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# EFFECTS OF DIETARY PROTEIN LEVELS WITH OR WITHOUT SYNTHETIC AMINO ACIDS AND ENZYME SUPPLEMENTATION ON PERFORMANCE OF BROILER CHICKENS

**R.M.S. Emam**

***Poultry Production Department, Faculty of Agriculture, Fayoum University, Egypt.***

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## SUMMARY

This experiment was conducted to study the effect of using two levels of (recommended (R), R-2%) crude protein (CP) supplemented with or without the requirements of methionine and lysine (Met. and Ly.) and each with two levels (0.00 and 0.10%) of Avizyme 1500 in 2 x 2 x 2 factorial arrangement (giving eight dietary treatments) on growth performance of broiler chickens (Ross strain). Accordingly, a total numbers of 192 one-day old unsexed Ross broiler chickens were initially fed a control diet for five days. At six days of age, chicks were randomly allotted to the dietary treatments, birds were divided into eight treatments (24 birds each), each treatment contained three replicates of eight birds each. Results obtained could be summarized in the following: The main effects of level of CP had insignificant effect on live body weight (LBW), live body weight gain (LBWG), feed intake (FI), feed conversion (FC) and performance index (PI) during finisher (25 to 41) and overall experimental periods (6 to 41 days). Level of CP had significant effect on crude protein conversion (CPC) during the period from 6 to 41 days, chicks fed diet containing R had the worst CPC during same period. Chicks fed adequate amounts of amino acids (AA) supplement diets recorded significantly higher PI value during the period from 6 to 41 days, while, recorded significantly the worst values of FC and CPC during the same period. Level of AA had insignificant effect on LBW, LBWG and FI during overall experimental period. Neither enzyme addition nor interaction between level of CP, AA and enzyme addition had any significant effect on LBW, LBWG, FC, CPC and PI during the period from 6 to 41 days. No significant differences due to level of CP or AA on blood constituents, except, red blood cells count (RBCs) which was significantly affected. Chicks fed diet containing R-2% CP or diet containing inadequate amounts of AA had higher value of RBCs. No significant differences due to enzyme addition and interaction effect of dietary treatments on blood constituents, except, interaction effect on neutrophils% (segment) which was significantly affected. Neither level of CP and enzyme addition nor interaction between level of CP, AA and enzyme addition had any significant effect on slaughter parameters% and tibia weight and ash% at the end of the finishing period. Level of CP, AA and interaction between level of CP, AA and enzyme addition had insignificantly affected chemical composition of broiler meat. Chicks fed diet containing R-2% supplemented with the requirements of Met. and Ly. with 0.10% of Avizyme had the best economical and relative efficiency values during the period from 6 to 41 days of age, as compared with those fed the control diet and other treatments. It can be concluded that, CP can be reduced from the recommended level by 2% and supplement these diets with either Met. and Ly or Avizyme without affecting performance. Besides, using such diets reduces feed cost and N pollution.

***Keywords**: crude protein, methionine, lysine, enzyme and broiler performance.*

## INTRODUCTION

Poultry production in Egypt has become one of the biggest agriculture industries and its improvement is a major goal of broiler producers. Also, the constant increase in demand for poultry meat by consumers requires an increase in production capacity (Recoules *et al.*, 2017). On the other hand, the rapid growth of modern broiler strains, coupled with lower prices, however, feed costs account for approximately 60–75% of broiler production costs, especially dietary protein sources (Khalaji *et al.*, 2016 and Asadi Kermani *et al.*, 2017).

So, efforts to reduce dietary protein level have been the subject of numerous investigators. Low protein diets supplemented with the requirements of essential amino acids (EAAs) are recommended for poultry reared in normal and heat stress conditions (Aftab *et al.*, 2006 and Vieira *et al.*, 2016). Using such diets reduces feed cost, reduces the risk of N pollution of the environment through reducing N excretion by the birds (Vieira *et al.*, 2016). Reduced N excretion lowers ammonia level in the poultry house (this may also reduce sticky and wet litter, hock burns and breast blisters), thus reducing excrete excess nitrogen in the form of uric acid (Mahmood *et al.*, 2017) and lowers the energetic efficiency of protein use as an energy source.

Ferguson *et al.* (1998) demonstrated with broilers that litter N could be reduced more than 16% when dietary crude protein (CP) was reduced by 2%, while maintaining adequate levels of dietary amino acids (AA). Although some investigators believed they do not maintain the same performance as high protein diets (Aletor *et al.*, 2000 and Abd El-Gawad *et al.*, 2004). Crude protein in the diets can be reduced from the recommended level by 3-4% than standard dietary recommendations level and supplement these diets with adequate amounts of synthetic AA (i.e., methionine and lysine (Met. and Ly.)) without affecting performance (Abdallah, 2005), this has been proven to be economical and has no negative impact on performance. Moreover, it reduces nitrogen pollution by approximately 24-34%. Also, the majorities of the published papers indicated that feeding poultry low protein diets supplemented with AA maintain the same performance as that obtained from high protein diets. However, Colonago *et al.* (1991) concluded that optimal performance of starter and grower could not be achieved with low protein diets supplemented with crystalline AA. Hence, great efforts will be directed to maximize the utilization of low protein diets. Consequently, supplementing low protein diets with mono or multi-enzymes may can be alternative way to improve broiler chicks performance and economical efficiency in the diet of broilers fed either a variety of cereals and protein sources or a nutrient deficient diet.

Feed enzymes have shown positive effects on animal performance, protein nutrition and physiology, animal welfare and the environment (Selle *et al.*, 2013) and the benefits of feed enzymes have proved to be more conspicuous in non conventional diets (Mahmood, *et al.*, 2017; Pan *et al.*, 2017 and Radfar *et al.*, 2017). Additionally, The use of exogenous enzymes improving feed efficiency, performance and environmental quality (Ding *et al.*, 2016). Improvement in feed conversion (FC) of broilers by supplemental xylanase, amylase and protease (Amerah *et al.*, 2017 and Singh e*t al.*, 2017).

In addition, exogenous proteases can improve AA utilization and enhance the digestibility of AA (Cowieson and Roos, 2014, Wang *et al.*, 2006 and Ding *et al.*, 2016) resulting in better growth performance. Ghazi *et al.* (2003) and Mahmood *et al.* (2017) reported that growth performance and protein utilization were improved in broilers fed exogenous protease supplemented diets. Pan *et al.* (2016) and Xu *et al.* (2017) reported that, a possible way to decrease the N pollution by enhancing N digestibility and reducing N excretion in animal production is supplementation of exogenous proteases. Previous studies have explored the use of exogenous proteases in low protein diets. In this respect, Freitas *et al.* (2011); Liu *et al.* (2015) and Liu *et al.* (2017) reported that protease supplementation in low CP diets resulted in improved feed efficiency in broilers, however, Freitas *et al.* (2011) reported that protease supplementation had no effects on performance, regardless of diet protein levels. However, other studies have reported inconsistent results (Naveed *et al.*, 1998) in broiler diets. Zanella *et al.* (1999) reported that enzyme supplementation should allow for a reduction in CP formulation and those AA individuals were not all improved by supplementation.

The objective of this study was to evaluate the effect of using two levels (recommended (R), R-2%) of CP supplemented with or without the requirements of Met. and Ly. and each with two levels (0.00 and 0.10%) of Avizyme 1500 on growth performance of broiler chickens (Ross strain).

## MATERIALS AND METHODS

This study was carried out at the Poultry Research Station, Ministry of Agric. and Land Recl., Regional Councils for Agricultural Research and Extension, Fayoum, Egypt, during the period from January to February, 2016. Chemical analyses were performed in the laboratories of Poultry Production Department, Faculty of Agriculture, Fayoum University according to the procedures outlined by A.O.A.C. (1990). This experiment was conducted to study the effect of using two levels of ((R), R-2%) CP supplemented with or without the requirements of Met. and Ly. and each with two levels (0.00 and 0.10%) of Avizyme 1500 in 2 x 2 x 2 factorial arrangement (giving eight dietary treatments) on growth performance of broiler chickens (Ross strain). Accordingly, a total numbers of 192 one-day old unsexed Ross broiler chickens were initially fed a control diet for five days. At six days of age, chicks were individually weighed to the nearest gram (start of experiment), wing-banded and randomly allotted to the dietary treatments, birds were divided into eight treatments (24 birds each), each treatment contained three replicates of eight birds each. Chicks were raised in electrically heated batteries with raised wire mesh floors and had a free access of mach feed and fresh water from nipple drinkers (2 nipples/cage) throughout the experiment.

The experimental treatments were as follows:

1. Chicks were fed the control diet ($T_1$). 2. $T_1$ + 0.1% Avizyme.

3.$T_1$ without the requirements of Met. and Ly. supplementation.

4.$T_1$ without the requirements of Met. and Ly. supplementation + 0.1% Avizyme. 5. $T_1$–2% CP. 6. $T_1$–2% CP + 0.1% Avizyme. 7. $T_1$–2% CP without the requirements of Met. and Ly. supplementation.

8.$T_1$–2% CP without the requirements of Met. and Ly. supplementation +0.1% Avizyme.

