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## Fortify low protein diet with supplemented essential amino acids on Performance , carcass characteristics, and whole-body female broiler chickens

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### ABSTRACT

*Effect of low protein diet supplemented with crystalline amino acids on broiler chickens was allocated. 96 female commercial broiler chicks were chosen at 10 days of age based on body weight and used for 21 days of this experiment. This study was conducted in completely randomized design with 20, 18, and 16% crude protein supplemented with crystalline amino acids. Treatments received the same basal diets (corn-soybean meal). The experimental units were allocated to 3 dietary treatments with 4 replicates and 8 chicks per replicate. Birds fed diets in which crude protein was in a stepwise manner from 20 to 16%. Ileal digestible quantities of all essential amino acids were almost equal in the diets and maintained at above NRC (1994) recommendations. Body weight gain, feed intake, feed conversion ratio were measured at the end experiment. At 31 days of age 8 birds from each treatment with a body weight close to the replicate mean were randomly taken for carcass weight, and abdominal fat, liver, gizzard, as percents of carcass weight. Significant effect ( $P < 0.05$ ), was observed among percentages of crude protein diets. Fortifying a low crude protein diet with essential amino acid resulted in more increase in body weight gain, feed intake and favorable decrease in feed conversion ratio. The relative weight of abdominal fat increased significantly ( $P < 0.05$ ) in low crude protein diets. The amount of excreted nitrogen was reduced ( $P < 0.05$ ), with decrease crude protein levels. This research indicated that dietary crude protein can be decrease until 16% (supplemented with crystalline amino acids).*

**Key words:** broiler chick, low protein diet, crystalline amino acid.

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### INTRODUCTION

Dietary protein is one of the most important factors affecting poultry production. Our information about feeding of amino acids has been progressed in two or three recent decades. This progress is undoubtedly due to the production of synthetic amino acids. Effects of supplementary amino acid in reducing protein intake, feed consumption and environmental pollution via reduce pollutants excretion (ammonia nitrogen) by farm animals, is main reasons for using of these supplements. With development of poultry industry, the problem of nitrogen excretion has been one of the biggest problems in developed and developing countries[1]. Lowering dietary protein levels and use of synthetic amino acids, while reduce cost of diet, will reduce environmental pollution of nitrogen. It is important to know which level of dietary protein is suitable for broiler performance. One problem with diets containing low levels of protein apart from its effect on performance of broiler[2,3]. In this study, by using low crude protein (CP) diets supplemented with amino acids, effect these diets on performance, carcass characteristics, whole-body composition, abdominal fat accumulation and reducing nitrogen pollution of female broiler in growing period were investigated.

## MATERIALS AND METHODS

**Birds and Housing:** This experiment was performed on 10-day-old female Ross 308 broiler chicks housed in unit testing group, on the litter. The experimental birds were given *ad libitum* access to water and diet. The ambient temperature was gradually decreased from 32 to 24°C over the period of 1 to 31 d of age. The birds were exposed to a 24 h light. Chicken vaccination was carried out according to schedule.

**Diet Formulation:** Corn, soybean meal and fish meal were sampled before diet formulation to determine crude protein as Kjeldahl nitrogen  $\times 6.25$ , moisture, metabolized energy[4]. To calculate the electrolyte balance, percentage of electrolytes in feed ingredients were extracted from Feedstuffs Table [5]. The dietary electrolyte balance was maintained at 250 mEq/kg in all dietary treatments by using of Calcium chloride, Calcium sulfate, and Sodium bicarbonate. All diets were formulated to be isoenergetic (3150 kcal/kg of AMEn). The concentration of dietary Calcium (Ca), available phosphorus (P), Sodium(Na) and Potassium(K) was maintained equal in all treatments (Table 1). Diets were formulated base on computer software, UFFDA(Table 1). Dietary amino acids were adjusted in higher levels than recommendations of NRC[6], and according to the rearing guide of Ross 308 strain. Dietary essential amino acids (threonine, tryptophan, arginine, valine and isoleucine) were balanced based on standardized ileal digestibility. Experimental design was a completely randomized design with 20, 18 and 16% crude protein(CP) levels on 96 broilers allocated in 3 treatments each with 4 replicated of 8 birds.

