

## The Effect of Different Levels of Choline and Betaine on Broilers Performance and Carcass Characteristics

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**Abstract:** An *in vivo* experiment was conducted to determine the effect of dietary betaine supplementation (Betafine) as a replacement for choline on broiler performance and carcass characteristics. The diets containing four betaine levels at 0, 33, 66 and 100% in replacement for choline and two basal diets (containing of 2.5% oil and lacking of oil) were used in a Completely Randomized Design (CRD) with a 2×4 factorial arrangement and four replicates of 10 birds/replicate. Three hundred twenty-day-old Ross broiler chicks were randomly distributed in cages and fed the experimental diets from 0-49 days of age. Feed and water were provided *ad-libitum*. Feed intake, body weight gain and feed conversion ratio were recorded weekly. At 49 days of age, one bird from each replicate was selected for blood sample collection and comparison of carcass characteristics. Betaine replacement for choline had no effect on feed intake, weight gain and feed conversion ratio but, a significant difference ( $p<0.05$ ) in body weight gain (at 0-3 and 3-6 weeks of age) and feed conversion ratio (at 0-3 weeks of age) was observed among different treatments. Betaine as a replacement for choline increased the breast weight and reduced the abdominal fat ( $p<0.05$ ), but had no significant effect on thigh, liver, fat of liver percentages and blood parameters. The present findings indicated that although replacement of betaine instead of choline in diets of broiler didn't improve significantly feed intake, weight gain, feed conversion ratio, percentage of carcass characteristics and blood parameters, but resulted in significant reduction of abdominal fat and significant increase of breast meat.

**Key words:** Betaine, choline, performance, carcass characteristics, broiler

### INTRODUCTION

Betaine, the common term for trimethylglycine, is a naturally occurring amino acid derivative found in a variety of foodstuffs of plant and animal origin. Betaine has 2 primary metabolic roles: it is a methyl group donor and it is an osmolyte that assists in cellular water homeostasis (Petronine *et al.*, 1992). Betaine, choline and methionine can be sources of methyl (-CH<sub>3</sub>) groups. It is well understood that choline may act as a methyl group donor but in order to function as a methyl group donor, it needs to be converted to betaine in the mitochondria. Betaine is synthesized from choline by choline oxidase and it can donate methyl groups to homocysteine to form methionine (Molitoris and Baker, 1976).

Many studies have examined the interrelationship between choline and methionine and between betaine and methionine to determine if these compounds can spare the needs of the chick for methionine with considerable variation in results. While, some studies have failed to demonstrate that the methionine content of the diet could

be reduced by supplementation with betaine (Rostango and Pack, 1996; McDevitt *et al.*, 2000). However, several studies suggest that addition of betaine may improve breast meat yield (Schutte *et al.*, 1997; McDevitt *et al.*, 2000). Betaine is indirectly involved in the synthesis of carnitine, which is required for transporting long chain fatty acids across the inner mitochondrial membrane for oxidation (McDevitt *et al.*, 2000) and therefore, may result in a leaner carcass.

The objective of this study was to examine the choline sparing effect of betaine and evaluation effect of betaine to enhancement of breast meat yield in male broiler.

### MATERIALS AND METHODS

A commercial basal diet was formulated to meet the nutritional requirements of the birds. Three hundred twenty-day-old Ross broiler chickens were randomly assigned to cages in a Completely Randomized Design (CRD) with a factorial arrangement and four replicates per

Table 1: Composition and nutrient contents of diets (%)