The composition and calculated analyses of the experimental diets (without Avizyme supplementation) are presented in Table (1). The experimental diets were supplemented with minerals and vitamins mixture, to cover the recommended requirements according to the strain catalog recommendations and were formulated to be iso-caloric. A commercial arabinoxylanase preparation (Avizyme 1500) was included in all dietary treatments, used at a rate of 1 kg/tonne (0.1%) of complete feed. Avizyme 1500 used in this study were purchased from Multi veta, Company, Egypt. It is a multi-enzyme preparation that includes 150 U/g endo-1,3(4)-beta-glucanase, 300 U/g endo-1,4 beta xylanase EC 3.2.1.8; 4000 U/g subtilisin (protease) EC 3.4.21.62; 400 U/g alpha amylase EC 3.2.1.1; 25 U/g polygalacturonase (pectinase) EC 3.2.1.15.

Batteries were placed into a room provided with a continuous light (23 h/d up to 41 days of age) and fans for ventilation. The experimental birds were reared under similar environmental conditions (open system), and were fed with broiler starter diet from six to 12 day, broiler grower diet from 13 to 24 day, and broiler finisher diet from 25 day to the end of the experiment at 41 day of age. Light was provided for 23 h/d. Room temperature on day 0 was 35ºC and 33ºC at the end of first week and decreased approximately 2ºC per week until 23ºC was reached, according to standard poultry rearing practices. Batteries were placed into a room provided with continuous fans for ventilation, the vaccination program adopted by recommended requirements according to standard commercial guidelines. Birds were individually weighed to the nearest gram at 6, 12, 24 and 41 days of age intervals during the experimental period.

At the same time, feed consumption was recorded and feed conversion (FC, g feed/g gain) and live body weight gain (LBWG) were calculated. Crude protein conversion (CPC), caloric conversion ratio (CCR) and performance index (PI) was calculated according to the equation described by North (1981) as follows: PI = (LBW, Kg/FC) x100. Accumulative mortality rate was obtained by adding the number of dead birds during the experiment divided by the total number of chicks at the beginning of the experimental period (mortality% was within normal limits and not related to treatments studied).

At the end of the finishing period (41 days of age), slaughter tests were performed using three chicks around the average LBW of each treatment. The birds were on feed withdrawal overnight (approximately 16h), then individually weighed to the nearest gram, and slaughtered by severing the jugular vein. After four minutes bleeding time, each bird was dipped in a water bath (80°) for 30s, and feathers were removed mechanically. After the removal of head, carcasses were manually eviscerated (by the same person to ensure uniformity of cuts) to determine some carcass traits, dressing% (eviscerated carcass without head, neck and legs) and total giblets% (gizzard empty, liver, heart and spleen). The eviscerated weight included the front part with wing and rear part. The abdominal fat was removed by hand from the parts around the viscera and gizzard, and was weighed to the nearest gram. The bone of front and rear were separated and weighed to calculate meat percentage. The meat without the skin from each part was weighed and blended using a kitchen blender. At the time of slaughter test, individual blood samples were taken from three birds of each treatment to determine hematological and biochemical characteristics of blood. Chemical analyses of representative samples of the carcass meat (without skin) were carried out to determine DM, CP (N x 6.25), EE and ash contents according to the methods of A.O.A.C.(1990). Nitrogen free extract (NFE) was calculated by difference.

**Table (1): Composition and analyses of the experimental diets.**

| Item, % | Starter (6-12 days of age) | | | | Grower (13-24 days of age) | | | | Finisher (25-41 days of age) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control (C) | C – amino acid (AA) | C- 2% CP[1] + AA | C- 2% CP - AA | C | C -AA | C- 2% CP[1] + AA | C- 2% CP - AA | C | C -AA | C- 2% CP[1] + AA | C- 2% CP - AA |
| Yellow corn, ground | 54.48 | 54.00 | 61.98 | 61.04 | 58.00 | 57.70 | 65.01 | 64.45 | 61.85 | 61.85 | 69.15 | 68.75 |
| Soybean meal (44%CP) | 31.22 | 31.79 | 24.78 | 25.90 | 26.70 | 27.10 | 20.47 | 21.25 | 22.62 | 22.65 | 16.25 | 16.78 |
| Broiler concentrate (48%CP[2]) | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| Calcium carbonate | 0.56 | 0.56 | 0.56 | 0.56 | 0.15 | 0.15 | 0.20 | 0.20 | 0.15 | 0.15 | 0.19 | 0.19 |
| Sodium chloride | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Vit. and Min. premix[3] | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Dicalcium phosphate | 0.50 | 0.50 | 0.50 | 0.50 | 0.30 | 0.30 | 0.40 | 0.40 | 0.42 | 0.42 | 0.45 | 0.45 |
| Vegetable oil[4] | 2.63 | 2.80 | 1.35 | 1.65 | 4.30 | 4.40 | 3.20 | 3.35 | 4.58 | 4.58 | 3.38 | 3.48 |
| DL–Methionine | 0.09 | 0.00 | 0.12 | 0.00 | 0.10 | 0.00 | 0.13 | 0.00 | 0.03 | 0.00 | 0.06 | 0.00 |
| L-Lysine | 0.17 | 0.00 | 0.36 | 0.00 | 0.10 | 0.00 | 0.24 | 0.00 | 0.00 | 0.00 | 0.17 | 0.00 |
| Total | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| Calculated analysis[5]: | | | | | | | | | | | | |
| Crude protein | 23.38 | 23.38 | 21.38 | 21.38 | 21.63 | 21.63 | 19.63 | 19.63 | 20.08 | 20.08 | 18.08 | 18.08 |
| Ether extract | 5.43 | 5.58 | 4.38 | 4.65 | 7.19 | 7.28 | 6.31 | 6.44 | 7.51 | 7.51 | 6.54 | 6.63 |
| Crude fiber | 3.53 | 3.56 | 3.25 | 3.31 | 3.30 | 3.32 | 3.01 | 3.06 | 3.10 | 3.11 | 2.82 | 2.85 |
| Calcium | 1.11 | 1.11 | 1.09 | 1.10 | 0.90 | 0.90 | 0.92 | 0.92 | 0.90 | 0.90 | 0.90 | 0.90 |
| Available phosphorus | 0.52 | 0.52 | 0.51 | 0.51 | 0.47 | 0.47 | 0.48 | 0.48 | 0.46 | 0.47 | 0.46 | 0.46 |
| Methionine | 0.50 | 0.41 | 0.50 | 0.39 | 0.49 | 0.39 | 0.49 | 0.37 | 0.40 | 0.37 | 0.40 | 0.35 |
| Methionine +Cystine | 0.86 | 0.78 | 0.84 | 0.73 | 0.83 | 0.73 | 0.80 | 0.68 | 0.72 | 0.69 | 0.70 | 0.64 |
| Lysine | 1.35 | 1.24 | 1.35 | 1.10 | 1.19 | 1.12 | 1.15 | 0.98 | 1.01 | 1.01 | 0.99 | 0.87 |
| Sodium | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.19 | 0.19 |
| Chloride | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 |
| ME, kcal./Kg | 3010.6 | 3011.4 | 3012.9 | 3012.4 | 3175.2 | 3174.8 | 3179.6 | 3176.0 | 3231.3 | 3230.8 | 3234.7 | 3232.1 |
| C/P ratio | 128.77 | 128.80 | 140.92 | 140.90 | 146.80 | 146.78 | 161.98 | 161.79 | 160.92 | 160.90 | 178.91 | 178.77 |
| Cost (£.E./ton)[6] | 6229.3 | 6171.1 | 5935.5 | 5843.5 | 6172.9 | 6114.9 | 5893.0 | 5801.0 | 5966.4 | 5950.8 | 5676.9 | 5628.3 |
| Relative cost[7] | 100.0 | 99.07 | 95.28 | 93.81 | 100.0 | 99.06 | 95.47 | 93.98 | 100.0 | 99.74 | 95.15 | 94.33 |

[1] *Crude protein* [2] *Broiler concentrate manufactured by Alpha Feed For Premix Production Company and contains: 48% CP; 1.5% crude fiber; 4.75% ether extract; 6.85% calcium; 3% available phosphorus; 1.2% methionine; 1.8% methionine + cystine; 2.4% lysine; 0.96% Sodium; 2415 K cal ME/kg.*

[3] *Each 3.0 Kg of the Vit. and Min. premix manufactured by Vetgreen Company and contains: Vit. A 10000000 IU; Vit. $D_3$ 2000000 IU; Vit. E 1000 mg; Vit. $K_3$ 1000 mg; Vit. B1 1000 mg; Vit. B2 500 mg; Vit. B6 1500 mg; Vit. B12 10 mg; biotin 50 mg; folic acid 1 g; niacin 3000 mg; Ca pantothenate 1000 mg; Zn 50 g; Cu 4 g; Fe 30 g; Co 0.1 g; Se 0.1 g; I 0.3 g; Mn 60 g and anti-oxidant 10 g, and complete to 3.0 Kg by calcium carbonate.* [4] *Mixture from 75% soybean oil and 25% sunflower oil.* [5] *According to NRC, (1994).* [6] *According to the local market price at the experimental time.* [7] *Assuming the price of the control group equal 100.*

The right tibia bone was collected to obtain bone ash values according to the method of Martinez *et al.* (2006). Tibias were pooled by replicate groups and autoclaved and adhering tissue was removed. Then bones were dried for 24 h at 100°C and weighed, tibia relative weight as a percentage of LBW was calculated, and then dry-ashed for 24 h in a 600°C muffle furnace. Ash weight was expressed as a percentage of dried tibia weight. The economic efficiency was calculated as the price of live body weight–total costs of raising a broiler as relative to total raising costs which was estimated based upon local current prices at the experimental time. Statistical analysis of results was performed using the General Linear Models (GLM) procedure of the SPSS software (version 16, SPSS Inc., Chicago, IL), according to the follow general model:

$$Y_{ijkl}= \mu+P_i+A_j+E_k+PA_{ij}+PE_{ik}+AE_{jk}+PAE_{ijk}+e_{ijkl}$$

Where:

$Y_{ijkl}$: observed value. μ: overall mean.
$P_i$: level of CP effect (i: recommended (R), R-2%)
$A_j$: level of AA (adequate and inadequate)
$E_k$: enzyme supplementation effect (k: 0.00 and 0.10%).
$PA_{ij}$: interaction effect of level of CP by level of AA addition.
$PE_{ik}$: interaction effect of level of CP by enzyme supplementation.
$AE_{jk}$: level of AA addition by enzyme supplementation effect.
$PAE_{ijk}$: level of CP by level of AA by enzyme supplementation effect.
$e_{ijkl}$: random error.

