The effect of synthetic essential amino acids supplementation to low crude protein diets on growth performance and serum metabolites in broiler chickens

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ABSTRACT
Objective: To assess the influence of reduction of crude protein (CP) by 5% and 10% with supplementation of crystalline synthetic essential amino acids (EAAs) on performance and carcass traits of broilers.

Design: Randomized controlled study

Animals: A total of 320 one-day-old broiler chicks, (Cobb 500) reared up to 35 days of age were randomly allocated into eight experimental groups with four replicates (10 each).

Procedures: The treatments as follow; the first group of chicks were fed on control protein to meet the CP requirement and total AAs according to Cobb 500 recommendation; the second group were fed idle EAAs (Id EAAs) to meet the recommended CP and the digestible EAAs. The third, fourth, and fifth groups were fed on diet 5% reduction of CP with EAAs supplementation exceed the digestible EAAs requirement of Cobb by 110, 120, and 130% (LCP6+110EAAs, LCP4+120EAAs, LCP5+130EAAs). However, the sixth, seventh, and eighth groups of chicks were fed on diet 10% reduction of CP with AA supplementation exceed the digestible EAAs requirement of Cobb by 110, 120, and 130% (LCP6+110EAAs, LCP7+120EAAs, LCP8+130EAAs).

Results: Broilers of group four, five, six, seven and eight showed a significant decrease in final body weight (BW), body weight gain (BWG) and feed intake at 21 and 35 days of age. However, the same variables were significantly enhanced in broilers of group one, two, and three (p <0.05). Additionally, there was a significant decrease of relative weight of abdominal fat pad of broilers in groups three, four, five compared to other treatment groups. Serum uric acid and cholesterol were linearly decreased with reduction of CP and supplementation with synthetic essential amino acids.

Conclusion and clinical relevance: The present results indicate that reduction of CP by 5% or 10% with crystalline EAAs supplementation by 110, 120 and 130% more than the digestible EAAs of broiler recommendation did not support the growth performance.

Keywords: Crude protein, Amino acids supplementation, Growth performance, Digestibility, Broiler.

1. INTRODUCTION

Crude protein (CP) and each amino acids are essential nutrients, which provide an optimal growth performance broilers [1]. About one-third of the feed ingredient’s cost comes from ingredients that purposely provide the nutritional content to supply the crude protein (CP) and amino acid (AA) requirements of broilers [2]. So, the important function of nutritionists is to reduce the cost of feed, while ensuring efficiency of utilization of supplemented low-protein diets with synthetic amino acids (AAs) to meet or exceed minimum amino acid standards suggested by NRC [3].

Supplementation of synthetic AAs to low-CP broiler chicks diets can provide significant way to utilize the least-cost formulations in order to decrease feed cost and subsequently to reduce nitrogen excretion to the environment [4]. Feeding on low CP diet even with supplementation of all EAAs adversely affect the growth performance due to insufficient amount of nitrogen to synthesize nonessential amino acids (NEAA) especially glycine, serine, proline, and glutamine; decreased level of electrolyte as potassium; and imbalances between certain amino acids as arginine to lysine, lysine to threonine. However, most of these factors cannot explain the real reason for adverse effect of such feeding regimen on growth performance [5].

. The ideal concept of amino acids is based on the level of dietary digestible amino acids levels, which provides a combination of EAAs that particularly meets an animal’s requirement for development and maintaining of protein [6]. To formulate diet based on idle protein, dietary EAAs levels is relative to the dietary lysine level, because lysine is a second limiting amino acid in corn-soybean meal based diet of poultry, and lysine functions as solely in protein synthesis [7].

There are many studies on the possible reduction of CP with supplementation of synthetic EAAs [4-6]. There were wide variation of the results of the supplementation of AAs because it is depending on the type of supplemented AA, such as first and second limiting AA, feed-grade AAs, all essential amino acids (EAAs) or both EAAs and non-essential amino acids (NEAAs) [4]. CP levels in broiler diets can be reduced by 3 to 4 percentage points without
sacrificing performance provided that free amino acids are supplemented in the diet to equal the amino acid levels in a standard diet [8].

The aim of this study was to assess the effect of feeding on reduced CP diet by 5% or 10% with supplementation of synthetic EAAs excess by 110%, 120%, and 130% more than Cobb nutrients requirement on growth performance, carcass traits, digestibility, and serum metabolites in broilers.

