

## Effects of amino acid dietary supplementation on growth performance and blood profiles of broiler chickens during the starter period

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### Abstract

*The aim of this study was to investigate the impact of dietary supplementation of amino acids on the blood profiles and growth performance of broiler chickens fed a low-protein diet during the starter period. One hundred and sixty unsexed broiler chicks, aged one day, were randomly divided into five groups, each with four replications and eight chicks per pen. The control group (T1) was fed a basal diet, while the other four groups were fed the basal diet supplemented with varying amounts of lysine, methionine, threonine, and valine. Growth performance and blood parameters were evaluated. Results indicated that there were significant differences in live body weight (LBW) and body weight gain (BWG) between T1, T2, and the other supplemented treatments (T3, T4, and T5), with the highest LBW observed in T5. However, there were no significant differences in the plasma levels of total protein, albumin, AST, uric acid, T3, T4, IgM, and IgG between the supplemented groups and the control. The findings suggest that the addition of extra amino acids to a low-protein diet during the starter period can enhance broiler chicken performance.*

**Keywords:** supplement, dietary, broilers, amino acids, proteins, growth

### Introduction

The poultry industry has been facing challenges in maximizing growth and productivity in broiler chickens. One possible solution is to provide nutritional supplements to enhance their growth performance and health. Among these supplements, amino acids have been shown to be crucial for the growth and development of broiler chickens. Amino acids are the building blocks of proteins, which are essential for muscle development and maintenance, immune function, and many other biological processes. However, the levels of amino acids in traditional broiler feed may not be optimal for maximizing growth and health. Thus, supplementation of amino acids in the diet may provide additional benefits to broiler chickens during the first few weeks.

The starter period, which is the first few weeks of a broiler chicken's life, is a critical time for growth and development. During this period, broiler chickens require high-quality nutrition to

support their rapid growth and development. Therefore, studying the effects of amino acid dietary supplementation on growth performance and blood profiles during this period is crucial to understanding the potential benefits of amino acid supplementation for broiler chickens

Improvement of animal health, welfare, and livestock productivity is dependent on the quality of feed composition. A critical component of feed is essential amino acids (EAA), which cannot be produced by the body and must be obtained from the diet (Jian et al., 2021). These amino acids are vital for the synthesis of proteins involved in physiological functions such as transmission, hormone signaling, cellular structure, and antioxidant systems, and their availability is a determining factor in the growth rate of chickens (Geraert and Adisseo, 2010).

Commercial chicken strains, known for their rapid growth, require sufficient amounts of amino acids to support optimal growth, especially in muscle development (Chen et al., 2016). However, high crude protein (CP) content in broiler feed can result in excess amino acids and increased nitrogen excretion. To enhance nitrogen retention efficiency, low-CP broiler diets can be supplemented with crystalline amino acids in a way that satisfies tissue growth and maintenance needs. L-threonine feed additives can also aid in reducing dietary crude protein, improving nitrogen use efficiency, decreasing nitrogen excretion, enhancing poultry tolerance to high ambient temperatures, and reducing litter ammonia levels (Shirisha et al., 2018).

In recent times, threonine has gained recognition as the third limiting amino acid in most plant-based poultry feeds. Along with lysine and methionine, its increasing usage in broiler rearing has been considered a crucial factor affecting poultry performance, as stated by Shirisa et al. (2018). Meanwhile, valine has been identified as the fourth limiting amino acid in corn-soybean meal diets for broiler chickens, and it plays a vital role in protein production, serving as a precursor for other amino acids and participating in glucose metabolism as a glycogenic amino acid, according to Wu (2009). Furthermore, Konashi et al. (2000) demonstrated that reducing valine in the diet led to a decrease in the relative weight of lymphoid organs in broilers.

Moreover, animals' skeletal muscles can synthesize glutamine with the aid of branched-chain amino acids such as leucine, isoleucine, and valine. Glutamine is considered a crucial component of the immune system (Newsholme and Calder, 1997). When selecting feed supplements, it is crucial to consider the health of high-yielding animals in addition to cost-effective production. Providing animals with high-quality protein supplementation can significantly enhance their immune systems and growth performance (Seifdavati et al., 2008). As a result, the primary aim of the present study was to investigate the effects of supplementing low-protein diets with extra amino acids during the starter period on the growth performance and biochemical parameters of broiler chickens.

