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Betaine Supplementation to Methionine Deficient Diet Improves Growth Performance, Carcass Characteristics, Blood Parameters and Economic Efficiency of Broilers

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ABSTRACT

The objective of this study was to evaluate the influence of supplementary betaine on growth performance, carcass traits and some blood parameters in broilers fed methionine deficient diets. a total of two hundred and seventy day-old commercial broiler chicks *Cobb* 400, were randomly allotted to nine treatments of six equal replicates and each replicate having five birds (9×6×5=270). Nine iso-nitrogenous and is-ocaloric diets consisting of a control diet (CD), 25% and 50% methionine deficient diet and with graded levels of betaine (1.0, 2.0 and 3.0% levels) were formulated and fed to chicks from 1 to 42 days of age. The results revealed that supplementation of betaine at 0.3% level improved (<0.05) performance of broilers fed methionine deficient diets. Betaine supplementation to methionine restricted diet increased immunity and tolerance to oxidative stress and better net profit over feed cost of broilers. It was concluded that betaine supplementation at 0.3% level in broiler diets containing sub-optimal concentrations of methionine was effective in enhancing the overall performance of commercial broilers.

Key words: Betaine, Broilers, Carcass yield, Breast yield, Body weight gain, Methionine

INTRODUCTION

Betaine is found ubiquitously in plants, animals, microorganisms, and rich dietary sources are seafood, spinach, and wheat bran. The chief physiological role of betaine is to function as a methyl donor and an osmolyte. Betaine helps to maintain the cellular water and ion balance, and to improve the capacity to combat heat stress via preventing dehydration and osmotic inactivation. Betaine is stable and is considered as a non-toxic feed additive (Yu *et al.,* 2004). Review of literature indicate that supplementation of betaine may improve hematology, serum biochemical and enzyme profile (Mishra *et al.,* 2019a, b), and reproductive per performance (Mishra *et al.,* 2019c) in pigs. Previous studies showed that dietary supplementation of betaine may improve digestibility, reduce abdominal fat weight, and increase breast meat yield (Rafeeq *et al.,* 2013, El-Shinnawy, 2015) in poultry. In addition, betaine has been reported to protect internal organs and boost their performance (Eklund *et al.,* 2006). Inclusion of betaine in poultry diets showed sparing effect on choline and methionine. As the methionine supplements are expensive it would be desirable to replace part of it to reduce feed cost. Considering the methionine sparing and other virtues of betaine it was hypothesized that supplementation of betaine would be beneficial to improve performance of broilers fed methionine deficient diets. The specific objective of this experiment was to study the effect of graded levels of beanie on performance of broilers fed diets deficient in methionine.

MATERIALS AND METHODS

A feeding trial of six weeks duration was conducted during February and March, 2017 to assess the feeding value of betaine on growth performance of commercial broilers fed diets deficient in methionine. a total of 270 day-old commercial broiler chicks *Cobb* 400, obtained from local hatchery were wing banded and weighed individually. They were divided into nine treatment groups with six replicates and five birds in each. (9x6x5). Dietary treatments consisted: of one maize - soya-based basal diet (BD), two experimental

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Table 1. Ingredients And Chemical Composition of Different Rations