Treatment means indicating significant differences (P≤0.01 and P≤0.05) were tested using Duncan's multiple range test (Duncan, 1955).

## RESULTS AND DISCUSSION

### *1-Productive performance:*

Effect of using two levels (R, R-2%) of CP supplemented with or without the requirements of Met. and Ly. and each with two levels (0.00 and 0.10%) of Avizyme 1500 on LBW and LBWG of Ross strain broiler chicks during the period from 6 to 41 days of age are presented in Table (2).

**Table (2): Effect of dietary protein levels with or without synthetic amino acids and enzyme supplementation on live body weight (LBW,g) and live body weight gain (LBWG,g) of broiler chickens.**

| Item | | | LBW, g (age, days) | | | | LBWG, g (age period, days) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 6 | 12 | 24 | 41 | 6-12 | 13-24 | 25-41 | 6-41 |
| Level of crude protein (CP)% | | | | | | | | | | |
| Recommended (R) | | | 142.89 | $267.89^{b}$ | 914.18 | 2003.8 | $126.59^{b}$ | 645.79 | 1037.8 | 1859.3 |
| R-2 | | | 142.18 | $285.14^{a}$ | 909.60 | 1993.6 | $143.41^{a}$ | 623.26 | 1037.7 | 1848.0 |
| SEM$^{1}$ | | | 1.58 | 2.83 | 11.73 | 31.91 | 1.93 | 10.15 | 26.95 | 31.40 |
| P-value | | | 0.607 | <0.001 | 0.805 | 0.835 | <0.001 | 0.163 | 0.998 | 0.814 |
| Amino acid addition (AA) | | | | | | | | | | |
| Adequate (A.) | | | 142.32 | 280.41 | $937.25^{a}$ | 2041.2 | $139.81^{a}$ | $655.15^{a}$ | 1065.3 | 1897.7 |
| Inadequate (Ina.) | | | 142.76 | 272.63 | $886.53^{b}$ | 1956.2 | $130.19^{b}$ | $613.90^{b}$ | 1010.3 | 1809.6 |
| SEM | | | 1.76 | 3.16 | 13.01 | 32.32 | 2.17 | 11.26 | 27.30 | 31.80 |
| P-value | | | 0.860 | 0.087 | 0.007 | 0.083 | 0.002 | 0.011 | 0.183 | 0.068 |
| Enzyme (En.)% | | | | | | | | | | |
| 0.00 | | | 142.37 | 272.22 | 910.64 | 1970.7 | $131.88^{b}$ | 636.54 | 1023.0 | 1826.0 |
| 0.10 | | | 142.70 | 280.82 | 913.14 | 2026.7 | $138.11^{a}$ | 632.51 | 1052.6 | 1881.3 |
| SEM | | | 1.76 | 3.17 | 13.00 | 32.32 | 2.15 | 11.25 | 27.30 | 31.80 |
| P-value | | | 0.894 | 0.059 | 0.893 | 0.252 | 0.045 | 0.802 | 0.472 | 0.249 |
| CP% × AA × En.% (treatments) | | | | | | | | | | |
| R | A. | 0.00 | 142.00 | 263.90 | 947.15 | 1994.9 | 124.25 | 680.55 | 1019.3 | 1850.1 |
| | | 0.10 | 140.48 | 281.29 | 942.05 | 2048.9 | 141.81 | 661.47 | 1062.1 | 1907.0 |
| | Ina. | 0.00 | 142.33 | 263.62 | 907.86 | 2020.4 | 121.29 | 644.24 | 1069.3 | 1874.9 |
| | | 0.10 | 143.76 | 262.76 | 859.67 | 1950.9 | 119.00 | 596.90 | 1000.6 | 1805.0 |
| R-2 | A. | 0.00 | 143.36 | 277.29 | 900.15 | 1940.2 | 138.46 | 618.08 | 994.6 | 1796.9 |
| | | 0.10 | 144.43 | 299.14 | 959.64 | 2180.8 | 154.71 | 660.50 | 1185.1 | 2036.6 |
| | Ina. | 0.00 | 141.79 | 284.08 | 887.38 | 1927.4 | 143.54 | 603.31 | 1008.8 | 1781.9 |
| | | 0.10 | 143.14 | 280.07 | 891.21 | 1926.1 | 136.93 | 611.14 | 962.4 | 1776.6 |
| SEM | | | 3.15 | 5.67 | 23.02 | 57.81 | 3.84 | 19.92 | 48.83 | 56.88 |
| P-value | | | 0.455 | 0.718 | 0.586 | 0.543 | 0.291 | 0.568 | 0.446 | 0.537 |

$^{a–b}$ *Means in a column with different superscripts differ significantly (P≤0.05).* $^{1}$ *Pooled SEM*

Data presented in Table (2) indicate the main effect of level of CP was significant ($P \leq 0.01$) for LBW at 12 days and LBWG during the period from 6 to 12 days, however, level of CP had insignificant effect on LBW and LBWG during the other periods studied. Chicks fed diet containing recommended level of CP -2% had heavier LBW and higher LBWG during the previous periods, while, chicks fed diet containing R have lower values (Table 2). In this respect, many strategies have been assessed to use the low CP diets for broiler chickens without negative effects on growth performance (Parr and Summers, 1991 and Si *et al.*, 2004). Also, in the studies of Kerr and Kidd (1999) suggest reduction of CP by two percentage units had no impact on daily LBWG or FC compared to birds fed the positive control diet since this level of CP reduction results in amino acid levels being relatively close to current recommendations. Garcia *et al.* (2000) reported that reducing dietary CP down to 19% insignificant effect on broiler performance.

Conversely, these results were unsupported by the findings of Asadi Kermani *et al.* (2017) who reported that lowering dietary CP concentration led to an increase in LBW ($P \leq 0.03$). Moreover, Angel *et al.* (2011); Freitas *et al* .(2011); Ragab *et al.* (2012) and Ding *et al.* (2016) indicated that growth performance significantly decrease with reduction of CP contents of the diet than those fed diets containing recommended level. Also, Abd El-Gawad *et al.* (2004) and Waldroup *et al.* (2005) reported that broiler chicks fed on optimum level of CP showed significantly ($P \leq 0.05$) higher LBW and LBWG value during the experimental periods, when compared with suboptimum level of CP, while, supplementing low protein diets with growth promoters or EAA partially improved the loss in LBWG. Further, in a study by Kamran *et al.* (2008) also noted that, although the birds consumed the same amount of protein and energy due to increased FI, there was a significant depression in LBWG of the birds with the reduction in dietary CP level during grower, finisher and overall experimental periods.

Concerning AA addition, the results cleared that chicks fed adequate amounts of AA supplement diets recorded significantly ($P \leq 0.01$) higher LBW at 24 days and LBWG values during the periods from 6 to 12 and 13 to 24 days. However, AA addition had insignificant effect on LBW and LBWG during the other periods studied. Similarly, Aletor *et al.* (2000) demonstrates that the growth of the grower broiler chicken is unaffected by decreasing dietary CP from 22.5 to 15.3% when such diets are supplemented with EAAs that meet the minimum NRC (1994) specifications. However, the increased feed consumption in the low protein diets led to a corresponding decrease in FC. Also, Schutte (1987) and Parr and Summers (1991) shown that optimal performance can be reached by supplementing diets with synthetic AA. By contrast, Moran *et al.* (1992) and Kamran *et al.* (2008) found that maximum performance cannot be reached by fortifying low protein diets with synthetic AA. However, Waldroup *et al.* (2006) demonstrated that reduction of more than 0.10% Met from current values could be tolerated without adversely affecting LBWG or FC.