2. MATERIALS AND METHODS

2.1. Experimental birds and management

Three hundred-twenty, 1-day-old broiler chicks (Cobb 500) were obtained from commercial hatchery (El Dakahilia Poultry Company). The chicks were weighed and randomly alienated into eight experimental groups in four replicates (each replicate, n=10). Feed and water were provided ad libitum throughout the experimental period. The chicks were reared under constant lighting and at a starting temperature 35-36 °C. Temperature was gradually decreased to between 25 and 28 °C over the following 2 wk.

2.2. Experimental diets

The first group is control diet; chickens were fed on a CP and essential amino acids (EAAs) according to the nutrients requirement of Cobb500. The second group was fed on a diet according to Cobb 500 to meet CP and the digestible EAAs requirements. The third, fourth, and fifth groups were fed on diet 5% reduction of CP with EAAs supplementation individually exceed the digestible EAAs requirement of Cobb by 110, 120, and 130% (LCP3+110EAAs, LCP4+120EAAs, LCP5+130EAAs), respectively. The sixth, seventh, eighth groups of chicks were fed on diet 10% reduction of CP with EAAs supplementation individually exceed the digestible EAAs requirement of Cobb by 110, 120, and 130% (LCP6+110EAAs, LCP7+120EAAs, LCP8+130EAAs) (Table 1 and 2).

2.3. Measurements

Chicks were individually weighted at the start of experiment to determine the initial body weight (IBW). Average final live body weight, body weight gain, feed intake, feed conversion ratio (g feed/g gain) were recorded at 21, and 35 days of age.

At the end of the experiment, all chickens were fasted for 12 h., five birds per experiment were weighed and slaughtered immediately. Blood samples were collected for serum analysis. Then carcasses were eviscerated and individually reweighed after the removal of head, neck, shanks, viscera, and giblets (liver, heart and gizzard) to obtain the dressed weight. The heart, liver, gizzard, and abdominal fat were weighed and expressed as a percentage of the live body weight (BW). Abdominal fat pad is the fat present around the gizzard and intestines, cloaca, and the bursa of Fabricius [9].

2.4. Serum metabolites measurement

Blood samples were collected into anticoagulant free tubes to obtain sera. Clean, non hemolyzed samples were kept at -20 °C until analysis. Total protein, albumin, aspartate aminotransferase (AST) activity, uric acid (UA), and cholesterol were analyzed using commercial kits [10, 11].

2.5. Digestibility trial

At the end of the experimental period, a digestibility trial was carried out for 5 days to determine the digestion coefficients of nutrients. The total excreted feces during the collection period were pooled, well mixed, weighed and sampled for analysis. The chemical analysis of feeds and feces was carried out according to AOAC [12]. Apparent digestibility nutrient was calculated for dry matter (DM), crude protein (CP), and ether extract (EE).

2.6. Statistical Analysis

The results were statistical analyzed by using ANOVA test to evaluate the impact of reduction of CP by 5% or 10% with essential amino acids supplementation excess the essential digestible amino acids (EAAs) recommendation of broiler chickens (Cobb 500) on performance, carcass traits, digestibility, and serum metabolites of broilers. Data were analyzed using statistical SPSS v20 (SPSS Inc., Chicago, IL, USA). Differences between dietary experimental groups were compared using Duncan’s multiple range test. Differences due to dietary treatments were considered significant if P-value for the effect was< 0.05.

3. RESULTS

The impact of CP levels and EAAs supplementation on BW, BWG, FCR, and feed consumption at 21 and 35 day of age is shown in Table 3. Briefly, the results revealed that the BW and BWG were significantly higher of chickens fed control, Idle EAAs, and LCP5%+110% EAAs compared to other experimental groups (p<0.05). Also, FCR was improved in control, Idle EAAs, and LCP 5% supplemented with EAAs than other experimental groups. Moreover, feed consumption in broiler fed on control, Idle EAAs, LCP 5% +110% EAAs diets was higher than other experimental groups.

The influence of low CP with supplementation of EAAs on carcass traits is presented in (Table 4). The dressing % was higher in broiler chickens fed control, Idle EAAs, and LCP5% plus 110EAAs than other experimental groups. There were not significant differences of relative weights of liver, heart, and gizzard between experimental groups. However, relative weight of abdominal fat pad of broilers fed control, Idle EAAs, LCP10% supplemented with 110%, 120, or 130% EAAs were higher than relative weight of abdominal fat pad of broilers fed LCP5% supplemented with 110, 120, or 130% EAAs.

