Original Research Article

Dietary addition of betaine on the performance of broilers fed with diets with a reduction in methionine and choline

ABSTRACT

A six-week experiment was conducted to evaluate the performance of broiler chicken(n=560) fed betaine added diets to reduce methionine and choline. There were five treatments, each with eight replicates comprising fourteen birds, each. The negative control groups (NC) were fed with betaine free basal diet. The positive control (PC) groups were fed with 0.05% betaine added basal diet. The three treatment groups (T1-T3) received 0.05% betaine added basal diet integrated with 20, 40, and 60% reduction in methionine and choline, respectively. Birds were reared on deep litter system with *ad-lib* feeding. The results showed PC bird groups had the highest body weight gain, followed by T1, NC, T2, and T3. The PC bird groups showed a significant (P<0.001) increase in the foot web index, serum antibody titer, and relative immune organ weights (bursa, spleen, and thymus) in the PC and T1 groups. The PC diet significantly (P<0.001) reduced shrinkage loss and fat pad thickness of chickens. Significantly higher (P<0.002) eviscerated and ready-to-cook yields with substantially better relative heart, breast, and neck weights were recorded in chickens fed PC and T1. Conclusively, betaine at a dietary level of 0.05% integrated with a 20% reduction in methionine and choline significantly improved growth performance, livability, immunity, and carcass traits of broiler chickens.

Keywords: betaine hydrochloride, methionine, choline, abdominal fat, ready-to-cook yield, FCR, feed intake

Introduction

It is common practice for commercial poultry farms to use antibiotic growth promoters (AGPs) such as virginiamycin and bacitracin methylene disalicylate. As a result of public concern about antibiotic residues and the development of antibiotic-resistant bacteria, all AGPs have been regulated or banned (Hafeez *et al.,* 2016). This has led many researchers to explore possible alternatives to AGPs. Several dietary strategies have been proposed to compensate for the loss of poultry productivity following the removal of AGPs from their diets. Several organic acids, minerals, bacteriophages, probiotics, prebiotics, and others may be included in the list (Jackson *et al.,* 2004; Khomayezi and Adewole, 2022). One of them, betaine can enhance production efficiency and the immune system (Attia *et al.,* 2005). Anhydrous and hydrochloride forms of betaine are available. Trimethylamine (TMA) is a by-product of chemicals that are used in the synthesizing process. It has been reported that betaine hydrochloride contains more TMA than natural betaine (Amerah, 2014).

Betaine metabolism occurs in liver and kidney cells through chemical reactions (transmethylation). Choline and betaine are the primary methyl group (CH_3) sources in animal diets; however, only betaine can act as a methyl group donor (Kettunen *et al.,* 2001). It functions as an osmolyte, a methyl donor (methionine sparing, choline sparing), an acid-base balancer, and a performance enhancer. As a result of rapid absorption in the duodenum, betaine is readily absorbed. A quarter of betaine is bioavailable, while most remain in the gastrointestinal tract (Jadhav *et al.,* 2020). Rather than excreting betaine, it is eliminated through metabolism. Various enzymatic reactions occur in liver and kidney cells to catabolize it. The compound also prevents fat accumulation in the liver and reduces abdominal fat (Rombola, 2016). In the hepatocyte, fatty acid oxidation occurs in the mitochondria of the inner mitochondrial membrane. During the transmethylation cycle, methyl functional groups must be added to carnitine for synthesis. A methyl group is found in betaine, which inhibits fatty liver development in birds by increasing carnitine levels (Rombola, 2016).

A diet containing betaine may reduce the need for other methyl donors, such as methionine and choline. Research conducted by Florou-Paneri *et al.* (1997), Matthews *et al.* (2001), and Zhan *et al.* (2006) showed that between 30% to 80% of the supplemental methionine can be substituted by betaine without adverse effects on performance. In Van der Klis and Fledderus (2007) study, choline was entirely replaced. Improving production by replacing choline and methionine with lower feeding costs is possible. It may be possible to replace choline and methionine in broiler diets with betaine hydrochloride, providing nutritionists with a new tool for composing an optimal diet. In addition to improving productive performance, betaine supplementation improves cell osmoregulation to reduce heat stress's harmful effects on viability and immune response (Attia *et al.*, 2005). A study by Nofal *et al.* (2015) showed that dietary betaine supplementation at 0.1 or 0.2% significantly improved carcass yield (carcass weight, dressing, thigh/breast percentage, and giblets percentage) decreasing abdominal fat (Jahanian and Rahmani, 2008), and reducing mortality rate (Lukic *et al.*, 2012). Enriching the diet with methionine, an essential amino acid for protein synthesis, is possible by adding betaine as a methyl group donor (Sun *et al.* 2008). As an organic osmolyte (dipolar zwitterion), betaine increases intracellular water retention, preventing intracellular enzymes from becoming inactivated by osmotic pressure.

