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Impact of Feed Low-Protein Diets During Starting Period of Diets Fortified with Amino Acids on Productive Performance and Blood Parameters of Broiler Chickens

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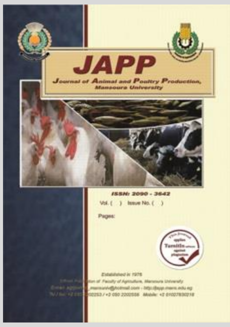

ABSTRACT

The purpose of this study was to examine how broilers' blood profiles and growth performance responded to dietary additional amino acid supplementation on a diet low in protein throughout the starter period. One hundred sixty 1-day-old, unsexed broiler chicks were divided into five groups at random, each with four replications and eight chicks per pen floor. The first group was fed a basal diet (the control group: T1), the second treatment was fed the basal diet (BD) after fasting for 24 h (T2), the third treatment was fed BD supplemented with high level of lysine 12.9 g/kg (T3), the fourth treatment was fed BD supplemented with lysine, methionine and threonine requirements of 13.7, 6.38 and 8.9 g/kg of diet, respectively (T4). The fifth treatment was fed BD supplemented with lysine, methionine, threonine, and valine requirements of 14.7, 6.8, 9.9, and 10.4 g/kg of diet, respectively (T5). Growth performance parameters and blood parameters were measured.) there were significant differences in live body weight (LBW) and body weight gain (BWG) between the T1, T2 and the other treatments (T3, T4 and T5) which supplemented with amino acid and the highest LBW were observed in T5. there were no significant differences in the plasma levels of total protein, albumin, AST, uric acid, T_3, T_4, IgM and IgG between amino acid-supplemented group and the control. The present outcomes show that extra amino acid supplements to the low–protein diet during the start period have improved chickens' performance.

Keywords: amino acids, blood parameters, immunity, broiler

INTRODUCTION

Improving feed composition is a key factor in improving animal health and welfare, as well as increasing livestock productivity. Essential amino acids (EAA) are amino acids that cannot be synthesized by the body and must be obtained from the diet (Jian *et al*., 2021). The availability of sufficient amounts of amino acids, which are needed to synthesize proteins involved in several physiological functions, such as transmission, hormone indicating, cellular structure, and antioxidant systems, determines the growth rate of chickens (Geraert and Adisseo, 2010) Geraert and Adisseo (2010). The availability of amino acids is essential for optimal growth, particularly muscle growth, as well as physiological function in commercial chicken strains because of their rapid growth Chen *et al.* (2016). High crude protein (CP) in broilers feed lead to excess amino acids and increased nitrogen excretion. Nitrogen retention efficiency can be improved when low-CP broiler diets are supplemented with crystalline amino acids in a manner that meets maintenance and tissue growth needs. The availability of L-threonine as a feed additive may enable poultry nutritionists to further reduce dietary crude protein. Reducing crude protein in the diet improves the nitrogen use efficiency, reduces nitrogen excretion, improves poultry tolerance to high ambient temperatures and reduces litter ammonia levels (Shirisha *et al*., 2018). Recently, threonine has been recognized as the third limiting amino acid in most poultry plant-based feeds. Due to the increasing use of lysine and methionine in broiler rearing, Shirisa *et al* (2018) also considered it to be one of the important factors affecting the performance of poultry. The fourth limiting amino acid in diets based on the corn-soybean meal for broiler chickens is valine (Corzo *et al*., 2009). Valine participates in the production of proteins, serves as a precursor for other amino acids, or participates in the metabolism of glucose as a glycogenic amino acid (Wu, 2009). Konashi *et al*. (2000) demonstrated that the relative weight of lymphoid organs in broilers was decreased by the reduction of valine in the diet. Additionally, branched-chain amino acids like leucine, isoleucine, and valine help animals' skeletal muscles make glutamine, which is thought to be a component of the immune system (Newsholme and Calder, 1997). In addition to cost-effective production, the health of high-growing animals is important when choosing feed supplements. Animals' immune systems and growth performance are mostly enhanced by high-quality protein supplementation (Seifdavati *et al*., 2008). Therefore, the main objective of the current trial was to investigate the impact of adding extra amino acids to low-protein diets during the starter period on growth performance and biochemical parameters of broiler chicken.

