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Effect of Betaine Supplementation to Methionine-deficient Diet on Growth Performance, Carcass Characteristics, Blood Parameters and Economic Efficiency of Broilers

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ABSTRACT

Background: Betaine is a protein found in shellfish, spinach and wheat bran. Betaine's main physiological function is to act as a methyl donor and an osmolyte. Betaine also functions as an osmolyte, ensuring that the avian's cellular water and ion balance are maintained. It may protect a variety of intestinal bacteria against osmotic changes resulting in increased microbial fermentation activity. Adding betaine to chicken diets has been shown to improve nutritional digestibility and lower abdominal fat weight.

Methods: A total of two hundred and seventy day-old commercial broiler chicks were randomly allotted to nine treatments of six equal replicates with 5 chicks each (9 × 6 × 5=270). Nine experimental isonitrogenous and isocaloric diets consisting of a control diet (CD), 25% and 50% methionine deficient diet and with graded levels of betaine (0.1, 0.2 and 0.3% levels) were formulated and fed to chicks from 1 to 42 days of age.

Result: The results of the study revealed that supplementation of betaine to broiler diets deficient in methionine at 25% and 50% resulted in better performance with ($p<0.05$) higher body weight gain and better FCR, higher percentages of livability, carcass yield, total edible parts, breast yield and breast meat while those of heart and abdominal fat were decreased but relative weights of giblets, liver and gizzard were not affected compared with their control counterparts. Betaine supplementation resulted in increased immunity against ND, better tolerance to oxidative stress and better net profit over feed cost of broilers at 42 days of age.

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

INTRODUCTION

Betaine is an organic chemical that helps birds avoid heat stress by preventing dehydration and maintaining cellular water and ion balance. Plants, animals, microorganisms and dietary sources including shellfish, spinach and wheat bran all contain betaine. Betaine is stable and is considered as a non-toxic feed additive (Yu *et al*., 2004). By substituting part of the choline and methionine that are added to animal feed with betaine, which has a comparable nutritional value, the price of animal feed might be brought down (Madan Kumar *et al*. 2021). Betaine may promote various intestinal microbes against osmotic variations and thus improve microbial fermentation activity. Previous studies showed that dietary supplementation of betaine in poultry diets could positively affect nutrients' digestibility, reduce abdominal fat weight and increase breast meat yield (Rama Rao *et al*. 2011). In addition, betaine has been reported to protect internal organs and boost their performance (Eklund *et al*. 2006). Its inclusion in poultry diet is sparing essential amino acids like choline and methionine (El-Shinnawy, 2015). In addition, it may play an important role in lean meat production by positively affecting the lipid metabolism with increased fatty acids catabolism and thus reducing carcass fat deposition. Moreover, it should be added to the diet as a natural anti-stressor through different routes (water/feed) to overcome the heat stress problem. The present study was conducted with an objective to study the effect of low methionine diet and supplementation of betaine at three different levels on the performance of broiler chicken. This article is aimed at throwing light at the methionine sparing effect of betaine when supplemented in methionine deficient broiler diets.

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MATERIALS AND METHODS

A biological trial of six weeks duration was conducted during February and March, 2017 at Poultry Unit, NTR College of Veterinary Science, Gannavaram as a part of PhD study to assess the feeding value of betaine on growth performance of commercial broilers. Dietary treatments included three levels of betaine (0.1 per cent, 0.2 per cent and 0.3 per cent) and one maize-soya based control diet, as well as two diets with 25% and 50% less methionine than the recommended (BIS Standards 2007). The experiment's basal diet (BD) was made from maize, soybean meal and DORB. To achieve equal protein and energy concentrations across all diets in each period, the quantities of soybean meal and DORB were modified. The crude protein content of broiler experimental pre-starter, starter and finisher diets was determined using the AOAC method (2005). In this experiment, two hundred and seventy day-old commercial broiler chicks (Cobb 400) bought from a local hatchery were used. Individual broiler chicks were wing banded and weighed. Each replicate received five broiler chicks. Each of the nine treatments received six replicates at random (9 × 6 × 5). The growth trial lasted 42 days, beginning on day one. Throughout the trial, all of the chicks were grown in well-ventilated elevated wire floor battery brooders under uniform management (brooding, feeding and watering) and normal hygienic settings. Fluorescent lamps supplied continuous illumination. Each battery brooder was made up of 16 cells. Each cell (one copy) houses five birds, with an average floor size of 0.9 square feet per bird. Each duplicate, which included 5 chicks, was housed independently using a fully randomised design (CRD) in a battery brooder that was heated by electricity. Up until day 7, the brooder temperature was kept at 34°C; after that, it was gradually lowered to 26°C until day 21, after which the chicks were kept at room temperature. Regardless of the treatments, all the chicks received ad libitum feedings of the appropriate broiler pre-starter, starter and finisher diets from day old to 14 days, 14 to 28 days and 29 to 42 days of age, respectively. Ad libitum access to clean, pure drinking water was available. The chicks were vaccinated with Mareks disease vaccine at 3 days, Lasota vaccine at 7^{th} (primary) and 21^{st} (booster) days of age and IBD vaccine at 14^{th} day of age with intermediate-Georgia strain. Except for the experimental diets, other management practices were followed uniformly throughout the experimental period.

