



Betaine Supplementation on Carcass Traits in Broilers

Shubhnish et al

Carcass Traits and Blood Biochemistry of Broiler Birds Fed Betaine Hydrochloride Supplemented Diets

Shubhnish*, Sajjan Sihag, Sushil Kumar, Radha Bai and Komal

Department of Animal Nutrition, College of Veterinary Sciences,

Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar, Haryana-125004, Haryana

*Correspondence: shubhnishbisla@gmail.com

ABSTRACT

A study was conducted to assess the effect of different levels of betaine hydrochloride on the carcass characteristics and different blood biochemical parameters of broiler chicken. A total of 200 birds were allocated into five different treatment groups of which T1 was taken as control while T2, T3, T4, and T5 were fed basal diet supplemented with 500, 1000, 1500 and 2000 mg betaine/kg feed, respectively. The percentage dressed, eviscerated and drawn carcass weight were significantly ($P < 0.05$) higher in T5 group followed by T4 in contrast with T1, T2, and T3 groups. Birds of T5 group had the lowest ($p < 0.05$) abdominal fat in comparison to control (T1). Both, T4 and T5 showed significantly higher breast and thigh percent weight, crude protein and moisture percentages and lowest ether extract, cholesterol and saturated fatty acid content in comparison to the other groups. Betaine supplementation resulted in increased haemoglobin and RBC count while decreased heterophils count in comparison to control group ($p < 0.05$). Birds in T5 group had higher total blood protein in comparison to the control. The groups supplemented with 2000 and 1500 mg betaine per kg feed had significantly ($P < 0.05$) lower serum cholesterol, triglyceride and LDL values while significantly ($P < 0.05$) higher HDL values in comparison to other groups. It was concluded that adding betaine @2g/kg feed in broilers diet enhanced the carcass yield with an increased protein, lowered cholesterol and fatty acid content along with improved blood biochemical parameters.

KEYWORDS: Betaine, Blood biochemistry, Carcass traits, Cholesterol, Triglyceride

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INTRODUCTION

Betaine a trimethyl derivative of glycine is a metabolite of plant and animal tissues and functions by protecting cells against osmotic inactivation which helps in maintaining cell volume under heat stress condition (Saeed et al., 2017) and by acting as a methyl group donor via trans methylation pathway to synthesize critical metabolites and regulate gene expression through DNA methylation (Anderson et al., 2012). Due to its methyl group donor function betaine is involved in protein and energy metabolism. The methionine sparing effect of betaine could enhance the efficient use of dietary protein. The higher levels of total protein have been found positively correlated with body tissue growth, confirming the essential protein sparing action of betaine in broilers (Park and Kim, 2019).

Thus betaine, with its dual function, has been evaluated for its various effects including nutrient sparing effect, improving performance efficiency, altering the body fat (Rao et al. 2011). Methionine is an important amino acid, which is essential for proper development of muscle (Waldroup et al., 2006) and immune response (Rama Rao et al., 2003) in chickens. Betaine is often considered as a carcass modifier due to methyl group donor property, which causes a higher availability of methionine and cystine for protein deposition, thus contributing to improving the carcass lean percentage (Yang et al., 2016). Keeping these facts in view, the present study was carried out to assess the effect of different doses of betaine on broilers carcass traits and different blood biochemical parameters related to immunity and body physiology.

MATERIALS AND METHODS

Ethical approval

The animal experiment was conducted in accordance with guidelines approved by the Institutional Animal Ethics Committee, 12/CPCSEA Dated 12.04.2022 in the Department of Animal Nutrition, Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar.

Experimental design

A study of 6 weeks duration was conducted on two hundred commercial broiler chicks randomly

distributed into five treatment groups with four replicates of ten birds each. The control group (T1) was offered only basal diet to fulfill the metabolizable energy (ME) and crude protein requirements of broilers and formulated as per BIS (2007) while groups T2, T3, T4 and T5 were supplemented with 500, 1000, 1500 and 2000 mg betaine hydrochloride/kg basal feed. The ingredients used in formulating basal diet and their chemical composition analyzed as per AOAC (2013) are presented in Table 1.