MATERIALS AND METHODS

Birds, Management and Experimental Design

From July to August 2017, private farms in Egypt's Dakranis Dakahlia Governorate served as the place for the experimental work for this study. The current study's objective would have been to study at whether broiler chicken growth performance and certain blood metabolites will be affected by delaying feeding and supplementing with high levels of important amino acids.

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Ross 308 one hundred sixty 1-day-old, unsexed broiler chicks were placed into 5 treatment groups, each of which had 4 replicates (floor pens) each replicate content 8 broiler chickens. The first group was fed a basal diet (T1: control) containing 21 CP% and 3000 k Cal/kg of ME supplemented with 100% essential amino acid contents recommended by NRC (1994), the second treatment was fed the basal diet after fasting for 24 h (T2), the third treatment was fed the basal diet supplemented with high level of lysine 12.9 g/kg (T3), the fourth treatment was fed the basal diet supplemented with lysine, methionine and threonine requirements of 13.7, 6.38 and 8.9 g/kg of diet, respectively (T4). The fifth treatment was fed the basal diet supplemented with lysine, methionine, threonine, and valine requirements of 14.7, 6.8, 9.9, and10.4 g/kg of diet, respectively (T5). These birds were fed the starter diet until 21 days of age, and then the experimental birds were fed on one growing diet until 35 days of age. Each pen's length and width were 70 and 70 cm, respectively, and it was used to raise birds. The pen's floor thus covered 0.49 m2 (70 x 70 cm). The starter diet was given to chicks from one day old to 21 days of age, and the grower diet was given to them from 22 to 35 days of age. According to NRC, diets were designed to meet or exceed broiler chicks recommended nutritional needs (1994). Water and mash-style food were readily available. (Table 1) displays the chemical and composition of the experimental diets.

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

Ingredients (%)	Starter,% (1-21 days of age) T1	T2	T3	T4	T5	Grower, % (22 - 35 days of age)
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[1]	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
Calculated analysis[2]						
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

[1]The following was delivered by 1Premix per kilogram of diet: VitaminA,2654µg; Vitamin D3,125µg; VitaminE9.9 mg;VitaminK3,1.7mg; VitaminB11.6mg; VitaminB1216.7 µg; VitaminB2 5.3mg; niacin36 mg; calcium pantothenate, 13 mg;folic acid,0.8mg; biotin,0.1mg; choline chloride,270g ; BHT,5.8g; Fe,50mg; Cu12mg; I 0.9mg; Zn 50mg; Mn 60mg; Se 0.2mg and Co0.2mg. 2Calculated from data provided by NRC (1994).

Performance of broiler chickens

Weekly measurements of the birds' live body weight (LBW), feed intake (FI), and body weight gain (BWG) were taken throughout the entire trial. The feed conversion ratio (FCR) was determined (feed: gain g). Prior to receiving any water or feed during the experiment, each bird was weighed to the closest gramme every morning. This was conducted out every week. Broiler live weights were recorded at the start of the trial and then every week after. Additionally, replicate group-based weekly data on the FI and BWG of broilers were kept. As a result, the feed consumed per unit of BWG was determined to be FCR.

Blood sampling and biochemical analysis

Three birds from each treatment were chosen at day 21, slaughtered, and blood samples were obtained in tubes that had been heparinized. The blood samples were centrifuged for 15 minutes at 4000 rpm in order to obtain plasma, which was stored at -20 °C until the biochemical analysis. By using commercially available kits, the following plasma biochemical components were measured: Commercial kits were used to assess total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and uric acid. Immunoglobulins (IgG and IgM) and triiodothyronine (T3), thyroxine (T4) were determined by ELISA technique

Statistical Analysis

One-way investigation of variance employing the least squares method of covariance was used to perform a measurable evaluation for the acquired data (SAS, 2006). To differentiate between significant changes in means, Duncan's multiple range test was used (Duncan, 1955).

