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Effect of Diets Formulated on the Basis of Four Critical Essential Amino Acids on Performance and Blood Biochemical Indices of Broiler Finisher Chickens Reared under Tropical Environment

Research Article

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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, carcass quality, nutrient digestibility, haematological and blood biochemical indices 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 with a gradual crude protein increase from 18 to 21% in 1% intervals. Diet 1, 2, 3 and 4 contained 18, 19, 20 and 21% dietary crude protein, respectively. Ileal digestible quantities of all essential amino acids (EAA) were almost equal in the diets and total amount of each EAA was maintained at or above NRC requirements. The performance of chickens fed 19% crude protein (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 resulted in significantly (P<0.05) lower final weight, weight gain, average daily weight gain and poorer feed conversion ratio (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. Chickens fed 21% CP fortified with balanced essential amino acids had the best values for apparent digestible crude protein, crude fibre (CF) and nitrogen free extract (NFE) compared with the chickens fed 18% CP with balanced essential amino acid. The observed means for most of the haematological and blood biochemical indices fell within the normal values for healthy chickens. 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, nutrient digestibility, carcass quality and haematological parameters of broiler finisher chickens reared under the tropical environment.

KEY WORDS broiler, carcass quality, essential amino acids, haematology, performance, tropical environment.

INTRODUCTION

It is well documented that nutrient requirements of poultry are significantly affected by seasons of rearing and ambient temperature. In the cool, hot and humid tropics, genetic potential of chickens, irrespective of the breed, might be affected adversely because of environmental constraints. Protein is generally considered as one of the major cost components of poultry diets (Firman and Boling, 1998). Therefore, placing more emphasis on crude protein requirement in broiler diet formulation becomes imperative to maximize the performance of broiler chickens as well as with economic considerations (Eits *et al.* 2004). Adding purified amino acids or amino acid precursors has been known for more than 50-years to allow for reduced levels of intact proteins to provide adequate levels of essential and non-essential amino acids (Pesti, 2009). NRC (1994) stated that poultry require a specific amount and balance of essential amino acids (EAA) and non-essential amino acids (NEAA), rather than the crude protein level *per se* to support growth, immune system and the whole body composition of broiler chickens. Some progress 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 occurred even with provision of all requirements for those amino acids considered as essential. Failure to obtain optimum results in performance may be attributed to one or more of the following factors: lack of a nitrogen pool to synthesize nonessential amino acids (NEAA); inability 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 (Namroud *et al.* 2008). The studies of Han *et al.* (1992); Dean *et al.* (2006) and Namroud *et al.* (2008) 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 acid (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 (Neto *et al.* 2000; Si *et al.* 2001; Pesti, 2009).

Excess dietary CP results in a lean bird but reduces feed efficiency whereas less than optimal protein content increases fat retention (Buyse *et al.* 1992). Therefore, importance of using appropriate amounts of balanced dietary CP and AA for poultry is a high priority issue for several reasons including feed efficiency, cost and environmental concerns (Buyse *et al.* 1992).

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 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 that will enhance growth performance, nutrient digestibility, carcass quality, haematological and blood biochemical indices 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 7 ˚5'N, longitude 6 ˚4'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 ˚C - 20˚C during the cool season while the maximum daily temperature is from 19 ˚C - 40 ˚C during the hot season. The mean relative humidity during dry and wet seasons is 21% and 72%, respectively.

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 one of four groups on the basis of approximately equal weights with fifteen (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 to 20 ˚C over the period of 28 to 56 d of age. The chicks were vaccinated in the hatchery against Marek's disease followed by vaccination at 5 and 21 days against Gumboro disease and on the 8^{th} day against Newcastle disease. Mash feed and water were supplied *ad libitum*.