## Methods

The experimental work for this study took place on private farms in Libya from July to August 2017. The study aimed to investigate the impact of delaying feeding and supplementing with high levels of essential amino acids on broiler chicken growth performance and certain blood metabolites. One hundred and sixty 1-day-old, unsexed broiler chicks of the Ross 308 breed were

divided into five treatment groups, each with four replicates (floor pens) containing eight broiler chickens. The first group (T1: control) was fed a basal diet with 21% crude protein and 3000 kCal/kg of metabolizable energy (ME), supplemented with 100% essential amino acid contents recommended by the National Research Council (NRC) in 1994. The second treatment (T2) involved feeding the basal diet after a 24-hour fasting period. The third treatment (T3) received the basal diet supplemented with a high level of lysine (12.9 g/kg). The fourth treatment (T4) received the basal diet supplemented with lysine, methionine, and threonine at 13.7, 6.38, and 8.9 g/kg of diet, respectively. The fifth treatment (T5) received the basal diet supplemented with lysine, methionine, threonine, and valine at 14.7, 6.8, 9.9, and 10.4 g/kg of diet, respectively. The starter diet was given to the chicks from one day old to 21 days of age, and the growing diet was given to them from 22 to 35 days of age. The diets were formulated to meet or exceed the recommended nutritional needs of broiler chicks according to NRC (1994). Each floor pen had a length and width of 70 cm, covering an area of 0.49 m<sup>2</sup>, and was used to raise the birds. Water and mash-style food were provided ad libitum.

Throughout the entire trial, the live body weight (LBW), feed intake (FI), and body weight gain (BWG) of the birds were measured on a weekly basis. The feed conversion ratio (FCR) was calculated as the ratio of feed consumed to weight gain in grams. Each bird was weighed to the nearest gram every morning before receiving any water or feed at the beginning of the experiment, and this was repeated every week. The initial live weight of the broilers was recorded at the start of the trial, followed by weekly measurements thereafter. Furthermore, weekly data on the FI and BWG of the broilers in replicate groups were collected. Consequently, the FCR was determined as the amount of feed consumed per unit of BWG.

On day 21, three birds were selected from each treatment group, slaughtered, and their blood samples were collected in heparinized tubes. The collected blood samples were then centrifuged at 4000 rpm for 15 minutes to obtain plasma, which was then stored at a temperature of -20°C until further biochemical analysis. The plasma biochemical components were measured using commercially available kits, including total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and uric acid. Additionally, the ELISA technique was utilized to determine the levels of immunoglobulins (IgG and IgM), triiodothyronine (T3), and thyroxine (T4).

The acquired data was subjected to a measurable evaluation using the least squares method of covariance in a one-way analysis of variance (SAS, 2006). To identify significant differences in means, Duncan's multiple range test (Duncan, 1955) was employed.

Table 1. Chemical composition (%) of starter and grower diets to broiler chicks

Ingredients (%)	Starter, % (1-21 days of age)					Grower, % (22 - 35 days of age)
	T1	T2	T3	T4	T5	
Yellow corn	56.23	56.23	56.23	56.23	56.23	55.94
Soybean meal 44	34.39	34.39	34.21	33.93	33.63	34.97
Corn Gluten Meal 60.2	2.53	2.53	2.50	2.50	2.50	2.00
Di calcium Phosphate	1.56	1.56	1.56	1.56	1.56	1.60
Limestone	1.17	1.17	1.17	1.17	1.17	1.20
DL-methionine	0.19	0.19	0.20	0.29	0.34	0.19
L-Lysine	0.00	0.00	0.20	0.29	0.39	0.10

Sodium chloride	0.50	0.50	0.50	0.50	0.50	0.50
Vit.+Min. Premix <sup>1</sup>	0.50	0.50	0.50	0.50	0.50	0.50
Soybean oil	2.93	2.93	2.93	2.93	2.93	3.00
L- Threonine	0.00	0.00	0.00	0.00	0.05	0.00
L -Valine	0.00	0.00	0.00	0.10	0.20	0.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
<b>Calculated analysis<sup>2</sup></b>						
Metabolizable energy,kcal/Kg	3003	3003	2998	2991	2985	2992
Crude Protein, %	21.67	21.67	21.78	21.9	22.1	21.6
Crude Fiber, %	3.67	3.67	3.66	3.64	3.62	3.7
Ether extract, %	2.47	2.47	2.47	2.47	2.46	2.45
Calcium, %	0.9	0.9	0.9	0.9	0.9	0.92
Av-Phosphorus, %	0.433	0.433	0.432	0.43	0.43	0.44
Methionine, %	0.542	0.5425	0.550	0.638	0.680	0.53
Meth, +Cys,(TSAA, %)	0.89	0.89	0.9	0.638	1.03	0.89
Lysine,%	1.1	1.1	1.29	1.37	1.46	1.2
L- Threonine,%	0.8	0.8	0.8	0.89	0.99	0.80
L -Valine,%	1	1	1	1	1.04	1

