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Effect of L-Leucine Supplementation on Growth Performance and Carcass Characteristics of Grower-Broiler Chickens Fed Low Protein Diets

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Abstract: Problem statement: Supplementation of broiler diets with cristalline amino acids (i.e. lysine, ethionine and threonine) may support equal broiler growth and improve overall amino acids balance and enable a reduction in CP level of diets. **Approach:** A trial was conducted to evaluate the effect of supplemental L-leucine in diets containing recommended levels and low crude protein (20 and 18%, respectively) with constant metabolizable energy (3200 kcal kg^{-1}) for broilers from 21-42 day of age. Six experimental diets were formulated with three levels of supplemental L-leucine, 0, 0.5 and 0.67% and two levels of crude protein. A total of 180 1 day-old Cobb broiler chickens were randomly divided into 36 experimental pens, 5 chickens in each pen, with each diet replicated 6 times. The dietary treatments were offered from 21-42 days of age. Feed intake, body weight gain and Feed Conversion Ratio (FCR) were measured on a weekly basis. At the end of the feeding trial the birds were slaughtered and carcass analyses conducted. **Results:** Feed intake, weight gain and FCR were not affected by increasing levels of L-leucine supplementation. Weight gain was significantly reduced ($p<0.05$), whereas feed intake and FCR were not significantly affected with decreasing dietary crude protein. A positive response in breast meat yield was achieved by the addition of L-leucine to levels up to 0.5% in the diet but a significant decrease was noted when the level reached 0.67% in diet. Supplementation of L-leucine significantly ($p<0.05$) decreased the relative weights of the liver and gizzard. However, the addition of L-leucine significantly reduced carcass weights when L-leucine was added at 0.67%. Lowering the dietary protein level also significantly reduced breast yield and carcass weight ($p<0.05$). However, abdominal fat, gizzard, liver and heart were not affected by protein level. **Conclusion/Recommendations:** It can be concluded that supplementation of L-leucine at levels up to 0.67% of the diet did not affect performance but deleteratious the carcass weight.

Key words: Leucine, low-protein diet, broiler, carcass characteristics

INTRODUCTION

Major changes in feeding strategies in poultry production have been proposed to alleviate environmental problems related to increasing nitrogen output in animal manure by large animal production units. During the last few decades, dietary formulations were aimed at maximizing broiler performance at least cost without concern for nutrient oversupply. Protein, having a major effect on growth performance, is the most expensive component in broiler diets[7]. Hence, the recent issues regarding the environment and the high cost of protein supplement has encouraged nutritionist to decrease Crude Protein (CP) levels in diets whilst sustaining performance. Since 1950's methionine and lysine supplementation have been successfully used in poultry ration formulations subsequently allowing the reduction in CP levels, whilst maintaining these amino acids levels. Moreover, with the development of additional feed-grade amino acids such as threonine and tryptopan, the possibility exists to further decrease the CP level in diets since it is known that increased performance in chicks can be achieved if Essential Amino Acids (EAA) in low CP diets are equal

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to those needed in higher CP diets[24]. However, the effects of replacing low CP with several amino acids on broiler have been shown to be quite variable[4,13,17,27]. The reason for the failure to achieve the performance on low protein diets supplemented with amino acids equivalent to that of higher CP diets are not clearly understood. Branched Chain Amino Acids (BCAAs) have been recognized as deleterious to many species when fed in excess[6,14]. Studies have shown that in some species excess BCAAs resulted in depressed feed intake and reduced growth. Excessive levels of leucine in the diet caused decreases in weight gain and feed intake during the starter period of broiler chickens[8,13,23,28] but increased carcass weight of growing broiler chickens[9,18].

Extreme excesses of dietary leucine also lead to feather problems similar in description to those occurring when valine is low[23]. No research has been reported concerning high levels of L-leucine in the diet of broiler chickens during the grower phase. The objective of this study was to investigate the effect of L-leucine supplementation on the performance of grower-broiler chickens and their carcass characteristics fed diets with two levels of protein. It was hypothesized that supplementation of L-leucine at levels above that recommended by NRC[21] to low protein diets could support growth performance.