Ingredient	Starter (0-21 day)		Grower (21-42 day)		Finisher (42-49 day)	
Corn	62.95	53.80	60.00	53.25	60.00	50.43
Soybean meal	30.95	30.00	21.73	20.86	20.63	20.22
Fish meal	3.00	3.00	3.00	3.00	3.00	3.00
Wheat	-	-	8.74	5.00	11.71	12.00
Wheat bran	-	7.59	4.28	12.89	4.90	11.00
Sunflower oil	-	2.50	-	2.50	-	2.50
Calcium carbonate	1.13	1.11	1.18	1.19	1.23	1.30
Salt	0.37	0.37	0.24	0.24	0.22	0.21
Dicalcium phosphate	1.00	0.99	0.57	0.54	0.78	1.00
Permixon without choline <sup>1</sup>	0.50	0.50	0.50	0.50	0.50	0.50
DL-methionine	0.10	0.11	0.03	0.03	0.03	0.03
Total	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient composition <sup>2</sup> ME (kcal kg <sup>-1</sup> )	2900.00	2900.00	2900.00	2900.00	2900.00	2900.00
Crude protein	20.83	20.83	18.12	18.12	16.30	16.30
Calcium	0.90	0.90	0.81	0.81	0.72	0.72
Avail P	0.40	0.40	0.31	0.31	0.27	0.27
Sodium	0.18	0.18	0.13	0.13	0.11	0.11
Lysine	1.13	1.13	0.92	0.91	0.78	0.78
Methionine	0.45	0.45	0.34	0.34	0.29	0.29
Met + Cys	0.94	0.94	0.68	0.68	0.55	0.55
Arginin	1.32	1.32	1.10	1.10	0.97	0.99
Threonine	1.12	1.10	0.97	0.96	0.96	0.95
Tripophane	0.41	0.40	0.37	0.36	0.29	0.29

<sup>1</sup>Supplied per kg of diet: Vitamin A, 10000 IU; Vitamin D<sub>3</sub>, 9790 IU; Vitamin E, 121 IU; B<sub>12</sub>, 20 g; riboflavin, 4.4 mg; calcium pantothenate, 40 mg; niacin, 22 mg; biotin, 30 g; thiamine, 4 mg; zinc sulphate, 60 mg; manganese oxide, 60 mg; <sup>2</sup>Calculated from NRC (1994) adjusted to crude protein and moisture content of ingredient used in formulation

treatment and 10 birds per cage (replicate). Dietary treatments consisted of a 2×4 factorial arrangement of treatments with 2 oil levels (without or 2.5% oil) and four betaine treatments (0, 33, 66 and 100% in replacement for supplemental choline (mg mg<sup>-1</sup>)). The dietary protein: energy ratio was formulated according to or in excess of NRC (1994). Feed and water were provided *ad-libitum* and chicks had access to 24 h light during the experiment. The experiment diets are shown in Table 1. From 1-49 days of age, feed consumption, body weight gain and feed conversion ratio were recorded weekly. On day 49, one bird from each replicate of treatments (close to mean body weight for each replicate) was selected for blood samples collection and comparison of carcass characteristics. The thigh, breast, abdominal fat pads, liver, fat of liver and blood parameters were recorded.

**Statistical analysis:** Data were analyzed according to General Linear Model (GLM) procedure of SAS software as a CRD factorial experiment. The data in percentage were first transformed to its Arc sin (%) and then analyzed. Duncan's multiple range tests was also used to compare means.

## RESULTS AND DISCUSSION

The effect of the treatments on feed consumption and body weight gain is shown in Table 2 and 3. Feed consumption within the different periods given was not affected by treatment. Body weight gain was reduced

significantly at 0-3 weeks of age in T<sub>1</sub> (without oil and 0% replacement) and at 3-6 weeks of age in T<sub>1</sub> (without oil and 0% replacement), T<sub>4</sub> (without oil and 100% replacement) and T<sub>8</sub> (with 2.5% oil and 100% replacement).

Since, the demand of broiler chicks for betaine increases by growth rate and stress. In addition to during the first weeks, the chicks are under environmental stresses, so it is obvious that the need betaine is high during this time. At growth phase the choline requirements of broiler chicks are stated in two forms: essential need and replaceable need. The need for choline not only decreases by age, but also can follow an increasing pattern. Because the size of all cells and parts of body enlarges and choline is present in the all membranes structures, so it is clear that the need for choline is high during the growth period. Therefore, the declines of body weight gain of these three experimental groups compared to the others are may justified by this explanation.

The effect of treatments on the feed conversion ratio is given in Table 4. There was significant effect of betaine replacement on feed to gain ratio at 0-3 weeks of age within T<sub>7</sub> (with 2.5% oil and 66% replacement). It may be mentioned that by using this amount of replacement, it can be achieved the benefits of betaine in the diet and supplied the amount of choline that is needs for particular and essential functions.