The diet contained the following premix per kilogram: 2654µg of Vitamin A, 125µg of Vitamin D3, 9.9mg of Vitamin E, 1.7mg of Vitamin K3, 1.6mg of Vitamin B1, 16.7µg of Vitamin B12, 5.3mg of Vitamin B2, 36mg of niacin, 13mg of calcium pantothenate, 0.8mg of folic acid, 0.1mg of biotin, 270g of choline chloride, 5.8g of BHT, 50mg of Fe, 12mg of Cu, 0.9mg of I, 50mg of Zn, 60mg of Mn, 0.2mg of Se, and 0.2mg of Co. The values were calculated based on data provided by NRC (1994)

## Results and Discussion

Table 2. Effect of supplementing amino acids in low protein diets during the starter phase on the live body weight of broiler chickens

	<u>LBW0</u>	<u>LBW7</u>	<u>LBW14</u>	<u>LBW21</u>	<u>LBW35</u>
Treatments					
T1	42.25	156.50 <sup>ab</sup>	403.25 <sup>b</sup>	722.25 <sup>c</sup>	2400.00 <sup>c</sup>
T2	42.25	145.75 <sup>b</sup>	405.50 <sup>b</sup>	688.50 <sup>c</sup>	2525.00 <sup>bc</sup>
T3	42.00	154.50 <sup>ab</sup>	416.75 <sup>ab</sup>	759.00 <sup>b</sup>	2670.00 <sup>b</sup>
T4	43.25	155.25 <sup>ab</sup>	432.50 <sup>ab</sup>	757.25 <sup>b</sup>	2690.00 <sup>b</sup>
T5	42.50	170.00 <sup>a</sup>	452.00 <sup>a</sup>	803.50 <sup>a</sup>	2957.50 <sup>a</sup>
SEM	0.673	5.28	12.37	11.22	59.10
Significance	NS	NS	NS	***	***

For each of the main effects, the means in the same column with different superscripts differ significantly ( $P < 0.05$ ).

Data in Table 2 displays the live body weight (LBW) of birds fed different experimental diets. There was no significant difference ( $P > 0.05$ ) in LBW between the experimental groups of broiler chicks from 0 to 14 days. However, the chicks that received amino acid-supplemented diets showed higher weights compared to those in T1 and T2. On the 3rd and final weighing, there was a significant difference in LBW between the control group (T1), the group that fasted for 24 hours after hatching (T2), and the other treatments that received amino acid supplementation. The highest LBW (803 and 2957 g, respectively) was observed in treatment T5, which was supplemented with Lysine, Methionine, Threonine, and valine requirements of 14.7, 6.8, 9.9, and 10.4 g/kg of diet.

These findings support the results of previous studies conducted by Aletor et al. (2000), which demonstrated that reducing dietary CP from 22.5 to 15.3 percent does not affect the growth of grower broiler chickens when supplemented with EAAs that meet the minimal NRC (1994) criteria. Nukreaw and Bunchasak (2015) also reported that adding Met + Lys to low-protein diets (Low-CP + Met + Lys) between 1 and 21 days of age significantly increased broiler chick body weight and FCR compared to those fed a low-CP diet. The chicks fed a conventional diet group still produced the most ( $P 0.01$ ). Purified amino acid supplementation led to greater body weights

in chickens than control birds (Wandita et al., 2018). Additionally, chicks fed a diet low in protein and high in amino acids (Met, Lys, and Thr) recorded the highest LBW (Saleh et al., 2021).