Individual body weights of birds were recorded at weekly intervals, while feed consumption was recorded replicate wise at weekly intervals and feed efficiency was determined as feed consumed per unit body weight growth. The reasons of avian mortality were determined for the entire study period. At the end of the growth experiment, one chick from each duplicate was chosen, weighing closer to the mean body weight of the corresponding treatment and slain. The electronic weighing balance was used to record the ready-to-cook yields, breast muscle yields, giblet (liver, heart and gizzard), abdominal fat and pancreas weights. Dressing % was estimated on a pre-slaughter weight basis using ready-to-cook yields/ carcass weight. Organ relative weights were given as a percentage of organ weight to clothed body weight of the bird. Thigh meat fat was estimated using the AOAC techniques for 100g of thigh meat. On the 42^{nd} day of the experiment, blood was taken from three birds per duplicate into a clean sterilised tube (CB Plus pro coagulation tube with clot activator) and maintained at room temperature in a tilted posture to allow serum separation. After that, blood samples were centrifuged at 3000 rpm for 5 minutes to separate the serum, which was then transferred to labelled 5ml eppendorf tubes and kept at -80°C until analysis.

The relative economics (feed cost per kg live weight gain) of raising broilers up to six weeks of age with betaine at various dietary levels, in low-methionine diets was studied. Feed cost per kg live weight gain was calculated based on the present actual input cost.

Data on BWG, FI, FCR, nutrient retention, economics, carcass traits, HI titer, serum and immune parameters were subjected to two-way ANOVA (factorial) in a completely randomized design (Snedecor and Cochran, 1994) and the significant difference were compared with Duncan's multiple range test as described by Duncan (1955) at P<0.05.

RESULTS AND DISCUSSION

The results revealed that 0.3% level supplementation of betaine to both 25% less and 50% less-methionine diets produced significantly (p<0.05) higher BWG, lower feed intake and better FCR at 42 days of age compared to control diet and other treatments (Table 1). The cumulative BWG was the highest in birds fed with 0.3 BET during the overall period indicating the methionine sparing effect of supplementing betaine in diets containing less methionine. These results were in concurrence with the results of Rama Rao *et al*. (2011). Methionine sparing activity and methyl donating property of BET at sub-optimal concentrations of the amino acid in the diet might be a reason for improved broiler performance with BET supplementation. Birds fed 0.3BET showed a lower cumulative feed intake. There was significantly (P<0.05) higher feed intake in birds fed basal diet. Results were in agreement with the report of (El-Shinnawy, 2015) who found that all birds consuming betaine-supplemented diets had somewhat lower FI values as compared to birds fed the control diet. However, (Honarbakhsh *et al.* 2007) indicated that betaine supplementation increased feed intake, which is in contrast to the present results. Feed conversion ratio (FCR) of birds was significantly (P<0.05) better in groups fed 0.3 BET supplemented diets and poor FCR values were recorded in birds fed diets with 25 and 50% less MET (1.87). The current findings corroborated with the findings of earlier researchers who also reported better FCR in broiler chicken fed diets with supplementation of betaine (Rafeeq *et al*., 2013,). Some researchers also reported that there was no effect of addition of betaine into low methionine diets on the FCR (Sahin *et al* 2020).

Highest livability (100%) was recorded in birds fed with 0.1% and 0.3% BET supplementation. The observed values of livability rate in this study are better than those obtained previously by (Tollba *et al.* 2007), who found that mortality rate was 5% for broiler chicks reared in normal conditions (24°C).

Better per cent carcass yield and liver weight were seen in 0.3 BET group and poor values in case of 50% less MET

Table 1: Effect of different levels of betaine (Bet) supplementation in diets with reduced methionine (Met) on performance of broiler chicken at 42d of age.