Neither enzyme addition nor interaction between level of CP, AA and enzyme addition had any significant effect on LBW, LBWG, FC, CPC and PI during all periods studied, except, enzyme addition with LBWG and PI during the period from 6 to 12 days which was significantly affected (Tables 2 and 3). Inclusion of Avizyme 1500 which contain (4000 U/g subtilisin (protease)) in broiler diet at 0.1% caused a significant ($P \leq 0.05$) increase in LBWG and PI during the previous period. Numerically, as shown in Tables (2 and 3), enzyme supplementation increase LBW at 24 and 41 days, LBWG, FI and PI during the periods from 25 to 41 and 6 to 41 days compared with those fed enzyme un-supplement diet (0.0%), however, these did not reach a level of statistical significance (the improvement in LBW and LBWG may be attributable to the effect of enzyme on the utilization of nutrients (protein, fat and carbohydrate digestibility) affecting growth). Many studies have reported adding enzyme blends containing proteases to the poultry diet (Rahman *et al.*, 2009). In this respect, Vieira *et al.* (2016) reported that, in the case of endogenous proteases, peptide bond specificity directly affects the rate of protein hydrolysis and the quantity of peptides and AA released and made available for absorption.

Data presented in Table (3) indicated that level of CP was insignificant effect of FI during all periods studies except the period from 6 to 12 days of age which was significantly affected. Chicks fed diet containing R-2% CP had higher value of FI only during the starter (6 to 12 days) period (Table 3). This observation was disagreement with reports by Kamran *et al.* (2008) who reported that FI was linearly increased with reduced CP diets during grower, finisher and overall periods. Likewise, Hidalgo *et al.* (2004), reported that birds fed the lowest dietary regimen with a constant ME:CP ratio had increased their FI during the finisher and overall experimental period. Feed intake depression is one of the earliest effects of dietary AA imbalance in broiler chickens (Alam *et al.*, 2014). Also, in this respect, Leung and Rogers (1969) suggest that, this reduction in FI has been indicated to be related with a fall in the most limiting AA in the plasma of broiler chickens fed on the imbalance diet. Abd El-Gawad *et al.* (2004) and Ragab *et al.* (2012) found that level of CP did not affect FI of the broiler chicks.

**Table (3): Effect of dietary protein levels with or without synthetic amino acids and enzyme supplementation on feed intake (FI, g) and feed conversion ratio (FC) of broiler chickens.**

| Item | | | FI, g (age period, days) | | | | FC (age period, days) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 6-12 | 13-24 | 25-41 | 6-41 | 6-12 | 13-24 | 25-41 | 6-41 |
| Level of crude protein (CP)% | | | | | | | | | | |
| Recommended (R) | | | 207.5$^{b}$ | 1058.3 | 2543.9 | 3810.9 | 1.67$^{a}$ | 1.68 | 2.57 | 1.92 |
| R-2 | | | 207.9$^{a}$ | 1055.3 | 2566.7 | 3825.3 | 1.49$^{b}$ | 1.73 | 2.54 | 1.87 |
| SEM[1] | | | 0.10 | 5.88 | 15.30 | 18.54 | 0.03 | 0.03 | 0.07 | 0.03 |
| P-value | | | 0.017 | 0.748 | 0.349 | 0.628 | <0.001 | 0.294 | 0.731 | 0.258 |
| Amino acid addition (AA) | | | | | | | | | | |
| Adequate (A.) | | | 207.3$^{b}$ | 1034.1$^{b}$ | 2555.0 | 3795.0 | 1.51$^{b}$ | 1.62$^{b}$ | 2.50 | 1.83$^{b}$ |
| Inadequate (Ina.) | | | 208.0$^{a}$ | 1079.5$^{a}$ | 2555.6 | 3841.2 | 1.64$^{a}$ | 1.80$^{a}$ | 2.61 | 1.95$^{a}$ |
| SEM | | | 0.12 | 6.57 | 17.11 | 20.93 | 0.03 | 0.03 | 0.07 | 0.03 |
| P-value | | | <0.001 | <0.001 | 0.982 | 0.121 | 0.001 | <0.001 | 0.319 | 0.007 |
| Enzyme (En.)% | | | | | | | | | | |
| 0.00 | | | 207.8 | 1051.3 | 2555.0 | 3801.0 | 1.62 | 1.69 | 2.54 | 1.90 |
| 0.10 | | | 207.6 | 1062.3 | 2555.6 | 3835.2 | 1.54 | 1.72 | 2.57 | 1.88 |
| SEM | | | 0.12 | 6.58 | 17.11 | 21.17 | 0.03 | 0.03 | 0.07 | 0.03 |
| P-value | | | 0.198 | 0.240 | 0.406 | 0.249 | 0.057 | 0.494 | 0.725 | 0.657 |
| CP% × AA × En.% (treatments) | | | | | | | | | | |
| R | A. | 0.00 | 204.3$^{e}$ | 1004.7$^{cd}$ | 2512.7$^{cd}$ | 3726.8$^{c}$ | 1.66 | 1.50 | 2.54 | 1.87 |
| | | 0.10 | 209.3$^{a}$ | 1026.0$^{c}$ | 2575.9$^{bc}$ | 3811.2$^{c}$ | 1.50 | 1.60 | 2.61 | 1.85 |
| | Ina. | 0.00 | 209.2$^{a}$ | 1134.4$^{a}$ | 2640.3$^{b}$ | 3983.8$^{b}$ | 1.75 | 1.80 | 2.51 | 1.96 |
| | | 0.10 | 207.0$^{c}$ | 1068.1$^{b}$ | 2446.7$^{de}$ | 3721.9$^{c}$ | 1.77 | 1.83 | 2.63 | 1.99 |
| R-2 | A. | 0.00 | 209.4$^{a}$ | 973.3$^{d}$ | 2362.8$^{e}$ | 3534.7$^{d}$ | 1.54 | 1.63 | 2.42 | 1.80 |
| | | 0.10 | 206.2$^{d}$ | 1132.6$^{a}$ | 2768.6$^{a}$ | 4107.4$^{a}$ | 1.36 | 1.74 | 2.44 | 1.80 |
| | Ina. | 0.00 | 208.1$^{b}$ | 1092.9$^{b}$ | 2665.0$^{b}$ | 3958.7$^{b}$ | 1.51 | 1.84 | 2.67 | 1.98 |
| | | 0.10 | 207.6$^{bc}$ | 1022.4$^{c}$ | 2470.2$^{d}$ | 3700.2$^{c}$ | 1.54 | 1.72 | 2.62 | 1.88 |
| SEM | | | 0.21 | 11.76 | 30.61 | 37.75 | 0.05 | 0.06 | 0.13 | 0.05 |
| P-value | | | <0.001 | <0.001 | 0.001 | <0.001 | 0.057 | 0.494 | 0.784 | 0.366 |

[a–e] *Means in a column with different superscripts differ significantly (P≤0.05).* [1] *Pooled SEM*

Concerning AA addition, as shown in Table (3), chicks fed diet supplemented with adequate amounts of AA had significantly lower (P≤0.01) FI values during the periods from 6 to 12 and 13 to 24 days. Enzyme supplementation had insignificant effect on FI during all periods studied (Table 3). Data presented in Table (3) indicated that interaction between level of CP, AA and enzyme addition had significant (P≤0.01) effect on FI during all periods studies. Chicks fed diet containing recommended level of CP -2% supplemented with AA plus 0.10% of Avizyme had higher value of FI during finisher and overall experimental periods

Numerically, as shown in Tables (2, 3 and 4), chicks fed diet containing recommended level of CP-2% supplemented with the requirements of Met. and Ly. plus 0.10% of Avizyme 1500 had heavier LBW at 41 days of age, higher LBWG, PI and the best FC and CPC values during the period from 6 to 41 days of age, while, those fed diet containing recommended level of CP -2% un-supplemented with AA plus 0.10% of Avizyme had lower values of LBW, LBWG and PI, but differences were not significant (Tables 2, 3 and 4). On the other hand, adequate amounts of AA or Avizyme supplementation to broiler diets enhanced LBW, LBWG, improved FC and PI compared with those fed un-supplemented diet, but differences were not significant (Tables 2, 3 and 4). Recent study by Liu *et al.* (2017) determined that, the low protein diet decreased (P = 0.01) LBW as compared with the high protein diet, when the CP level decreased from 22.5% to 20.5%, there were no effects on FI and FC, but reduced LBWG of broilers at 1 to 21 d of age. While both the low and high protein diet with multienzyme supplementation increased (P≤0.01) FI and LBWG, with a greater effect in the high CP diet (P≤0.001), there was no effect of protein or enzymes on FC. In this respect, Ghazi *et al.* (2003) reported that the use of a mono component protease resulted in an increase of LBWG and FI, while FC was either negatively affected or not affected at all, depending on the protease concentration used. Conversely, Hidalgo *et al.* (2004) found that LBWG and FCR were adversely affected when the broilers were fed diets formulated to contain suboptimum concentrations of CP and ME. Nutritionists can employ protease and synthetic Val. supplementation to achieve additional CP reduction (Vieira *et al.*, 2016).

