	68.99	0.408
42 d	73.60	73.78	74.27	0.652
BMY (%)				
21 d	23.02	23.23	23.25	0.360
42 d	24.65[b]	25.19[ab]	26.01[a]	0.373
AFY (%)				
21 d	1.08	1.01	0.96	0.064
42 d	1.55[a]	1.39[ab]	1.17[a]	0.105

[z] Please see Table 2 for the description of dietary treatment
[y] SEM, standard error of means
[a-b]: Within a row, means without a common superscript letter differ (P<0.05)

summarized in Table 5. Carcass yield of birds consuming Diet 3 was higher (p<0.05) than that of birds consuming Diet 2 at 42 d of age, but was similar (p>0.05) to other groups at 21 d of age. All birds slaughtered had similar SEY, EY regardless of dietary treatments or slaughter ages. Supplementation of BET to diet of 25% Met deficiency increased (p<0.05) BMY but decreased (p<0.05) AFY (Diet 3 vs. Control) at 42 d of age only. The similar trend, however, was not observed when BET was supplemented to diet of 15% Met deficiency (Diets 2 vs. Control).

Dietary treatment had no effect (p>0.05) on contents of CP and EE in breast meat of birds at 21 d of age (Table 6). At 42 d of age, however, breast meat of birds consuming Diet 3 had higher (p<0.05) contents of CP and EE than the birds consuming Control diet. Similarly, CP content in the liver sample of broilers fed Diet 3 was also higher (p<0.05) than that of broilers fed Control diet, Diet 2 at both ages. On the contrary, the EE content of the liver was lower (p<0.05) for broilers fed Diet 3 than for broilers fed other diets at the age of 42 d.

Table 6: Contents (%, DM basis) of crude protein and ether extract in Breast Meat (BM) and liver of broiler chickens slaughtered at 21 or 42-d age

	Diet 1(Control)[z]	Diet 2	Diet 3	SEM[y]
Crude protein				
BM (21 d)	78.60	79.32	79.02	0.807
BM (42 d)	78.96[b]	80.26[ab]	81.94[a]	0.734
Liver (21 d)	71.93[b]	72.11[b]	75.39[a]	0.398
Liver (42 d)	67.58[c]	69.01[b]	69.45[a]	0.376
Ether extract				
BM (21 d)	4.39	4.09	5.54	0.539
BM (42 d)	3.45[b]	3.18[b]	4.56[a]	0.263
Liver (21 d)	10.50[a]	9.66[a]	5.41[b]	0.871
Liver (42 d)	17.40[a]	16.16[b]	13.90[c]	0.324

[z] Please see Table 2 for the description of dietary treatment;
[y] SEM, standard error of means
[a-c]: Within a row, means without a common superscript letter differ (P<0.05)

DISCUSSION

The growth performance of the broiler chickens as affected by dietary treatments in this study was consistent with observations of Saunderson and Mckinlay[18] and Guo *et al.*[8] who showed no difference in body weight between broilers fed diets supplemented with DL-methionine or DL-methionine + betaine. However, this experiment revealed that supplementation of BET to the diet of 15% Met deficiency increased growth rate of broilers at the starter phase as compared to that of chickens consumed the diet with normal level of Met (Control). Other studies also showed that supplementation of choline or BET increased ADG of birds fed diets marginally deficient in Met[19-20]. Furthermore, betaine is an osmolyte that could improve intestinal structure and function to increase growth performance[21,22]. It appears, thus, that BET and Met in marginally Met-deficient diets could lead to an equivalent growth response in broilers and that BET could spare a small portion of the Met.

