Effect of Diets Formulated on the Basis of Four Critical Essential Amino Acids on Crude Protein Requirement, Carcass Quality and Haematological Parameters of Broiler Finisher Chickens Reared under Tropical Environment

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Key words: Broiler, essential amino acids, crude protein, carcass quality, haematology, tropical environment

Abstract: A study was conducted in a completely randomized design to evaluate the effect of diets formulated on the basis of four critical essential amino acids (lysine, methionine, tryptophan and threonine) on the crude protein requirement of broiler finisher chickens (28-56 days) reared under tropical environment. One hundred and eighty chickens were used in this experiment. There were four experimental diets each with three replicates (15 birds per replicate). The experimental diets were formulated in a gradual crude protein increase from 18-21 by 1% interval. Diet 1-4 contained 18-21% dietary crude protein respectively. All the diets were formulated to have an additional 10% for the four critical essential amino acids. The performance of chickens fed 19% CP was similar to chickens fed 20 and 21% CP diets in terms of final weight, weight gain and feed conversion ratio. Feeding 18% CP with essential amino acids diet resulted in significantly ($p<0.05$) lower final weight, weight gain, average daily weight gain and poor FCR than those fed diets higher crude protein diets. Generally, it was observed that chickens fed 19, 20 and 21% CP supplemented with balanced essential amino acids were statistically similar in terms of the carcass weight, dressing percentage, thighs, drumsticks, heart, lung and back weights compared to the chickens fed 18% CP supplemented with balanced essential amino acids. It can be concluded that crude protein requirement of broiler finisher chickens (28-56 days) can be reduced to 19% with essential amino acids supplementation without having any adverse effect on growth, carcass quality and haematological parameters of broiler finisher chickens reared under the tropical environment.

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INTRODUCTION

It is well documented that nutrient requirements of poultry is significantly affected by seasons of rearing and ambient temperature. In the cool, hot and humid tropics, genetic potential of chickens irrespective of the breeds might be affected adversely because of environmental constraints. Protein is generally considered as one of the major cost components of the poultry diets[1]. Therefore, placing more emphasis on crude protein requirement in broilers diet formulation becomes imperative to maximize the performance of broiler chickens with the economic considerations[2]. Adding purified amino acids or amino acid precursors has been known for >50 years to allow for reduced levels of intact proteins to provide adequate levels of essential and nonessential amino acids (CP)[3]. NRC[4] stated that poultry requires a specific amount and balance of Essential Amino Acids (EAA) and Non Essential Amino Acids (NEAA), rather than the crude per se to support growth, immune system and the whole body composition of broiler chickens. Some achievements has been made to lower the Crude Protein (CP) content of broiler diets by providing all those amino acids considered to be critical. However, some results from several researchers still showed that reduction of CP has some negative effects on performance and meat quality. This failure was pointed out even with providing all requirements for those amino acids considered as essential. Failure to obtain optimum result in performance may be attributed to one or more of the following factors: lack of nitrogen pool to synthesize Non Essential Amino Acids (NEAA); insufficiency of body capacity to meet all NEAA requirements, especially Gly, Ser, Pro and Glu, decreased level of potassium or altered ionic balance and imbalances among certain amino acids such as arginine to lysine, lysine to threonine[51]. The works of Han *et al*.[6]; Dean *et al*.[17] showed that standard dietary recommendations can be met by supplementing the lower protein diets with EAA and NEAA to meet the exact requirement. It is well known that the CP and Amino Acids (AA) status of a diet affects the carcass composition of broilers with increased carcass protein and decreased carcass fat accompanying increases in dietary protein or essential AA contents because protein has the ability to act as a lipotrophic agent[3, 8, 9]. Excess dietary CP results in a lean bird but reduces feed efficiency whereas less than optimal protein content increases fat retention[10]. Therefore, importance of using appropriate amounts of balanced dietary CP and AA for poultry is a high priority issue for several reasons like feed efficiency, cost and environmental concerns[10].

In the tropics, it is still unclear as to what extent the amino acid supplementation could be used to replace the dietary protein in broiler rations in the tropics as most of the previous studies of replacing dietary protein were conducted under the temperate condition. Therefore, this study aims at evaluating the effect of diets formulated on the basis of four critical essential amino acids on the crude protein requirement of broiler chickens reared under tropical environment.