Ingredient	Pre -Starter Diets (0 - 2 Weeks						Starter Diets (3-4weeks)						Finisher Diets (5-6weeks)					
	BD	MET		BETAINE %			BD	MET		BETAINE %			BD	MET		BETAINE %		
		25% less	50% less	0.1	0.2	0.3		25% less	50% less	0.1	0.2	0.3		25% less	50% less	0.1	0.2	0.3
Maize	55.8	55.8	55.8	55.8	55.8	55.8	55.5	55.5	55.5	55.5	55.5	55.5	62.8	62.8	62.8	62.8	62.8	62.8
Soybean meal	36.1	36.1	36.1	36.1	36.1	36.1	33.9	33.9	33.9	33.9	33.9	33.9	29	29	29	29	29	29
DORB	4.2	4.2	4.2	4.2	4.2	4.2	7	7	7	7	7	7	4.8	4.8	4.8	4.8	4.8	4.8
DL-Methionine	0.2	0.15	0.1	0.15	0.15	0.15	0.22	0.17	0.11	0.17	0.17	0.17	0.16	0.12	0.08	0.12	0.12	0.12
Betaine	0	0	0	0.1	0.2	0.3	0	0	0	0.1	0.2	0.3	0	0	0	0.1	0.2	0.3
Salt	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38
Di calcium phosphate	1	1	1	1	1	1	1.2	1.2	1.2	1.2	1.2	1.2	0.7	0.7	0.7	0.7	0.7	0.7
Limestone powder	2	2	2	2	2	2	1.45	1.45	1.45	1.45	1.45	1.45	1.8	1.8	1.8	1.8	1.8	1.8
Pre-mix[1]	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42
Nutrient levels, on dry matter basis																		
ME (kcal/kg)*	3000	3000	3000	3000	3000	3000	3000	3000	3000	3000	3000	3000	3100	3100	3100	3100	3100	3100
Crude protein (%)**	22.2	22.2	22.1	22.2	22.2	22.2	21.6	21.5	21.5	21.5	21.5	21.5	19.51	19.49	19.46	19.49	19.49	19.49
Lysine (%)*	1.17	1.17	1.17	1.17	1.17	1.17	1.13	1.13	1.13	1.13	1.13	1.13	1	1	1	1	1	1
Methionine (%)*	0.46	0.41	0.36	0.41	0.41	0.41	0.48	0.42	0.37	0.42	0.42	0.42	0.41	0.37	0.33	0.37	0.37	0.37
Threonine (%)*	0.82	0.82	0.82	0.82	0.82	0.82	0.79	0.79	0.79	0.79	0.79	0.79	0.72	0.72	0.72	0.72	0.72	0.72
Calcium (%)*	0.17	0.17	0.17	0.17	0.17	0.17	0.95	0.95	0.95	0.95	0.95	0.95	0.97	0.97	0.97	0.97	0.97	0.97
d. M+C (%)*	0.8	0.75	0.7	0.75	0.75	0.75	0.8	0.75	0.7	0.75	0.75	0.75	0.71	0.67	0.63	0.67	0.67	0.67

Calculated values, **Analyzed values; 1 Pre-mix contains AB2D3K (0.015 kg): Vit-A 82,500IU, Vit-B2 50mg, Vit-D3 12,000IU, Vit-K 10mg; B-complex- DS (0.015 kg), Trace minerals (0.1 kg): Fe- 80mg, Cu- 25mg, Mn- 65mg, Co- 50ppm and I- 1.2mg per kg diet; Toxin binder (0.1 kg), CC-60% (0.1 kg): choline chloride Coccidiostat (0.05 kg): 3-5, Dinitro-O-Toluamide: 25 per cent W/W.

diets having 25 and 50% less methionine than the basal diet. Each of these main treatment groups were fed three graded (0.1%, 0.2%, and 0.3%) levels of betaine. The basal diet (BD) was prepared by using maize, soybean and de-oiled rice bran (Table 1). All the chicks were reared in well ventilated raised wire floor battery brooders under uniform management (brooding, feeding and watering) and standard hygienic conditions throughout the experiment. During the experiment, light was provided continuously by using fluorescent bulbs. Each battery brooder consisted of 16 cells. Each cell (one replicate) accommodated five birds providing an average floor space of 0.9 sq. ft. per bird. Brooder temperature was maintained at 34 ± 1°C up to 7 days of age and then gradually reduced to 26 ± 1°C by 21 days of age after which chicks were maintained uniformly at room temperature. Irrespective of treatments all the chicks were fed *ad lib.* with respective broiler pre-starter diet from day old to 14 days, broiler starter diet from 14 to 28 days and finisher diet from 29 to 42 days of age. Clean and fresh drinking water was provided *ad lib.* daily. The individual body weight of birds was recorded at weekly intervals up to six weeks of age, while feed consumption was recorded replicate wise at weekly intervals and the feed efficiency was calculated as feed consumed per unit body weight gain. Mortality among the birds during entire experimental period was recorded. At the end of growth trial, one bird from each replicate and thus a total of six birds weighing nearer to the mean body weight of respective treatment groups were selected, weighed and slaughtered. The ready-to-cook yields, breast muscle yield, and proportionate weight of giblet (liver, heart and gizzard), abdominal fat and pancreas were recorded with the help of electronic weighing balance. Thigh meat fat for 100 g of thigh meat was analysed as per the AOAC (2005) methods for estimation of fat. On 42nd day, 5 ml of blood from three birds per replicate was collected into a clean sterilized tube (CB Plus pro coagulation tube with clot activator) and kept in a slanted position at room temperature to facilitate separation of serum. Further, blood samples were centrifuged at 3000 rpm for 5 minutes to separate the serum and serum was transferred to labeled 5 ml Eppendorf tubes which were stored at -80°C until analysis. Serum concentrations of albumin, uric acid, cholesterol and triglycerides were determined using the Erba Chem-5 plus V2 clinical chemistry semi auto analyzer with commercially available diagnostic kit (M/S Excel Diagnostics Pvt. Ltd., Hyderabad, India). Feed cost per kg live weight gain was calculated based on the present actual input cost. Data obtained were subjected to analysis of variance following SPSS. Treatment means were separated and significance was declared at $P<0.05$.