Moreover, betaine inhibits cellular apoptosis and reduces energy expenditure in Gastro Intestinal tract cells. Several studies have found that betaine can boost growth rates, feed efficiency, and breast yields (Waldroup *et al.,* 2006; Ramarao *et al.,* 2011). However, scanty research was undertaken to see the effect of the dietary addition of betaine on the performance of broilers fed with diets with reduced methionine and choline. Accordingly, the present study was designed to study the effect on growth performance, liveability, immune response, and carcass traits in broiler chickens.

MATERIALS AND METHODS

The experiment was approved by the Institutional Animal Ethical Committee (Resolution No. XII/2022- Ref. No. VCU/IAEC/CPCSEA/2022; Dated: 06/05/2022) and the Board of Studies. The experiments were conducted for six weeks at the Experimental Broiler Shed and Paraclinical Laboratories of the College of Veterinary and Animal Sciences, Udgir, Maharashtra (India).

Broiler management

The biological experiment of six weeks was conducted on day-old 560 straight-run commercial broiler chicks of the "Ven Cobb 430Y" strain. The day-old chicks were equally distributed into five treatment groups randomly. There were eight replicates (14 chicks each) under each treatment group; thus, 112 broiler chicks were under each treatment. Following standard managemental practices, the birds were reared under a deep litter system using paddy husk as litter material. The feed and water were made available to the birds round the clock.

Experimental diets

The feed ingredients required for formulating the broiler diet were procured from the local market. The betaine hydrochloride (98% betaine hydrochloride) was procured from Taian Havay Chemicals Co Ltd., China. The proximate analysis of the experimental diets was carried out as per AOAC (2016). The ingredient and nutrient composition of the different dietary treatments at different growth stages (Pre-starter (0-7 days); starter (7-21 days) and finisher (21-42 days) is presented in Table 1, 2 and 3 respectively.The negative control group (NC) received a basal diet, according to BIS (2007). Group positive control group (PC) was fed a basal diet with 0.05% betaine. The treatment groups T1, T2, and T3 received the basal diet containing 0.05% betaine with 20, 40, and 60% reduction in methionine and choline, respectively. The birds were fed with broiler pre-starter (0-7 days), broiler starter (8-21 days), and broiler finisher (22-42 days) feed.

Performance study

The growth performance and other related parameters were measured. The birds were weighed weekly individually to record their body weights. A replicate-wise gain in weight, feed intake, cumulative weekly feed intake, and cumulative weekly feed conversion ratio was calculated under each treatment group. The feed intake was recorded by offering the weighed quantity and subtracting the leftover residue. The mortality, if any, was recorded and expressed in percentage.

Immune response

The cell-mediated (CMI) and antibody-mediated (AMI) immune responses of broiler chicks were accessed by *in vivo* foot web index (cutaneous basophilic hypersensitivity test)to phytohemagglutinin (a lectin from *Phaseolus Vulgaris*- PHA-P) and serum antibody titers to *Newcastle disease virus* (NDV), respectively. The foot web index to PHA-P was calculated by Corrier and Deloach (1990). On the 22^{nd} day post-hatch, eight birds from each treatment were randomly selected, and the toe thickness of both the left and right foot at the 3^{rd} and 4^{th} interdigital spaces were measured by a digital micrometer. Immediately after measurements, 0.1 ml PHA-P (1 mg per ml) was intradermally injected into the right foot web. In contrast, 0.1 ml of phosphate buffer saline (PBS) was injected into the left foot web as a placebo. The web swellings of both feet were measured after 24 hours. The *in vivo* response to PHA-P was expressed as a web index.

