

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 bio-assay was conducted to evaluate the performance of broiler chicken (n=560) fed with diets with the reduction in methionine and choline with the dietary addition of betaine. There were five treatments, each with eight replicates comprising fourteen birds, each. The negative control group (NC) received a basal diet, according to BIS (2007). The positive control (PC) group was fed a basal diet with 0.05% betaine. The three treatment groups received the basal diet containing 0.05% betaine with 20, 40, and 60% reduction in methionine and choline, respectively. Standard management practices were followed in the rearing of the birds. Study parameters included growth performance, immune response, and carcass traits. The PC group recorded the highest body weight gain (), followed by T1, NC, T2, and T3. The birds fed 0.05% betaine 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 dietary addition of 0.05% of betaine significantly reduced shrinkage loss and fat pad thickness. Significantly higher eviscerated and ready-to-cook yields with substantially better relative heart, breast, and neck weights were recorded in PC and T1. Betaine hydrochloride at a dietary level of 0.05% 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 (Leeson, 2007). 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, and prebiotics may be included in the list (Jackson *et al.*, 2004). It has been shown that betaine can enhance production efficiency and the immune system (Attia *et al.*, 2005). Anhydrous and hydrochloride versions 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).

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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 (Jahani 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 GI 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 research work 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 is presented in Table 1. 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 as per](#) Corrier and Deloach (1990). On the 22nd day post-hatch, eight birds from each treatment were randomly selected, and the toe thickness of both the left and right foot at the 3rd and 4th 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 5th day of age and booster on the 21st 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 bodyweights 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 different growth stages

Sr.	Ingredient (Kgs)	Phase	NC	PC	T1	T2	T3
1.	Maize	Pre-starter	54.655	54.655	54.643	54.634	54.639
		Starter	55.62	55.62	55.597	55.582	55.574
		Finisher	59.655	59.655	59.641	59.631	59.623
2.	Soybean Meal	Pre-starter	39.273	39.273	39.333	39.39	39.435
		Starter	36.887	36.887	36.96	37.027	37.087
		Finisher	31.83	31.83	31.888	31.94	31.99
3.	Vegetable Oil	Pre-starter	2.46	2.46	2.4474	2.4348	2.4202
		Starter	3.913	3.913	3.901	3.888	3.874
		Finisher	4.94	4.94	4.927	4.916	4.905
4.	Dicalcium Phosphate	Pre-starter	1.78	1.78	1.78	1.78	1.78
		Starter	1.83	1.83	1.83	1.83	1.83
		Finisher	1.9	1.9	1.9	1.9	1.9
5.	Limestone Powder	Pre-starter	0.89	0.89	0.89	0.89	0.89
		Starter	0.87	0.87	0.87	0.87	0.87
		Finisher	0.85	0.85	0.85	0.85	0.85
6.	Salt	Pre-starter	0.3	0.3	0.3	0.3	0.3
		Starter	0.3	0.3	0.3	0.3	0.3
		Finisher	0.3	0.3	0.3	0.3	0.3
7.	Trace Min. Premix*	Pre-starter	0.11	0.11	0.11	0.11	0.11
		Starter	0.1	0.1	0.1	0.1	0.1
		Finisher	0.1	0.1	0.1	0.1	0.1
8.	Vitamin Premix**	Pre-starter	0.15	0.15	0.15	0.15	0.15