Table 1. Ingredients and Chemical Composition (% DM Basis) of Experimental Diets in Different Growth Phases of Broiler Chicks

Ingredients (Kg/100kg)	Pre-starter	Starter	Finisher
Maize	50	52.6	58.2
Soybean Meal	25	22.3	16.8
Fish Meal	6	6	6
Groundnut cake	10	10	10
Vegetable Oil	4.67	5.48	5.9
Mineral Mixture	2	2	2
Common salt	0.5	0.5	0.5
Proximate chemical Composition (%DMB)			
Moisture	11.6	12.3	12.5
Dry Matter	88.4	87.6	87.5
Crude Protein	22.8	21.8	20.1
Ether Extract	7.78	8.38	8.61
Crude Fibre	4.89	4.45	4.15
Ash	9.83	9.51	8.92
Nitrogen Free Extract	43.1	43.5	45.7
Metabolizable Energy(Kcal/Kg)	3010.67	3104.64	3195.56

The birds were housed in deep litter system from day-old to 42 days of age following standard management practices. The birds were fed pre starter, starter and finisher diets for 1 to 7, 8 to 21 and 22 to 42 days of age, respectively. Feed and water were provided *ad-lib*. All the birds were vaccinated against Ranikhet disease on 4th day and IBD on 13th day of age. At the end of the feeding trial (42 days), about 2 ml blood sample was collected from one bird per replicate via brachial wing vein puncture into vacutainer tubes containing EDTA for blood biochemistry. Samples of breast and thigh muscles were taken from each of the slaughtered

birds and stored in deep-freeze separately for further analysis. These samples were analyzed for moisture, protein, ether extract, cholesterol and fatty acid composition as per AOAC (2013).

RESULTS AND DISCUSSION

Carcass Traits

The results of present study showed that dressed mean percentage weight was significantly ($p < 0.05$) increased in all betaine supplemented treatment groups in comparison to control group (T1) and ranged from 75.67% to 77.42%. The percent mean weight of dressed, eviscerated and drawn carcass

were seen highest in T5 group supplemented with 2000 mg betaine per kg feed followed by T4 group which showed non-significant difference with T5 group (Table 2). The weight of giblets (liver, heart and gizzard) was observed highest in T5 group. However, no marked difference ($p>0.05$) was observed in comparison to control (T1) group. The current findings are in line with the findings of Susmita et al., (2020) who stated that replacement of 50% methionine with 0.3% betaine in feed resulted in significant increase in carcass yield in comparison to basal diet control group. Our results

corroborate with the findings of Chand et al., (2017) which depicted that significantly ($p<0.05$) higher dressing percentage was recorded in group containing betaine @2g/kg feed followed by group containing betaine @1.5g/kg feed in comparison to control. The increase in dressing percentage may be due to the osmotic effects of betaine, which increases water retention (Waldroup and Fritts, 2005). Our results are in line with El-Shinnawy (2015) also; who reported that supplementation of betaine significantly increased the dressing percentage in chicken.

Table 2: % Mean values of dressed, eviscerated, drawn yield and weight of giblets in broiler chicken under different dietary treatments

Treatment	Dressed %	Eviscerated %	Drawn %	Liver %	Heart %	Gizzard %	Giblet %
T1	75.6 ^a ±0.30	66.3 ^a ±0.28	72.5 ^a ±0.26	2.74±0.05	0.71±0.01	2.20±0.03	5.65±0.04
T2	76.1 ^{ab} ±0.29	66.8 ^{ab} ±0.26	72.98 ^{ab} ±0.30	2.71±0.05	0.70±0.01	2.22±0.02	5.63±0.05
T3	76.7 ^b ±0.36	66.8 ^{ab} ±0.37	73.2 ^b ±0.39	2.73±0.04	0.71±0.01	2.25±0.01	5.69±0.04
T4	76.9 ^{bc} ±0.37	67.45 ^{bc} ±0.38	73.9 ^{bc} ±0.41	2.76±0.03	0.75±0.01	2.21±0.02	5.72±0.04
T5	77.4 ^c ±0.20	67.9 ^c ±0.16	74.8 ^c ±0.20	2.79±0.03	0.73±0.01	2.24±0.04	5.76±0.07

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

The results pertaining to abdominal fat percentage showed that groups (T3, T4, T5) receiving 1000, 1500 and 2000 mg betaine per kg feed resulted in significant ($p<0.05$) abdominal fat reduction in comparison to control group (Table 3). Perusal of data related to weight of lymphoid organs (spleen, bursa & thymus) showed no significant difference among betaine supplemented groups and control group (T1). The percent mean weight of breast and thigh were highest ($p<0.05$) in T5 group supplemented with betaine at 0.2% inclusion level (2000mg/kg) in comparison to control group (T1). Previous studies by Susmita et al. (2020) indicated that betaine @ 0.3% inclusion level in diet reduced abdominal fat significantly in comparison to control group. Zhan et al. (2006) reported that activity of hormone sensitive lipase enzyme in abdominal fat was increased by

betaine (0.5g/kg) supplementation resulting in significant reduction in abdominal fat content. Betaine supplementation also enhances the synthesis of methylated compounds in body such as carnitine and creatine. Carnitine is responsible for the transport of fatty acids through the inner mitochondrial membrane where fatty acid oxidation takes place (Stryer, 1988) ultimately leading to less fatty acids deposition in the body. However, our results are in contrast with Attia et al. (2005) who stated that there is an increase in abdominal fat pad by the supplementation of betaine. Another findings by Nofal et al. (2015) corroborating with our results stated that inclusion of 0.2% betaine significantly increased carcass weight, dressing percentage, giblets weight, thigh and breast muscle yield in growing broilers under heat stress conditions.