Experimental diets

Four broiler finisher diets were formulated. Diets 1, 2, 3 and 4 contained 18, 19, 20 and 21% crude protein respectively (Table 1). The diets were formulated to be isocaloric. Diets were also formulated to meet the NRC (1994) nutrient requirements for essential amino acids. All the diets were chemically analyzed according to the standard of AOAC (1990) 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 diets formulated on the basis of four critical essential amino acids on the crude protein requirement (5-8 weeks)

Ingredients	**Treatments**			
	18% CP	**19% CP**	**20% CP**	**21% CP**
Corn grain	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[1]	0.30	0.30	0.30	0.30
L-lysine HCl	0.49	0.45	0.40	0.34
DL-methionine	0.30	0.28	0.25	0.22
L-tryptophan	0.11	0.10	0.09	0.07
L-threonine	0.32	0.31	0.28	0.25
Total	**100**	**100**	**100**	**100**
Calculated analysis				
Metabolizable energy (kcal/kg)	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[2] (%)	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

[1] Biomix premix supplied per kg of diet: vitamin A: 10000 IU; vitamin D_3: 2000 IU; vitamin E: 23 mg; vitamin K: 2 mg: vitamin B_1: 1.8; vitamin B_2: 5.5 mg; Niacin: 27.5 mg; Pantothenic acid: 7.5 mg; vitamin B_{12}: 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 and Antioxidant: 1.25 mg.
[2] TSAA: total sulphur amino acid.

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)

Parameters (%)	**Level of crude protein (%)**			
	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 included: final body weight and feed intake. From the primary data collected for feed intake and weight gain, data for feed conversion rate were generated. Mortality was checked twice daily; birds that died were weighed with the weight used to adjust the feed conversion.

Nutrient digestibility trial

A nutrient digestibility trial was carried out at the end of the experiment, using metabolic cages. This was done by randomly selecting 3 birds of approximately equal weight from each replicate. The birds were placed in alternate cages, with polythene bags attached beneath the cages. The birds were fed with the control diet for 7 days of the adjustment period and fasted for 24 hours with only water provided. Forty gram of the experimental diet was allocated to each bird by 8:00 a.m. daily.

Faecal samples were collected daily, separated from feed and other extraneous materials, weighed, bulked together and kept in a deep freezer. At the end of the 6th day, the birds were not fed. The remaining faeces were then collected by 8.00 a.m. on the 7th day. The total samples were thawed and weighed which they were thoroughly mixed together and oven dried for 72 hours at 65 ˚C. The dried samples were then weighed and ground, after which samples were taken for proximate analysis along with the sample of the feed fed at the Department of Animal Science Laboratory, Ahmadu Bello University, Zaria. Nutrient retention was determined for crude protein, crude fibre, ether extract, ash and nitrogen free extract.

Nutrient retention= ((nutrient intake-nutrient output)/(nutrient intake)) × 100

Carcass characteristics and whole-body analyses

At the end of the experimental period (d 56), three 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 organ weights (i.e., liver, intestine, kidney, spleen and gizzard) were recorded at this stage.

Haematological and blood serum evaluation

The collection of blood was carried out on d 56 of the experiment. Two mL of blood samples was collected from three birds per replicate via the wing veins and put into ethylenediaminetetraacetic acid (EDTA) treated Bijou bottles (1 mg/mL) for haematological assay. Blood samples were analyzed within one hour of collection. The packed cell volume (PCV) was determined by microhaematocrit method (Schalm *et al.* 1975), haemoglobin concentration (Hb) was measured spectrophotometrically by cyanomethaemoglobin method (Schalm *et al*. 1975) using a SP6-500UV spectrophotometer (Pye UNICAM England), red blood cells and white blood cells counts were estimated using haemocytometer (Schalm *et al*. 1975), the mean cell haemoglobin, mean cell volume and mean cell haemoglobin count were calculated from Hb, PCV and red blood cell (RBC) (Jain, 1986).