Table 3. Effect of low protein diets supplemented with amino acids during starter phase on body weight gain of broiler chicken

	<b>BWG0:7</b>	<b>BWG8:14</b>	<b>BWG15:21</b>	<b>BWG22:35</b>	<b>BWG0:35</b>
Treatments					
T1	114.25 <sup>ab</sup>	246.75	319.00 <sup>ab</sup>	1677.75 <sup>c</sup>	2357.75 <sup>c</sup>
T2	103.50 <sup>b</sup>	259.75	283.00 <sup>b</sup>	1836.50 <sup>bc</sup>	2482.75 <sup>bc</sup>
T3	112.50 <sup>ab</sup>	262.25	342.25 <sup>a</sup>	1911.00 <sup>b</sup>	2628.00 <sup>b</sup>
T4	112.00 <sup>ab</sup>	277.25	324.75 <sup>ab</sup>	1932.75 <sup>b</sup>	2646.75 <sup>b</sup>
T5	127.50 <sup>a</sup>	282.00	351.50 <sup>a</sup>	2154.00 <sup>a</sup>	2915.00 <sup>a</sup>
SEM	5.17	12.36	16.72	59.31	59.04
Significance	NS	NS	NS	***	***

Table 3 presents the results regarding the effect of amino acid supplementation on body weight gain (BWG) in broiler chickens at 35 days of age. The data suggests that dietary amino acid supplementation did not have a significant impact on BWG ( $P > 0.05$ ) during the 0-21 day period. However, among the treated groups, the birds receiving extra amino acids from lysine, methionine, threonine, and valine (T5) had a better BWG compared to other groups. During the 22-35 day period, a significant difference in BWG was observed between broilers fed amino acid-supplemented diets and those on control or fasting diets ( $P < 0.05$ ). The broilers receiving extra amino acids from lysine, methionine, threonine, and valine (T5) had the best BWG. These findings align with previous studies by Cheng et al. (1997) who reported improved BWG and feed conversion ratio (FCR) in broiler chicks when methionine was added to low-protein diets. Additionally, Wandita et al. (2018) reported that the average daily weight gain in broilers supplemented with 0.5% purified amino acids was higher than in the control group.

Table 4. Effect of low protein diets supplemented with amino acids during starter phase on feed intake of broilers chicken

	<b>FI0:7</b>	<b>FI7:14</b>	<b>FI15:21</b>	<b>FI22:35</b>	<b>FI0-35</b>
Treatments					
T1	188.00 <sup>b</sup>	486.75 <sup>bc</sup>	393.50 <sup>ab</sup>	2409.5 <sup>b</sup>	3477.8 <sup>c</sup>
T2	198.25 <sup>ab</sup>	596.25 <sup>ab</sup>	365.25 <sup>b</sup>	2669.5 <sup>a</sup>	3829.3 <sup>b</sup>
T3	191.50 <sup>b</sup>	416.25 <sup>c</sup>	419.75 <sup>ab</sup>	2598.8 <sup>ab</sup>	3626.3 <sup>bc</sup>
T4	215.00 <sup>ab</sup>	484.50 <sup>bc</sup>	446.25 <sup>ab</sup>	2661.3 <sup>a</sup>	3807.0 <sup>b</sup>
T5	227.25 <sup>a</sup>	661.50 <sup>a</sup>	473.00 <sup>a</sup>	2802.5 <sup>a</sup>	4164.3 <sup>a</sup>
SEM	9.22	35.71	25.92	70.98	91.52
Significance	*	**	*	*	**

The results of the impact of amino acid-enriched diets on feed intake in broiler chicks over the entire study period are presented in Table 4. The data reveal significant differences in feed intake between the control group fed on the basal diet or group fed the basal diet after fasting for 24 hours and the other groups receiving amino acid supplementation ( $P > 0.05$ ) during the 0-35 day period. Moreover, broiler chickens fed a diet fortified with extra amino acids from lysine, methionine, threonine, and valine (T5) had a significantly higher feed intake than the other experimental groups during the entire experimental period. This finding is consistent with Wandita et al. (2018), who reported that the feed efficiency in broilers supplemented with 0.5% purified

amino acids was the highest value among all treatments.

Table 5. Effect of low protein diets supplemented with amino acids during starter phase on feed conversion ratio (FCR, g feed: g gain) of broiler chickens

	<b>FCR0:7</b>	<b>FCR7:14</b>	<b>FCR15:21</b>	<b>FCR22:35</b>	<b>FCR0-35</b>
Treatments					
T1	1.64	1.96 <sup>bc</sup>	1.23	1.43	1.47 <sup>a</sup>
T2	1.91	2.30 <sup>ab</sup>	1.30	1.46	1.54 <sup>a</sup>
T3	1.70	1.60 <sup>d</sup>	1.24	1.36	1.38 <sup>a</sup>
T4	1.95	1.75 <sup>cd</sup>	1.38	1.38	1.44 <sup>a</sup>
T5	1.79	2.34 <sup>a</sup>	1.35	1.30	1.43 <sup>a</sup>
SEM	0.10	0.11	0.09	0.05	0.05
Significance	NS	**	NS	NS	NS