		Starter	0.15	0.15	0.15	0.15	0.15
		Finisher	0.15	0.15	0.15	0.15	0.15
9.	DL-Methionine	Pre-starter	0.117	0.117	0.0936	0.0702	0.0468
		Starter	0.130	0.130	0.104	0.078	0.052
		Finisher	0.105	0.105	0.084	0.063	0.042
10.	L-Lysine	Pre-starter	0.08	0.08	0.078	0.076	0.074
		Starter	0.035	0.035	0.033	0.03	0.028
		Finisher	0.00	0.00	0.00	0.00	0.00
11.	Choline chloride	Pre-starter	0.05	0.05	0.04	0.03	0.02
		Starter	0.05	0.05	0.04	0.03	0.02
		Finisher	0.05	0.05	0.04	0.03	0.02
12.	Toxin binder	Pre-starter	0.07	0.07	0.07	0.07	0.07
		Starter	0.05	0.05	0.05	0.05	0.05
		Finisher	0.055	0.055	0.055	0.055	0.055
13.	Coccidiostat	Pre-starter	0.05	0.05	0.05	0.05	0.05
		Starter	0.05	0.05	0.05	0.05	0.05
		Finisher	0.05	0.05	0.05	0.05	0.05
14.	B-Complex***	Pre-starter	0.015	0.015	0.015	0.015	0.015
		Starter	0.015	0.015	0.015	0.015	0.015
		Finisher	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 from various treatment groups were analyzed statistically using a completely randomized design (one-way ANOVA) following standard methods (Snedecor and Cochran, 1994) to test the effect of dietary reduction in methionine and choline with the dietary addition of betaine. The analysis of 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 2. 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 2: Effect of dietary addition of betaine on weekly and phase-wise 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
II	BWG	341.3 ^{ab}	344.6 ^b	344.9 ^b	340.3 ^{ab}	338.8 ^a	0.69	0.017
	FI	392.1 ^a	385.3 ^a	383.7 ^a	402.2 ^b	405.2 ^b	1.84	0.001
	FCR	1.15 ^b	1.12 ^a	1.11 ^a	1.18 ^c	1.20 ^c	0.006	0.001
III	BWG	448.3 ^{bc}	459.3 ^d	456.3 ^{cd}	442.0 ^b	429.6 ^a	1.38	0.001
	FI	570.3	570.6	568.7	570.7	567.5	1.94	0.983
	FCR	1.27 ^b	1.24 ^a	1.25 ^a	1.29 ^c	1.32 ^d	0.005	0.001
IV	BWG	509.4 ^c	521.5 ^c	514.8 ^c	493.3 ^b	475.0 ^a	2.59	0.001
	FI	733.8	730.0	721.6	731.7	723.6	4.51	0.905
	FCR	1.44 ^b	1.40 ^a	1.40 ^a	1.48 ^c	1.52 ^d	0.008	0.001
V	BWG	626.2 ^{bc}	636.6 ^c	622.4 ^{bc}	609.4 ^{ab}	593.5 ^a	3.88	0.005
	FI	1043.7	1045.1	1025.3	1036.4	1025.9	7.87	0.897
	FCR	1.67 ^b	1.64 ^a	1.65 ^a	1.70 ^c	1.73 ^d	0.006	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 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 (PC).

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), Pirompuet *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).

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In the current study, the increase in body weight gain in the birds fed with the dietary addition of 0.05% betaine to the standards 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 Awadet *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), 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), Awadet *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 (Alirezai *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 (Alirezai *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-Mohaseset *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) and El-Shinnawy (2015), who reported

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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 3. Effect of dietary addition of betaine on the 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 4: 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.

REFERENCES

- Alirezaei, M., Gheisari, H. R. Ranjbar V. R. and Hajibemani, A. 2012. Betaine: a promising anti-oxidant agent for enhancement of broiler meat quality. *British Poultry Science*, **53**(5):699-707.
- Amer, S. A. A. E. Omar, W. A. Mohamed, H. S. Gharib and W. A. El-Eraky 2018. Impact of betaine supplementation on the growth performance, tonic immobility, and some blood chemistry of broiler chickens fed normal and low energy diets during natural summer stress. *Zagazig Veterinary Journal*, **46**(1):37-50.
- Amerah, A. M. 2014. The differences between natural betaine and betaine hydrochloride. *International Poultry Science*, **22**:11-13.
- AOAC (Association of the analytical community) International 2016. Official Methods of Analysis of Association of Analytical Community International. 20th edition.
- Arif, M., R. Baty, S. Alhubaiti, E. H. Ijaz, M. Fayyaz, M. E. Shafi and S. S. Elnesr, 2022. The impact of betaine supplementation in quail diet on growth performance, blood chemistry, and carcass traits. *Saudi Journal of Biological Sciences*, **29**(3):1604-1610.
- Attia, P. G. Q. T. M. Phan, A. V. Maker, M. R. Robinson, M. M. Quezado, J. C. Yang and S. A. Rosenberg 2005. Autoimmunity correlates with tumor regression in patients with metastatic melanoma treated with anti-cytotoxic T-lymphocyte antigen-4—*Journal of Clinical*

Oncology, **23**(25):6043.

Awad, A. L. H. N. Fahim, A. F. Ibrahim and M. M. Beshara 2014. Effect of dietary betaine supplementation on productive and reproductive performance of domyati ducks under summer conditions. *Egyptian Poultry Science Journal*, **34**(2): 228-235.