MATERIALS AND METHODS

Experimental site: The experiment was carried out at the Poultry Unit of Kogi State Ministry of Agriculture, Kabba, located within the Southern Guinea Savannah Zone on latitude 705'N, longitude 604'E and altitude of 640 m above sea level. It has an annual rainfall of 1500 mm and rain starts between late April and early May to mid October. The dry season begins around the middle of November with cool weather that ends in February. This is followed by relatively hot-dry weather between March and April just before the rain begins. The minimum daily temperature is from 14-20°C during the cool season while the maximum daily temperature is from 19-40°C during the hot season. The mean relative humidity during dry and wet seasons is 21 and 72%, respectively. Kabba, College of Agriculture Metrological Section, Ahmadu Bello University, Nigeria.

Experimental design and management of birds: One hundred and eighty mixed sex Arbor Acre broiler chickens were housed in a deep litter system and had free access to water and a common diet for 7 days. On d 7 they were randomly re-allocated to four groups on the basis of approximately equal weights with (15) chickens per replicate in a completely randomized design. The experimental birds were given ad libitum access to water and diet. The ambient temperature was gradually decreased from 30-20°C over the period of 28-56 days of age. The chicks were vaccinated in the hatchery against Marek, Gumboro and Bouba disease, followed by vaccination at 5 and 21 days against Gumboro disease and on the 8th day against Newcastle disease. Mash feed and water were supplied *ad libitum*.

Experimental diets: Four broiler finisher diets were formulated. Diets 1-4 contained 18-21% crude protein, respectively (Table 1). The diets were formulated to be isocaloric. Diets were also formulated to meet the NRC[4] nutrient requirements for essential amino acids. All the diets were chemically analyzed according to the standard of AOAC[11] methods for their proximate compositions (Table 2). Diets were supplemented with complete vitamin and trace mineral premixes. The l-lysine HCl, dl-methionine, l-tryptophan and l-threonine used in the diets were feed grade, (minimum 98% purity) and purchased from Ajinomoto Incorporation Japan.

Table 1: Composition of broiler finisher diet formulated on the basis of four critical essential amino on the crude protein requirement (5-8 weeks)

	Treatments			
	1	2	3	4
Ingredients	18% CP	19% CP	20% CP	21% CP
Maize	65.78	62.76	58.01	53.28
Groundnut cake	14..00	16.56	20.50	24.50
Soya cake	8.00	8.00	8.00	8.00
Fish meal	3.00	3.00	3.00	3.00
Palm oil	3.50	4.10	4.97	5.87
Limestone	1.00	1.00	1.00	1.00
Bone meal	2.90	2.90	2.90	2.90
Common salt	0.30	0.30	0.30	0.30
Premix**	0.30	0.30	0.30	0.30
Lysine	0.49	0.45	0.40	0.34
Methionine	0.30	0.28	0.25	0.22
Tryptophan	0.11	0.10	0.09	0.07
Threonine	0.32	0.31	0.28	0.25
Total	100.00	100.00	100.00	100.00
Calculated analysis (%)				
ME (Kcal kg^{-1})	3151	3152	3150	3151
Crude protein	18.00	19.00	20.00	21.00
Ether extract	7.53	7.56	7.65	7.79
Crude fibre	3.00	3.07	3.12	3.25
Calcium	1.33	1.33	1.34	1.35
Lysine	1.15	1.15	1.15	1.15
Methionine	0.60	0.57	0.60	0.60
Available P	0.57	0.57	0.57	0.57
TSAA	0.85	0.85	0.85	0.85
Tryptophan	0.21	0.21	0.21	0.21
Threonine	0.83	0.83	0.83	0.83
Glycine	1.16	1.26	1.42	1.52
Arginine	1.17	1.25	1.40	1.54
Phenylalanine	0.81	0.85	0.91	0.98
Leucine	1.50	1.54	1.62	1.70
Isoleucine	0.76	0.79	0.83	0.88
Valine	0.82	0.85	0.90	0.95

Total sulfur amino acid = TSSA; Try = Tryptophan; P = Phosphorus; ME = Metabolizable energy;**Biomix Premix Supplied per kg of diet: Vit. A, 10,000 iu; Vit.D3, 2000 iu; Vit E, 23 mg; Vit. K, 2 mg: Vit. B1,1.8; Vit. B2, 5.5 mg; Niacin, 27.5 mg; Pantothenic acid, 7.5 mg; Vit. B12, 0.015 mg: Folic acid, 0.75 mg; Biotin, 0.06 mg; Choline Chloride, 300 mg; Cobalt, 0.2 mg; Copper, 3 mg; Iodine, 1 mg; Iron, 20 mg; Manganese, 40 mg; Selenium, 0.2 mg; Zinc, 30 mg; Antioxidant, 1.25 mg

Table 2: Proximate composition of broiler finisher chickens fed diets formulated on the basis of four critical essential amino acids on crude protein requirement (5-8 weeks)