Treatment	Body wt. gain, (g)	Feed intake, (g)	FCR
Basal diet (BD)	1905.14[a]	3396.70[a]	1.78[c]
25% less Met (25% Met)	1811.30[c]	3399.12[a]	1.87[a]
25% met+0.1% BET	1835.15[bc]	3390.91[b]	1.84[b]
25% met+0.2% BET	1850.09[b]	3306.63[c]	1.78[c]
25% met+0.3% BET	1926.14[a]	3110.41[d]	1.61[d]
50% Less MET (50% met)	1825.14[c]	3433.40[a]	1.88[a]
50% met+0.1% Bet	1852.66[b]	3467.10[a]	1.87[a]
50% met+0.2% Bet	1879.47[b]	3386.67[b]	1.80[b]
50% met+0.3% Bet	1952.47[a]	3256.36[c]	1.66[d]
S E M	16.91	39.503	0.029
N	30	30	30
P- value	0.041	0.001	0.015

Means bearing different superscripts in a column differ significantly (P<0.05, P<0.01).

Table 2: Effect of different levels of betaine (Bet) supplementation in diets with reduced methionine (Met) on carcass parameters of broilers.

Treatment	%Carcass yld	Liver (g)	Heart (g)	Gizzard (g)	Spleen (g)	Breast meat yld (g)	Abd fat(g)	Thigh meat fat (g/100 g)	Bursa (g)	Thymus (g)
Basal diet (BD)	67.25[b]	2.50	4.11	3.00	1.90	32.12[a]	1.190[ab]	0.587	0.072	0.187
25% less Met	63.75[b]	3.50	3.79	3.75	1.00	28.23[c]	1.285[b]	0.600	0.077	0.160
25% met+0.1% Bet	68.15[ab]	2.75	4.98	3.00	1.90	30.50[c]	1.285[b]	0.600	0.092	0.281
25% met+0.2% Bet	69.00[ab]	3.00	4.65	3.00	2.75	31.50[ab]	0.159[c]	0.407	0.065	0.267
25% met+0.3% Bet	69.50[ab]	3.75	4.38	3.75	2.75	33.50[a]	0.112[c]	0.422	0.094	0.290
50% less met	62.00[b]	3.00	4.93	3.00	1.25	28.23[c]	1.332[a]	0.560	0.081	0.195
50% met+0.1% Bet	70.15[ab]	2.75	4.73	2.75	1.93	31.50[ab]	1.250[ab]	0.578	0.073	0.286
50% met+0.2% Bet	70.25[ab]	3.15	4.68	2.00	2.00	32.00[a]	0.146[c]	0.400	0.066	0.217
50% met+0.3% Bet	71.25[a]	3.25	4.42	2.75	3.00	33.00[a]	0.115[c]	0.455	0.096	0.297
S E M	0.715	0.188	0.083	0.179	0.002	0.917	0.003	0.003	0.002	0.001
N	6	6	6	6	6	6	6	6	6	6
P- value	0.052	0.500	0.405	0.672	0.094	0.057	0.047	0.175	0.062	0.096

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

group. These results are in concurrence with (Esteve and Mack 2000), who also reported that addition of BET to diets of broilers improves the percent carcass yield. A higher % breast meat yield was found in 0.3 BET and lowest in 50% less MET groups and all other groups showed the intermediate values. Similar to the present study, increased breast yield with BET supplementation was reported in broilers (Waldroup *et al*., 2006). Enhanced muscle protein accretion due to improved utilization of dietary MET with BET supplementation might have contributed to higher breast yields with BET supplementation. The % abdominal fat and thigh meat fat were found to be the lowest in 0.3 BET group and 0.2 BET. As found in the study, significant decrease in % abdominal fat in betaine fed broilers was also reported in literature (El-Shinnawy 2015) Table 2.

In the present study, the lipid peroxidation, which is estimated by the level of SOD in serum was found to be lower in the betaine supplemented groups and higher in the BD and 25 and 50% less MET groups, which is an indication of the lowered oxidative stress in the betaine treated groups. The T_3 and TSH concentration in the serum of the BET supplemented groups was numerically better than the BD group and 25 and 50 % less MET groups as an indication of enhanced metabolism. The serum corticosteroids levels were significantly lower in the betaine supplemented groups compared to the BD and 25% less MET and 50% less MET groups indicating the protective property against oxidative stress. These results corroborated with the findings of Nasiroleslami *et al*. (2018) that betain supplementation decreased liver MDA level and SOD activity which are responsible for the destruction of peroxides and have specific roles in protecting tissues from oxidative damage activity. Singh *et al*. (2015) reported a linear increase in the concentration of T3 and TSH in serum and reduced plasma corticosteroid with betaine supplementation. This might be due to the protective effect of betaine against low methionine -induced oxidative stress observed, besides the restoration of S-adenosyl methionine (SAM), which contributes to an

Table 3: Effect of different levels of betaine (Bet) supplementation in diets with reduced methionine (Met) on serum biochemical parameters of broilers.