For AMI response, the broiler birds were vaccinated with the LaSota vaccine on the 5^{th} day of age and booster on the 21^{st} day. The AMI was determined by estimating serum antibody titers against the NDV. The blood was collected from one bird per replicate and eight birds per treatment group. The blood was allowed to clot at room temperature and centrifuged at 1500 rpm for 10 minutes. The clear upper serum layer was carefully extracted and stored at -20°C until further use. A haemagglutination inhibition (HI) test was performed in the U bottom microtitre plate using 4 HA unit ND antigens. The reciprocal of the highest dilution of serum showing 50% haemagglutination inhibition (button formation) was taken as the HI titer (log 2).

The weight of immune organs (bursa of fabricius, thymus, and spleen) was taken using a digital weighing balance at the end of the trial by slaughtering one bird per replicate and expressed in percentage of live weight.

Carcass traits

At the end of the experiment, eight birds with body weights closer to the mean of the dietary treatment group were selected from each group. The birds were kept off feed for eight hours before slaughter, but clean, potable drinking water was offered. The parameters like weight loss due to shrinkage, blood loss weight, de-feathered weight, dressed weight, eviscerated weight, cut-up part (breast, thigh, drumsticks, back, neck, and wing) weight, giblet (heart, liver, and gizzard) weight, and abdominal fat pad thickness was recorded and expressed as % of live weight.

Table 1: Ingredients composition (%) of different dietary treatments at the pre-starter stage (0-7 days)

Sr.	Ingredient (Kgs)	NC	PC	T1	T2	T3
1.	Maize	54.655	54.655	54.643	54.634	54.639
2.	Soybean Meal	39.273	39.273	39.333	39.39	39.435
3.	Vegetable Oil	2.46	2.46	2.4474	2.4348	2.4202
4.	Dicalcium Phosphate	1.78	1.78	1.78	1.78	1.78
5.	Limestone Powder	0.89	0.89	0.89	0.89	0.89
6.	Salt	0.3	0.3	0.3	0.3	0.3
7.	Trace Min. Premix*	0.11	0.11	0.11	0.11	0.11
8.	Vitamin Premix**	0.15	0.15	0.15	0.15	0.15
9.	DL-Methionine	0.117	0.117	0.0936	0.0702	0.0468
10.	L-Lysine	0.08	0.08	0.078	0.076	0.074
11.	Choline chloride	0.05	0.05	0.04	0.03	0.02
12.	Toxin binder	0.07	0.07	0.07	0.07	0.07
13.	Coccidiostat	0.05	0.05	0.05	0.05	0.05
14.	B-Complex***	0.015	0.015	0.015	0.015	0.015
	Total	100	100	100	100	100
	Betaine Hydrochloride	---	0.05	0.05	0.05	0.05
	*Trace mineral premix supplied Mg- 300, Mn- 55, I- 0.4, Fe- 56, Zn-30, and Cu- 4 mg/Kg diet. **The vitamin premixes supplied Retinol 2475 mcg, Cholecalciferol 30 mcg; Menaquinone 1mg; Tocopherol 26.8 mg d-alpha tocopherol /Kg diet. ***B-complex provided Thiamine 2mg, Riboflavin 4mg, Cyanocobalamin 10mcg; Niacin 60mg; Pantothenic acid 10mg/Kg diet					

Table 2: Ingredients composition (%) of different dietary treatments at the starter stage (7-21 days)

Sr.	Ingredient (Kgs)	NC	PC	T1	T2	T3
1.	Maize	55.62	55.62	55.597	55.582	55.574
2.	Soybean Meal	36.887	36.887	36.96	37.027	37.087
3.	Vegetable Oil	3.913	3.913	3.901	3.888	3.874
4.	Dicalcium Phosphate	1.83	1.83	1.83	1.83	1.83
5.	Limestone Powder	0.87	0.87	0.87	0.87	0.87
6.	Salt	0.3	0.3	0.3	0.3	0.3
7.	Trace Min. Premix*	0.1	0.1	0.1	0.1	0.1
8.	Vitamin Premix**	0.15	0.15	0.15	0.15	0.15
9.	DL-Methionine	0.130	0.130	0.104	0.078	0.052
10.	L-Lysine	0.035	0.035	0.033	0.03	0.028
11.	Choline chloride	0.05	0.05	0.04	0.03	0.02
12.	Toxin binder	0.05	0.05	0.05	0.05	0.05
13.	Coccidiostat	0.05	0.05	0.05	0.05	0.05
14.	B-Complex***	0.015	0.015	0.015	0.015	0.015
	Total	100	100	100	100	100
	Betaine Hydrochloride	---	0.05	0.05	0.05	0.05
	*Trace mineral premix supplied Mg- 300, Mn- 55, I- 0.4, Fe- 56, Zn-30, and Cu- 4 mg/Kg diet. **The vitamin premixes supplied Retinol 2475 mcg, Cholecalciferol 30 mcg; Menaquinone 1mg; Tocopherol 26.8 mg d-alpha tocopherol /Kg diet. ***B-complex provided Thiamine 2mg, Riboflavin 4mg, Cyanocobalamin 10mcg; Niacin 60mg; Pantothenic acid 10mg/Kg diet					