**Performance, Carcass Characteristics and Whole-Body Analyses:** At the end of grower period(d31), feed intake and body weight gain of chickens were measured to determine feed conversion ratio. At the end of the experimental period(d31), two birds per replicate(with a body weight close to the replicate mean), were slaughtered. After slaughter heart, liver, gizzard, abdominal fat, breast and thigh relative percentage of total carcass weight were determined. Also thigh and breast muscles were isolated from the bone and four uniform samples from each treatment were elected to measure carcass protein, fat and dry matter(%). At the end of the experimental period(d 31) one birds per replicate(with a body weight close to the replicate mean), were slaughtered by cervical dislocation for determination of whole-body composition according to procedures described by Barker and Sell [7]. Two birds from each treatment At 31 days of age were selected and kept in individual cages and excreta were collected and lyophilized, and were sent to the laboratory for determining nitrogen by Kjeldahl method to determine the nitrogen excretion.

**Statistical Analysis:** Data were analyzed using the general linear model ANOVA (SAS Institute. [8].) in a completely randomized design. Means were compared by using Duncan's multiple range test. In all cases, significance coefficient was set at  $P < 0.05$ .

## RESULTS AND DISSCUTION

Reducing of dietary protein up to 16% in diets supplemented with synthetic essential amino acids in growing period affected body weight gain, feed intake and feed conversion(Table 2). The highest body weight gain and food intake achieved in 16% CP diets. The best feed conversion ratio observed in the low-protein diets (18%). In growing period, since in this experiment balance among certain amino acids, balance between nutritionally essential and nonessential amino acids,the more feed intake, and was used of the optimum dietary electrolyte balance (250 mEq/kg), reducing dietary protein level supplement with essential amino acids up to 16% in female broiler chickens, had positive effect on birds performance. The results of this experiment is in agreement with other studies[9,10,11].

Effects of different levels of dietary protein supplemented with essential amino acids on the gizzard, heart, liver, breast, thigh and abdominal fat relative percentage of total carcass weight are given in Table 2. The relative weight of abdominal fat increased significantly in low CP diets( $P < 0.05$ ) and highest amount of abdominal fat obtained in diet with 16% CP, but relative weight of heart, liver, breast and thigh were not significant difference( $P > 0.05$ ) among the dietary treatments. Maximum relative weight of breast and thigh, respectively, obtained in 16% CP supplemented with amino acids and 20% CP diets. This result was in agreement with others researchs[1,11,12]. One of the mechanisms involved in increasing carcass fatness by feeding low CP diets is the lower heat increment involved in deamination and transamination of surplus amino acids to other metabolites and finally uric acid. Breast percentage of total carcass weight of chicks fed low CP supplemented diets was increased compared to the chicks that fed control diet(Table 2). Darsi *et al.*[13] observed similarly results. Thigh relative percentage of total carcass weight decreased linearly that was not significant and are in agreement with Namroud *et al.* [14], Darsi *et al.*[13].

We found an increase in whole body fat and fat content of breast muscle when dietary CP levels were reduced but difference was not significant ( $P > 0.05$ ), while fat content of drumstick + thigh muscle increased significantly ( $P < 0.05$ ) for birds fed low CP diets (Table 2). Carcass dry matter was increased significantly and protein content of total carcass decreased by reducing dietary CP level (Table 2). Aletor *et al.* [1], Bregendahl *et al.* [2] observed same result. In chicks fed low CP diets fat content of total carcass increased that our results about fat deposition in different part of carcass are in complete agreement with others experiments [13-15]. Dry matter of breast and drumstick + thigh muscle affected by dietary treatment in linear manner that was in agreement with Darsi *et al.* [13] observation. Breast and drumstick + thigh muscle protein not affected by dietary treatment that was in agreement with Horniakova and Abas and Darsi *et al.* [13,16]. Fat content of breast and drumstick + thigh muscle was lower for birds fed low-CP diet (Table 2) and this result was obtained by others researches [16,17]. Lipase phosphorylation begin the process of fat breakdown (lipolyse). Produced fatty acids are removed from fat cells as oxidative fuels for other tissues.