**Table (4): Effect of dietary protein levels with or without synthetic amino acids and enzyme supplementation on crude protein conversion (CPC) and performance index (PI) of broiler chickens.**

| Item | | | CPC (age period, days) | | | | PI (age period, days) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 6-12 | 13-24 | 25-41 | 6-41 | 6-12 | 13-24 | 25-41 | 6-41 |
| Level of crude protein (CP) % | | | | | | | | | | |
| Recommended (R) | | | 0.387$^{a}$ | 0.364$^{a}$ | 0.517$^{a}$ | 0.411$^{a}$ | 16.56$^{b}$ | 56.90 | 81.97 | 54.21 |
| R-2 | | | 0.347$^{b}$ | 0.340$^{b}$ | 0.459$^{b}$ | 0.371$^{b}$ | 19.98$^{a}$ | 54.73 | 81.79 | 54.43 |
| SEM$^{1}$ | | | 0.01 | 0.01 | 0.010 | 0.006 | 0.41 | 1.55 | 3.35 | 1.53 |
| P-value | | | <0.001 | 0.018 | 0.006 | <0.001 | <0.001 | 0.337 | 0.971 | 0.924 |
| Amino acid addition (AA) | | | | | | | | | | |
| Adequate (A.) | | | 0.353$^{b}$ | 0.334$^{b}$ | 0.478 | 0.379$^{b}$ | 19.26$^{a}$ | 60.36$^{a}$ | 86.85 | 57.54$^{a}$ |
| Inadequate (Ina.) | | | 0.381$^{a}$ | 0.371$^{a}$ | 0.497 | 0.404$^{a}$ | 17.28$^{b}$ | 51.28$^{b}$ | 76.90 | 51.11$^{b}$ |
| SEM | | | 0.01 | 0.01 | 0.010 | 0.006 | 0.47 | 1.72 | 3.39 | 1.55 |
| P-value | | | 0.002 | <0.001 | 0.357 | 0.006 | 0.003 | <0.001 | 0.054 | 0.007 |
| Enzyme (En.)% | | | | | | | | | | |
| 0.00 | | | 0.375 | 0.348 | 0.484 | 0.394 | 17.61$^{b}$ | 56.19 | 80.26 | 53.17 |
| 0.10 | | | 0.359 | 0.356 | 0.492 | 0.389 | 18.93$^{a}$ | 55.45 | 83.50 | 55.48 |
| SEM | | | 0.01 | 0.01 | 0.010 | 0.006 | 0.46 | 1.72 | 3.39 | 1.55 |
| P-value | | | 0.063 | 0.476 | 0.712 | 0.591 | 0.049 | 0.762 | 0.526 | 0.322 |
| CP% × AA × En.% (treatments) | | | | | | | | | | |
| R | A. | 0.00 | 0.390 | 0.326 | 0.511 | 0.402 | 16.38 | 64.52 | 82.22 | 55.93 |
| | | 0.10 | 0.345 | 0.348 | 0.524 | 0.395 | 19.19 | 61.97 | 87.42 | 58.60 |
| | Ina. | 0.00 | 0.400 | 0.389 | 0.505 | 0.420 | 15.42 | 52.29 | 83.10 | 52.32 |
| | | 0.10 | 0.414 | 0.396 | 0.528 | 0.427 | 15.24 | 48.82 | 75.15 | 49.99 |
| R-2 | A. | 0.00 | 0.360 | 0.319 | 0.437 | 0.359 | 18.78 | 58.25 | 82.29 | 55.46 |
| | | 0.10 | 0.317 | 0.342 | 0.441 | 0.358 | 22.67 | 56.69 | 95.49 | 60.15 |
| | Ina. | 0.00 | 0.353 | 0.361 | 0.483 | 0.394 | 19.86 | 49.69 | 73.42 | 48.95 |
| | | 0.10 | 0.360 | 0.338 | 0.474 | 0.375 | 18.60 | 54.30 | 75.94 | 53.17 |
| SEM | | | 0.01 | 0.01 | 0.030 | 0.010 | 0.85 | 3.04 | 6.07 | 2.77 |
| P-value | | | 0.063 | 0.476 | 0.791 | 0.383 | 0.468 | 0.143 | 0.904 | 0.626 |

$^{a-b}$ *Means in a column with different superscripts differ significantly (P≤0.05).* $^{1}$ *Pooled SEM*

However, Ding *et al.* (2016), reported that the protease supplementation (300 mg/kg) had no effects on LBWG or FI, while, FC was slightly improved (1.4%) between 1 and 21 days of age.

Data presented in Tables (3 and 4) indicate the main effect of level of CP was significant (P≤0.01) for FC and PI during the period from 6 to 12 days and CPC during all periods studies. Chicks fed diet containing recommended level of CP had the worst FC and the lower PI value during the period 6 to 12 days, and those fed R-2% CP had the best FC and the higher value of PI during the same period. Also, chicks fed diet containing recommended level of CP had the worst CPC during all periods studies. It can be concluded that CP can be reduced from the recommended level by 2% without affecting LBW, LBWG, FI, FC and PI (Tables 2, 3 and 4).

In this regard, Aletor *et al.* (2000) suggest that, protein efficiency ratio were generally significantly improved with decreasing dietary CP. In contrast to previous reports from our results Ragab *et al.* (2012) found that chicks fed diets containing recommended level of CP had the best FC and CPC during the over all period studied, while, feeding the sub-optimal CP level diet resulted in the worst FC and CPC values during the same period.

Concerning AA addition, data presented in Tables (3 and 4) indicate the main effects of AA addition significantly affected FC, CPC and PI during all periods studied, except, the finisher period which was insignificantly (P≥0.05) affected. The results cleared that chicks fed adequate amounts of AA supplement diets recorded significantly (P≤0.01) the best values of FC, CPC (this may be partially attributed to lower FI of AA supplemented groups) and the higher PI during all periods studies (Tables 3 and 4).

***2- Blood constituents:*** Impact of CP, AA and enzyme supplementation on blood constituents of Ross broiler chicks are presented in Table (5). The results indicated no significant differences due to level of CP and AA addition on blood constituents, except, red blood cells count (RBCs) which was significantly (P≤0.05) affected. Chicks fed R-2% CP had higher value of RBCs, while, chicks fed R had lower value and chicks fed diet containing inadequate amounts of AA had higher value of RBCs, while, chicks fed adequate amounts of AA had lower value at the end of the experiment. Enzyme addition had insignificantly affected blood constituents. The results indicated no significant differences due to interaction effect of dietary treatments on blood constituents, except, neutrophils% (segment) which was significantly (P≤0.05) affected (Table 5). Chicks fed control diet had higher value of segment while, chicks fed diet containing R-2% supplemented with the requirements of Met. and Ly. plus 0.10% of Avizyme had lower value of segment at the end of the experiment (Table 5). Similar results were previously observed by Ragab *et al.* (2012) who found that chicks fed diet containing suboptimal CP level unsupplemented with organic acids had higher RBCs value.

**Table (5): Effect of dietary protein levels with or without synthetic amino acids and enzyme supplementation on some blood parameters of broiler chickens.**

| Item | | | Hemoglobin (g/dL) | Hematocrit (Ht) % | Red blood cells (RBCs) | Mean Cell Volume (MCV µ3) | Total leucocytes (TLC)/ cmm | Lymphocyte (LYMP%) | Neutrophils% | | Eosinophils (ESINO%) | Basophils (BASO%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | SEGMENT | STAFF | | |
| **Level of crude protein (CP) %** | | | | | | | | | | | | |
| **Recommended (R)** | | | **10.77** | **35.82** | **2.83$^{b}$** | **138.48** | **137.92** | **54.437** | **41.44** | **1.688** | **2.375** | **0.063** |
| **R-2** | | | **10.60** | **34.79** | **3.05$^{a}$** | **137.33** | **133.53** | **60.042** | **35.21** | **2.042** | **2.500** | **0.208** |
| **SEM$^{1}$** | | | **0.20** | **0.58** | **0.05** | **1.91** | **3.79** | **2.890** | **2.450** | **0.290** | **0.520** | **0.130** |
| **P-value** | | | **0.552** | **0.248** | **0.019** | **0.691** | **0.446** | **0.211** | **0.109** | **0.416** | **0.873** | **0.445** |
| **Amino acid addition (AA)** | | | | | | | | | | | | |
| **Adequate (A.)** | | | **10.52** | **35.07** | **2.82$^{b}$** | **137.35** | **132.92** | **53.562** | **41.81** | **1.938** | **2.500** | **0.188** |
| **Inadequate (Ina.)** | | | **10.85** | **35.54** | **3.05$^{a}$** | **138.46** | **138.94** | **60.917** | **34.83** | **1.792** | **2.375** | **0.083** |
| **SEM** | | | **0.20** | **0.58** | **0.05** | **1.91** | **3.79** | **2.890** | **2.450** | **0.290** | **0.520** | **0.130** |
| **P-value** | | | **0.278** | **0.596** | **0.015** | **0.701** | **0.271** | **0.109** | **0.077** | **0.734** | **0.873** | **0.583** |
| **Enzyme (En.)%** | | | | | | | | | | | | |
| **0.00** | | | **10.65** | **35.17** | **2.96** | **137.69** | **137.18** | **54.938** | **40.81** | **1.813** | **2.250** | **0.188** |
| **0.10** | | | **10.72** | **35.44** | **2.92** | **138.13** | **134.26** | **59.542** | **35.83** | **1.917** | **2.625** | **0.083** |
| **SEM** | | | **0.20** | **0.61** | **0.06** | **1.96** | **3.99** | **3.040** | **2.580** | **0.300** | **0.550** | **0.130** |
| **P-value** | | | **0.819** | **0.755** | **0.624** | **0.879** | **0.610** | **0.299** | **0.192** | **0.808** | **0.633** | **0.583** |
| **CP% × AA × En.% (treatments)** | | | | | | | | | | | | |
| **R** | **A.** | **0.00** | **10.55** | **35.53** | **2.69** | **138.75** | **139.13** | **44.750** | **51.75$^{a}$** | **1.750** | **1.500** | **0.250** |
| | | **0.10** | **10.43** | **35.48** | **2.52** | **140.67** | **136.80** | **44.000** | **51.00$^{ab}$** | **2.000** | **3.000** | **0.000** |
| | **Ina.** | **0.00** | **11.40** | **37.20** | **3.06** | **138.50** | **136.35** | **60.000** | **37.50$^{abcd}$** | **1.000** | **1.500** | **0.000** |
| | | **0.10** | **10.70** | **35.10** | **3.05** | **136.00** | **139.40** | **69.000** | **25.50$^{cd}$** | **2.000** | **3.500** | **0.000** |
| **R-2** | **A.** | **0.00** | **10.20** | **33.60** | **3.04** | **132.50** | **135.15** | **54.000** | **40.00$^{abcd}$** | **2.000** | **3.500** | **0.500** |
| | | **0.10** | **10.90** | **35.70** | **3.05** | **137.50** | **118.95** | **71.500** | **24.50$^{d}$** | **2.000** | **2.000** | **0.000** |
| | **Ina.** | **0.00** | **10.45** | **34.35** | **3.04** | **141.00** | **138.10** | **61.000** | **34.00$^{bcd}$** | **2.500** | **2.500** | **0.000** |
| | | **0.10** | **10.83** | **35.50** | **3.05** | **138.33** | **141.90** | **53.667** | **42.33$^{abc}$** | **1.667** | **2.000** | **0.333** |
| **SEM** | | | **0.31** | **1.01** | **1.00** | **3.04** | **6.03** | **4.590** | **3.900** | **0.460** | **0.830** | **0.200** |
| **P-value** | | | **0.819** | **0.755** | **0.621** | **0.777** | **0.524** | **0.064** | **0.031** | **0.365** | **0.873** | **0.445** |

