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Effect of L-Glycine on Carcass Parameters, Blood Biochemicals, and Jejunal Histomorphometry of Commercial Broiler Chicken

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A study was conducted for duration of six weeks on 240, day-old Cobb-430Y broiler strain chicks and randomly distributed to four groups each containing three replicates and 20 chicks per replicate. Group A was Negative Control (NC) contained 3% Low Protein (LP) diet containing 19.50,

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18.00 and 16.50% crude protein (CP) for broiler pre-starter, starter and finisher phases, respectively. Positive Control (PC) group B contained standard protein 22.50, 21.00, and 19.50% CP as per breeder recommendation. No L-Glycine was added to NC and PC diets. Group C (NC+Gly1) was formulated by adding 0.640, 0.810, and 0.630% L-Glycine in NC diet to arrive 2.30, 2.30, and 2.00% digestible (Dig.) Gly + Ser levels while group D (NC+Gly2) was formulated by supplementation of 0.755, 0.985, and 0.740% L-Glycine in NC diet to arrive 2.40, 2.40, and 2.10% digestible Gly + Ser levels in broiler pre starter, starter and finisher diets respectively. Digestible Gly + Ser levels of NC group were 1.71, 1.56 and 1.43%; and of PC group were 2.02, 1.91 and 1.76%, respectively for broiler pre starter, starter and finisher diets. Ratio of dig. Met, Thr, Trp, Arg, and Val. ratioed to Lys. were balanced in all diets. All diets were isocaloric (3010, 3100, and 3200 kcal ME /kg) for broiler pre starter, starter and finishaer phases, respectively. Results showed that the L-Glycine supplementation in AA-balanced 3% LP diet did not affect per cent eviscerated yield, giblet yield, ready-to-cook yield, and breast meat yield but helped to reduce abdominal fat without altering serum biochemical parameters. Dig. Gly + Ser levels of 2.30, 2.30, and 2.00% (L-Glycine levels of 0.640, 0.810, and 0.630%) in 3% low protein (19.50, 18.00, and 16.50% CP) for broiler pre starter, starter and finisher diets helps to improve jejunal crypt depth leads to increasing cell renewal rate and maturation in the gut. Incorporation of L-Glycine is recommedded to maintain Dig. Gly + Ser levels of 2.30, 2.30, and 2.00% in broiler pre starter, starter and finisher phases with reduction of 3% CP in breeder recommended commercial broiler diet. Reducing dietary crude protein using L-Glysine without harming broiler performance could be economical.

Keywords: Broiler; L-glycine; dig. gly + ser.; low-CP.

1. INTRODUCTION

The broiler feed cost accounts for 70-75% of the total cost of production. Poultry nutritionist have been made many attempts to reduce feed cost by formulating a balanced diet with incorporation of various amino acids. Glycine is the smallest proteinogenic non-essential amino acid and has no isomeric form. Glycine and serine are interconvertible, and their interconversion is not limited to metabolism. Therefore, glycine and serine are evaluated together in poultry nutrition studies. Insufficient glycine levels in feed impair growth rate. Hence, it is considered as conditionally essential in poultry diets [1]. The reduction of the CP content in poultry diets with a desirable effect on performance was shown to be limited even when the requirement of essential amino acids was considered [2].

Glycine is deficient in plant-based diets compared to chickens' ability to synthesize protein [3,4]. Corn-soya based broiler diets for 7-14 and 35-42 days provide 30.8% and 28.2% of the glycine required for weight gain and the production of uric acid in the body, respectively [5]. This suggests that chicken-fed conventional diets cannot produce enough glycine to meet metabolic needs [6]. A practical corn-soya diet responds to glycine addition [7]. Glycine enhances protein utilization and essential amino acids' metabolic and functional efficiency, particularly sulfur-containing amino acids like cysteine, threonine, and arginine [8]. Therefore further decrease in CP content in the broiler diet, non-essential amino acids like glycine and serine need to be considered. Feeding broilers with reduced CP in all vegetable diets limit the concentration of glycine [9,10,11]. Hence, the currently formulated corn-soy diet must be supplemented with synthetic glycine (L-glycine), which is available. Glycine has a positive impact on increasing the effectiveness of protein utilization in low CP diets. A low CP diet supplemented with L-glycine improves broiler performance [10,12]. Glycine can directly or indirectly influence the ntestinal mucosa function and improve dietary energy utilization [13]. Studies on biochemical parameters found that the glycine supplementation up-regulated the metabolic processes [14]. The addition of glycine to diluted protein diets improved the meat quality by increasing the protein and decreasing the fat content of meat [15]. Positive impact of L-Glycine on gut health was also reported [16,17-19]. Considering the importance of Glycine in corn-soy diet, the present experiment was carriedout to study the L-glycine supplementation in low protein diet on carcass parameters, serum biochemical parameters, and jejunal histomorphometry.