It was interesting that all birds which had similar body weight at the end of 42-day experiment had the lower ADFI consuming Diet 3 compared to birds consuming Control diet and Diet 2. The reason for the reduced feed intake by BET when supplemented to diet of 25% Met deficiency but not of 15% Met deficiency is not known. The results, however, showed that the reduction of feed intake by BET did not negatively affect the growth of broiler chickens and therefore led to an improved feed efficiency by 4.34%. Emmert *et al.*[19] found that feeding Met-deficient diets to chickens increased the activity of Betaine-Homocysteine-Methyltransferase (BHMT) that specifically catalyses the transport of the preformed labile methyl group from the BET molecule to homocysteine that can be irreversibly transformed to cysteine for body protein synthesis and/or can be re-methylated by other methyl

sources to form Met. It was also showed that supplementation of BET to chickens increased BHMT activity as well[23,24]. Therefore the improved feed efficiency with supplementation of BET to the diet of 25% Met deficiency is partially due to the joint action of Met deficiency and BET supplementation. The effect of BET supplementation on improving feed efficiency was consistent with its effect on increasing serum concentrations of GH and IGF-1 that have been demonstrated to be positively related to feed efficiency[25,26]. Esteve-Garcia and Mack[12] also reported that BET supplementation improved feed efficiency. In contrast, Schutte *et al*.[27] did not found positive effect of BET supplementation to the diets containing 0.05 or 0.10% added DL-Met on feed efficiency. Collectively, these results suggest that the effect of BET supplementation on feed efficiency may depend on diet composition, dietary level of Met and level of BET supplementation.

There are great variations regarding to what extent of dietary Met could be replaced by BET in broiler chickens, ranging from none to complete replacement. Differences in dietary composition especially level of Met, feeding management and health condition of the experimental birds may all attribute to the difference of chicks in responses to the replacement of Met with BET observed from these studies. The present study showed that up to 25% of dietary total Met could be replaced by BET without negatively affecting birds' growth performance.

The higher BMY of birds consuming Diet 3 compared to that of birds consuming Diet 2 or Control indicated that BET supplemented at the level of replacing 25% of the dietary Met increased the BMY at the end of 42-d growth period. This compared to the similar body weight and carcass yield of all treatments at the same age of the birds suggests that BET supplemented at the level of replacing 25% of dietary Met may have the positive effect on partitioning nutrients towards protein synthesis in breast meat. This hypothesis is supported by the elevated serum levels of GH and IGF-1 and increased protein content of the breast meat by this level of BET supplementation as that shown in Tables 4 and 6 and by the observation of Zhan[28] who reported that BET supplementation increased GH and IGF-1 concentration in the blood. Growth hormone and IGF-1 have been well recognized to promote protein synthesis[29-31]. Other researchers also observed that BET improved breast meat yield[13]. On the contrary, Abdominal Fat Yield (AFY) as well as fat content in the liver was reduced, whereas fat content in the breast meat was increased by the supplementation of BET at the level of replacing 25% of dietary Met. These results were consistent with other reports[10,32]. However, Esteve-Garcia and Mack[12] reported that the effects of BET on breast yield and abdominal fat were small and non-significant while BET can significantly increase carcass yield. All of these indicated that growths of different tissues in broiler chickens responded to BET supplementation differently.

The exact mechanism that BET affects carcass composition is not clear. It has been proposed that the improvement of carcass lean percentage could be attributed to the increased availability of Met and cystine for protein deposition in BET-supplemented diets[13]. Xu and Zhan[23] showed that BET supplementation enhanced the synthesis of methylated compounds such as carnitine that is required for the transport of fatty acids through the inner mitochondrial membrane where fatty acid oxidation takes place and that has been shown to reduce carcass and liver lipid content in pigs [33]. In addition, dietary supplementation of BET has been shown to support the synthesis of phosphatidylcholine that is a limiting element in the synthesis of Very Low Density Lipoprotein (VLDL)[34, 35] and VLDL prevents the deposition of fat in the liver and accelerates the removal of fat from the liver[36]. All of these could contribute to the alteration of carcass compositions observed in this study.

CONCLUSION

Broiler chickens fed diets with supplementation of BET at the level in replacing 15 or 25% of dietary Met had similar growth performance to the chickens fed diet of normal Met content over a 42-d feeding period. However, supplementation of BET at the level in replacing 25% of dietary Met increased concentrations of GH and IGF-1 in the serum and increased breast meat yield (%) and its protein and fat content, but reduced the fat content in the liver as compared to the non-BET supplemented diet of normal Met content. This study demonstrated that 25% of total Met in the diet that was formulated to meet the Met requirement based the Feeding Standard of P. R. China could be replaced by BET.

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