RESULTS AND DISCUSSION

Growth performance of broiler chicks:

Live body weight:

Table 2 shows data on live body weight (LBW) of birds fed different experimental diets. Live body weight was found non-significant (P>0.05) between the experimental groups of broiler chicks from 0 to 14 days but the treatments of amino acid -supplementation were highest weight compared with the T1and T2. In the 3rd and final live body weight there were a significant difference between the control group (T1), fed fasting 24 h after hatching (T2) and the other treatments which amino acid- supplemented and the highest live body weight (803 and 2957 g, respectively) were observed in the treatment (T5) amino acid-supplemented with Lysine, Methionine, Threonine, and valine requirements of 14.7, 6.8, 9.9, and 10.4 g/kg of diet. The current research confirms the findings of Aletor *et al.* (2000) who demonstrated that the growth of the grower broiler chicken is unaffected by dietary CP reduction from 22.5 to 15.3 percent when such diets are supplemented with EAAs that meet the minimal NRC (1994) criteria. Similarly, Nukreaw and Bunchasak (2015) demonstrated that, between 1 and 21 days of age, adding Met + Lys to low-protein diets (Low-CP + Met + Lys) significantly increased broiler chick body weight and FCR compared to those fed Low-CP diet, even while feeding the conventional diet group still produced the most (P 0.01).. Purified amino acid-supplemented chickens had greater body weights than control birds (Wandita *et al*., 2018). Chicks fed a diet low in protein and high in amino acids (Met, Lys and Thr) significantly recorded the highest BW (Saleh *et al*., 2021).

Table 2. Effect of low protein diets supplemented with amino acids during starter phase on live body weight (LBW, g) of broiler chickens

	LBW0	LBW7	LBW14	LBW21	LBW35
Treatments					
T1	42.25	156.50^{ab}	403.25^{b}	722.25^{c}	2400.00^{c}
T2	42.25	145.75^{b}	405.50^{b}	688.50^{c}	2525.00^{bc}
T3	42.00	154.50^{ab}	416.75^{ab}	759.00^{b}	2670.00^{b}
T4	43.25	155.25^{ab}	432.50^{ab}	757.25^{b}	2690.00^{b}
T5	42.50	170.00^{a}	452.00^{a}	803.50^{a}	2957.50^{a}
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).

Body weight gain: -

Table 3 displays the findings on the impact of amino acid supplementation on body weight gain at 35 days of age. Dietary supplementation of amino acids had no discernible effect on BWG. (P > 0.05) during the periods from 0 to 21 day, however, the treatment (T5) who extra amino acid from lysine, methionine, threonine, and valine was the better in BWG as compared to other groups. A significant difference was observed in BWG (during the periods from 22 to 35 days) of broilers fed amino acid-supplemented diets compared with the control and fasting treatments (P < 0.05).

Table 3. Effect of low protein diets supplemented with amino acids during starter phase on body weight gain (BWG, g) of broiler chickens

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

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

The best BWG was detected in broiler chickens fed the dietary extra amino acid from lysine, methionine, threonine, and valine (T5). Our results agree with Cheng *et al.* (1997) who found that broiler chicks' BWG and FCR were improved when Met was added to low-protein diets. Moreover, the group's average daily increase of bird's supplemented with 0.5% purified amino acids was higher than in the control group (Wandita *et al*., 2018).

Feed intake: -

Table 4 shows the impact of feeding broiler chicks diets enriched with amino acids over the entire study period on feed intake. Between the control group fed on the basal diet or group fed the basal diet after fasting 24 h, and the other groups supplemented with dietary amino acids, there were significant differences in the feed intake of broiler chicks aged 0 to 35 days (P > 0.05). Moreover, broilers fed the diet fortified with extra amino acid from lysine, methionine, threonine, and valine (T5) significantly enhanced FI than the other experimental groups during whole experimental periods. This finding supports Wandita *et al.* (2018) conclusion that birds' feed efficiency in the group supplemented with 0.5% purified amino acids was the highest value among all treatments.