Also, two millilitres of 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. Serum total protein was determined by the Kjedahl method as described by Kohn and Allen (1995). Albumin was determined using the bromocresol green (BCG) method as described by Peters *et al*. (1982). Aspartate amino-transferase (AST) and alanine amino-transferase (ALT), alkaline phosphatase (ALP) and creatine kinase (CK) were determined using spectrophotometric methods described by Rej and Holder (1983). Cholesterol was determined according to the method of Roschlan *et al*. (1974) while urea was determined as described by Kaplan and Szabo (1979).

Statistical analyses

All data obtained were statistically analyzed using the general linear models (GLM) procedure of SAS software, (SAS, 2001) 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

Dietary treatment had effects on final body weight, weight gain, average daily weight gain, feed intake, average daily feed intake and feed conversion ratio. Broiler chickens fed 19% CP, 20% CP and 21% CP had similar weight gains (Table 3).

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)

Parameters	Crude protein level (%)				SEM
	18	19	20	21	
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]	80.37[a]	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
Feed conversion ratio	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

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

This result is similar to the research conducted by Han *et al*. (1992), Moran and Bushong (1992) and Moran (1994). They reported that there were no adverse effects on weight gain of low crude protein diets supplemented with essential amino acids during the first six weeks of age. 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. Chickens fed 18% crude protein with essential amino acids had the least weight gain.

This result is similar to the report of Bregendahl *et al.* (2002), who reported a significant depression in body weight when the crude protein in diets supplemented with essential amino acids were reduced from 23 to 18%. The poor performance with low crude protein (18%) supplemented with the four critical essential amino acids observed in this study could be associated to 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*. (2002) and Elmutaz *et al*. (2014), 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*. (2001) 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. However, it was reported by Han *et al.* (1992) that equal feed intakes may be expected if low crude protein diets with the same metabolizable energy are supplemented with limiting amino acid.

It is obvious in this present study that decreasing dietary CP below 19%, even with maintained EAA levels, retarded growth and feed intake and increased FCR.

This means that, in agreement with many researchers, crystalline amino acids are not able to completely re-place CP in diets (Si *et al*. 2004; Waldroup *et al*. 2005; Yamazaki *et al*. 2006). Furthermore, research conducted by Neto *et al*. (2000) revealed a 13% increase in FCR of birds fed 17% CP diets as compared to those fed 24% CP diets. The poor performance and high feed conversion ratio observed for birds fed 18% CP, even with added essential amino acids, might be attributed to low CP and imbalance of essential amino acids.

Dietary treatments had no significant effect on mortality, which shows that the diets up to the highest level tested did not have any adverse effect on the health of birds. 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.* (2001) and Baker *et al.* (1993), who reported that lowering the dietary CP level may not affect carcass characteristics yield, breast meat and thigh yield of birds.

Moran and Stillborn (1996) found no effect on the carcass yield of broiler 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 (1999) who also reported a significant decrease in the carcass yield of the birds fed low CP diets supplemented with EAA. Deficiency of dietary protein is known to increase the fat deposition in broilers (Yamazaki *et al*. 2006). Many researchers have reported the effect of supplementing several amino acids such as Met + Cys (Bunchasak *et al*. 1996), Arg (Leclercq *et al*. 1994), Trp and Glu on decreasing carcass and abdominal fat and hepatic lipid content. In our study, inclusion of adequate Met, Lsy, Try and Thre did not result in any significant reduction in fat deposition in abdominal cavity and carcass across the treatments.

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)

Parameters	Crude protein levels (%)				SEM
	18	19	20	21	
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 (% 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

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

This result is in agreement with previous studies of Yamazaki *et al*. (2006) and Han *et al*. (1992), their argument was that NEAA biosynthesis was also a limiting factor in a low CP diet and this could affect broiler performance and body composition. However, 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.* (2002), Hai and Blaha (2000) and Neto *et al*. (2000) who observed that abdominal fat pad weight increased with low CP diets.