$^{a–d}$ *Means in a column with different superscripts differ significantly (P≤0.05).* $^{1}$ *Pooled SEM*

**3-Slaughter parameters%:** Impact of CP, AA and enzyme supplementation on slaughter parameters% of Ross broiler chicks are presented in Table (6). Neither level of CP and enzyme addition nor interaction between level of CP, AA and enzyme addition had any significant effect on slaughter parameters% at the end of the finishing period (Table 6). It may be mentioned that perhaps the carcass weight and breast meat weight were not affected due to adequate levels of essential AA particularly Lys. and Met. in low CP diets, because these two AA are exclusively used for protein accretion in the body (Si *et al.*, 2001 and Baker *et al.*, 2002).

**Table (6): Effect of dietary protein levels with or without synthetic amino acids and enzyme supplementation on slaughter parameters% of broiler chickens.**

| Item | | | Live body weight (g) | Blood &feather | Total giblets | Abdominal fat | Half breast | Half rear | Breast meat | Rear meat | Carcass weight after evisceration | Dressing | Tibia | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | Weight | Ash |
| Level of crude protein (CP) % | | | | | | | | | | | | | | |
| Recommended (R) | | | 1962.2 | 9.27 | 5.04 | 1.80 | 17.49 | 13.5 | 88.22 | 85.29 | 62.77 | 67.80 | 0.236 | 46.03 |
| R-2 | | | 1982.4 | 8.66 | 4.76 | 2.07 | 17.16 | 14.5 | 87.13 | 86.59 | 63.03 | 67.79 | 0.247 | 46.22 |
| SEM$^{1}$ | | | 71.03 | 0.45 | 0.17 | 0.22 | 0.28 | 0.30 | 0.47 | 0.84 | 0.66 | 0.62 | 0.010 | 0.75 |
| P-value | | | 0.850 | 0.372 | 0.279 | 0.42 | 0.438 | 0.05 | 0.141 | 0.314 | 0.792 | 0.986 | 0.474 | 0.862 |
| Amino acid addition (AA) | | | | | | | | | | | | | | |
| Adequate (A.) | | | 2036.9 | 8.27 | 4.76 | 1.89 | $17.87^{a}$ | 13.97 | 88.40 | 86.34 | $64.09^{a}$ | $68.84^{a}$ | 0.234 | 46.40 |
| Inadequate (Ina.) | | | 1907.6 | 9.65 | 5.04 | 1.98 | $16.79^{b}$ | 14.09 | 86.94 | 85.54 | $61.71^{b}$ | $66.75^{b}$ | 0.249 | 45.48 |
| SEM | | | 71.03 | 0.45 | 0.17 | 0.22 | 0.28 | 0.30 | 0.47 | 0.84 | 0.66 | 0.62 | 0.010 | 0.75 |
| P-value | | | 0.242 | 0.063 | 0.278 | 0.781 | 0.025 | 0.795 | 0.058 | 0.531 | 0.034 | 0.044 | 0.311 | 0.621 |
| Enzyme (En.)% | | | | | | | | | | | | | | |
| 0.00 | | | 1967.2 | 8.32 | 5.01 | 1.88 | 17.43 | 14.14 | 87.75 | 85.97 | 63.58 | 68.59 | 0.243 | 45.78 |
| 0.10 | | | 1977.3 | 9.61 | 4.79 | 1.99 | 17.23 | 13.93 | 87.60 | 85.91 | 62.21 | 67.00 | 0.240 | 46.48 |
| SEM | | | 71.03 | 0.45 | 0.17 | 0.22 | 0.28 | 0.30 | 0.47 | 0.84 | 0.66 | 0.62 | 0.010 | 0.75 |
| P-value | | | 0.924 | 0.078 | 0.380 | 0.72 | 0.631 | 0.65 | 0.835 | 0.959 | 0.187 | 0.111 | 0.835 | 0.534 |
| CP% × AA × En.% (treatments) | | | | | | | | | | | | | | |
| R | A. | 0.00 | 1965.8 | 7.83 | 5.06 | 1.51 | 17.36 | 14.34 | 87.73 | 86.79 | 64.71 | 69.77 | 0.243 | 47.33 |
| | | 0.10 | 2052.9 | 8.24 | 4.74 | 1.88 | 18.31 | 12.98 | 88.99 | 85.50 | 63.75 | 68.50 | 0.221 | 46.21 |
| | Ina. | 0.00 | 2025.0 | 9.12 | 5.34 | 1.61 | 17.71 | 13.89 | 88.95 | 85.52 | 63.15 | 68.49 | 0.224 | 45.71 |
| | | 0.10 | 1805.0 | 11.89 | 5.00 | 2.19 | 16.60 | 12.96 | 87.20 | 83.37 | 59.45 | 64.46 | 0.256 | 44.87 |
| R-2 | A. | 0.00 | 1945.0 | 7.64 | 4.84 | 2.29 | 18.11 | 14.07 | 87.90 | 85.74 | 64.16 | 69.00 | 0.237 | 45.36 |
| | | 0.10 | 2184.0 | 9.38 | 4.39 | 1.88 | 17.69 | 14.49 | 88.99 | 87.33 | 63.72 | 68.10 | 0.234 | 46.72 |
| | Ina. | 0.00 | 1933.0 | 8.69 | 4.80 | 2.09 | 16.54 | 14.24 | 86.40 | 85.85 | 62.32 | 67.11 | 0.267 | 44.70 |
| | | 0.10 | 1867.5 | 8.91 | 5.01 | 2.02 | 16.31 | 15.28 | 85.22 | 87.44 | 61.92 | 66.93 | 0.248 | 48.12 |
| SEM | | | 107.39 | 0.68 | 0.25 | 0.33 | 0.42 | 0.46 | 0.71 | 1.27 | 1.00 | 0.94 | 0.014 | 1.13 |
| P-value | | | 0.955 | 0.172 | 0.492 | 0.92 | 0.201 | 0.92 | 0.787 | 0.864 | 0.490 | 0.364 | 0.233 | 0.692 |

[a–b] *Means in a column with different superscripts differ significantly (P≤0.05).* [1] *Pooled SEM*

In the studies of Kerr and Kidd (1999) suggest that abdominal fat% was unaffected when dietary CP levels were reduced by 2%. Moreover, decreasing dietary CP from 22.5 to 15.3% has no significant influence on liver, total body protein content and abdominal fat, conversely, total body fat content was increased significantly (P≤0.01) by 51% (Aletor *et al.*, 2000). Also, Abd El-Gawad *et al.* (2004) and Nawaz *et al.* (2006) found that there were no significant differences in carcass characteristics or abdominal fat weight in broilers fed low CP diets. In addition, no differences in carcass weight, breast meat weight and abdominal fat (Hidalgo *et al.,*2004 and Kamran *et al.,*2008) thigh weight, liver and heart weights (Kamran *et al.*, 2008) in broilers fed low CP diets with a constant ME:CP ratio. But, Dozier and Moran (2002) reported that feeding broiler chickens diet formulated to contain suboptimum level of CP and ME impaired the amount and yields of carcass parts. Si *et al.* (2004) reported significant increase in abdominal fat with decrease in the dietary CP level. Carcass composition becomes inferior in broilers fed diets in which CP has been lowered by more than 3%, even when all known nutrient requirements are met (Sterling *et al.*, 2005 and Waldroup *et al.*, 2005).