MATERIALS AND METHODS

In a 3×2 factorial design experiment, 180 straight run day-old broiler chicks (Cobb) were used to evaluate the effects of three levels of L-leucine supplementation and two levels of crude protein on growth performance and carcass characteristics of grower-broiler chicks fed a corn-soyabean meal based diets. Initially, all birds were placed in four stainless steel cages measuring 2 feet ×2 feet ×1 feet high, fitted with feeders and waterers. The chicks were fed a standard commercial starter ration till 21 days of age. Feed and water were given *ad libitum* and they were subjected to continuous lighting. At day 21 the chicks were individually weighed and randomly allotted to six dietary treatments with each treatment replicated five times, with 5 birds in each replicate.

Table 1 shows the composition of the experimental diets with 3 levels of L-leucine supplementation (0, 0.5 and 0.67%) and two levels of dietary CP (20 and 18%). All diets were formulated using corn, soybean meal and DL-Methionine to meet or exceed the NRC[21] nutrient requirements. Fishmeal, soyabean meal and corn were analyzed for CP prior to diet formulation. The CP was determined using Kjedahl procedure[2]. The calculated ME was 3200 kcal per kg of all diets. Diet 1 was considered a positive control and calculated to contain 20% CP and 3200 kcal kg^{-1} of ME. Birds were given the experimental diets from 21-42 days of age. Feed and fresh water was provided *ad libitum* and a continuous lighting regime was subjected to the chicks throughout the experiment. Body weight and feed consumption were recorded weekly. Feed consumption was calculated as a difference between the amount of feed offered to the birds and the amount of feed that remained at the end of each feeding period. In cases where there was mortality, feed consumption was corrected for body weight of mortality. Body weight gain was calculated as the difference between the final and initial weights for each weighing periods. Feed conversion ratio (feed: Gain ratio) was calculated as a ratio between feed consumption and weight gain for each period and calculated for the growing period between 21 and 42 day. On 42nd day of the experiment, six birds from each treatment were randomly selected, slaughtered, defeathered and viscera removed to determine the carcass percentage. The data on carcass weight, abdominal fat and organ weight i.e., heart, liver and gizzard were also recorded. Abdominal fat comprised of leaf fat surrounding the cloaca and abdominal muscles, excluding fat surrounding the gizzard. Statistical analyses were conducted using General Linear Models procedure of SAS[26], based on a 3×2 factorial design. The differences among means were separated using Duncan's new multiple range test[30].

RESULTS

Proximate analyses of experimental diets are shown in Table 1. The CP of diets were as expected ranging from 18.00-18.04% for the low protein diets, while the high protein diets had values ranging from 20.00-20.02%. The calculated ME content ranged from 3204-3207 Mcal kg^{-1}. The mean of total L-leucine contents of the diets supplemented with 0, 0.5 and 0.67% L-leucine, were 1.63, 2.12 and 2.28%, respectively.

Growth performance: The weight gain, feed consumption and Feed Conversion Ratio (FCR) of broiler chickens are presented in Table 2. There was no significant interaction between protein level and L-leucine supplementation, therefore only the main effect means are presented. The main effect of L-leucine supplementation showed that there was no significant differences ($p>0.05$) in body weight gain, feed intake and FCR among birds fed different levels of L-leucine.

Table 1: Composition (%) and nutrient content of experimental diets

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Corn (8 %)	61.000	65.31	61.00	65.51	61.00	65.54
Soya bean meal (44%)	24.000	25.00	24.00	25.00	24.00	25.00
Fish meal (57%)	6.990	2.10	6.49	1.60	6.32	1.40
Palm oil	5.420	5.00	5.42	4.80	5.42	4.80
Limestone	1.260	1.26	1.26	1.26	1.26	1.26
Salt	0.280	0.28	0.28	0.28	0.28	0.28
Dicalcium phosphate	0.100	0.10	0.10	0.10	0.10	0.10
Mineral mix	0.250	0.25	0.25	0.25	0.25	0.25
Vitamin mix[1]	0.250	0.25	0.25	0.25	0.25	0.25
L-lysine	0.200	0.20	0.20	0.20	0.20	0.20
DL-methionine	0.150	0.15	0.15	0.15	0.15	0.15
L-leucine	0.000	0.00	0.50	0.50	0.67	0.67
Choline cloride	0.100	0.10	0.10	0.10	0.10	0.10
Calculated analysis						
ME Kcal kg^{-1}	3,204	3,207	3,208	3,202	3,207	3,204
CP (%)	20.000	18.00	20.01	18.05	20.02	18.04
Ca (%)	0.920	0.68	0.89	0.66	0.89	0.65
Available P (%)	0.330	0.20	0.32	0.19	0.31	0.18
Fiber (%)	3.140	3.27	3.13	3.27	3.13	3.27
Mehionine + Cystine (%)	0.840	0.76	0.83	0.75	0.83	0.75
Lysine (%)	1.281	1.09	1.25	3,207	1.25	1.06
Leucine (%[1])	1.710	1.58	2.18	2.06	2.34	2.22