Table 4. Effect of low protein diets supplemented with amino acids during starter phase on feed intake (FI, g) of broiler chickens

	FI0:7	FI 7:14	FI 15:21	FI22:35	FI 0-35
Treatments					
T1	188.00^{b}	486.75^{bc}	393.50^{ab}	2409.5^{b}	3477,8^{c}
T2	198.25^{ab}	596.25^{ab}	365.25^{b}	2669.5^{a}	3829.3^{b}
T3	191.50^{b}	416.25^{c}	419.75^{ab}	2598.8^{ab}	3626.3^{bc}
T4	215.00^{ab}	484.50^{bc}	446.25^{ab}	2661.3^{a}	3807.0^{b}
T5	227.25^{a}	661.50^{a}	473.00^{a}	2802.5^{a}	4164.3^{a}
SEM	9.22	35.71	25.92	70.98	91.52
Significance	*	**	*	*	**

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

Feed conversion ratio:

Table 5 shows how treatments for amino acid supplementation affected the feed conversion ratio in broiler chicks 35 days old. The feed conversion ratio of broiler chicks did not substantially differ between the control group and the other treatments from 0 to 35 days of age. But broilers fed the diet fortified with high level of lysine 12.9 g/kg (T3) displayed significantly better FCR (1.6 feed: gain) than the other experimental groups during the period from 7 to 14 days of age. Similarly, Ospina-Rojas *et al.* (2017) noted that serum ammonia was reduced by about 30% by valine, isoleucine, or a combination when compared to broilers fed low protein, it was reported that FCR was only quantitatively enhanced by additional valine and isoleucine, however BWG was increased by 11% by valine + isoleucine.

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

	FCR 0:7	FCR7:14	FCR15:21	FCR22:35	FCR 0-35
Treatments					
T1	1.64	1.96^{bc}	1.23	1.43	1.47^{a}
T2	1.91	2.30^{ab}	1.30	1.46	1.54^{a}
T3	1.70	1.60^{d}	1.24	1.36	1.38^{a}
T4	1.95	1.75^{cd}	1.38	1.38	1.44^{a}
T5	1.79	2.34^{a}	1.35	1.30	1.43^{a}
SEM	0.10	0.11	0.09	0.05	0.05
Significance	NS	**	NS	NS	NS

Blood Constituents and Immune Response: -

As presented in Table 6, the plasma levels total protein, albumin, AST, uric acid, thyroid hormones (T3 and T4), IgM and IgG were not significantly affected by feeding low-CP diet with amino acids (lysine, methionine, threonine and valine) supplementation. But the experimental groups supplemented with amino acids and group fed the basal diet after fasting 24 h were higher concentration for ALT compared with control group. This result has agreed with that illustrated by Dairo *et al*. (2010) who claimed that a 1% reduction in dietary protein had no impact on the blood plasma levels of AST and ALT. Similar, study for broiler chicken was reported by Ndazigaruye *et al*. (2019) demonstrated that diets supplemented with exogenous protease enzyme, low 1 percent CP, and 8–12 amino acids did not impact blood serum albumin, AST, or ALT. According to Badawi *et al*. (2019), no discernible differences have been found between various treatments based on low CP diets that are low in total protein, albumin, ALT, AST, and uric acid (2, 4 and 6 percent of the standard CP control diet), and they are supplemented with synthetic essential and non-essential amino acids ($p < 0.05$). Dietary protein especially amino acid influences immune system components by decreasing serum IgA and IgG levels (Abbasi *et al*., 2014). Wandita *et al*. (2018) Immunoglobulin levels measured in the treatment group revealed that broiler chickens' inflammation was reduced by the addition of pure amino acids. Therefore, it might be inferred that adding synthetic amino acids (Met + Lys) to the low-CP diet may have reduced plasma T3 due to Met rather than Lys supplementation, and that the effect of dietary Met insufficiency on plasma T3 will depend on the degree of deficiency (Carew *et al*., 2003). On the other hand, plasma total T3 was unaffected by the methionine and threonine insufficiency. When compared to the control group given unlimited access to food, only the lysine deficiency resulted in decline in T4 (Carew *et al*., 1997).