	Level of crude protein (%)			
Parameters (%)	18	19	20	21
Dry matter	90.14	89.49	92.84	90.90
Crude protein	17.01	18.22	18.99	20.64
Crude fibre	2.04	2.91	2.98	3.01
Ether extract	6.68	7.41	8.41	8.98
Ash	4.11	5.21	3.14	4.89
Nitrogen free extract	70.16	66.25	66.48	62.48

Parameters measured: The parameters measured include: final body weight and feed intake. From the primary data collected for feed intake and weight gain, data for feed conversion rate was generated. Mortality was checked twice daily; birds that died were weighed with the weight used to adjust the feed conversion (FCR = total feed consumed÷(weight of live birds+weight of dead birds).

Carcass characteristics and whole-body analyses: At the end of the experimental period (d 56), 3 birds per replicate with weights closest to the mean body weight of the replicate were used for the carcass study. The birds were slaughtered by cervical dislocation after being kept off-feed for twenty four hours (with free access to water). The birds were weighed, de-feathered and eviscerated. Weights of the conventional cut up parts (breast muscle, thigh muscle, abdominal fat,) and the data on and organs weight (i.e., liver, intestine, kidney, spleen and gizzard) were recorded at this stage.

Haematological and blood serum evaluation: Two mls of blood samples was collected from each of three birds per replicate via the wing veins and put into ethylene Di-amine Tetra Acetic Acid (EDTA) treated Bijou bottles (1 mg mL^{-1}) for haematological assay. Blood samples were analyzed within three hours of collection for Packed Cell Volume (PCV), Hemoglobin concentration (Hb) and Total Protein (TP) according to the methods described by Lamb (1991) at the Haematology Laboratory, Veterinary Teaching Hospital, Ahmadu Bello University, Zaria. Each sample was determined in triplicates. Also, two millilitres (2 mL sec) blood from three birds per replicate were allowed to clot and then centrifuged and serum was separated and stored at -20°C until analyzed for serum parameters (albumin, total protein, glucose, cholesterol, triglyceride, urea, uric acid, Aspartate Amino-Transferase (AST), Alanine Amino-Transferase (ALT), Alkaline Phosphatase (ALP) and Creatine Kinase (CK) according to the methods described by Bush at the Haematology Laboratory, Veterinary Teaching Hospital, Ahmadu Bello University, Zaria. Each sample was analyzed in triplicates.

Statistical analyses: All data obtained were statistically analyzed using the General Linear Models (GLM) procedure of SAS[12] for the analysis of variance. Duncan's multiple range tests were used to determine differences among treatment means. Means were considered different at $p<0.05$.

General linear model:

$$Y_{ij} = \mu + K_i + e_{ij}$$

Where:

Y_{ij} = Observation of the ith level of crude protein as shown by broilers performance

μ = Overall mean

K_i = ith effect of crude protein

e_{ij} = Random error

RESULTS AND DISCUSSION

An important application of ideal amino acid concept is the formulation of low protein diets, allowing for the same animal performance if compared to unbalanced high protein diets. Dietary treatment had effects on final body weight, weight gain, average daily weight gain, feed intake, average daily feed intake, feed conversion ratio and feed cost per kg weight gain. Broiler chickens fed 19, 20 and 21% CP had similar weight gains (Table 3). This result is similar to the research conducted by Han *et al*.[6], Moran and Bushong[13] and Moran and Stilborn[14]. They reported that there were no adverse effects on weight gain with low crude protein diets supplemented with essential amino acids during the first six weeks of age.

Chickens fed 18% crude protein with essential amino acids had the least weight gain. This result is similar to the report of Colnago *et al*.[15] who reported a significant depression in body weight when crude protein diets supplemented with essential amino acids were reduced from 23-18%. The increase in weight gain observed for chickens fed diets high in crude protein with the four most critical essential amino acids may be due to the efficient protein and amino acid utilizations, sufficient body capacity to meet all NEAA requirements and adequate nitrogen pool to synthesize NEAA. The poor performance with low crude protein (18%) supplemented with the four critical essential amino acids observed in this study could be associated with the differences in amino acids digestibility of diets because of the slight variation in the levels of the feed ingredients, insufficient body capacity to meet all NEAA requirement and inadequate nitrogen pool to synthesize NEAA. The significant effect observed for feed intake disagreed with the findings of Bregendahl *et al*.[16] and Awad *et al*.[17] who reported no significant differences across the treatments fed low crude protein versus high crude protein but similar to the findings of Kidds *et al*.[18] who reported an increase in feed intake of chickens fed diets containing 19% CP as compared to those fed diets containing 22.5% CP with essential amino acids. The reason may be because of the changes in amino acid contents of low crude protein diets. Although it was reported by Han *et al*.[6] that equal feed intakes may be expected if low crude protein diets with the same metabolizable energy are supplemented with limiting amino acid.