BIS-Bureau of Indian Standard 2007. Indian standard of poultry feed specification.

Casarin, A., M. Forat and B. J. Zabarar-Krick 1997. Interrelationships between betaine (Betain-BCR) and level of feed intake on the performance parameters and carcass characteristics of growing-finishing pigs. *Journal of Animal Science*, **75**(11):75.

Chand, N., S. Naz, H. Maris, R. U Khan, S. Khan and M.S. Qureshi 2017. Effect of betaine supplementation on the performance and immune response of heat-stressed broilers. *Pakistan Journal of Zoology*, **49**(5):1857-1862.

Corrier, D.E. and J. R. Deloach 1990. Evaluation of cell-mediated, cutaneous basophil hypersensitivity in young chicken by an interdigital skin test. *Poultry Science*, **69**(3):403-408.

Cromwell, G. L., M.D. Lindemann, J. R. Randolph, H.J. Monegue, K.M. Laurent and G. R. Parker 1999. Efficacy of betaine as a carcass modifier in finishing pigs fed normal and reduced energy diets. *Journal of Animal Science*, **77**(1):179.

De Ridder, J. J.M. and K. Van Dam 1973. The efflux of betaine from rat-liver mitochondria a possible regulating step in choline oxidation. *Biochimica et Biophysica Acta*, **291**:557-563.

Duncan D.B. 1955. Multiple range and multiple F tests. *Biometrics*, **11**(1):1-42.

El-

Shinnawy, A.M. 2015. Effect of betaine supplementation to methionine adequate diet on growth performance, carcass characteristics, some blood parameters and economic efficiency of broilers. *Journal of Animal and Poultry Production*, **6**(1):27-41.

El-Shinnawy, M. M., K. Sherif, H. F. A Motawe and M. A. Osman 2017. Effect of partial or total inclusion of betaine and/or choline instead of added methionine in broiler diets on 2-carcass characteristics, some blood parameters, and sensory evaluation of meat. *Egyptian Journal of Nutrition and Feeds*, **20**(2):139-155.

Esteve-Garcia, E. and S. Mack 2000. The effect of DL-methionine and betaine on growth performance and carcass characteristics in broilers. *Animal Feed Science and Technology*, **87**(1-2):85-93.

Ezzat, W., M. S. Shoeib, S. M. Mousa, A. M. Bealish and Z. A. Ibrahiem 2011. Impact of betaine, vitamin C and folic acid supplementations to the diet on productive and reproductive performance of Matrouh poultry strain under Egyptian summer condition. *Egyptian Poultry Science Journal*, **31**(3):521-537.

Florou-

Paneri, P., D. C. Kufidis, V. N. Vassilopoulos and A. B. Spais 1997. Performance of broiler chicks fed a low choline and methionine diet supplemented with betaine. *Epitheorese Zootechnikes Epistemes*, **24**:103-111.

Ghasemi, H. A. and N. Nari 2020. Effect of supplementary betaine on growth performance, blood biochemical profile, and immune response in heat-stressed broilers fed different dietary protein levels. *Journal of Applied Poultry Research*, **29**(2):301-313.

Gudev, D., S. Popova-Ralcheva, I. Ianchev and P. Moneva 2011. Effect of betaine and air ammonia concentration on broiler performance, plasma corticosterone level, lymphoid organ weights, and some hematological indices. *Biotechnology in Animal Husbandry*, **27**(3):687-703.

Hassan, R. A., Y. A. Attia And E. H. El-Ganzory 2005. Growth, carcass quality, and serum constituents of slow-growing chicks as affected by betaine addition to diets containing 1. Different levels of choline. *International Journal of Poultry Science*, **4**(11):840-850.

Huang, Q. C., Z. R. Xu, X. Y. Han and W. F. Li 2006. Changes in hormones, growth factor, and lipid metabolism in finishing pigs fed betaine. *Livestock Science*, **105**:78-85.

Jackson, M. E., K. Geronian, A. Knox, J. McNab and E. McCartney 2004. A dose-response study with the feed enzyme beta-mannanase in broilers provided with corn-soybean meal-based diets in the absence of antibiotic growth promoters. *Poultry Science*, **83**(12):1992-1996.