With reduction of crude protein in diets supplemented with essential amino acids, N excretion significantly ( $P < 0.05$ ) reduced (Table 2). For every one percent decrease of dietary CP, there was about 0.3% less nitrogen excreted in the excreta. As a result, a 13% reduction of nitrogen excretion was observed without affecting growth performance, which is in close agreement with Schutte [18]. He concluded that in broiler chick diets based on corn-soybean meal with adequate lysine and methionine, the protein level could be reduced 1.5 to 2% and the nitrogen excretion would be reduced by 15-20%. Result in our study, is in agreement with studies of Namroud *et al.* [14].

**Table 1) Ingredient and Nutritional composition of experimental diets (%)**

Ingredient	Treatments <sup>1</sup>			Item	Diet 1	Diet 2	Diet 3
	Diet 1	Diet 2	Diet 3				
Yellow corn	57.66	63.38	68.6	AMEn(Kcal.Kg)	3150	3150	3150
Soybean meal	30.54	24.72	18.87	Crude protein,%	20	18	16
Anchovy meal	4	4	4	Ca (%)	0.9	0.9	0.9
Soybean oil	4.52	4	3.68	Available P (%)	0.45	0.45	0.45
Di calcium phosphate	1.25	1.28	1.30	Chlorine (%)	0.21	0.16	0.2
Sodium bicarbonate	0.0	0.3	0.65	Potassium (%)	0.81	0.72	0.62
Calcium chloride	0.15	0.1	0.1	DEB(mEq/kg)	250	250	250
Calcium sulfate	0.0	0.06	0.0	Na (%)	0.1	0.14	0.24
Limestone	0.89	0.87	0.95	Solfor (%)	0.21	0.20	0.17
Sodium chloride	0.1	0.0	0.01	Total and standardized ileal digestible amino acids <sup>4</sup>			
Mineral premix <sup>2</sup>	0.25	0.25	0.25	L-Lys (%)	1.1	1.1	1.1
Vitamin premix <sup>3</sup>	0.25	0.25	0.25	DL-Met (%)	0.56	0.61	0.63
DL-Methionine	0.26	0.31	0.35	Met+Cys (%)	0.84	0.84	0.84
L-Lys Hcl	0.13	0.30	0.47	L-Thr (%)	0.75	0.73	0.73
L-Thr	0.0	0.07	0.15	L-Ile (%)	0.88	0.76	0.75
L-Arg	0.0	0.07	0.22	L-Arg (%)	1.22	1.14	1.14
L-Trp	0.0	0.0	0.03	L-Trp (%)	0.2	0.18	0.18
L-Ile	0.0	0.0	0.08	L-Leu (%)	1.57	1.46	1.34
L-Val	0.0	0.0	0.04	L-Val (%)	0.89	0.81	0.75

<sup>1</sup>Treatments: 1) 20% CP, 2) 18% CP + 100% EAA, 3) 16% CP + 100% EAA.

<sup>2</sup>mineral premix Added (mg/kg) to the diet: Manganese, 110.60; Zinc, 110.40; Iron (ferrous sulfate), 50; Copper, 8.30; Selenium (sodium selenite), 0.30; I, 1.08; Co, 0.1; Mo, 0.05.

<sup>3</sup>Vitamin premix added (per kg of diet): A (retinyl acetate), 11,023 IU; D (cholecalciferol), 118 IU; E (DL- $\alpha$ -tocopheryl acetate), 23.54 IU; K (menadione), 1.47 mg; B<sub>12</sub>, 0.0151 mg; riboflavin, 5.895 mg; niacin, 42.93 mg; D-Pantothenic acid, 12.11 mg; Choline, 477.7 mg; Folic acid, 1.15 mg; Pyridoxine, 4.17 mg; Thiamin, 1.23 mg and D-Biotin, 0.075 mg.

<sup>4</sup>Includes amino acids from intact protein and crystalline sources. Crystalline amino acids were assumed 100% truly digestible.