RESULTS AND DISCUSSION

The results revealed that supplementation of betaine 0.3% produced significantly ($P<0.05$) higher body weight gain, lower feed intake, and better feed conversion ratio (FCR) in broilers fed 25 and 50% methionine restricted diets (Table 2). The results clearly demonstrate the methionine sparing effect of betaine. These results corroborate with the with the existing literature (Virtanen and Rosi, 1995; Kidd *et al.,* 1997; Rama Rao *et al.,* 2011) which indicate methionine sparing capability of betaine at sub-optimal concentrations of the amino acid in the diet. A part of methionine is utilized by the bird for single carbon transfer in absence of inadequate amount of methyl donors. Thus, supplementation of betaine to the methionine restricted diets contributed to the methyl donating property (Kidd *et al.,* 1997), and thereby making methionine available for other vital functions like protein synthesis and immune modulation. As a result, supplementation of betaine to methionine restricted diet improved the performance of broilers and the best response was obtained when betaine was supplemented at 0.3%. Contrary to the present findings, some other research workers (Rostagno and Pack, 1996; Schutte *et al.,* 1997) reported lack of improvement in broiler performance with betaine supplementation. The variation in response could be due to lower level of crude protein and betaine used in the above cited studies.

Table 2. Effect of graded levels of betaine supplementation on performance of broilers fed methionine deficient diets

Treatment	Body weight gain	Feed Intake	FCR
Basal Diet (BD)	1905.14^{a}	3396.70^{a}	1.78^{c}
25% Less MET	1811.30^{c}	3399.12^{a}	1.87^{a}
0.1BET	1835.15^{bc}	3390.91^{b}	1.84^{b}
0.2BET	1850.09^{b}	3306.63^{c}	1.78^{c}
0.3BET	1926.14^{a}	3110.41^{d}	1.61^{d}
50% Less MET	1825.14^{c}	3433.40^{a}	1.88^{a}
0.1BET	1852.66^{b}	3467.10^{a}	1.87^{a}
0.2BET	1879.47^{b}	3386.67^{b}	1.80^{b}
0.3BET	1952.47^{a}	3256.36^{c}	1.66^{d}
Statistical significance			
Effect of methionine	1818.22	3413.53	1.87
Methionine × betaine	1882.66	3319.68	1.76

Means bearing different superscripts in a column differ significantly (P<0.05)

Honarbakhsh *et al.* (2007) indicated that betaine supplementation increased feed intake, which is in contrast to the present results. The FCR was improved due to betaine supplementation in birds fed methionine deficient diets. The current findings corroborated with the findings of earlier researchers who also reported better FCR in broiler chicken fed diets with supplementation of betaine (Baghaei *et al.,* 2009; Rama Rao *et al.,*2011; Rafeeq *et al.,* 2013; El-Shinnaw, 2015). Some researchers also reported that there was no effect of addition of betaine into low methionine diets on the FCR. (Schutte *et al.,* 1997; Modirsanei *et al.,* 2002).