2. MATERIALS AND METHODS

An experiment was conducted for a period of six weeks duration on 240 day-old straight-run commercial broiler chicks of Cobb-430Y strain to study the effect of L-Glycine supplementation in low protein diet on the carcass parameters, blood Biochemicals, and jejunal histomorphometry of commercial broiler chicken. Chicks were randomly distributed to four groups, *viz*., A, B, C and D, containing 60 chicks in each group subdivided into three replicates of 20 chicks each. Group A was the Negative Control (NC) diet containing of 3% Low Protein (LP) 19.50, 18.00 and 16.50% CP for broiler pre-starter, starter, and finisher phases, respectively. Positive Control (PC) group B contained standard protein 22.50, 21.00 and 19.50% CP for pre starter, starter and finisher phases, respectively as per the breeder recommendation. No L-Glycine was added to NC and PC diets. Group C (NC+Gly1) was formulated by incorporation of 0.640, 0.810 and 0.630% L-Glycine in pre starter, starter and finisher respectively, in the NC diet to arrive at 2.30, 2.30 and 2.00% digestible Gly + Ser levels in respective diets. Group D (NC+Gly2) was formulated by incorporation of 0.755, 0.985 and 0.740% L-Glycine in pre starter, starter and finisher diets respectively. In NC diet to arrive at 2.40, 2.40 and 2.10% digestible Gly + Ser levels in respective diets. The dig. Gly + Ser levels for pre starter, starter and finisher diets of NC group (A) were 1.71, 1.56 and 1.43%, and of PC group (B) were 2.02, 1.91 and 1.76% respectively. The ratio of dig. Met, Thr, Trp, Arg and Val to Lys. were balanced in all the experimental diets. All the experimental diets were isocaloric, containing 3010, 3100, and 3200 kcal/kg of metbabolizable energy (ME) for pre starter, starter and finisher feed respectively. During the experimental period following parameters were studied.

2.1 Biochemical Parameters

At the end of experiment on 35th day, blood samples were collected two birds from each replicate. The blood samples were collected in a sterile dry labeled glass tube and kept in a slanted position at room temperature to facilitate the serum separation. The serum total protein and albumin were estimated calorimetrically using the Biuret method, utilizing available diagnostic kits in the market. The serum globulin levels were calculated mathematically by subtracting serum albumin from total serum protein. The serum Albumin: Globulin ratio (A/G) were calculated. The Serum Urea Nitrogen and Serum Uric Acid was estimated using commercial diagnostic kits.

2.2 Carcass Parameters

At the end of experiment one male and one female bird were randomly selected from each replicate and slaughtered to study the carcass parameters. The broilers were fasted for four hours, and pre-slaughter weights were recorded. The eviscerated weight, Giblet weight, breast weight and ready-to-cook (RTC) yield weight were recorded. The per cent eviscerated yield, giblet yield and ready-to-cook yield were calculated on live weight basis. The abdominal fat weights were taken and the percentage of fat pad was calculated.

2.3 Histomorphological Studies of Jejunum

At the end of experiment on 42 day age Jejunum of six birds from each treatment was collected at the time of slaughter. Three pieces of each jejunal sample (one from the top, one from the middle, and one from the bottom of the jejunal loop) were collected for the histo-morphometry. The collected samples were fixed and preserved in 10% neutral buffer formalin. After fixation, the collected tissue pieces were processed using the alcohol-xylene protocol in an automated tissue processor and embedded in paraffin (60 degrees Celsius). The tissue sections were cut at 3-5 micrometer thickness on automated tissue microtome, and slides were stained by routine Haematoxylin and Eosin methods [20]. The jejunal tissue sections were observed for villus height, width, and crypt depth measurements. Villus height was measured by the distance from the crypt opening to the tip of the villus, whereas crypt depth was measured at the villus's base, middle, and top to arrive at the average width of the villus.

2.4 Statistical Analysis

The data analyzed in a completely randomized design by using one-way ANOVA with the help of IBM SPSS Software-20. The Duncan Multiple Range Test (DMRT) post-hoc analysis was done to test the significant mean differences between the groups with significance levels defined at $P < .05$ [21].