Nutrients digestibility of broilers fed different dietary treatments is shown in (Table 5). There were not differences (p>0.05) of digestibility of DM, protein, and ether extract (EE) between treatment groups.

The effect of control, Idle EAAs, and CP levels supplemented with EAAs on serum metabolites are shown in (Table 6). There were not significant (p > 0.05) difference of serum total protein, albumin, and globulin between experimental groups. However, there was significantly decreased of UA of broilers fed LCP supplemented with EAAs compared to UA in blood of chickens fed control diet. There was linearly decreased of cholesterol level with groups of LCP supplemented with EAAs.
### Table 1. Ingredients % and chemical composition of starter diet for broiler chickens.

| Ingredients | Control | Idle EAAs | LCP5% +110 EAAs | LCP5% +120 EAAs | LCP5% +130 EAAs | LCP10% +110 EAAs | LCP10% +120 EAAs | LCP10% +130 EAAs |
|-------------|---------|-----------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Y.Corn % (8.5) | 62.8 | 62.5 | 64.1 | 64.5 | 63.7 | 66.3 | 66.2 | 66.5 |
| SBM% (48%) | 28.1 | 28.1 | 28 | 27 | 28 | 27 | 26.5 | 25.8 |
| Corn gluten% | 4 | 4 | 2 | 2 | 1 | 0.5 | 0.5 | 0.5 |
| oil | 1.2 | 1.3 | 1.5 | 1.6 | 2.1 | 1.6 | 1.75 | 1.8 |
| limestone | 1.07 | 1.07 | 1.07 | 1.07 | 1.07 | 1.07 | 1.07 | 1.07 |
| Dicalcium phosphate | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 |
| Vita&min premix* | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Salt | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| DL-Methionine | 0.11 | 0.24 | 0.31 | 0.36 | 0.42 | 0.33 | 0.39 | 0.42 |
| DL-Lysine | 0.31 | 0.31 | 0.47 | 0.66 | 0.77 | 0.52 | 0.69 | 0.86 |
| L-Tryptophane | 0 | 0.00 | 0 | 0.00 | 0 | 0 | 0.012 | 0.03 |
| L-Threonine | 0.08 | 0.08 | 0.18 | 0.28 | 0.35 | 0.22 | 0.31 | 0.4 |
| L-Valine | 0.00 | 0.00 | 0.00 | 0.14 | 20.69 | 0.07 | 0.18 | 0.29 |
| CP % | 21.7 | 21.77 | 20.8 | 20.7 | 30.37 | 19.7 | 19.77 | 19.7 |
| ME (Kcal/kg) | 3038 | 3036 | 3033 | 3034 | 30.90 | 3036 | 3037 | 3038 |
| Ca % | 0.91 | 0.91 | 0.90 | 0.90 | 0.45 | 0.90 | 0.90 | 0.90 |
| P % | 0.46 | 0.46 | 0.45 | 0.45 | 0.45 | 0.45 | 0.45 | 0.45 |
| Methionine | 0.50 | 0.45 | 0.49 | 0.54 | 0.49 | 0.49 | 0.54 | 0.57 |
| D-Methionine | 1.32 | 1.18 | 1.29 | 1.41 | 1.29 | 1.41 | 1.53 |
| Lysine | 0.26 | 0.21 | 0.21 | 0.21 | 0.20 | 0.21 | 0.23 |
| L-Tryptophane | 0.87 | 0.77 | 0.84 | 0.92 | 0.84 | 0.92 | 1.00 |
| Threonine | 1.00 | 0.96 | 1.07 | 20.69 | 0.98 | 1.07 | 1.16 |

1Experimental diets were corn-soybean based diet supply nutrients to meet requirement, positive control (PC). Idle EAAs meet digestible amino acids requirement, LCP, corn-soybean meal based diet with reduction of CP by 5% and 10% with supplementation of digestible amino acids excess requirement by 110%, 120%, 1.30%.

2 Vitamins and minerals premixed to cover the required vitamins and minerals per kilogram diet (Vitamin A: 10,000 I. U.; Vitamin D3: 1,500 I. U.; Vitamin E: 10 mg; Vit. K3: 2 mg; Vit. B1: 2 mg; Vit. B2: 5 mg; Vit. B6: 3 mg; Vit. B12: 0.01 mg; Niacin: 27 mg; Folic acid: 1 mg; Biotin: 0.05 mg; Pantothenic acid: 10 mg; Mn: 60 mg; Zn: 50 mg; Cu: 10 mg; I: 0.1 mg; Se: 0.1 mg; Co: 0.1 mg; Fe: 50 mg.