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Table 3. Percentage Mean values of abdominal fat, spleen, thymus, bursa, thigh and breast weight percentage in broiler chicken under different dietary treatments

Treatment	Abdominal fat %	Spleen %	Bursa %	Thymus %	Thigh %	Breast %
T1	0.94 ^b ±0.03	0.13±0.04	0.15±0.04	0.37±0.02	9.31 ^a ±0.15	25.2 ^a ±0.17
T2	0.91 ^b ±0.01	0.12±0.03	0.17±0.05	0.38±0.01	9.43 ^a ±0.13	26.3 ^b ±0.24
T3	0.87 ^a ±0.01	0.15±0.01	0.17±0.03	0.41±0.02	10.14 ^b ±0.16	26.8 ^{bc} ±0.21
T4	0.85 ^a ±0.02	0.14±0.02	0.19±0.02	0.40±0.03	9.85 ^{ab} ±0.14	27.2 ^c ±0.14
T5	0.85 ^a ±0.04	0.14±0.01	0.18±0.01	0.39±0.01	10.4 ^b ±0.17	27.4 ^c ±0.23

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

Breast and thigh meat composition as presented in Table 4 & 5 shows that betaine supplementation had a positive effect on the meat quality of broilers. The highest percent crude protein and lowest ether extract content of both breast and thigh meat was observed in T5 group differing significantly with the control (T1) group. Our results are supported by the study of Popova et al. (2016) which reported that betaine supplemented @1g/kg and 2g/kg feed had resulted in significantly increased protein content of breast muscle of broilers in comparison to control group. Studies conducted by Chen et al. (2022) also

showed an increase in crude protein of breast muscles in betaine supplemented groups in comparison to control group. These results suggest that the dietary betaine supplementation may have favorable influence on protein deposition and fat reduction, due to its capability of methyl donor and increased activity of lipase enzyme. The improvement of lean carcass with increased protein percentage can be attributed to the higher availability of methionine and cystine for protein deposition in betaine-supplemented diets (McDevitt et al. 2000).

Table 4. Composition of breast meat of the experimental birds under different dietary treatments

Treatment	Moisture %	CP %	EE %
T1	72.17 ^a ±0.25	21.38 ^a ±0.12	6.12 ^b ±0.19
T2	72.32 ^a ±0.27	21.45 ^a ±0.16	5.82 ^b ±0.14
T3	72.94 ^{ab} ±0.19	21.69 ^a ±0.17	5.36 ^{ab} ±0.12
T4	73.48 ^b ±0.22	22.25 ^b ±0.14	4.71 ^a ±0.21
T5	73.78 ^b ±0.28	22.34 ^b ±0.20	4.68 ^a ±0.09

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

Table 5. Composition of thigh meat of the experimental birds under different dietary treatments

Treatment	Moisture %	CP %	EE %
T1	73.5 ^a ±0.14	18.4 ^a ±0.17	7.31 ^b ±0.23
T2	73.6 ^a ±0.12	18.7 ^a ±0.27	7.21 ^b ±0.17
T3	73.5 ^a ±0.21	19.1 ^{ab} ±0.25	6.86 ^{ab} ±0.19
T4	74.4 ^b ±0.11	19.6 ^b ±0.16	6.62 ^a ±0.24
T5	74.1 ^b ±0.19	19.8 ^b ±0.22	6.53 ^a ±0.13

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

Fatty acid and cholesterol composition of breast and thigh muscle as depicted in Table 6 & 7 respectively showed that cholesterol and saturated fatty acid (SFA) levels were decreased ($P<0.05$) while mono unsaturated fatty acid levels were increased significantly ($P<0.05$) in group supplemented betaine @2000 mg/kg feed (T5) in comparison to the control group. Though, there were

increments in PUFA level by betaine supplementation but the changes were non-significant to control. These results are supported by Yang et al. (2022) that Intra-muscular saturated fatty acids decreased, while total monounsaturated fatty acids and polyunsaturated fatty acids increased ($P<0.05$) by supplementation of betaine in broiler birds.