Table 5 presents the effects of amino acid supplementation treatments on the feed conversion ratio of 35-day-old broiler chicks. From day 0 to 35, there was no substantial difference in the feed conversion ratio between the control group and the other treatment groups. However, broilers fed a diet fortified with a high level of lysine (12.9 g/kg) (T3) showed a significantly better feed conversion ratio (1.6 feed: gain) than the other experimental groups during the period from day 7 to day 14. A study by Ospina-Rojas et al. (2017) also found that serum ammonia was reduced by approximately 30% by valine, isoleucine, or a combination when compared to broilers fed low protein. Although additional valine and isoleucine only had a quantitative effect on feed conversion ratio, valine + isoleucine increased body weight gain by 11%.

Table 6. Effect of low protein diets supplemented with amino acids during starter phase on plasma biochemical indices of broiler chickens at 21 days of age

	<b>Total Protein</b> g/dl	<b>Albumin</b> g/dl	<b>AST</b> U/L	<b>ALT</b> U/L	<b>Uric Acid</b> mg/dl	<b>IgG</b> mg/dl	<b>IgM</b> mg/dl	<b>T3-Total</b> ng/ml	<b>T4-Total</b> ng/dl
Treatments									
T1	1.87	0.700 <sup>b</sup>	329.0 <sup>a</sup>	17.5 <sup>b</sup>	4.00	2.17	2.82	0.840	1.10 <sup>b</sup>
T2	2.05	0.675 <sup>b</sup>	326.0 <sup>a</sup>	25.25 <sup>a</sup>	4.17	2.62	2.65	0.850	1.26 <sup>ab</sup>
T3	2.30	0.725 <sup>b</sup>	283.0 <sup>ab</sup>	23.65 <sup>a</sup>	3.50	2.67	2.06	0.795	1.46 <sup>a</sup>
T4	1.99	0.800 <sup>ab</sup>	221.0 <sup>b</sup>	23.35 <sup>a</sup>	2.82	2.44	2.70	1.09	1.30 <sup>ab</sup>
T5	1.87	1.00 <sup>a</sup>	282.0 <sup>ab</sup>	25.25 <sup>a</sup>	3.07	2.75	2.35	0.907	1.13 <sup>b</sup>
SEM	0.191	0.067	26.27	1.81	0.42	0.262	0.443	0.095	0.083
Significance	NS	NS	NS	*	NS	NS	NS	NS	NS

Table 6 presents the findings that feeding a low-CP diet supplemented with amino acids (lysine, methionine, threonine, and valine) did not significantly affect the plasma levels of total protein, albumin, AST, uric acid, thyroid hormones (T3 and T4), IgM, and IgG. However, the groups that received amino acid supplementation and the group fed the basal diet after 24 hours of fasting showed higher concentrations of ALT compared to the control group. This result is consistent with the study by Dairo et al. (2010), which showed that a 1% reduction in dietary protein did not affect the plasma levels of AST and ALT. Ndazigaruye et al. (2019) also reported that diets supplemented with exogenous protease enzyme, low 1% CP, and 8-12 amino acids did not affect the blood serum albumin, AST, or ALT in broiler chicken. Similarly, Badawi et al. (2019) found no discernible differences in the plasma levels of total protein, albumin, ALT, AST,

and uric acid when broiler chickens were fed low-CP diets supplemented with synthetic essential and non-essential amino acids (2%, 4%, and 6% of the standard CP control diet). However, dietary protein, especially amino acid, can affect the immune system components by decreasing the serum levels of IgA and IgG (Abbasi et al., 2014). Wandita et al. (2018) reported that the addition of pure amino acids to broiler chicken diets reduced inflammation and the levels of immunoglobulins.

Thus, adding synthetic amino acids (Met + Lys) to a low-CP diet may reduce plasma T3 due to Met supplementation, and the effect of dietary Met insufficiency on plasma T3 will depend on the degree of deficiency (Carew et al., 2003). However, plasma total T3 was not affected by methionine and threonine insufficiency. Only lysine deficiency resulted in a decline in T4 when compared to the control group given unlimited access to food (Carew et al., 1997).

### Conclusion

Recent research on broiler production indicates that initiating diets with lower CP and additional essential amino acids such as lysine, methionine, threonine, and valine during the start phase can improve chicken performance. However, raising the quantity of dietary amino acids does not significantly affect blood measurements in broiler chickens that are fed low protein diets.

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