Concerning AA addition, the results indicated that no significant differences due to AA on slaughter parameters%, except, half breast, carcass weight after evisceration and dressing which were significantly (P≤0.05) affected (Table 6). It can be concluded that, chicks fed diet containing adequate amounts of AA had higher values of half breast, carcass weight after evisceration and dressing%, while, chicks fed inadequate amounts of AA had lower values at the end of experiment. The carcass composition responses were similar to that of LBW and LBWG, which emphasis this conclusion, no obvious increase in abdominal fat content was recorded. However, Swennen *et al.* (2006) as a consequence of enhanced de novo lipogenesis in the liver of birds fed the low CP diets, birds are expected to have increased liver weights and deposit more abdominal fat due to increased ME:CP ratio. In this regard, Lipstein and Bornstein (1975) showed that the effect of dietary CP on carcass composition is basically an AA effect. Similarly increasing dietary Met. levels have shown to reduce abdominal fat content (Mendonca and

Jensen, 1989) and increase breast meat yield (Hilckling *et al.*, 1990). Also, Holsheimer and Veerkamp (1992) demonstrated that dietary Lys. content resulted in an increase of breast meat yield. These results are in harmony with those obtained by Waldroup *et al.* (2006) who reported that reduction of more than 0.10% Met from current values have shown to negatively affected breast meat yield in a linear manner. Similar results were reported by Zaman *et al.* (2008) who concluded that lowering the dietary CP with EAA supplementation resulted in breast meat yield similar to those birds fed on control diet. It seems that low CP slightly increased the digestibility of AA required for carcass formation.

***4- Tibia parameters***: Data presented in Table (6) showed that neither level of CP, AA and enzyme addition nor interaction between level of CP, AA and enzyme addition had any significant effect on tibia weight and ash% at the end of the finishing period. This finding disagreed with Ragab *et al.* (2012) who found that the highest ash ($P\leq0.01$) value was observed for chicks fed diets containing optimal CP level, while, those fed diet containing suboptimal level of CP had significantly lower value.

***5- Chemical composition of broiler meat:*** Level of CP, AA and interaction between level of CP, AA and enzyme addition (experimental treatments) had insignificantly affected chemical composition of broiler meat (Table 7). Inclusion of enzyme in broiler diet at 0.1% caused a significant ($P\leq0.01$) increase in moisture% of broiler meat. Carcass part significantly influenced ($P\leq0.01$) chemical composition of broiler meat except, ash and NFE% which was insignificantly affected. Rear part had higher fat% than the breast part (5.89 vs. 0.82%). However, breast part had higher moisture and protein% (consequently lower fat%) than rear part (Table 7).

These results agree with the those of Neto *et al.* (2000) who reported that chicks fed low CP diets supplemented with EAAs had similar total carcass protein contents as those fed a 24% dietary CP. By contrast, Zaman *et al.* (2008) reported that increases in dietary CP resulted in increased dry matter, CP and fat contents of carcass. Also, Si *et al.* (2001) demonstrated that the CP and AA status of a diet influences the carcass composition of broilers and decrease in dietary CP causes a decrease in carcass protein and an increase in carcass fat content. Moreover, Ragab *et al.* (2012) reported that level of CP significantly affected protein, fat and ash of chicks meat. The highest fat% (the lowest protein and ash%) were observed for chicks fed diets containing sub-optimal CP level, while those fed diet containing recommended level of CP had lower fat% (the highest protein and ash%), however, insignificantly affected moisture and NFE% (Ragab *et al.*, 2012). The inverse relationship found between moisture% and fat% values obtained in the present study is in agreement with that reported by Marks (1993) and Ragab *et al.* (2012) in chemical composition of Japanese quail meat and broiler chicks.

Generally, the discrepancies in responses often observed in the researches in chicks fed protein AA supplemented diets can be partly attributed to other factors. Such as the degree of CP restriction, the inclusion of the CP and ME contributions of the AA supplements, diets composition, the class (or strain) and age of the chickens used and rearing conditions, whether or AA imbalances. Also perhaps differences in experimental designs could be responsible for the variations observed.

***6- Economical efficiency (EEf):***

Results in Table 8 show that EEf values during the period from 6 to 41 days of age was improved of chicks fed diet containing R-2% supplemented with the requirements of Met. and Ly. plus 0.10% of Avizyme had the best economical and relative efficiency values being 0.2928 and 129.92%, respectively followed by chicks fed control diet -2% CP supplemented with the requirements of Met. and Ly. plus 0.00% of Avizyme (0.2617 and 116.11%, respectively) then chicks fed control diet supplemented with 0.1% Avizyme (0.2345 and 104.04%, respectively) as compared with those fed the control diet and other treatments. Whereas, chicks fed control diet -2% CP inadequate amounts of AA plus 0.00% of Avizyme had the lowest corresponding values, being 0.1811 and 80.36%, respectively). The relative efficiency varied between 80.36%, to 129.92%, which is of minor importance relative to other factors of production. In this respect, Vieira *et al.* (2016) stated that feeds formulated with reduced CP are less expensive. The results of this study are disagreement with those of Abd El-Gawad *et al.* (2004) and Ragab *et al.* (2012) who found that broiler chicks fed diets containing either optimum level of CP or adding probiotics, EEf values were increased.

**Table (7): Effect of dietary protein levels with or without synthetic amino acids and enzyme supplementation on chemical composition of broiler meat %.**

| Item | | | Moisture | Protein | Fat | Ash | NFE |
|---|---|---|---|---|---|---|---|
| Level of crude protein (CP)% | | | | | | | |
| Recommended (R) | | | 74.52 | 18.78 | 3.18 | 2.29 | 1.23 |
| R-2 | | | 74.48 | 18.71 | 3.53 | 2.05 | 1.23 |
| SEM$^1$ | | | 0.26 | 0.63 | 0.75 | 0.17 | 0.01 |
| P-value | | | 0.908 | 0.942 | 0.745 | 0.322 | 0.938 |
| Amino acid addition (AA) | | | | | | | |
| Adequate (A.) | | | 74.49 | 18.89 | 3.08 | 2.31 | 1.23 |
| Inadequate (Ina.) | | | 74.51 | 18.60 | 3.63 | 2.03 | 1.23 |
| SEM | | | 0.26 | 0.63 | 0.75 | 0.17 | 0.01 |
| P-value | | | 0.951 | 0.747 | 0.612 | 0.251 | 0.817 |
| Enzyme (En.)% | | | | | | | |
| 0.00 | | | 73.95$^b$ | 18.75 | 3.72 | 2.35 | 1.23 |
| 0.10 | | | 75.05$^a$ | 18.74 | 2.99 | 1.99 | 1.23 |
| SEM | | | 0.26 | 0.63 | 0.75 | 0.17 | 0.01 |
| P-value | | | 0.006 | 0.988 | 0.499 | 0.132 | 0.817 |
| Carcasse part | | | | | | | |
| Breast | | | 75.09$^a$ | 20.79$^a$ | 0.82$^b$ | 2.07 | 1.24 |
| Rear | | | 73.91$^b$ | 16.70$^b$ | 5.89$^a$ | 2.28 | 1.22 |
| SEM | | | 0.30 | 0.21 | 0.20 | 0.19 | 0.01 |
| P-value | | | 0.009 | <0.001 | <0.001 | 0.438 | 0.070 |
| CP% × AA × En.% (treatments) | | | | | | | |
| R | A. | 0.00 | 72.65 | 19.38 | 3.97 | 2.77 | 1.23 |
| | | 0.10 | 75.15 | 18.49 | 2.38 | 2.75 | 1.24 |
| | Ina. | 0.00 | 75.12 | 18.32 | 3.27 | 2.05 | 1.23 |
| | | 0.10 | 75.16 | 18.93 | 3.10 | 1.59 | 1.22 |
| R-2 | A. | 0.00 | 74.54 | 19.04 | 3.18 | 2.00 | 1.23 |
| | | 0.10 | 75.61 | 18.65 | 2.80 | 1.72 | 1.22 |
| | Ina. | 0.00 | 73.46 | 18.27 | 4.44 | 2.60 | 1.23 |
| | | 0.10 | 74.30 | 18.89 | 3.69 | 1.89 | 1.23 |
| SEM | | | 0.52 | 1.26 | 1.49 | 0.33 | 0.01 |
| P-value | | | 0.138 | 0.891 | 0.674 | 0.991 | 0.321 |

$^{a–b}$ *Means in a column with different superscripts differ significantly (P≤0.05).* $^1$ *Pooled SEM*