Table 3: Ingredients composition (%) of different dietary treatments at the finisher stage (21-42 days)

Sr.	Ingredient (Kgs)	NC	PC	T1	T2	T3
1.	Maize	59.655	59.655	59.641	59.631	59.623
2.	Soybean Meal	31.83	31.83	31.888	31.94	31.99
3.	Vegetable Oil	4.94	4.94	4.927	4.916	4.905
4.	Dicalcium Phosphate	1.9	1.9	1.9	1.9	1.9
5.	Limestone Powder	0.85	0.85	0.85	0.85	0.85
6.	Salt	0.3	0.3	0.3	0.3	0.3
7.	Trace Min. Premix*	0.1	0.1	0.1	0.1	0.1
8.	Vitamin Premix**	0.15	0.15	0.15	0.15	0.15
9.	DL-Methionine	0.105	0.105	0.084	0.063	0.042
10.	L-Lysine	0.00	0.00	0.00	0.00	0.00
11.	Choline chloride	0.05	0.05	0.04	0.03	0.02
12.	Toxin binder	0.055	0.055	0.055	0.055	0.055
13.	Coccidiostat	0.05	0.05	0.05	0.05	0.05
14.	B-Complex***	0.015	0.015	0.015	0.015	0.015
	Total	100	100	100	100	100
	Betaine Hydrochloride	---	0.05	0.05	0.05	0.05
	*Trace mineral premix supplied Mg- 300, Mn- 55, I- 0.4, Fe- 56, Zn- 30, and Cu- 4 mg/Kg diet. **The vitamin premixes supplied Retinol 2475 mcg, Cholecalciferol 30 mcg; Menaquinone 1mg; Tocopherol 26.8 mg d-alpha tocopherol /Kg diet. ***B-complex provided Thiamine 2mg, Riboflavin 4mg, Cyanocobalamin 10mcg; Niacin 60mg; Pantothenic acid 10mg/Kg diet					

Statistical analysis

Data analysis of variance (ANOVA) was performed using standard methods (Snedecor and Cochran, 1994) to test the effect of dietary reduction in methionine and choline with the dietary addition of betaine. Data was done using SPSS software package version 20.0. Variables with unequal observations were analyzed using the least square design method and Duncan's multiple range test (Duncan, 1955). Results were considered significant at 95% ($P<0.05$) for comparison.

RESULTS AND DISCUSSION

Growth performance:

The effect of betaine on body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) in all treatment groups is given in Table 4. The weekly BWG in the positive control (PC) group (dietary addition of betaine 0.05% to the basal diet) and T1 (0.05% betaine with the reduction in 20% methionine and choline in basal diet) was significantly ($P<0.05$) higher compared to the negative control (NC) group. The PC group's overall BWG was higher ($P<0.05$), followed by T1, NC, T2, and T3. The dietary addition of betaine hydrochloride has a significant ($P<0.05$) effect on BWG during the starter (0-21 days), finisher (21-42 days), and overall (0-42 days) growth phases. The chicks receiving feed containing betaine hydrochloride at 0.05% in a standard diet (PC) had higher BWG ($P<0.001$) during starter (0-21 days), finisher (21-42 days), and overall (0-42 days) growth phases.