**Table 2) Effect of dietary CP level supplementation with excess essential amino acids(EAA) on performance, visceral organs, abdominal fat, breast, thigh<sup>1</sup>, Nitrogen excretion<sup>2</sup>, and Whole-body, breast and thigh composition<sup>3</sup>(% dry matter) and Nitrogen excretion during 10 to 31 d of age**

Assy Item	Levels of Crude Protein			Significance level(P< 0.05)	
	20% CP	18% CP + EAA <sup>4</sup>	16% CP + EAA		
BWG <sup>5</sup> (g) ± SE <sup>6</sup>	586 <sup>b</sup> ± 51	938 <sup>a</sup> ± 41	998 <sup>a</sup> ± 34		
FI(g) ± SE	1016 <sup>c</sup> ± 50	1427 <sup>b</sup> ± 53	1550 <sup>a</sup> ± 42		
FCR ± SE	1.73 <sup>a</sup> ± 0.12	1.52 <sup>b</sup> ± 0.05	1.55 <sup>b</sup> ± 0.03		
Gizzard <sup>7</sup> ± SE	3.3 <sup>a</sup> ± 0.2	2.9 <sup>b</sup> ± 0.1	2.6 <sup>b</sup> ± 0.1		
Liver ± SE	2.1 ± 0.1	2.3 ± 0.1	2.4 ± 0.1	NS	
Heart ± SE	0.5 ± 0.1	0.6 ± 0.2	0.5 ± 0.1	NS	
Abdominal Fat ± SE	2.3 <sup>b</sup> ± 0.3	2.5 <sup>a</sup> ± 0.2	2.7 <sup>a</sup> ± 0.3		
Breast ± SE	15.3 ± 0.4	15.5 ± 0.3	15.8 ± 0.2	NS	
Thigh ± SE	14.7 ± 0.3	14.5 ± 0.3	14.2 ± 0.2	NS	
Whole body composition <sup>8</sup> (dry matter)	DM (%) ± SE	36 <sup>b</sup> ± 1.1	36.1 <sup>b</sup> ± 0.7	36.1 <sup>b</sup> ± 0.7	
	Fat (%) ± SE	13.7 ± 0.6	14.0 ± 1.1	14.1 ± 0.6	NS
	CP (%) ± SE	20.2 <sup>a</sup> ± 0.2	19.2 <sup>b</sup> ± 0.3	18.7 <sup>c</sup> ± 0.4	
EN <sup>9</sup> (%) ± SE	75.8 <sup>a</sup> ± 0.1	54.8 <sup>b</sup> ± 0.8	50.1 <sup>b</sup> ± 2.3		
Breast <sup>10</sup>	DM (%) ± SE	27.2 ± 0.3	27.3 ± 0.2	27.3 ± 0.3	NS
	Fat (%) ± SE	3.2 ± 0.3	3.3 ± 0.2	3.5 ± 0.2	NS
	CP (%) ± SE	22.5 ± 0.2	22.4 ± 0.2	22.2 ± 0.1	NS
Dru+Thi <sup>11</sup>	DM (%) ± SE	29.5 ± 0.2	30.4 ± 0.6	30.1 ± 0.5	NS
	Fat (%) ± SE	7.1 <sup>c</sup> ± 0.1	8.3 <sup>b</sup> ± 0.1	11.3 <sup>a</sup> ± 0.1	
	CP (%) ± SE	18.5 ± 0.2	18.4 ± 0.1	18.2 ± 0.1	NS

<sup>a-d</sup> Values within columns without a common letter differ significantly

<sup>1</sup>Results are the means of 4 replicates (8 chicks per replicate) per treatment.

<sup>2</sup>Results are the means of 2 replicates (2 chicks per treatment).

<sup>3</sup>Results are the means of 4 replicates (1 chicks per replicate) per treatment.

<sup>4</sup>EAA = Amino acids status: 100% total essential amino acids(L-Thr, L-Arg, L-Trp, L-Ile, and L-Val (%)) above NRC (1994).

<sup>5</sup>BWG = Body Weight gain, FI = Feed intake, and FCR = feed conversion ration. <sup>6</sup>SE = Standard error.

<sup>7</sup>Relative weight Percentage of visceral organs, abdominal fat, breast, and thigh

<sup>8</sup>CP = crude protein., DM = dry matter., and Fat(% dry matter).

<sup>9</sup>EN = Excretion Nitrogen (% dry matter)

<sup>10</sup>Breast = chemical composition (% dry matter).

<sup>11</sup>Dru+Thi = drumstick + thigh (% dry matter)

## CONCLUSION

The results of this study suggested that low protein(18 and 16%) amino acids-supplemented diets have been balanced based on standardized ileal digestibility and appropriate electrolyte balance(250 mEq/Kg Diet) had no adverse effect on female broiler performance in 10 to 31 days of age, moreover decreased N excretion.

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