### Table 2. Ingredients (%) and chemical composition of grower diet of broiler chickens.

| Ingredients | control | Idle EAAs | LCP5% +110 EAAs | LCP5% +120 EAAs | LCP5% +130 EAAs | LCP10% +110 EAAs | LCP10% +120 EAAs | LCP10% +130 EAAs |
|-------------|---------|-----------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Y.Corn % (8.5) | 67.4 | 66 | 69.6 | 68.3 | 68.6 | 72.6 | 71.7 | 71.7 |
| SBM% (48%) | 25 | 26 | 23.5 | 24 | 23.5 | 21 | 21 | 20.5 |
| Corn gluten% | 2 | 2 | 0.6 | 0.6 | 0.5 | 0.5 | 0.5 | 0.5 |
| oil | 2 | 2.3 | 2.2 | 2.5 | 2.5 | 1.7 | 2 | 2.1 |
| limestone | 1 | 1 | 1.04 | 1.02 | 1.02 | 1.01 | 1.01 | 1.03 |
| Dicalcium phosphate | 1.65 | 1.65 | 1.64 | 1.65 | 1.67 | 1.7 | 1.7 | 1.7 |
| Vita&mineral premix* | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Salt | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| DL-Methionine | 0.13 | 0.24 | 0.30 | 0.35 | 0.40 | 0.3 | 0.35 | 0.39 |
| DL-Lysine | 0.28 | 0.24 | 0.45 | 0.58 | 0.72 | 0.53 | 0.67 | 0.82 |
| L-Tryptophane | 0 | 0 | 0 | 0.02 | 0.04 | 0.013 | 0.03 | 0.05 |
| L-Threonine | 0.08 | 0.06 | 0.18 | 0.25 | 0.32 | 0.22 | 0.29 | 0.36 |
| L-Valine | 0.00 | 0.00 | 0.05 | 0.13 | 0.22 | 0.1 | 0.19 | 0.29 |
| Chemical composition (calculated) | 19.37 | 19.74 | 18.29 | 18.64 | 18.59 | 17.40 | 17.57 | 17.52 |
| ME (Kcal/kg) | 3107 | 3108 | 3111 | 3108 | 3106 | 3109 | 3107 | 3106 |
| Ca % | 0.84 | 0.84 | 0.84 | 0.84 | 0.84 | 0.84 | 0.84 | 0.84 |
| P % | 0.42 | 0.42 | 0.42 | 0.42 | 0.42 | 0.42 | 0.42 | 0.42 |
| Methionine | 0.48 | 0.42 | 0.46 | 0.50 | 0.55 | 0.46 | 0.50 | 0.55 |
| Lysine | 1.19 | 1.06 | 1.15 | 1.26 | 1.36 | 1.155 | 1.26 | 1.36 |
| L-Tryptophane | 0.23 | 0.20 | 0.188 | 0.21 | 0.22 | 0.187 | 0.20 | 0.22 |
| D-Threonine | 0.87 | 0.69 | 0.76 | 0.83 | 0.89 | 0.76 | 0.83 | 0.89 |
| Valine | 0.9 | 0.92 | 0.89 | 0.97 | 1.05 | 0.89 | 0.97 | 1.05 |

1Experimental diets: Refer to Table 1 for key.
Table 3. Effect of reduction crude protein with amino acids supplementation of broiler chickens on performance.