Note: The NRC (1994) recommends minimum levels of 1.09% Leucine for diets with 3200 ME kcal kg^{-1} and 20% crude protein on broiler from 21-42 days of age. [1]: Premix provided the following per kg or diet: Vitamin A 50.000 MIU, Vitamin D3 10.000 MIU, Vitamin E 75.000, Vitamin K 20.000 g, Vitamin B1 10.000 g, Vitamin B2 30.000 g, Vitamin B6 20.000 g, Vitamin B12 0.100 g, Calcium D-Panthothenate 60.000 g, Nicotinic acid 200.000 g, folic acid 5.000 g, Biotin 235.000 mg

Table 2: Weight gain, feed intake and Feed Conversion Ratio (FCR) of broilers fed diets with two levels of protein and supplemented with L-leucine from 21-42 day

Treatments	Feed intake (g)	Weight gain (g)	FCR
Main effect of protein level (%)			
20	2596.1	1275.5^{a}	2.13
18	2578.3	1170.3^{b}	1.92
Main effect of leucine suppl. (%)			
0.0	2608.3	1247.2	2.18
0.5	2582.6	1232.3	2.20
0.67	2570.8	1189.1	2.28
Level of significance			
Crude protein	NS	*	NS
L-leucine	NS	NS	NS
Crude protein × L-leucine	NS	NS	NS

Means within columns with different superscripts are significantly different ($p<0.05$). NS: Non Significant

Similarly, there were no significant differences ($p>0.05$) in the feed intake and FCR among chickens fed two levels protein. However, chickens fed low-CP diets gained significantly less weight ($p<0.05$) than chickens fed the control diets.

Relative organ weight and carcass characteristics: Table 3 shows the carcass weight, relative organ weights as affected by dietary concentration of L-leucine. The carcass weight, relative breast weight, gizzard and liver weights were significantly ($p<0.05$) reduced with increasing L-leucine supplementation. However, the abdominal fat and heart weights were not affected by increasing L-leucine supplementation. The relative liver weights ranged from 41.50-49.35 g, heart weight from 9.90-11.77 g, gizzard from 42.61-52.55 g and abdominal fat from 11.14-16.58 g. It was noted that there was an increase in breast meat yield with addition of 0.5% L-leucine, but decreased significantly when L-leucine was added at 0.67% of the diet. Supplementation of L-leucine significantly ($p<0.05$) decreased the relative weight of the liver and gizzard. However, addition L-leucine had not significant effect on carcass and heart. Decreasing dietary protein significant ($p<0.05$) decreased carcass weight and breast meat. However, lowering dietary protein had no significant effect on abdominal fat, gizzard, liver and heart weights.

DISCUSSION

The corn-soya bean meal based diets used in this experiment were formulated using supplements of lysine and DL-methionine to contain nutrient levels similar to those used in commercial broiler industry and were not designed to be deficient in L-leucine. Consequently, levels of leucine were in excess of their minimum requirements (Table 1). Many amino acids, when fed in excess to broiler growing chickens cause symptoms of toxicity such as decreased feed intake, weight gain and increased mortality[8,15,29]. L-leucine in excess may cause toxicity in chicks fed low protein diets as reported by Farran *et al*.[14,28].

Table 3: Weights (g) of Carcass, abdominal fat, breast meat, gizzard, liver and heart of broiler fed diets supplemented with L-leucine with two levels of protein at 42 days of age