Dietary crude protein supplemented with essential amino acids had significant (P<0.05) effects on the digestibility of crude protein, crude fibre, ash and NFE (Table 5). Chickens fed 21% CP fortified with balanced essential amino acids had the best values for apparent digestible crude protein, crude fibre and NFE compared with the chickens fed 18% CP with balanced essential amino acid. This result agreed with the findings of Neto *et al*. (2000) and Ratriyanto *et al.* (2012) who reported that efficiency of nutrient utilization was better for chickens fed higher level of CP diets supplemented with essential amino acids. The reasons may be as a result of increased proventricular acid secretions, increased pancreatic and intestinal mucosa secretions at this phase of rearing.

Digestible ash however, was influenced by dietary treatment; the high value suggests that more minerals were available to the birds as none of the birds showed any mineral deficiency symptoms throughout the experimental period.

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 6), although the observed means for all the parameters fell within the normal values for healthy chickens as reported by Jain (1993) and Suchy (2000). The non significant differences observed across the treatment groups on the plasma glucose is similar to the studies of Swennen *et al*. (2005) and Swennen *et al.* (2006). It indicated that carbohydrate metabolism was not affected by the diet. 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 obtained in this experiment are similar to the values of 11.30 ± 1.82 g/dL reported by Oladele and Ayo (1999). This means that the chickens in all the treatment groups were not anaemic. The high values of 10.70 - 16.07 × 10^{6} / L for red blood cells in this study are contrary to the values of 1.58 - 3.82 × 10^{6} / L reported by Mitruka and Rawnsley (1977). This implies that chickens were polycythaemic. Chickens fed diets containing 18% CP had the highest uric acid value of 250.33 (mg/dL) compared to other treatments with higher levels of CP. This result is contrary to the findings of Collins *et al*. (2003), Malheiros *et al.* (2003) who reported considerably lower uric acid levels in the plasma of low crude protein.

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

Parameters (%)	Crude protein levels (%)				SEM
	18	19	20	21	
Dry matter	88.18	87.33	84.56	87.41	1.87
Crude protein	64.57^d	74.07^b	70.88^c	77.28^a	1.98
Crude fibre	59.29^c	67.93^b	67.34^b	75.31^a	3.27
Ether extract	85.58	90.20	88.07	88.08	1.38
Ash	75.19^c	87.53^a	56.90^d	82.35^b	0.98
Nitrogen free extract	59.16^d	70.33^b	66.80^c	78.15^a	0.62

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 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)

Parameters	Crude protein levels (%)				SEM
	18	19	20	21	
PCV (%)	32.33^a	30.00^b	30.00^b	29.67^b	0.84
Hb (g/dL)	9.67^a	8.80^b	8.67^b	9.37^a	0.45
RBC (×10^6/L)	3.53	3.57	3.73	3.83	0.53
WBC (×10^6/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)	29.96	29.34	28.91	31.70	1.73
Urea (mmol/L)	4.03	3.43	3.53	3.37	0.31
Uric acid (mg/dL)	250.33^a	47.67^b	60.67^b	85.67^b	25.24
Creatine (mg/dL)	49.33	53.33	50.00	35.32	9.08
Glucose (mg/dL)	3.77	4.34	4.50	5.33	1.45
AST (UI/L)	38.67	47.33	32.00	34.33	8.38
ALT (UI/L)	23.67	21.00	25.33	29.33	5.64
ALP (UI/L)	45.33	53.33	66.33	65.67	8.88
Cholesterol (mg/dL)	3.10	3.20	3.73	2.47	0.66
Triglycerides (mg/dL)	2.30	2.33	1.53	2.20	0.52
Albumin (g/dL)	3.20^b	4.23^a	4.20^a	3.12^b	3.04
Total protein (g/dL)	3.20	3.80	4.07	3.67	0.70

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 and ALP: alkaline phosphatase.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The high level of uric acid observed for chickens fed 18% CP could be attributed to the quality and quantity of the protein, indicating an imbalance of amino acids in the diet. The mean values of 3.12-4.23 g/dL of albumin are similar to the value reported by Jain (1993).