The carcass characteristics of experimental chicks are shown in Table 5. Carcass composition was not affected, except breast weight and abdominal fat pad, by betaine

replacement. Breast weight showed a significant increase effect ( $p < 0.05$ ) with an increase in the amount of betaine within  $T_8$  (with 2.5% oil and 100% replacement). Also the percentage of abdominal fat pad to body weight showed a significant reduction ( $p < 0.05$ ) within

$T_3$  (without oil and 66% replacement). Blood parameters were not affected by different treatments (Table 6).

There is a noticeable variation in the published literature regarding the efficacy of betaine, choline for methylation. It is reported that betaine methylates

**Table 2: Effect of different levels of choline replaced with betaine on body weight gain (g) from 0-49 days of age**

Levels of replacement (%)	Probability>F								SE	Measurement		
	Diet without of oil				Diet with 2.5% oil					Basal diet	Replacement	Basal diet× replacement
	0	33	66	100	0	33	66	100				
0-21 day	457.25 <sup>a</sup>	477.75 <sup>b</sup>	477.00 <sup>b</sup>	471.75 <sup>b</sup>	472.75 <sup>b</sup>	479.75 <sup>b</sup>	491.05 <sup>b</sup>	492.25 <sup>b</sup>	17.10	0.165	0.047	0.786
21-42 day	1482.50 <sup>a</sup>	1572.00 <sup>b</sup>	1579.75 <sup>b</sup>	1550.00 <sup>a</sup>	1581.25 <sup>b</sup>	1609.25 <sup>b</sup>	1607.00 <sup>b</sup>	1538.25 <sup>a</sup>	22.42	0.241	0.023	0.653
42-49 day	372.75	347.25	367.75	366.00	378.00	350.25	352.50	394.75	16.09	0.172	0.135	0.427
0-49 day	2312.00	2426.00	2423.00	2343.25	2432.00	2439.25	2451.00	2405.00	30.81	0.134	0.119	0.352

Means with common superscripts do not differ significantly ( $p < 0.05$ )

**Table 3: Effect of different levels of choline replaced with betaine on feed consumption (g) from 0-49 days of age**

Leavels of replacement (%)	Probability>F								SE	Measurement		
	Diet without of oil				Diet with 2.5% oil					Basal diet	Replacement	Basal diet× replacement
	0	33	66	100	0	33	66	100				
0-21 day	828.25	824.25	824.25	820.00	880.25	874.25	871.00	879.00	18.510	0.928	0.128	0.704
21-42 day	3115.00	3178.25	3120.50	3180.00	3176.00	3195.00	3111.00	3137.25	59.110	0.347	0.489	0.271
42-49 day	1195.00	1160.25	1191.00	1198.25	1267.00	1252.00	1264.00	1270.00	43.730	0.351	0.169	0.608
0-49 day	5133.50	5162.75	5135.75	5198.25	5332.25	5321.25	5246.00	5286.25	43.73	0.531	0.274	0.403

Means with common superscripts do not differ significantly ( $p < 0.05$ )

**Table 4: Effect of different levels of choline replaced with betaine on feed conversion ratio from 0-49 days of age**

Levels of replacement (%)	Probability>F								SE	Measurement		
	Diet without of oil				Diet with 2.5% oil					Basal diet	Replacement	Basal diet× replacement
	0	33	66	100	0	33	66	100				
0-21 day	1.775 <sup>a</sup>	1.732 <sup>a</sup>	1.735 <sup>a</sup>	1.762 <sup>a</sup>	1.830 <sup>b</sup>	1.795 <sup>a</sup>	1.830 <sup>b</sup>	1.760 <sup>a</sup>	0.038	0.318	0.031	0.314
21-42 day	2.050	1.930	1.920	1.939	2.010	1.932	1.901	2.030	0.317	0.224	0.412	0.284
42-49 day	3.271	3.308	3.218	3.318	3.258	3.292	3.187	3.230	0.047	0.263	0.247	0.183
0-49 day	2.352	2.230	2.279	2.318	3.390	3.305	2.172	2.297	0.037	0.199	0.139	0.343

Values with common superscripts do not differ significantly ( $p < 0.05$ )