Table 4: Effect of dietary addition of betaine on body weight gain (g), Feed intake (FI), and Feed conversion ratio (FCR) of the broiler birds

Week	Parameter	NC	PC	T1	T2	T3	Pooled SEM	P Value
I	BWG	150.1[b]	153.2[c]	152.3[c]	147.1[a]	146.0[a]	0.35	0.001
	FI	153.1[a]	155.6[ab]	154.9[ab]	158.5[bc]	160.4[c]	0.68	0.002
	FCR	1.02[a]	1.02[a]	1.02[a]	1.08[b]	1.10[b]	0.007	0.001
VI	BWG	709.6[ab]	734.5[b]	721.2[b]	692.4[ab]	668.4[a]	6.61	0.016
	FI	1421.9	1421.5	1411.7	1429.0	1402.5	15.36	0.988
	FCR	2.00[b]	1.94[a]	1.96[a]	2.06[c]	2.10[d]	0.011	0.001
Phase (days)								
0-21 days	BWG	939.7[c]	957.2[d]	953.6[d]	929.1[b]	914.4[a]	1.38	0.001
	FI	1115.4[a]	1111.6[a]	1107.4[a]	1131.4[b]	1133.1[b]	2.58	0.001
	FCR	1.19[b]	1.16[a]	1.16[a]	1.22[c]	1.24[d]	0.006	0.001
21-42 days	BWG	1845.2[c]	1886.0[d]	1858.4[cd]	1795.1[b]	1730.8[a]	6.61	0.001
	FI	3199.4	3196.7	3158.5	3197.1	3152.0	14.08	0.718
	FCR	1.73[b]	1.69[a]	1.70[a]	1.78[c]	1.82[d]	0.008	0.001
0-28 days	BWG	1449.1[c]	1478.7[d]	1468.4[d]	1422.7[b]	1389.3[a]	2.60	0.001

	FI	1849.2	1841.6	1829.0	1863.1	1856.7	4.77	0.183
	FCR	1.28[b]	1.25[a]	1.25[a]	1.31[c]	1.34[d]	0.007	0.001
0-35 days	BWG	2075.2[c]	2115.4[d]	2090.9[c]	2032.1[b]	1982.9[a]	3.71	0.001
	FI	2892.9	2886.7	2854.3	2899.4	2882.6	8.23	0.485
	FCR	1.39[b]	1.36[a]	1.37[a]	1.43[c]	1.45[d]	0.007	0.001
0-42 days	BWG	2784.8[c]	2843.2[d]	2812.0[cd]	2724.4[b]	2651.8[a]	6.55	0.001
	FI	4314.8	4308.2	4266.0	4328.4	4285.1	14.06	0.665
	FCR	1.55[b]	1.52[a]	1.52[a]	1.59[c]	1.62[d]	0.009	0.001

Values bearing different superscripts differed significantly (P<0.05); NS-Non-Significant (P>0.05)

The birds fed with standard basal diet (NC), basal diet plus 0.05% betaine (PC), and 0.05% betaine with a 20% reduction in methionine and choline (T1) from the basal diet resulted in significantly reduced feed consumption compared to treatment T2 and T3. The feed consumption was lower (P<0.001) in NC, PC, and T1 groups compared to T2 and T3 during the starting (0-21 days) phase. At the end of the 6th week, the overall (0-42 day) feed consumption was numerically higher in treatment group T2 compared to the NC, PC, T1, and T3 groups.

During the first week, FCR was better (P<0.001) in NC, PC, and T1 than in T2 and T3. Birds fed a basal diet containing 0.05% betaine (PC) and a 20% reduction in methionine and choline from the standard basal diet with 0.05% of betaine (T1) had better FCR compared to birds fed the basal diet without betaine (NC). The birds fed the diet containing 0.05% betaine with 40 and 60% reduction in methionine and choline levels in basal diet (T2 and T3) had poor FCR compared to birds fed 0.05% betaine in standard basal diet (Azadmanesh and Jahanian, 2014).

The overall mortality during the experimental trial was 2.86%, within the normal range. Dietary addition of 0.05% betaine in the standard basal diet (PC), dietary addition of 0.05% betaine with a 20% reduction in methionine and choline from the basal diet (T1) had better livability compared to birds fed without betaine, and the birds provided with 40 and 60% reduction in methionine and choline levels. The percent mortality was highest when birds were fed a 60% reduction in methionine and choline plus a 0.05% betaine-containing diet.