3. RESULTS AND DISCUSSION

3.1 Carcass Parameters

The percent of eviscerated yield, giblet yield, ready-to-cook yield, abdominal fat, and breast meat yield of broiler birds fed L-Glycine in a low protein diet is presented in Table 1. The broiler birds' per cent eviscerated yield, giblet yield, ready-to-cook yield, and breast meat yield did not differ significantly among NC, PC, NC+Gly1, and

NC+Gly2 groups. No notable effects on dressing percentage by reducing dietary CP compared to the control diet were reported [22]. They concluded that feeding Cobb 500 broilers on low protein diets at persistent ME with the same amino acid concentrations does not adversely affect carcass parameters. There was no significant difference in dressing yield and breast meat yield in glycine-supplemented groups [23,24], as observed in the present findings. In contrast, Kriseldi et al. [25] observed positive response of L-Gly on carcass characteristics of broiler chicken fed with Low-CP diet.

Though the abdominal fat percentage did not differ significantly, there was a numerical reduction in abdominal fat percentage in group C and D compared to group A and B. Hejdysz et al. [26] reported that glycine supplementation decreased fat deposition. Cave [27] and Paschal et al. [28] reported that adding 3% glycine in a low protein diet (18.2%CP) decreased carcass fat. Supplementation of L-Glycine to LP diets in the present study seems beneficial in reducing the abdominal fat percentage. A significant interaction between CP and glycine levels for abdominal fat was reported [28,29,30]. Increased abdominal fat in the NC diet might be due to reduced dietary protein. The reduced protein diet promotes fatty acid synthesis. The reduced protein diet changed lipid metabolism and improved abdominal fat deposition [31].

The overall results of the present study indicated that the AA-balanced LP diet with or without the addition of L-Glycine did not affect eviscerated yield, giblet yield, ready-to-cook yield, and breast meat yield of broiler birds. The addition of L-Glycine in AA balanced LP diets might be beneficial in reducing abdominal fat.

3.2 Serum Biochemical Parameters

The statistical analysis of the data on various serum biochemical parameters estimated at the end of the 35th day of age is depicted in Table 2. The statistical analysis of the data showed that the serum total protein, albumin, globulin, albumin: globulin ratio, serum uric acid, and serum urea nitrogen values did not differ significantly among NC, PC, NC+Gly1, and NC+Gly2 groups. The LP diet, NP diet, and LP diet with the addition of L-glycine were statistically similar indicating that addition of L-Gycine in AA balanced LP diet did not affect serum biochemical parameters in broiler at the end of 35th day age. These results corroborated the findings of [32], who reported that L-Glycine supplementation in the LP diet did not alter blood biochemical parameters. In contrast to the present findings, Hernandez et al. [33] reported that the higher fortification with Gly in their NC diet consistently increased the serum UA. Gly is directly involved in the synthesis of UA by providing two carbons and one nitrogen atom, which might be the reason behind the observed increase in serum UA in higher Gly fortification levels. However, supplemental Glycine numerically increased serum urea nitrogen values. Yuan et al. [34] reported that supplemental Gly significantly increased the serum urea nitrogen.

Table 1. Effect of L-Glycine supplementation in low protein diet on carcass parameters on the basis of live weight

Treatment	Body weight (g)	Eviscerated yield (%)	Giblet yield (%)	RTC yield (%)	Abdominal Fat (%)	Breast yield (%)
A	2412.33^{a} ±72.24	68.86 ±1.05	3.88 ±0.09	72.74 ±1.08	2.14 ±0.12	30.30 ±1.37
B	2697.50^{b} ±18.79	68.61 ±0.42	3.68 ±0.10	72.29 ±0.37	2.25 ±0.06	30.32 ±0.41
C	2638.00^{b} ±25.83	69.00 ±0.26	3.73 ±0.04	72.74 ±0.29	1.96 ±0.04	31.87 ±0.33
D	2577.83^{b} ±37.80	68.39 ±0.24	3.80 ±0.06	72.19 ±0.24	1.97 ±0.07	31.31 ±0.28
SEm	30.148	0.282	0.040	0.287	0.046	0.377
P-value	0.01	0.890	0.369	0.877	0.064	0.388

Means bearing different superscripts within the column differs significantly (P < .05)

Table 2. Effect of L-Glycine supplementation in low protein diet on serum biochemical parameters