Treatments	Level of leucine (%)	Level of protein (%)	Carcass weight (g)	Abdominal Fat (g)	Breast (g)	Gizzard (g)	Liver (g)	Heart (g)
Level of L-leucine (%)								
0.0			1675.1[a]	13.86	448.55[ab]	49.40[a]	46.91[a]	9.90
0.5			1637.7[ab]	12.74	486.68[a]	44.92[b]	42.82[b]	9.96
0.67			1580.0[b]	14.48	428.22[b]	43.94[b]	41.99[b]	13.21
Crude protein (%)								
20			1678.8[a]	13.06	462.58[a]	45.18	45.22	10.28
18			1583.0[b]	14.33	434.38[b]	46.99	42.60	11.77
L-leucine × crude protein Interactions								
	0.00	18	1611.3	16.58	435.42	52.55	44.74	9.63
	0.00	20	1738.9	11.14	461.77	46.25	49.35	10.16
	0.67	18	1524.0	13.10	408.66	45.26	41.50	15.79
	0.67	20	1635.9	15.87	447.77	42.61	42.48	10.63
	0.50	18	1613.6	13.30	459.16	43.17	41.83	9.89
	0.50	20	1661.8	12.18	478.20	46.67	43.82	10.04
Level of significance								
L-leucine			*	NS	*	*	*	NS
Crude protein			*	NS	*	NS	NS	NS
L-leucine × crude protein interaction		NS	*	NS	*	NS	NS	

Means within columns with different superscripts are significantly different ($p<0.05$); NS: Non Signifiant

However, in this study, the addition of 0.5 and 0.67% L-leucine to the diets resulted in a total of 2.06-2.34% in the respective diets, which was not sufficiently high to cause a significant reduction in growth. This finding agrees with results of Penz *et al.*[23] who showed that the addition of 1.6% L-leucine (total of 3.23%) in a 22.9% protein diet did not cause a significant decrease in growth performance of chicks. Similarly, in an earlier study, Erwan *et al.*[9] found that addition 0.5% L-leucine to an iso-nitrogenous diet (20% CP) with either 3200 or 3000 kcal kg^{-1} ME did not show significant effect on feed intake, weight gain and FCR in grower broiler chickens. Moreover, Edmonds and Baker[8], showed that addition of up to 4% L-leucine to a basal diet did not effect weight gain of broiler chicks. On the other hand, addition of 4.8% L-leucine caused a significant reduction on the growth performance of broiler chicks[23]. Farran *et al.*[14] reported that addition of 40 g L-leucine kg^{-1} did not induce toxicity in starter chicks fed high protein diet (255 g kg^{-1}). Similarly, excessive amount of L-leucine (70-100 g kg^{-1}) in a high protein diet did not affect performance of the rats[5]. D'mello and Lewis[6] indicated a decreasing trend in feed intake with increasing supplementation of dietary L-leucine. The reason why excessive L-leucine is toxic to chicks fed low protein diets is not well understood. It is speculated that the consumption of high dietary protein results in an abundance of all amino residues at the absorptive sites of the small intestines. Therefore, L-leucine being the least available amino acids would still be fairly absorbed in adequate amounts. In low protein diets, L-leucine may also accelerate the degradation of other BCAAs, mainly in muscles through the stimulated activity of branched-chain α-ketoacid dehydrogenase[16], which lead to the lowering of valine and iso-leucine in rat tissues. The results of this study indicate that addition of L-leucine in low protein diet may have toxic effects on grower broiler chickens as reflected by reduced feed intake, feed consumption and increase FCR even though they were not significant.

Liver and gizzard weights (Table 3), were significantly decreased in chicks fed low-CP diets. However, the finding of this study was not in agreement with Penz *et al.*[23] who found that liver weight was slightly increased by the addition of 5.4% L-leucine to the diet of broiler chickens. The negative-control, low CP diets in the experiment did not support weight gain similar to that of normal CP diets (control) despite meeting or exceeding the NRC recommendations. The ratio of essential and non-essential amino acids has also been suggested as a potential problem with low dietary CP in diets. However, studies evaluating the additions of L-leucine to low CP diets failed to show the same growth performance as those fed the recommended CP levels in broiler grower chickens. Other studies also reported that supplementation of non-essential amino acids to low CP diets have been unable to show equal growth performance of broiler fed control diets[1,4,10-12,19,24,25].

There were no significant difference on abdominal fat, gizzard, liver and heart due to level of protein. The results of the present study were in agreement with those Moran and Etches[20,22] who reported that chickens fed

varying protein levels to grower-finishing broiler did not show significant effect on the weights of gizzard, liver and heart. Lowering the dietary CP level significantly reduced carcass weight ($p<0.05$) agree with the findings of[31] who observed significant decrease in carcass responses of broiler fed on low CP diets.

CONCLUSION

Broiler chikens fed diets with supplementation of L-leucine up to level 0.67% in low CP had similar growth performance to the chickens fed diet of normal leucine content over a 21-42 d feeding period. However, carcass weights reduced significantly when L-leucine was added at 0.67%. Furthermore, lowering the crude protein level significantly reduced the weight gains and carcass weights.

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