According to our findings, adding betaine to broiler chicken diets without reducing methionine and choline levels significantly influences their performance. Reducing 20% levels of methionine and choline with 0.05% betaine per kg of feed also improved the broilers' body weight. In a previous study, Rafeeq *et al.* (2011) reported that the dietary addition of 0.07% and 0.14% betaine to the basal diet significantly improves weight gain. According to Sahin *et al.* (2020), dietary betaine addition at 0.8 g/kg had the highest body weight gain. Popova *et al*. (2016) and Amer *et al.* (2018) reported that dietary betaine (2g/kg) significantly improves body weight gain. Cromwell *et al*. (1999) also noted that adding betaine positively affected body weight gain.

In contrast, to our findings, Esteve-Garcia and Mack (2000), Pirompud*et al.* (2005), and Uzunoglu and Yalcin (2019) did not find any significant difference in body weight gain due to betaine addition. The dietary addition of betaine in broiler chicken may have benefited growth performance as it spared dietary methionine and choline for other vital functions like protein synthesis and immune modulation. In the treatment groups T2 and T3, methionine and choline were low in the diet (40 and 60% reduction from the standard basal diet); thus, betaine did not appear to be able to spare methionine and choline. Similarly, the dietary replacement of methionine with betaine had no positive impact on broiler productivity in previous studies conducted by Saunderson and Mac Kinlay (1990) and Park and Ryu (2011).

In the current study, the increase in body weight gain in the birds fed with the dietary addition of 0.05% betaine to the standard diet (PC) and the dietary addition of 0.05% betaine with a 20% reduction in methionine and choline (T1) might be due to considerable improvement in digestion and absorption of nutrients by protecting and developing the intestinal morphological characteristics and reducing the intestinal pH. Methionine is an essential amino acid for poultry. In the present study, broilers fed on the 40 and 60% reduction in methionine and choline from the standard basal diet (T2 and T3) had lower weight gain. These were improved when birds were fed a standard basal diet with the dietary addition of 0.05% betaine. Ratriyanto*et al.* (2017) reported increased crude protein and fiber digestibility at 0.06% and 0.12% doses. The dietary addition of betaine improved the digestion of crude protein and ether extract in laying chickens (Ezzat *et al.,* 2011). Increased digestibility of crude protein, crude fiber, and ether extract may have contributed to the increased body weight gain in groups PC and T1.

According to Awad*et al.* (2014), feed consumption was barely raised by adding various levels of betaine to the feed. Rafeeq *et al.* (2011) reported that considerable increase in feed consumption when broilers were fed a diet containing 0.14% and 0.07% of betaine. Amer *et al.* (2018) found that dietary betaine (2g/kg diet) significantly increased feed intake ($P<0.05$) for both normal-energy and low-energy diets. The findings were in line with the earlier work of Casarin *et al*. (1997), Kitt *et al.* (1999), Azadmanesh and Jahanian, (2014), and Sahin *et al.* (2020). They reported that overall feed consumption was not affected by dietary betaine addition. The reduction in feed intake may contribute to an improvement in nutrient absorption and availability in the gut. Better utilization and absorption of nutrients in the treatment group (PC) led to an earlier satisfaction of energy requirements.

The dietary addition of 0.05% betaine in the basal diet significantly improved the FCR of broilers. Also, our results suggest that betaine can partially replace methionine and choline in maize-soybean-based diets of meat-type chicken, resulting in better growth performance. The results are consistent with earlier research work of Virtanen and Rosi (1995), Rostagno and Pack (1996), Matthews and Southern (2000), Hassan *et al.* (2005), Waldroup *et al.* (2006), Ramarao *et al.* (2011), Gudev *et al.* (2011), Rafeeq *et al.* (2011), Awad*et al.* (2014) who reported that dietary addition of betaine improved FCR compared to control. In contrast, to our findings, El-Shinnawy (2015), Amer *et al.* (2018), Park and Kim (2019),and Sahin *et al.* (2020) reported that dietary betaine addition had no significant effect on FCR.

The methyl donor function of methionine is crucial for body protein synthesis, thereby making it desirable to spare its role (Sun *et al.* 2008). In addition, betaine contains three methyl groups, which are donated during several metabolic reactions, making it an effective compound to spare methionine as a methyl donor group (Alirezaei *et al.* 2012). Methionine can be directed to protein synthesis when betaine acts as a methyl group donor in remethylation. Betaine is further converted into dimethylglycine by an enzyme called betaine-homocysteine methyltransferase (Alirezaei *et al.* 2012).