Treatment	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	A/G ratio	Serum Uric Acid (mg/dL)	Serum Urea Nitrogen (mg/dL)
A	2.86 ±0.98	1.51 ±0.42	1.35 ±1.03	1.78 ±1.32	3.24 ±0.60	4.13 ±0.92
B	2.36 ±0.40	1.63 ±0.34	0.73 ±0.38	3.33 ±2.90	2.69 ±0.45	3.78 ±1.10
C	2.72 ±0.44	1.83 ±0.41	1.87 ±2.25	1.93 ±1.31	2.62 ±0.24	4.27 ±0.87
D	3.62 ±2.08	1.62 ±0.31	1.13 ±0.67	1.88 ±1.11	2.89 ±0.82	4.21 ±0.61
SEm	1.207	0.371	1.282	1.812	0.588	0.858
P-value	0.344	0.523	0.503	0.420	0.265	0.781

Means bearing different superscripts within the column differs significantly (P < .05)

Table 3. Histomorphological observations of jejunum of sixth-week broiler birds supplemented with different L-Glycine levels in Low protein diet

Treatment	Villi hight (µm)	Villi width (µm)	Crypt depth (µm)	Villi height to crypt depth ratio
A	1349.04±9.43	181.15±10.93	202.01^{a}±12.10	6.72^{ab}±0.41
B	1322.79±48.81	208.46±5.42	201.01^{a}±4.52	6.59^{ab}±0.35
C	1381.79±22.48	172.60±7.98	240.28^{b}±5.29	5.75^{a}±0.10
D	1362.30±20.75	194.19±27.10	182.33^{a}±8.34	7.50^{b}±0.31
SEm	14.0026	12.104	7.236	0.230
p-value	0.568	0.427	0.006	0.029

Means bearing different superscripts within a column differ significantly (P < .05)

3.3 Histomorphological Studies of Jejunum

The statistically analyzed data on the measurement of jejunal villus height, width, and crypt depth is depicted in Table 3. The biometrical measurement of jejunal villi height and width among NC, PC, NC+Gly1 and NC+Gly2 groups did not differ significantly. In agreement with the present findings, Ospina-Rojas et al. [13] reported that Gly+Ser did not alter the height of the intestinal villus. Similarly, Salim et al. [32] observed no significant difference in villi height in broilers fed an LP diet fortified with Glycine. Laudadio et al. [35] examined histological parameters in the jejunum were not affected by dietary protein level. Glycine can directly or indirectly influence the proper function of the intestinal mucosa and improve the utilization of dietary energy [13].

The jejunal villus crypt depth was significantly higher (P < .05), while the Villi Height to crypt depth ratio (VH:CD) was significantly less (P < .05) in broilers from NC+Gly1 group compared to broilers from NC, PC and NC+Gly2 groups. These present findings are in agreement with those of [36]. Tsirtsikos et al. [36] reported that the increase in crypt depth of chicken supplemented with Gly and Thr might provide more surface area for nutrient absorption by increasing enterocyte proliferation and intestinal mucin secretion because mucin-producing goblet cells are present mainly in the crypt. Geyra et al. [37] reported that crypt development is essential to increase cell renewal rate and maturation in the gut. The overall findings of the present study indicated that addition of L-glycine having dig. Glycine levels of 1.39, 1.48, 1.25 with respective dig Gly + Ser levels of 2.30, 2.30, and 2.00 in pre-starter, starter, and finisher diet respectively, help to improve jejunal crypt depth at the sixth week of age in broilers that increase cell renewal rate and maturation in the gut.

4. CONCLUSIONS

The findings of the present research concludes that supplementation of L-Glycine in commercial broiler chicken fed with AA-balanced 3% low-protein diets without changing the dietary ME did not affect per cent eviscerated yield, giblet yield, ready-to-cook yield, and breast meat yield but helped to reduce abdominal fat without altering serum biochemicals parameters. Moreover, dig. Gly + Ser levels of 2.30, 2.30, and 2.00% (L-Glycine levels of 0.640, 0.810, and 0.630%) in 3% low protein (19.50, 18.00, and 16.50% CP) for respective pre starter, starter and finisher broiler diets helps to improve jejunal crypt depth leads to increasing cell renewal rate and maturation in the gut in commercial broiler chicken. Supplementation of L-Glycine is recommended to maintain dig. Gly + Ser levels of 2.30, 2.30, and 2.00% in pre starter, starter and finisher phases with a reduction of 3% CP in a standard commercial broiler diet. Reducing dietary crude protein using L-Glysine without harming broiler performance could be economical.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ETHICAL APPROVAL

The research is approved by Institutional Animal Ethics Committee (IAEC) [Protocol No IAEC13/23/KNPCVS/2024].

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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