**Table (8): Effect of dietary protein levels with or without synthetic amino acids and enzyme supplementation on economical efficiency (EEf) of broiler chickens.**

| Item | Level of crude protein % | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Recommended | | | | Recommended -2 | | | |
| Amino acid addition | Accurate | | Inaccurate | | Accurate | | Inaccurate | |
| Enzyme % | 0.00 | 0.10 | 0.00 | 0.10 | 0.00 | 0.10 | 0.00 | 0.10 |
| $a_1$ | 0.2043 | 0.2093 | 0.2092 | 0.2070 | 0.2094 | 0.2062 | 0.2081 | 0.2076 |
| $b_1$ | 622.93 | 627.93 | 617.11 | 622.11 | 593.55 | 598.55 | 584.35 | 589.35 |
| $a_1$ x $b_1$=$c_1$ | 127.26 | 131.43 | 129.10 | 128.78 | 124.29 | 123.42 | 121.60 | 122.35 |
| $a_2$ | 1.0047 | 1.0260 | 1.1344 | 1.0681 | 0.9733 | 1.1326 | 1.0929 | 1.0224 |
| $b_2$ | 617.29 | 622.29 | 611.49 | 616.49 | 589.30 | 594.30 | 580.10 | 585.10 |
| $a_2$ x $b_2$=$c_2$ | 620.19 | 638.47 | 693.67 | 658.47 | 573.57 | 673.10 | 633.99 | 598.21 |
| $a_3$ | 2.5127 | 2.5759 | 2.6403 | 2.4467 | 2.3628 | 2.7686 | 2.6650 | 2.4702 |
| $b_3$ | 596.64 | 601.64 | 595.08 | 600.08 | 567.69 | 572.69 | 562.83 | 567.83 |
| $a_3$ x $b_3$=$c_3$ | 1499.2 | 1549.8 | 1571.2 | 1468.2 | 1341.3 | 1585.5 | 1499.9 | 1402.7 |
| ($c_1$+$c_2$+$c_3$)=$c_{total}$ | 2246.6 | 2319.7 | 2394.0 | 2255.5 | 2039.2 | 2382.1 | 2255.5 | 2123.2 |
| D | 1497.8 | 1497.8 | 1497.8 | 1497.8 | 1497.8 | 1497.8 | 1497.8 | 1497.8 |
| e = $c_{total}$ + d | 3744.4 | 3817.4 | 3891.7 | 3753.2 | 3536.9 | 3879.8 | 3753.3 | 3621.0 |
| F | 1.9949 | 2.0489 | 2.0204 | 1.9509 | 1.9402 | 2.1808 | 1.9274 | 1.9261 |
| G | 2300.0 | 2300.0 | 2300.0 | 2300.0 | 2300.0 | 2300.0 | 2300.0 | 2300.0 |
| f x g=h | 4588.3 | 4712.5 | 4646.9 | 4487.1 | 4462.5 | 5015.8 | 4433.0 | 4430.0 |
| h - e =i | 843.88 | 895.05 | 755.20 | 733.85 | 925.51 | 1136.0 | 679.73 | 809.07 |
| EEf = i / e | 0.2254 | 0.2345 | 0.1941 | 0.1955 | 0.2617 | 0.2928 | 0.1811 | 0.2234 |
| R | 100.00 | 104.04 | 86.10 | 86.757 | 116.11 | 129.92 | 80.36 | 99.142 |

*$a_1$, $a_2$ and $a_3$ ………………..average feed intake (Kg/bird) during the periods of starter, grower and finisher, respectively.*

*$b_1$, $b_2$ and $b_3$ ……………… price / Kg feed (P.T.) during the periods of starter, grower and finisher, respectively (based on average local market price of diets during the experimental time).*

*$c_1$, $c_2$ and $c_3$ ………………..feed cost (P.T.) during the periods of starter, grower and finisher, respectively.*

*Total feed cost (P.T.) = $c_{total}$ = ($c_1$+$c_2$+$c_3$)*

*d ………… other costs (including chick pries and other management costs (based on feed cost = 60% of total cost))*

*Total cost = $c_{total}$ + d = e*

*Average LBWG (Kg/ bird) f*

*Price / Kg live weight (P.T.) g………………(according to the local market price at the experimental time).*

*Total revenue (P.T.) = f x g = h*

*Net revenue (P.T.) = h - e =i*

*Economical efficiency = (i / e) ………………..(net revenue per unit feed cost).*

*Relative efficiency r……………..(assuming that economical efficiency of the control group (1) equals 100).*

In general, the optimum performance in the present experiment was obtained by broilers fed diets formulated to contain 21.38, 19.63, and 18.08% CP supplemented with Met. and Ly. for starter, grower and finisher periods, respectively.

## CONCLUSIONS

The results of the present study indicated that Ross broiler chicks fed low CP diets supplemented with EAAs or Avizyme maintain the same performance as that obtained from chicks fed diets containing recommended level of CP. On the other hand, it can be concluded that CP can be reduced from the recommended level by 2% and supplement these diets with either EAAs or Avizyme without affecting performance. Besides, using such diets reduces feed cost.

2-Formulation based on bird amino acid requirements rather than CP can achieve optimal performance of broiler chickens and minimize N excretion by simply reducing total dietary N intake.

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# مستويات بروتين العليقة مع إضافة أو عدم إضافة الأحماض الأمينية المصنعة والإنزيم علي أداء بداري التسمين

**رمضان محمد سلامة إمام**

**قسم إنتاج الدواجن - كلية الزراعة - جامعة الفيوم – مصر**

تم إجراء التجربة لدراسة تأثير إضافة مستويين من البروتين (المستوي الموصي به (ص)، ص -٢ %) مع إضافة أو عدم إضافة الاحتياجات من المثيونين والليسين وكل منها مع مستويين من الافيزيم ١٥٠٠ (صفر و ٠.١%) في تجربة عامليه ٢ x ٢x ٢( للحصول علي ٨ معاملات غذائية) علي أداء بداي التسمين (سلالة روس). غذيت الكتاكيت عمر يوم ولمدة خمسة أيام علي عليقة المقارنة وفي اليوم السادس تم توزيع ١٩٢ كتكوت غير مجنس بصورة عشوائية إلى ٨ معاملات (٢٤ طائر/معاملة) كل معاملة مقسمة إلي ثلاثة مكررات (٨ طائر/مكرر).

وتم تلخيص النتائج المتحصل عليها كما يلي :

الأداء الإنتاجي: لم يكن لمستوي البروتين أي تأثيرات معنوية علي وزن الجسم الحي، الزيادة في وزن الجسم، كمية الغذاء المستهلكة، كفاءة تحويل الغذاء والأداء الإنتاجي خلال فترة الناهي (٢٥-٤١ يوم) والفترة الجمالية للتجربة (٦–٤١ يوم). هناك تأثير معنوي لمستوي البروتين علي كمية الغذاء المأكول، كفاءة تحويل البروتين خلال الفترة من ٦-٤١ يوم. الكتاكيت المغذاه علي عليقة تحوي المستوي الموصي به من البروتين كانت الأسوأ معنويا في كفاءة تحويل البروتين خلال نفس الفترة. الكتاكيت المغذاه علي عليقة مضاف إليها كمية كافية من الأحماض الامينية كانت الاعلي معنويا في معدل الأداء الإنتاجي خلال الفترة من ٦-٤١ يوم، بينما سجلت أقل قيم معنوية لكفاءة تحويل الغذاء وكفاءة تحويل البروتين خلال نفس الفترة. بينما لم يكن هناك أي تأثير معنوي لمستوي الأحماض الامينية علي وزن الجسم الحي، الزيادة في وزن الجسم، كمية الغذاء المستهلكة خلال الفترة الجمالية للتجربة. لم يكن هناك أي تأثير معنوي لإضافة الإنزيم ولا للتداخل بين مستوي البروتين، وإضافة الأحماض الامينية والإنزيم علي وزن الجسم الحي، الزيادة في وزن الجسم، كفاءة تحويل الغذاء، كفاءة تحويل البروتين، ومعدل الأداء الإنتاجي خلال الفترة من ٦-٤١ يوم.

قياسات الدم: لم يكن لمستوي البروتين أو الأحماض الامينية أي تأثيرات معنوية علي قياسات الدم فيما عدا عدد كرات الدم الحمراء والتي تأثرت معنوياً. الكتاكيت المغذاه علي عليقة تحوي المستوي الموصي به من البروتين -٢% أو المغذاة علي العليقة غير المضبوطة بالنسبة للأحماض الامينية كانت الاعلي معنويا في عدد كرات الدم الحمراء. لم يكن لإضافة الإنزيم أو للتداخل بين المعاملات التجريبية أي تأثيرات معنوية علي قياسات الدم فيما عدا تأثير التداخل علي (segment) neutrophils% والتي تأثرت معنوياً.

قياسات الذبيحة والتبيا: لم يكن هناك أي تأثير معنوي لأي من مستوي البروتين ولإضافة الإنزيم أو للتداخل بين مستوي البروتين ولإضافة الأحماض الامينية والإنزيم علي قياسات الذبيحة% أو وزن التبيا أو نسبة الرماد بها في نهاية الفترة التجريبية.

التحليل الكيمائي للحم: لم يؤثر معنوياً مستوي البروتين، الأحماض الامينية والتداخل بين مستوي البروتين ولإضافة الأحماض الامينية والإنزيم علي التحليل الكيمائي للحم.

الكفاءة الاقتصادية: كانت أحسن قيم للكفاءة الاقتصادية والنسبية للكتاكيت المغذاه علي عليقة تحوي المستوي الموصي به من البروتين -٢% مضاف إليها الاحتياجات من المثيونين والليسين مع 0.10 % أفيزيم خلال الفترة من ٦-٤١ يوم من العمر، مقارنة بتلك التي غذيت على عليقة المقارنة والمعاملات الاخري.

يمكن استنتاج انه يمكن خفض نسبة البروتين ٢% عن المستوي الموصي به مع إضافة الاحتياجات من المثيونين والليسين مع 0.10 % أفيزيم بدون حدوث آي تأثير علي الأداء. بالإضافة إلى أن استخدام هذه العلائق يقلل من تكلفة الغذاء والتلوث بالنيتروجين.