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

	Total Protein *g/dl*	**Albumin** *g//dl*	**AST** U/L	**ALT** U/L	**Uric Acid** mg/dl	**IgG** mg/dl	**IgM** mg/dl	**T3-Total** ng/ml	**T4-Total** ng/dl
Treatments									
T1	1.87	0.700^{b}	329.0^{a}	17.5^{b}	4.00	2.17	2.82	0.840	1.10^{b}
T2	2.05	0.675^{b}	326.0^{a}	25.25^{a}	4.17	2.62	2.65	0.850	1.26^{ab}
T3	2.30	0.725^{b}	283.0^{ab}	23.65^{a}	3.50	2.67	2.06	0.795	1.46^{a}
T4	1.99	0.800^{ab}	221.0^{b}	23.35^{a}	2.82	2.44	2.70	1.09	1.30^{ab}
T5	1.87	1.00^{a}	282.0^{ab}	25.25^{a}	3.07	2.75	2.35	0.907	1.13^{b}
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

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

CONCLUSION

According to recent studies on broiler production, chicken performance was enhanced by starting diets with less CP and additional doses of essential amino acids such lysine, methionine, threonine, and valine during the start period. On the other hand, increasing the amount of dietary amino acids has no appreciable effect on the blood measurements in broiler chickens fed low protein diets.

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تأثير العلائق المنخفضة البروتين المدعمة بالاحماض الأمينية الأساسية خلال فترة البادئ على الأداء الإنتاجي ومقاييس الدم لدجاج التسمين

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الملخص

الهدف من هذه الدراسة هو معرفة مدي تأثير أضافة الأحماض الأمينية الأساسية بمستويات عالية لعليقة منخفضة البروتين خلال فترة البادئ علي الأداء الإنتاجي ومقاييس الدم. حيث أجريت هذه التجربة علي ١٦٠ كتكوت عمر يوم من سلالة الروس ٣٠٨ غير محدد الجنس حيث قسمت التجربة الي خمس معاملات بحيث تحتوي كل معاملة علي أربع مكررات وكل مكررة تحتوي علي ثمانية كتاكيت تسمين.. حيث تم تغذية المعاملة الأولي علي عليقة أساسية أما المعاملة الثانية تمت تغذية الكتاكيت علي العليقة الأساسية بعد ٢٤ ساعة من وصول الكتاكيت الي العنبر أما المجموعة الثالثة فكانت مدعمة بحمض أميني الليسين بمستوي ١٢,٩جم/كجم والمجموعة الرابعة غذيت على العليقة الاساسية مدعمة بالاحماض الأمينية الليسين و الميثونين والثيرونين بمستويات ١٣,٧ و ٦,٣٨ و ٨,٩ علي الترتيب أما المجموعة الخامسة غذيت على العليقة الاساسية مدعمة بالأحماض الأمينية الليسين والميثونين والثيرونين والفالين بمستويات ١٤,٧ و ٦,٨ و ٩,٩ و ١٠,٤ علي الترتيب. وتم قياس معدلات النمو وبعض مقاييس الدم كان هناك فرق معنوي بين المجموعة الكنترول (T1) ، التغذية الصيام ٢٤ ساعة بعد الفقس (T2) والمعاملات الأخرى التي تحتوي على الأحماض الأمينية وأعلى LBW لوحظ في المعاملة الخامسة. كما وجد أنه لا يوجد أختلافات معنوية في مقاييس الدم لكل من البروتين الكلي والألبومين و AST وحمض اليوريك و T3 و T4 و الجلوبيولين المناعي . (IgM, IgG) تظهر النتائج الحالية أن مكملات الأحماض الأمينية الإضافية الي العلائق التي تحتوي على نسبة منخفضة من البروتين في النظام الغذائي خلال فترة البادئ قد حسنت أداء الدجاج.