Table 3: Crude protein requirements and performance of broiler finisher chickens fed diets formulated on the basis of four critical essential amino acids (5-8 weeks)

	Crude protein level (%)				
Parameters	18	19	20	21	SEM
Initial weight (g)	1401.83	1402.45	1402.81	1402.02	1.02
Final weight(g)	2857.33[b]	3090.16[a]	3248.81[a]	3263.49[a]	51.49
Weight Gain (g)	1455.50[b]	1687.71[a]	1846.00[a]	1861.48[a]	51.32
Ave daily gain (g)	69.31[b]	80.37a	87.91[a]	88.64[a]	2.44
Feed Intake (g)	4012.20[b]	4012.90[b]	4274.80[a]	4212.50[a]	77.75
Feed Intake (g/b/d)	191.08[b]	191.90[b]	203.56[a]	200.60[a]	3.70
FCR	2.77[b]	2.38[a]	2.32[a]	2.27[a]	0.09
Mortality (%)	0.00	0.00	0.00	1.75	0.88

[a, b]Means with different superscript on the same row differ significantly ($p<0.05$); SEM = Standard Error of Means; FCR = Feed conversion ratio

Broiler finisher chickens fed the lowest crude protein supplemented with amino acid (18% CP) had the poorest value for feed conversion ratio. This result is similar to the finding of Garcia *et al*.[8] who reported 13% increase in FCR of birds fed 17% CP diets as compared to those fed 24% CP diets. The poor feed conversion ratio might be the result of the birds fed low CP diets consuming more feed and growing more slowly. A linear decrease in feed cost per kg weight gain was observed as the levels of crude protein supplemented with amino acids increased. It was observed that chickens fed 19, 20 and 21% CP with essential amino acids were similar compared to chickens fed 18% CP diet. This means that the overall cost of producing 1kg of lean meat was minimal for birds fed diets high in crude protein supplemented diets as compared to birds fed low crude protein supplemented with amino acid. Dietary treatments had no significant effect on mortality.

Dietary treatments had significant effects on live weight, carcass weight, dressing percentage, back, gizzard, lungs, kidney, heart, breast weight, wings, thigh and drumstick (Table 4). Chickens fed 19, 20 and 21% CP with the supplementation of amino acids had the best results in most of the parameters measured for carcass characteristics. The results disagreed with the findings of Si *et al*.[9]; Baker *et al*.[19] who reported that lowering the dietary CP level may not affect carcass characteristics yield, breast meat and thigh yield of birds. Moran and Stillborn[14] found no effect on the carcass yield of broiler

Table 4: Carcass characteristics of broiler finisher chickens fed diets formulated on the basis of four critical essential amino acids at each protein level (5-8 weeks)

	Crude protein levels (%)				
Parameters	18	19	20	21	SEM
Live weight (g)	2916.67[C]	3150.00[b]	3296.67[a]	3283.33[a]	0.03
Carcass weight (g)	2280.00[b]	2626.67[a]	2740.00[a]	2736.67[a]	0.03
Dressing (%)	78.21[b]	83.37[a]	83.13[a]	83.34[a]	1.26
Prime cuts and organ weights expressed as % of dressed weight					
Breast	16.75[b]	20.92[ab]	20.74[ab]	23.05[a]	1.43
Wings	5.94[C]	7.50[b]	7.56[b]	8.81[a]	0.29
Thigh	8.69[b]	11.58[a]	12.19[a]	12.21[a]	0.36
Drumsticks	5.80[b]	7.84[a]	7.28[a]	8.49[a]	0.41
Back	9.73[b]	11.09[a]	11.30[a]	11.90[a]	0.41
Liver	1.05	1.29	1.52	1.60	0.20
Heart	0.25[b]	0.52[a]	0.41[a]	0.48[a]	0.05
Kidney	0.36[b]	0.48[a]	0.46[ab]	0.46[ab]	0.03
Gizzard	1.07[b]	1.27[ab]	1.51[a]	1.59[a]	0.12
Abdominal fat	0.70	0.68	0.56	0.69	0.11
Spleen	0.08	0.10	0.11	0.08	0.02
Lung	0.29[b]	0.44[a]	0.43[a]	0.53[a]	0.09

[a-c]Means with different superscript on the same row differ significantly ($p<0.05$), SEM = Standard Error of Means