Treatment	T3 nmol/ml	TS HuU/ml	LP nmol/ml	SCS ng/ml
Basal diet (BD)	1.42[b]	0.15[a]	2.03[a]	189.26[a]
25% less Met (25% Met)	1.30[c]	0.06[b]	2.13[a]	189.22[a]
25% met+0.1% Bet	1.51[a]	0.16[a]	1.97[ab]	180.32[c]
25% met+0.2% Bet	1.55[a]	0.15[a]	1.86[ab]	181.14[b]
25% met+0.3% Bet	1.56[a]	0.18[a]	1.74[b]	182.19[b]
50% less met (50% met)	1.20[c]	0.06[b]	2.20[a]	188.72[a]
50% met+0.1% Bet	1.54[b]	0.15[a]	1.93[ab]	180.97[a]
50% met+0.2% Bet	1.57[b]	0.16[a]	1.86[ab]	181.14[c]
50% met+0.3% Bet	1.57[b]	0.17[a]	1.65[b]	182.99[a]
S E M	0.042	0.01	0.023	0.193
N	6	6	6	6
P- value	0.034	0.001	0.001	0.057

Means bearing different superscripts in a column differ significantly($P<0.05, P<0.01$).: LP-Lipid peroxidation, SCS-Serum corticosteroids.

Table 4: Influence of different levels of betaine (Bet) supplementation in diets with reduced methionine (Met) over feed cost and gain / loss of broilers at market age (42 d).

Treatment	Cost of the diet for total feed intake (Rs.)			Cost for cumFI	Other input cost of prod	Total cost of prod per bird (Rs.)	B.wt a 6 wks t (g/bird)	Sale amount	Net revenue over feed cost
	PS	St	Fn	(Rs./bird)	(Rs./bird)			(Rs./bird)	(Rs./bird)
Basal diet (BD)	10.22	25.60	53.45	89.26	90	179.26	1949.14	214.41	125.14
25% less Met (25% Met)	7.21	26.29	55.85	89.35	90	179.35	1856.30	204.19	114.84
25% met+0.1% BET	8.84	26.18	54.11	89.13	90	179.13	1879.15	206.71	117.57
25% met+0.2% BET	8.56	26.49	51.90	86.95	90	176.95	1895.09	208.46	121.51
25% met+0.3% BET	8.03	25.36	48.43	81.81	90	171.81	1971.14	216.83	135.02
50% Less MET (50% met)	8.84	25.85	55.54	90.23	90	180.23	1870.97	205.81	115.58
50% met+0.1% Bet	7.98	25.70	57.42	91.10	90	181.10	1897.66	208.74	117.65
50% met+0.2% Bet	7.61	25.51	56.15	89.27	90	179.27	1924.47	211.69	122.43
50% met+0.3% Bet	7.61	25.85	52.16	85.62	90	175.62	1997.47	219.72	134.10

PS-pre starter, St-starter, Fn-finisher, cum FI-cumulative feed intake.

- Day-old chick cost Rs. 36/- per chick ; Day old-chick weight-5 g.
- Vaccination and medication cost Rs. 2/- per bird.
- Labour charges Rs. 50/- per bird.
- Miscellaneous Rs. 2/- per bird.

The sale price of broilers for farm gate was taken as Rs 110/- per kg live weight.

increase in the supply of substrate needed for the synthesis of glutathione that protects the cell from reactive metabolites (Alirezaei *et al.*, 2012) (Table 3).

Net revenue over feed cost per bird obtained by the sale of birds was higher in the birds fed with 0.3 BET compared to birds fed with 25 and 50% less MET (Table 4). The results were in accordance with the observations of El -Shinnawy (2015) who also reported improved economic efficiency with supplementation of betaine to broiler diets.

CONCLUSION

Based on the data obtained from the present study it can be concluded that betaine supplementation at 0.3% level in diets was effective in enhancing growth, feed conversion efficiency, breast yield and lymphocyte proliferation in broilers fed a diet containing sub-optimal concentrations of Methionine.

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