Jadhav, R. J., A. Bhattacharyya and P. K. Shukla 2020. Role of betaine in poultry production. *Poultry Planner*, **22**:8-13.

Jahanian, R. and H. R. Rahmani 2008. The effect of dietary fat level on the response of broiler chicks to betaine and choline supplements. *Journal of Biological Science*, **8**(2):362-367.

Kettunen, H., S. Peuranen, K. Tiitonen and M. Saarinen 2001. Intestinal uptake of betaine *in vitro* and the distribution of methyl groups from betaine, choline, and methionine in the body of broiler chicks. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, **128**(2):269-278.

Kitt, S. J., P. S. Miller, A. J. Lewis and H. Y. Chen 1999. Effects of betaine and pen space allocation on growth performance, plasma urea concentration and carcass characteristics of growing and finishing barrows. *Journal of Animal Science*, **77**:53.

Klasing, K. C., K. L. Adler, J. C. Remus and C. C. Calvert 2002. Dietary betaine increases intraepithelial lymphocytes in the duodenum of coccidia-infected chicks and increases functional properties of phagocytes. *The Journal of Nutrition*, **132**(8):2274-

2282.

Leeson, S. 2007. Balancing science versus societal issues in poultry nutrition. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, **2**: 71.

Lukic, M., Z. Jokic, V. Petricevic, Z. Pavlovski, Z. Skrbic and L. Stojanovic 2012. The effect of full substitution of supplemental methionine with betaine in broiler nutrition on production and slaughter results. *Biotechnology in Animal Husbandry*, **28**(2):361-368.

Matthews, J. O. And L. L. Southern 2000. The effect of dietary betaine in *Eimeria acervulina*-infected chicks. *Poultry Science*, **79**:60-65.

Matthews, J. O., L. L. Southern, T. D. Bidner and M. A. Persica 2001. Effects of betaine, pen space, and slaughter handling method on growth performance, carcass traits, and pork quality of finishing barrows. *Journal of Animal Science*, **79**(4):967-974.

Mostashari-Mohases, M., A. A. Sadeghi, J. Ahmadi and S. Esmailkhanian 2017. Effect of betaine supplementation on performance parameters, betaine homocysteine S-methyltransferase gene expression in broiler chickens consume drinking water with different total dissolved solids. *Kafkas Universitesi Veteriner Fakultesi Dergisi*, **23**(4):563-569.

Nofal, M. E., A. G. Magda, S. M. M. Mousa, M. M. Doaa and A. M. A. Bealsh 2015. Effect of dietary betaine supplementation on productive, physiological, and immunological performance and carcass characteristics of growing developed chicks under the condition of heat stress. *Egyptian Poultry Science Journal*, **35**:237-259.

Nutautaitė, M., S. Alijosius, S. Bliznikas, V. Sasyte, V. Viliene, A. Počekvicius and A. Raceviciute-Stupeliene 2020. Effect of betaine, a methyl group donor, on broiler chicken growth performance, breast muscle quality characteristics, oxidative status, and amino acid content. *Italian Journal of Animal Science*, **19**(1):621-629.

Park, J. H. and I. H. Kim 2019. The effects of betaine supplementation in diets containing different levels of crude protein and methionine on the growth performance, blood components, total tract nutrient digestibility, excreted noxious gas emission, and meat quality of the broiler chickens. *Poultry Science*, **98**(12):6808-6815.

Park, J. H. and K. S. Ryu 2011. Relationship between dietary protein levels and betaine supplementation in laying hens. *The Journal of Poultry Science*, **48**(4):217-222.

Pirompuđ, P., S. Attamangkune, C. Bunchasak and A. Promboon 2005. Effect of feeding betaine to broilers reared under tropical conditions on performance and carcass traits. In *Proceedings of 43rd Kasetsart University Annual Conference, Thailand, Animals* (pp. 254-261).