A variety of factors may contribute to the improvement in performance caused by dietary betaine addition in meat-type chicken, including improved intestinal integrity (Sakomura*et al.*, 2013), stimulating growth hormone and insulin-like growth factor-I (Huang *et al.,* 2006), and increasing intestinal immunity (Klasing *et al.,* 2002). Also, betaine's osmolyte function positively influences intestinal cells' growth, survivability, and integrity (Mostashari-Mohases*et al.*, 2017). Consequently, betaine could enhance growth in broilers on a marginal methionine-low diet and spare a small amount of methionine, in agreement with Zhan *et al.* (2006). The findings are congruent with the earlier work of Lukic *et al.* (2012)andEl-Shinnawy (2015), who reported a lower mortality rate in broilers fed diets containing betaine with optimum methionine and choline levels.

Immunity:

There was a significant (P<0.001) increase in foot web index when birds were fed 0.05% of betaine. Birds provided 0.05% of betaine than the control (NC) had better cell-mediated responses. There was a significant (P<0.001) increase in serum antibody titer when birds were fed 0.05% betaine in the standard basal diet. The treatment T1 in which birds have provided the 0.05% betaine with a 20% reduction in methionine and choline levels, showed a significant increase in serum antibody titer compared to control (NC) and the birds fed with 40 and 60% reductions in methionine and choline levels. The significant difference in the relative weights of the bursa (P<0.01), spleen (P<0.001), and thymus (P<0.001) were observed in different treatment groups. As compared to other dietary treatments, birds fed with 0.05% of betaine in a standard basal diet having adequate methionine and choline (PC) and 0.05% betaine with a reduction in 20% methionine and choline (T1) had a better relative weight of immune organs compared to 0.05% dietary betaine with a reduction in 40 and 60% methionine and choline (T2 and T3). The control diet (NC) with no betaine had lower relative weights of immune organs than supplemented groups (PC, T1, T2, and T3).

The previous study conducted by Remus *et al.* (2004),Chand *et al.* (2017), Ghasemi and Nari (2020) reported that dietary supplementation of betaine enhances immunity. In contrast to our findings, Ramarao *et al.* (2022) reported that methionine supplementation and dietary betaine addition did not affect antibody titers against NDV. In the present study, the good immune response indicates that the betaine may have spared methionine from being used for other vital functions, like protein synthesis and immune modulation, which enhanced broiler growth.

Table 5. Effect of dietary addition of betaine on Immune-Competence at 42 days of age

Immune-Competence	NC	PC	T1	T2	T3	Pooled SEM	P Value
CMI PHA-P foot web index (mm)	0.25^{a}	0.30^{b}	0.29^{b}	0.28^{b}	0.27^{b}	0.004	0.001
Humoral-HI titer against ND (log 2)	5.25^{a}	6.75^{b}	6.63^{b}	5.63^{a}	5.50^{a}	0.164	0.003
Lymphoid Organ Weight							
Bursa (% of live wt.)	0.15^{a}	0.17^{b}	0.17^{b}	0.15^{a}	0.15^{a}	0.003	0.010
Spleen (% of live wt.)	0.20^{a}	0.24^{b}	0.23^{b}	0.22^{b}	0.20^{a}	0.003	0.001
Thymus (% of live wt.)	0.39^{a}	0.45^{d}	0.44^{cd}	0.42^{bc}	0.41^{b}	0.005	0.001

Values bearing different superscripts differed significantly (P<0.05)

Carcass traits:

The dietary addition of 0.05% of betaine significantly reduces shrinkage loss compared to control (NC) birds. The blood loss and feather loss were comparable when birds were fed the diet with or without betaine. The eviscerated and ready-to-cook yield was significantly higher when birds were fed a standard basal diet containing 0.05% betaine (PC) and in treatment group T1 when birds were fed 0.05% betaine with a 20% reduction in methionine and choline from the basal diet. Dietary 40% and 60% reduction in methionine and choline with 0.05% betaine addition had lowered eviscerated and ready-to-cook yield. The relative weights of the liver, gizzard, and giblet were comparable when birds were fed a diet containing betaine. Also, adding betaine to the diet did not significantly increase the relative weights of the back, drumstick, thigh, and wing. The relative weights of heart, breast, and neck were substantially better in birds fed 0.05% betaine (PC) in the standard basal diet. Similarly, the dietary addition of 0.05% betaine with a 20% reduction of methionine and choline from the basal diet had also shown a significant effect on relative weights of the heart, breast, and neck compared to a 40% reduction in methionine and choline levels. The abdominal fat pad thickness was significantly reduced when birds were fed 0.05% of betaine with either 20, 40, or 60% reduction in methionine and choline levels from the basal diet compared to the control group (NC).