**Table 5: Effect of different levels of choline replaced with betaine on carcass characteristics at 49 d (%)**

Levels of replacement (%)	Probability>F								SE	Measurement		
	Diet without of oil				Diet with 2.5% oil					Basal diet	Replacement	Basal diet× replacement
	0	33	66	100	0	33	66	100				
Abdominal fat	2.332 <sup>a</sup>	2.332 <sup>a</sup>	1.890 <sup>b</sup>	2.220 <sup>a</sup>	2.274 <sup>a</sup>	2.199 <sup>a</sup>	2.160 <sup>a</sup>	2.285 <sup>a</sup>	0.253	0.170	0.045	0.218
Thigh	19.902	19.590	19.385	20.281	19.598	20.101	19.280	19.352	0.569	0.839	0.511	0.560
Breast	18.547 <sup>a</sup>	18.707 <sup>a</sup>	19.050 <sup>a</sup>	19.200 <sup>a</sup>	19.020 <sup>a</sup>	19.041 <sup>a</sup>	19.080 <sup>a</sup>	20.271 <sup>b</sup>	0.708	0.163	0.021	0.879
Liver	2.345	2.320	2.362	2.280	2.230	2.223	2.189	2.205	0.121	0.318	0.960	0.084
Fat of liver	13.874	14.051	14.133	13.901	14.013	14.518	14.390	14.612	3.224	0.306	0.570	0.464

Values with common superscripts do not differ significantly ( $p < 0.05$ )

**Table 6: Effect of different levels of choline replaced with betaine on some of blood parameters at 49 days**

Levels of replacement (%)	Probability>F								SE	Measurement		
	Diet without of oil				Diet with 2.5% oil					Basal diet	Replacement	Basal diet× replacement
	0	33	66	100	0	33	66	100				
Cholesterol	137.75	144.00	141.00	138.50	135.25	133.50	130.75	138.25	7.97	0.280	0.780	0.173
Triglycerides	48.50	48.25	54.50	50.25	52.25	51.50	49.50	46.25	8.33	0.381	0.674	0.154
LDL (mg dL <sup>-1</sup> )	95.05	102.55	100.15	97.25	93.75	96.30	91.45	97.85	6.02	0.115	0.652	0.207
VLDL (mg dL <sup>-1</sup> )	9.73	9.70	10.85	10.65	10.35	10.44	9.62	9.49	1.64	0.312	0.761	0.116
HDL (mg dL <sup>-1</sup> )	28.00	33.75	34.25	30.20	31.50	28.50	29.00	32.75	3.20	0.477	0.822	0.097

Values with common superscripts do not differ significantly ( $p < 0.05$ )

homocysteine to methionine about three times more efficiently than choline (Saunderson and Mackinlay, 1990). Conversely, other experiments showed that betaine, choline and methionine appear to be equivalent as sources of methyl group (Rostango and Pack, 1996). Garcia *et al.* (2000) with using a chick growth model concluded that the sparing of methionine by the methylation of homocysteine to methionine is increased by adding choline or betaine. These results are also confirmed by other who evaluated the effect of supplemental betaine and choline in duck (Wang *et al.*, 2004).

The results of this experiment are in agreement with Rostango and Pack (1996) and McDevitt *et al.* (2000), who also showed dietary with adding different level of betaine increase breast weight. It can stated that betaine can increase the breast meat by synthesis of methionine from homocystin and improving the lysine and methionine absorption. Otherwise researchers reported reduction of abdominal fat in result of effect of betaine on lipid metabolism such as other role of betaine. The result of present study confirms results of Garcia *et al.* (2000) and Wang *et al.* (2004), who reported similar results about of betaine role on lipid metabolism. Probably betaine can do this function by its specific role in carnitine synthesis and induces decrease of carcass fat.

## CONCLUSION

The results of this study indicate little or no positive benefit in terms of body weight gain, feed conversion from the addition of betaine to corn-soybean meal based diets for broiler chicks. At 21 and 42 day of age improvement in body weight gain and conversion ratio feed to gain from betaine supplementation but not 49 day of age. Therefore, age of bird might be a consideration for using these supplements as birds processed at younger ages might be more responsive to these nutrients. Further studies are needed to evaluate the potential effect of age on response to choline and betaine.

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