Percentage of chick survived, carcass yield and

Table 3. Effect of graded levels of betaine supplementation on dressing percentage and relative organ weight in broilers fed methionine deficient diets

	% Live weight							
Treatment	Carcass yield	Liver	Heart	Gizzard	Spleen	Breast yield	Abdominal fat	Thymus
Basal Diet (BD)	67.25^{b}	2.50	4.11	3.00	1.90	32.12^{a}	1.190^{ab}	0.187
25% Less MET	63.75^{b}	3.50	3.79	3.75	1.00	28.23^{c}	1.285^{b}	0.160
0.1BET	68.15^{ab}	2.75	4.98	3.00	1.90	30.50^{c}	1.285^{b}	0.281
0.2BET	69.00^{ab}	3.00	4.65	3.00	2.75	31.50^{ab}	0.159^{c}	0.267
0.3BET	69.50^{ab}	3.75	4.38	3.75	2.75	33.50^{a}	0.112^{c}	0.290
50% Less MET	62.00^{b}	3.00	4.93	3.00	1.25	28.23^{c}	1.332^{a}	0.195
0.1BET	70.15^{ab}	2.75	4.73	2.75	1.93	31.50^{ab}	1.250^{ab}	0.286
0.2BET	70.25^{ab}	3.15	4.68	2.00	2.00	32.00^{a}	0.146^{c}	0.217
0.3BET	71.25^{a}	3.25	4.42	2.75	3.00	33.00^{a}	0.115^{c}	0.297
Effect of methionine	62.88	3.25	3.76	3.88	1.13	27.12	1.31	0.18
Methionine × betaine	69.72	3.11	4.64	2.88	2.39	32.00	0.51	0.27

Means bearing different superscripts in a column differ significantly (P<0.05), weight of liver, heart, spleen, breast meat yield, abdominal fat,(abd fat) bursa, thymus expressed as % of dressed wt.

Table 4. Effect of graded levels of betaine supplementation on some serum metabolites in broilers fed methionine deficient diets

Treatment	Alb g/dl	Chol mg/dl	UA mg/dl	TG mg/dl	T_3 nmol/ml	TSH uU/ml	CORT ng/ml
Basal Diet (BD)	2.97^{a}	108.66^{a}	14.33	119.06^{ab}	1.42^{b}	0.15^{a}	189.26^{a}
25% Less MET	2.70^{ab}	98.73^{b}	17.61	135.00^{b}	1.30^{c}	0.06^{b}	189.22^{a}
0.1BET	2.00^{a}	70.50^{c}	17.33	96.33^{bc}	1.51^{a}	0.16^{a}	180.32^{c}
0.2BET	2.33^{ab}	86.83^{bc}	17.33	95.67^{bc}	1.55^{a}	0.15^{a}	181.14^{b}
0.3BET	2.13^{ab}	62.17^{c}	11.00	85.50^{c}	1.56^{a}	0.18^{a}	182.19^{b}
50% Less MET	2.30^{ab}	105.92^{a}	22.30	145.91^{a}	1.20^{c}	0.06^{b}	188.72^{a}
0.1BET	1.67^{b}	91.50^{b}	26.33	102.33^{bc}	1.54^{b}	0.15^{a}	180.97^{a}
0.2BET	1.50^{b}	93.33^{b}	19.33	97.50^{bc}	1.57^{b}	0.16^{a}	181.14^{c}
0.3BET	1.10^{b}	66.33^{c}	16.50	89.17^{c}	1.57^{b}	0.17^{a}	182.99^{a}
Effect of methionine	2.5	102.33	19.96	140.46	1.25	0.06	188.97
Methionine × betaine	1.79	78.44	17.97	94.42	1.55	0.16	181.46

Means bearing different superscripts in a column differ significantly (P<0.05), Alb, albumin; Chol, cholesterol, UA, uric acid; TG, triglycerides; SOD, TSH, thyroid stimulating hormone; CORT, serum corticosteroids.

proportionate weight of liver was higher in birds fed supplemental betaine at 0.3 %. Other workers (Esteve and Mack, 2000; Mc Devitt *et al.,* 2000; El- Shinnawy, 2015) also reported that addition of betaine to diets of broilers improves the percent carcass yield. On the contrary no significant difference was found in live weights and carcass weights due to supplementation of betaine at 0.1% of the diet. Breast yield was lower in birds fed 50% less methionine as compared to those fed a standard diet, but it improved when betaine was supplemented to such diet at 0.3% level. Similar to the present study, increased breast meat yield with betaine supplementation was reported in broilers by Waldroup *et al.,* (2006). Enhanced muscle protein accretion (Saunderson and McKinlay, 1990) due to improved utilization of dietary methionine with betaine supplementation (Augustine and Danforth, 1999) might have contributed to higher breast yields with betaine