- Popova, T., M. Todorova, M. Ignatova and E. Petkov 2016. Effect of dietary betaine supplementation on the growth performance, carcass traits and meat quality in broiler chickens. In *Çitiñazootehnica–factor important pentru o agricultura detipeuropean*(pp. 247-253).
- Rafeeq, M., N. P. Talat, M. T. Muhammad and M. A. Bajwa 2011. Performance of broiler chicken in early life on methionine-deficient feed with added choline and betaine. *International Journal of Livestock Production*, **2**(9):142-144.
- Ramarao S. V., V. L. N. R Mantena S. S. P Prakash Bhukya and N. Devanaboyina 2022. Effect of methyl donor's supplementation on performance, immuneresponses and antioxidant variables in broiler chicken fed diet without supplemental methionine. *Animal Bioscience*, **35**(3):475.
- Ramarao, S. V., M. V. L. N. Raju, A. K. Panda, P. Saharia and G. S. Sunder 2011. Effect of supplementing betaine on performance, carcass traits, and immuneresponses in broiler chicken fed diets containing different concentrations of methionine. *Asian-Australasian Journal of Animal Sciences*, **24**(5):662-669.
- Ratriyanto, A., R Indreswari and A. M. P. Nuhriawangsa 2017. Effects of dietary protein level and betaine supplementation on nutrient digestibility and performance of Japanese quails. *Brazilian Journal of Poultry Science*, **19**(3):445-454.
- Remus, J.C., E. E. M. Pierson and M. Hruby 2004. The evaluation of betaine and enzymes in coccidian challenged broilers. XXII Poultry Congress, Istanbul, Turkey 8-13 June, 2004.
- Rombola, L.G. (2016). Benefit of betaine in laying hen diets. *All About Feed*, 1:1-3.
- Rostagno, H.S. and M. Pack 1996. Can betaine replace supplemental DL-methionine in broiler diets? *Journal of Applied Poultry Research*, **5**(2):150-154.
- Sahin, T., O. C. Ozel and M. Olme 2020. The effect of supplementation of betaine on performance, carcass yield, and some blood parameters in broilers. *Erciyes Universitesi Veteriner Fakültesi Dergisi*, **17**(3):260-267.
- Sakomura, N.K., N.A.A. Barbosa, F.A. Longo, E.P. Da Silva, M. A. Bonato and J.B. K. Fernandes 2013. Effect of dietary betaine supplementation on the performance, carcass yield, and intestinal morphometrics of broilers submitted to heat stress. *Brazilian Journal of Poultry Science*, **15**: 105–112.
- Saunderson, L. C and J. Mackinlay 1990. Changes in body weight, composition, and hepatic enzyme activities in response to dietary methionine, betaine, and choline levels in growing chicks. *British Journal of Nutrition*, **63**:339–349.
- Schutte, J. B., J. De Jong, W. Smink and M. Pack 1997. Replacement value of betaine for DL-methionine in male broiler chicks. *Poultry Science*, **76**:321-325.
- Singh A. K., K. Tapan, C. C. David and H. Sudipto 2015. Effects of supplementation of

betaine hydrochloride on physiological performances of broilers exposed to thermal stress. *Open Access Animal Physiology*, **7**:111-120.

Snedecor, G. W. and W. G. Cochran 1994. "Statistical methods" 8th Edition, Iowa State University Press, Ames, State of America.

Sun, H., W. R. Yang, Z. B. Yang, Y. Wang, S. Z. Jiang and G. G. Zhang 2008. Effects of betaine supplementation to methionine deficient diet on growth performance and carcass characteristics of broilers. *American Journal of Animal and Veterinary Sciences*, **3**(3):78-84.

Uzunoglu, K. and S. Yalcin 2019. Effects of dietary supplementation of betaine and sepiolite supplementation on performance and intestinal health in broilers. *Ankara Universitesi Veteriner Fakultesi Dergisi*, **66**(3):221-230.

Van Der Klis, J. D. and J. Fledderus 2007. Evaluation of raw materials for poultry: what's up? In *Proceedings of the 16th European Symposium on Poultry Nutrition*. World Poultry Science Association, Strasbourg, France.

Virtanen, E. and L. Rosti 1995. Effects of betaine on methionine requirement of broilers under various environmental conditions. Pages 88-92 in *Proceedings of the Australian Poultry Science Symposium*, University of Sydney, Sydney NSW, Australia.

Waldroup, P. W., M. A. Motl, F. Yan and C. A. Fritts 2006. Effects of betaine and choline on response to methionine supplementation to broiler diets formulated to industry standards. *Journal of Applied Poultry Research*, **15**(1):58-71.

Zhan, X. A., X. Li, J. Z. R. Xu and R. Q. Zhao 2006. Effects of methionine and betaine supplementation on growth performance, carcass composition and metabolism of lipids in male broilers. *British Poultry Science*, **47**(5):576-580.