Table 6. Fatty acids and cholesterol content of breast meat of the experimental birds under different dietary treatments

Treatment	SFA %	MUFA %	PUFA %	Cholesterol (mg/100g fat)
T1	37.5 ^c ±0.16	42.7 ^a ±0.20	21.5±0.04	57.2 ^c ±0.26
T2	37.2 ^c ±0.14	42.8 ^a ±0.17	21.6±0.05	56.5 ^{bc} ±0.27
T3	36.6 ^b ±0.19	43.3 ^{ab} ±0.13	21.6±0.01	55.7 ^b ±0.27
T4	35.9 ^a ±0.14	43.2 ^b ±0.22	21.7±0.07	54.7 ^a ±0.27
T5	35.8 ^a ±0.12	43.5 ^b ±0.19	21.8±0.09	54.8 ^a ±0.27

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

Table 7. Fatty acids and cholesterol content of thigh meat of the experimental birds under different dietary treatments

Treatment	SFA %	MUFA %	PUFA %	Cholesterol (mg/100g fat)
T1	36.4 ^b ±0.30	43.5 ^a ±0.23	19.3±0.14	61.6 ^c ±0.21
T2	36.1 ^b ±0.28	44.2 ^b ±0.24	19.4±0.16	60.8 ^{bc} ±0.24
T3	35.7 ^{ab} ±0.14	45.7 ^c ±0.25	19.4±0.11	59.6 ^a ±0.27
T4	35.3 ^a ±0.24	45.7 ^c ±0.29	19.6±0.11	59.7 ^a ±0.21
T5	35.2 ^a ±0.26	45.9 ^c ±0.24	19.8±0.12	59.4 ^a ±0.26

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

BLOOD BIOCHEMISTRY

In hematological analysis the mean values of Hb were significantly higher in T3, T4 and T5 group in comparison to control (Table 8). The total RBC count was significantly higher in all betaine supplemented groups in comparison to control group. TLC and lymphocyte count was increased with betaine supplementation and was seen significantly elevated in T5 and T4 groups in comparison to other groups. The percent heterophil count was significantly reduced in all betaine supplemented groups in comparison to control (T1) while being lowest in 2000mg betaine per kg feed supplemented group (T5). However, among betaine supplemented groups there wasn't any significant difference observed.

These findings are in agreement with Nofal et al., (2015) who showed that supplementation of betaine in diet significantly decreased heterophil percentage but lymphocyte percentage was significantly increased, whereas, H/L ratio was significantly reduced.

Exposure to heat stress in poultry leads to reduction in the number of lymphocytes (L), increment in the number of heterophils (H), and consequently increasing the H:L ratio. Decreased level of heterophil percent by betaine supplementation is an indication of reduced stress and is related to the increase in inflammatory cytokines production and consequently stimulation of corticotrophin-releasing hormones in the hypothalamus (Nofal et

al., 2015; Mashaly et al., 2004). Dietary supplementation of betaine can decrease the H:L ratio and stimulate the humoral immune response via regulation of cytokines production in the macrophages of the liver cell, inhibition of prostaglandin synthesis (Zhang et al., 1996), as well as increasing the release of nitric oxide from heterophils and macrophages (Klasing et al., 2002).

Gudev et al. (2011) also reported that supplementing betaine at the level of 1.5g/kg in feed significantly increased the lymphocyte and significantly decreased heterophil percentage. Another study by Chand et al., (2017) showed that the treatment groups having betaine had significantly lower heterophil number and significantly higher lymphocytes number than the control group.

Table 8. Mean values of hematological parameters of broiler birds under different dietary treatments

Treatments	Hemoglobing/dl	TEC×10 ⁶ /μl	TLC×10 ³ /μl	Heterophil %	Lymphocyte %
T1	10.4 ^a ±0.12	2.06 ^a ±0.17	26.2 ^a ±0.14	28.7 ^b ±0.02	58.3 ^a ±0.11
T2	10.9 ^{ab} ±0.17	2.63 ^b ±0.13	26.3 ^a ±0.12	28.2 ^a ±0.03	58.5 ^a ±0.08
T3	11.3 ^{bc} ±0.11	2.81 ^b ±0.07	26.4 ^{ab} ±0.17	28.2 ^a ±0.08	58.5 ^a ±0.12
T4	11.6 ^c ±0.24	2.73 ^b ±0.09	26.6 ^b ±0.07	28.3 ^a ±0.02	58.9 ^b ±0.05
T5	11.5 ^c ±0.09	2.94 ^b ±0.15	26.5 ^b ±0.14	28.2 ^a ±0.05	58.8 ^b ±0.12