Table 5. Effect of graded levels of betaine supplementation on feed cost broilers fed methionine deficient diets

Treatment	CFI (g)	Body weight(g)	Feed cost(₹)	Other cost(₹)	Total cost(₹)	Sale proceed(₹)	Net return(₹)
Basal Diet (BD)	3396.70	1949.14	89.26	90	179.26	214.41	125.14
25% Less MET	3399.07	1856.30	89.35	90	179.35	204.19	114.84
0.1BET	3390.91	1879.15	89.13	90	179.13	206.71	117.57
0.2BET	3306.63	1895.09	86.95	90	176.95	208.46	121.51
0.3BET	3110.41	1971.14	81.81	90	171.81	216.83	135.02
50% Less MET	3433.40	1870.97	90.23	90	180.23	205.81	115.58
0.1BET	3467.10	1897.66	91.10	90	181.10	208.74	117.65
0.2BET	3386.67	1924.47	89.27	90	179.27	211.69	122.43
0.3BET	3256.36	1997.47	85.62	90	175.62	219.72	134.10

CFI, cumulative feed intake; Day- old chick cost ₹ 36/- per chick ; Day old-chick weight – 45 g,Vaccination and medication cost ₹ 2/- per bird, Labour charges ₹ 50/- per bird, Miscellaneous ₹ 2/- per bird, The sale price of broilers for farm gate was taken as ₹ 110/- per kg live weight.

supplementation. The % abdominal fat and thigh meat fat (g/100g) was also found to be lower in 0.3% betaine supplemented groups. Similarly, decrease in % abdominal fat in betaine fed broilers was also reported by El- Shinnawy (2015). Significantly (P<0.05) lower values of serum albumin, cholesterol, uric acid, triglycerides were observed in the 0.3% betaine supplemented group (Table 4). The results of this study are similar to the results mention in the literature that uric acid and triglyceride concentration in serum was significantly decreased by addition of betaine in the diet (Zhan *et al.,* 2006; El-Shinnawy, 2015). In contrast, an increase in the above mentioned serum parameters was observed by Rama Rao *et al.,* (2011) and Baghaei *et al., (*2009) when betaine was supplemented to diets having low levels of methionine or adequate levels of methionine. The decrease in uric acid and triglyceride concentration in serum may be due to increase in the hormone sensitive lipase activity in abdominal fat which initiates the catabolism of triglycerides in adipocytes causing a decrease in serum concentration of triglyceride.

The T_3 and TSH concentration in serum of the betaine supplemented groups was similar among the groups. The serum corticosteroids levels were significantly lower (P<0.05) in the betaine supplemented groups compared to the basal diet and 25% less MET and 50% less MET groups indicating the protective property of betaine against oxidative stress. These results corroborated with the findings of Mahmoudi *et al.* (2018) who reported that betaine have specific roles in protecting tissues from oxidative damage. Singh *et al.,* (2015) reported a linear increase in the concentration of T_3 and TSH in serum and reduced plasma concentration of corticosteroid due to betaine supplementation. This might be due to the protective effect of betaine against low methionine -induced oxidative stress. that is associated with the restoration of S-adenosyl methionine (SAM), which contributes to an increase in the supply of substrate needed for the synthesis of glutathione that protects the cell from reactive metabolites (Ganesan *et al.,* 2011; Alirezaei *et al.,* 2012).

Net revenue over feed cost per bird obtained by the sale of birds were higher in the birds fed 0.3% betaine containing diets (₹135.02/ bird, 134.10/ bird) compared to birds fed with 25 and 50% less MET. (Table 5).

CONCLUSION

Based on the findings of the present study it can be concluded that betaine supplementation at 0.3% level in broiler diets containing sub-optimal concentrations of methionine was effective in enhancing growth, feed conversion efficiency and carcass yield. The supplementation of betaine did not exert any adverse effect on biochemical attributes and aided in lowering the serum concentration of corticosteroids. The net revenue over feed cost per bird was enhanced due to supplementation of betaine to broiler diets having sub-optimal methionine concentration.

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