| Ingredients | control | Idle [EAAs] | LCP5% +110 [EAAs] | LCP5% +120 [EAAs] | LCP5% +130 [EAAs] | LCP10% +110 [EAAs] | LCP10% +120 [EAAs] | LCP10% +130 [EAAs] |
|-------------|---------|-------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Initial BW, g | 44.25±0.91 | 42.42±0.77 | 43.07±0.5 | 44.35±0.78 | 44.28±0.80 | 43.07±0.83 | 43.76±1.14 | 42.42±0.78 |
| 1-21 d BW, g | 751.08±67.46 | 725.84±70.11 | 688.16±50.53 | 629.50±23.42 | 588.75±95.80 | 617.18±182.40 | 620±180.31 | 570.92±70.05 |
| BWG, g | 708.81±0.61 | 675.69±22.47 | 591.83±22.07 | 569.41±72.19 | 567.72±114.29 | 565.13±128.23 | 519.50±126.29 |
| Feed consumption, g | 1261.63±51.63 | 1216.07±53.39 | 1148.31±51.35 | 1088.19±78.98 | 1151.61±52.78 | 1076.67±188.24 | 1159.91±56.88 | 1083.72±28.90 |
| FCR | 1.76±0.051 | 1.83±0.076 | 1.69±0.062 | 1.91±0.074 | 2.00±0.094 | 1.94±0.100 | 1.86±0.094 | 2.14±0.108 |
| 21-35 d FBW, g | 1855.00±1126.81 | 1906.51±1150.33 | 1782.16±1099.41 | 1627.08±1077.27 | 1636.27±1070.45 | 1562.35±1024.00 | 1598.30±1030.28 | 1522.71±1036.09 |
| BWG, g | 1126.81±49.69 | 1160.45±74.20 | 1150.33±50.99 | 1150.41±42.71 | 1190.1±58.45 | 1170.34±34.82 | 1180.21±29.09 | 1182.56±21.25 |
| Feed consumption, g | 2317.38±28.49 | 2538.89±54.22 | 2340.52±18.05 | 2190.27±76.91 | 2357.47±68.05 | 2170.83±29.64 | 2342.64±30.82 | 2264.93±22.2 |
| FCR | 2.21±0.058 | 2.21±0.081 | 2.12±0.112 | 2.05±0.107 | 2.17±0.105 | 2.0±0.105 | 2.2±0.105 | 2.2±0.105 |
| Over all 1-35 BWG, g | 1829.32±1179.44 | 1881.61±1163.74 | 1787.15±149.54 | 1608.83±164.45 | 1563.75±151.50 | 1518.91±170.01 | 1452.00±146.59 | 1496.10±1158.85 |
| Feed consumption, g | 3578.96±33.2 | 3754.96±149.0 | 3488.83±103.1 | 3278.47±95.0 | 3258.59±76.0 | 3124.47±180.0 | 3152.56±143.68 | 3348.66±100.0 |
| FCR | 1.97±0.18 | 2.03±0.23 | 2.1±0.23 | 1.97±0.20 | 2.22±0.20 | 2.25±0.21 | 2.20±0.21 | 2.11±0.17 |

1experimental diets were corn-soybean based diet supply nutrients to meet Cobb requirement, positive control (PC), Idle [EAAs] meet digestible amino acids requirement, negative control, corn-soybean meal based diet with reduction of CP by 1% and 2% with supplementation ofIdle amino acids excess requirement by 110%, 120%, 130% (NC1, NC2).

Table 4. Effect of low crude protein diet with supplementation of amino acids on carcass characteristics in broiler chickens.

| Ingredients | control | Idle [EAAs] | LCP5% +110 [EAAs] | LCP5% +120 [EAAs] | LCP5% +130 [EAAs] | LCP10% +110 [EAAs] | LCP10% +120 [EAAs] | LCP10% +130 [EAAs] |
|-------------|---------|-------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Dressing | 74.69±0.66 | 75.19±1.86 | 74.75±1.49 | 72.40±0.88 | 71.61±0.62 | 69.07±1.92 | 71.85±0.22 | 70.86±0.25 |
| Heart | 0.48±0.03 | 0.49±0.05 | 0.50±0.04 | 0.50±0.00 | 0.51±0.03 | 0.50±0.03 | 0.47±0.02 | 0.51±0.01 |
| Liver | 2.47±0.19 | 2.21±0.07 | 2.23±0.33 | 2.19±0.13 | 2.56±0.24 | 2.49±0.16 | 2.48±0.07 | 2.24±0.04 |
| Gizzard | 2.32±0.08 | 2.67±0.16 | 2.67±0.12 | 2.83±0.16 | 2.85±0.20 | 2.31±0.05 | 2.90±0.15 | 2.34±0.08 |
| Fat | 2.15±0.31 | 2.50±0.05 | 1.03±0.03 | 1.11±0.005 | 1.00±0.04 | 2.48±0.06 | 2.44±0.02 | 2.01±0.14 |

1experimental diets were corn-soybean based diet supply nutrients to meet Cobb requirement, positive control (PC), Idle [EAAs] meet digestible amino acids requirement, LCP5% and LCP10%, corn-soybean meal based diet with reduction of CP by 5% and 10% with supplementation of individually synthetic crystalline amino acids excess the requirement of digestible EAAs by 110%, 120%, 130%.