Table5: Haematological Parameters and serum biochemical indices of broiler finisher chickens fed graded diets formulated on the basis of four critical essential amino acids at each protein level (5-8 weeks)

	Crude Protein Levels (%)				
Parameters	18	19	20	21	SEM
PCV (%)	32.33[a]	30.00[b]	30.00[b]	29.67[b]	0.84
Hb (g dL^{-1})	9.67[a]	8.80[b]	8.67[b]	9.37[a]	0.45
RBC (×106/l)	3.53	3.57	3.73	3.83	0.53
WBC (×106/l)	16.07[a]	13.02[b]	10.70[c]	14.67[ab]	0.34
MCH (Pg)	27.46	26.42	23.33	24.47	2.87
MCV (fl)	91.75	88.92	80.56	77.75	7.89
MCHC (g dL^{-1})	29.96	29.34	28.91	31.70	1.73
Urea (mmol L^{-1})	4.03	3.43	3.53	3.37	0.31
Uric acid (mg dL^{-1})	250.33[a]	47.67[b]	60.67[b]	85.67[b]	25.24
Creatine (mg dL^{-1})	49.33	53.33	50.00	35.32	9.08
Glucose (mg dL^{-1})	3.77	4.34	4.50	5.33	1.45
AST (u iL^{-1})	38.67	47.33	32.00	34.33	8.38
ALT (u iL^{-1})	23.67	21.00	25.33	29.33	5.64
ALP (u iL^{-1})	45.33	53.33	66.33	65.67	8.88
Cholesterol (mg dL^{-1})	3.10	3.20	3.73	2.47	0.66
Triglycerides (mg dL^{-1})	2.30	2.33	1.53	2.20	0.52
Albumin (g dL^{-1})	3.20[b]	4.23[a]	4.20[a]	3.12[b]	3.04
Total protein (g dL^{-1})	3.20	3.80	4.07	3.67	0.70

[a-c]Means with different superscript on the same row differ significantly ($p<0.05$); SEM = Standard Error of Means; PCV = Packed Cell Volume; HC = Haemoglobin Count; RBC = Red Blood Cells; WBC = White Blood Cells; MCH = Mean Cell Haemoglobin; MCV = Mean Cell Volume; MCHC = Mean Cell Haemoglobin Count; AST = Aspartate Amino-Transferase; ALT = Alanine Amino-Transferase; ALP = Alkaline Phosphatase

fed low CP diets adequate in essential amino acids (EAA). Chickens fed 18%CP with diets adequate in EAA had the least carcass yield; this report is similar to the findings of Kerr and Kidd[20] who also reported a significant decrease in the carcass yield of the birds fed low CP diets supplemented with EAA. Abdominal fat pad was observed to be higher in birds fed 18% CP diet with adequate EAA. This result is similar to the findings of Sterlings *et al*.[21] and Garcia *et al*.[8] who observed that abdominal fat pad weight increased with low CP diets.

The evaluations of red blood cells, mean cell haemoglobin, mean cell volume, mean cell haemoglobin counts, urea, creatine, glucose, AST, ALT, ALP, cholesterol, triglycerides and total protein did not reveal any significant differences across the treatment groups (Table 5), although the observed means for all the parameters fell within the normal values for healthy chickens as reported by Jain[22]; Kral and Suchy[23]. Chickens fed 18% CP with balanced essential amino acids had the highest value for PCV compared to those fed higher levels of CP. The reasons could as a result of amino acid imbalance from the variations in amount of the feed ingredient used and physiological status of chickens. The mean values for haemoglobin count of 8.67-9.67 g dL^{-1} obtained in this experiment is similar to the values of 11.30±1.82 g dL^{-1} reported by Oladele and Ayo[24]. This means that the chickens in all the treatment groups were not anaemic. The high values of 10.70-16.07×6/l for red blood cells in this study are contrary to the values of 1.58-3.82 ×6/l reported by Mitruka and Rawnsley[25]. This implies that chickens were polycytheamic. Chickens fed diets containing 18% CP had the highest uric acid value of 250.33 compared to other treatments with higher levels of CP. This result is contrary to the findings of Collin *et al*.[26], Malheiros *et al*.[27] who reported considerably lower uric acid levels are measured in the plasma of low crude protein. The mean values of 3.12-4.23 g dL^{-1} of albumin are similar to the value reported by Jain[22].

CONCLUSION

It was concluded that the crude protein requirement for broiler finisher chickens can be reduced from 21-19% CP as long as the essential amino acids and all other nutrients meets the requirements of broiler chickens without having any adverse effects on growth, carcass quality and haematological and blood serum indices.

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