Table 6: Effect of dietary addition of betaine on carcass traits and organ weight (% of live wt.) of broiler birds at 42 days of age

Traits	NC	PC	T1	T2	T3	Pooled SEM	P Value
Shrinkage	5.61^{b}	4.35^{a}	4.44^{a}	4.50^{a}	4.53^{a}	0.080	0.001
Blood loss	3.08	3.15	3.10	3.09	3.07	0.020	0.757
Feather loss	4.53	4.52	4.54	4.55	4.56	0.026	0.993
Eviscerated yield	69.44^{a}	70.95^{b}	70.78^{b}	70.16^{ab}	69.89^{a}	0.147	0.002
Ready to cook yield	74.71^{a}	76.39^{b}	76.21^{b}	75.53^{ab}	75.21^{a}	0.162	0.002
Heart	0.76^{a}	0.84^{c}	0.84^{c}	0.82^{bc}	0.80^{b}	0.006	0.001
Liver	2.40	2.45	2.43	2.40	2.39	0.018	0.872
Gizzard	2.10	2.16	2.16	2.14	2.13	0.011	0.433
Giblet	5.26	5.44	5.43	5.36	5.33	0.027	0.209
Breast	21.60^{a}	22.64^{c}	22.60^{c}	22.16^{b}	21.97^{b}	0.082	0.001
Back	15.86	15.91	15.90	15.88	15.88	0.039	0.997
Drumstick	10.55	10.70	10.65	10.59	10.58	0.021	0.189
Thigh	10.57	10.62	10.61	10.58	10.57	0.028	0.977
Neck	3.61^{a}	3.73^{c}	3.70^{bc}	3.67^{ab}	3.63^{a}	0.012	0.003
Wing	7.24	7.36	7.33	7.28	7.26	0.024	0.553
Abdominal Fat pad Thickness	0.75^{b}	0.69^{a}	0.69^{a}	0.69^{a}	0.71^{ab}	0.007	0.030

Values bearing different superscripts differed significantly ($P<0.05$); NS-Non-Significant ($P>0.05$)

According to Arif *et al.* (2022), adding betaine to the diet improves the carcass traits of Japanese quail. Further, Waldroup *et al.* (2006) reported increased breast meat yield in broilers after choline or betaine additions at 1,000 mg/kg or 500 mg/kg. According to El-Shinnawy*et al.* (2017), adding methionine to betaine, choline, or both could improve broiler carcass yield and total edible parts yield. Betaine included in the diet at a rate of 2 g/kg increased the proportion of breast muscle and its output, according to Nutautaite*et al.* (2020). A study by Arif *et al.* (2022) revealed that dietary betaine addition altered carcass traits in Japanese quail.

The previous study by Virtanen and Rosi (1995) reported that the body fat percentage decreased when birds were fed the betaine-containing diet. As betaine is involved in synthesizing carnitine, essential for the oxidation of long-chain fatty acids across mitochondrial membranes, it may help reduce carcasses' fat content and reveal leaner carcasses (De Ridder and Van Dam, 1973; Saunderson and Mac Kinlay, 1990). Despite betaine being implicated in the metabolism of lipids, there is no clear evidence that it decreases carcass fat in poultry. It is consistent with the findings of Virtanen and Rosi (1995). They reported that betaine is more effective than DL-methionine in supporting breast meat growth. In contrast, Schutte *et al.* (1997) reported increased breast meat production when DL-methionine was added to the basal diet without betaine supplementation. Singh *et al.* (2015) found that dietary betaine did not influence carcass traits.

Conclusion

Betaine hydrochloride at a dietary level of 0.05% with a 20% reduction in methionine and choline significantly improved broiler chickens' growth performance, livability, immunity, and carcass traits.

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