Table 5. Effect of low crude protein with supplementation of EAAs on digestibility in broiler chickens.

| Ingredients | control | Idle [EAAs] | LCP5% +110 [EAAs] | LCP5% +120 [EAAs] | LCP5% +130 [EAAs] | LCP10% +110 [EAAs] | LCP10% +120 [EAAs] | LCP10% +130 [EAAs] |
|-------------|---------|-------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| DM% | 78.02±2.81 | 75.89±4.05 | 78.77±3.39 | 77.50±3.15 | 76.28±2.61 | 75.17±3.67 | 73.16±3.39 | 72.72±2.69 |
| CP% | 77.18±2.92 | 73.91±4.50 | 76.51±3.63 | 75.15±4.05 | 73.41±3.39 | 72.64±4.14 | 69.39±3.15 | 72.72±3.15 |
| EE% | 85.66±1.28 | 83.40±2.80 | 84.50±2.84 | 83.53±2.42 | 85.01±2.59 | 83.16±3.16 | 81.84±3.13 | 82.12±2.22 |

1experimental diets were corn-soybean based diet supply nutrients to meet Cobb requirement, positive control (PC), Idle [EAAs] meet digestible amino acids requirement, LCP5% and LCP10%, corn-soybean meal based diet with reduction of CP by 5% and 10% with supplementation of individually synthetic crystalline amino acids excess the requirement of digestible EAAs by 110%, 120%, 130%.
The results of carcass traits revealed that there were non-significant differences in the relative weight of liver, gizzard, and heart between experimental treatments. These results are in accordance with those of earlier studies [15, 20, 22, 23]. Our findings may be due to the vital organs being of similar size and weight. However, abdominal fat deposition was significantly decreased in broiler chickens fed LCP5% with EAAs compared to other experimental groups (p<0.05). In the same context, it has been postulated that feeding 19% CP diets supplemented with high level of essential amino acids (EAs) to non-essential amino acids (NEAA) prevents excess fat deposition [24]. Certain amino acids such as methionine, and arginine may have the ability to reduce body fat deposition in broiler chicks and genetically fat chickens [25, 26]. However, Si et al. [16] found that supplementation of amino acids to low CP diets increased the carcass and abdominal fat. Likewise, chicks fed diets low CP and supplemented with amino acids have a significant increase in abdominal fat weight compared to the chicks fed control diet [18].

There were non-significant difference in digestibility of DM, CP, and EE. However, the digestibility of DM, protein, and EE were higher in broiler chickens fed LCP (19%) supplemented with 2EAAs diet or 5 EAAs than control diet (21.5%CP) [27].

It has been reported that plasma total protein and albumin are the main transport proteins in avian species, and they reflect the avian nutritional condition [28]. Our data showed that there was non-significant difference in serum total protein, albumin, and globulin. The same findings have been recorded in broiler chickens fed on control diet (22.2% CP) and low CP (16.2%) supplemented with EAAs under tropical climate [15]. However, serum total protein was significantly decreased in chickens fed on control diet according to NRC compared with those fed low CP and supplemented with lysine, methionine and threonine above the recommended level of NRC [29].

Uric acid is the product of protein catabolism and turnover in the body. In diets with low CP with supplementation of EAAs, glycine deficiency may explain the decreased serum uric acid level [5, 14]. Uric acid has been found higher in serum of broiler fed control diet (21% CP) than those fed low CP (19.5%) and supplemented with 115% lysine, methionine and 108% threonine above NRC requirement [29]. Moreover, the higher uric acid with high dietary protein level maybe due to the high consumption of AA and the low level of serum uric acid maybe due to the poor AA ingestion particularly glycine [5, 14].

Regarding to AST activity, there were significant differences among treatment groups. Similarly, Attia et al. [27] found that AST reported a wide variation among groups. Mitochondria, liver and skeletal muscles are the main source of AST, so it is considered a less specific for liver function than other enzymes [30, 31].

Conflict of interest
The authors declare that they have no conflict of interest.

Research ethics committee permission
All methods used in the study were performed in accordance with the ethical guidelines and recommendations of the Research Ethics Committee, Faculty of Veterinary Medicine, Mansoura University.

Author contribution
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