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

In serological examinations, the data presented in Table 9 shows that cholesterol, triglyceride and LDL mean values of serum were significantly reduced by betaine supplementation in broilers and the lowest values were in T5 group followed by T4, T3, and T2 in comparison to the control group (T1). The mean values of HDL were improved by betaine supplementation and found maximum in T5 group followed by T4, T3, T2 in comparison to control (T1). Research conducted by Singh et al. (2015) also revealed that betaine at 2.0 g/kg feed decreased (P<0.01) serum triacylglycerol compared with the other dietary groups (control & 1.3g/kg) and this response was linear with the inclusion level of betaine in diet (P<0.01). Mishra et al. (2019) reported that concentrations of cholesterol (mg/dl) and triglyceride (mg/dl) differed significantly in 3g/kg

feed supplemented group from control group for the whole length of gestation period in sows showing lipolytic action of betaine on body fat. The lipase enzyme activity which catabolises triglycerides and long chain fatty acids to small chain fatty acids and glycerol by its lipolytic action in α -oxidation process might be responsible for the reduction in cholesterol and saturated fatty acid content. In another study by Saleh et al. (2023) the dietary inclusion of betaine and organic minerals resulted in reduction (P<0.01) in the levels of total cholesterol and LDL-cholesterol values in broilers. However, contrary to our findings, Rao et al. (2011) observed significant increase in serum cholesterol levels when betaine was fed at 800 mg/kg level at different levels of methionine supplementation.

Table 9. Mean values of serum cholesterol, triglycerides, HDL and LDL of broiler birds under different dietary treatments

Treatments	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
T1	131.25 ^d ±2.24	76.7 ^d ±1.23	81.4 ^a ±0.37	39.1 ^c ±0.66
T2	128.75 ^c ±1.25	71.3 ^c ±1.71	83.4 ^b ±0.44	38.4 ^b ±0.36
T3	126.75 ^b ±1.39	68.1 ^b ±1.55	85.5 ^c ±0.45	37.9 ^b ±0.41
T4	124.50 ^a ±2.12	65.6 ^a ±2.12	86.3 ^d ±0.44	36.9 ^a ±0.64
T5	123.75 ^a ±1.84	64.8 ^a ±1.91	86.4 ^d ±0.50	36.1 ^a ±0.51

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

Serum proteins albumin, globulin and total protein mean values were significantly higher in T3, T4 and T5 groups in comparison to the control group and were maximum in the T5 group as can be seen in Table 10. Park and Kim (2019) reported that total protein and albumin concentrations were elevated in broiler chickens fed a diet containing 0.12% betaine compared with those fed no betaine diet ($P < 0.05$). They also mentioned that total protein is positively related to the synthesis of tissue and can be used as an indicator of health, nutritional status and functional effectiveness of some organs and immune efficiency of animals. But, it can also reflect any underlying conditions like dehydration, kidney

disease, liver disease or any other infection. So, if total protein is abnormal, further tests must be performed to identify which protein fraction is abnormal, so that a specific diagnosis can be made to rule out the cause of elevated or decreased levels. Our results are also in line with study conducted by Afrin et al. (2018) which revealed a significant increase in total protein at 0.45% inclusion level when compared with control group. However, contrary to our findings, Susmita et al. (2020) found the decreased ($p < 0.05$) levels of albumin in comparison to the control group when half of the methionine was replaced with 0.1%, 0.2% or 0.3 % inclusion levels in betaine supplemented groups.

Table 10. Mean values of serum proteins of broiler birds under different dietary treatments

Treatments	Albumin (g/dl)	Globulin(g/dl)	Albumin/Globulin ratio	Total protein (g/dl)
T1	1.84 ^a ±0.01	1.72 ^a ±0.01	1.06±0.01	3.56 ^a ±0.02
T2	1.92 ^a ±0.02	1.81 ^{ab} ±0.04	1.06±0.03	3.73 ^a ±0.03
T3	2.21 ^b ±0.04	1.93 ^b ±0.03	1.14±0.03	4.14 ^{bc} ±0.04
T4	2.59 ^c ±0.02	1.92 ^b ±0.01	1.34±0.01	4.51 ^c ±0.01
T5	2.62 ^c ±0.03	1.94 ^b ±0.08	1.35±0.06	4.55 ^c ±0.04

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

CONCLUSION

Based on the findings, it can be concluded that supplementation of broilers diet with betaine @2000mg/kg feed resulted in better production of leaner carcass with improved fatty acid profile and protein composition of meat in comparison to other betaine doses and control group. Supplementation of betaine at the level of 2000mg/kg feed had the most favourable outcome in blood biochemistry related parameters also, when compared with rest of the treatment groups.

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