CONCLUSION

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

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REFERENCES

AOAC. (1990). Official Methods of Analysis. Vol. I. 15th Ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Baker D.H., Becker D.H., Norton H.W., Jenson A.H. and Harmon, B.G. (1993). Lysine imbalance of corn protein in the growing pig. *J. Anim. Sci*. **28,** 23-26.

Bregendahl K., Sell J.L. and Zimmerman D.R. (2002). Effect of low protein diets on growth performance and body composi tion of broiler chicks. *Poult. Sci*. **81,** 1156-1167.

Bunchasak C., Tanaka K., Ohtani S. and Collado C.M. (1996). Effect of Met + Cys supplementation to low-protein diet on the growth performance and fat accumulation of broiler chicks at starter period. *Japanese J. Anim. Sci. Technol*. **67,** 956-966.

Buyse J., Decuypere E., Berghman L., Kuhn E.R. and Vandesande F. (1992). The effect of dietary protein content on episodic growth hormone secretion and on heat production of male broilers. *British Poult. Sci*. **33,** 1101-1109.

Collin A., Malheiros R.D., Moraes V.M.B., Van As P., Darras M., Taouis V.M., Decuypere E. and Buyse J. (2003). Effects of dietary macronutrient content on energy metabolism and uncoupling protein mRNA expression in broiler chickens. *British J. Nutr*. **90,** 261-269.

Dean D.W., Bidner T.D. and Southern L.L. (2006). Glycine supplementation to low protein, amino acid-supplemented diets supports optimal performance of broiler chicks. *Poult. Sci*. **85,** 288-296.

Eits R.M., Meijerhof R. and Santoma G. (2004). Economics determine optimal protein levels in broiler nutrition. *World Poult. J.* **20,** 21-22.

Elmutaz A.A., Mohamad F., Idrus Z., Abdoreza S.F. and Loh T.C. (2014). Amino acids fortification of low-crude diet for broiler under tropical climate: ideal essential amino acids profile. *Italian J. Anim. Sci*. **13,** 3166-3172.

Firman J.D. and Boling S.D. (1998). Ideal protein in turkeys. *Poult. Sci*. **77,** 105-110.

Hai D.T. and Blaha J. (2000). Effect of low-protein diets adequate in levels of essential amino acids on broiler chicken performance. *Czech J. Ani. Sci*. **45,** 429-436.

Han Y., Suzuki V., Parsons C.M. and Baker D.H. (1992). Amino acid fortification of a low protein corn and soybean meal diet for chicks. *Poult. Sci*. **71,** 1168-1178.

Jain N.C. (1986). Schalm’s Veterinary Haematology. Lea and Febriger Publisher, Philadelphia, USA.

Jain N.C. (1993). Essentials of Veterinary Haematology. Lea and Febiger Publisher, Philadelphia, USA.

Kaplan A. and Szabo I.I. (1979). Clinical Chemistry Interpretation and Technique. Henry Kumpton Publisher, London, United Kingdom.

Kerr B.J. and Kidd M.T. (1999). Amino acid supplementation of low-protein broiler diets: glutamic acid and indispensable amino acid supplementation. *J. Appl. Poult. Res*. **8,** 298-309.

Kidd M.T., Gerard P.D., Heger J., Kerr B.J., Rowe D., Sistani K. and Burnham D.J. (2001). Threonine and crude protein responses in broiler chicks. *Anim. Feed Sci. Technol*. **94,** 57-64.

Kohn R.A. and Allen M.S. (1995). Enrichment of proteolysis activity relative to nitrogen in preparations from the rumen for *in vitro* studies. *Anim. Feed Sci. Technol*. **52,** 1-4.

Leclercq B., Chagneau A.M., Cochard T. and Khoury J. (1994). Parative responses of genetically lean and fat chickens to lysine, argentine and non-essential amino acid supply. *Br. Poult. Sci*. **35,** 687-696.

Malheiros R.D., Moraes V.M.B., Collin A., Janssens G.P.J., Decuypere E. and Buyse J. (2003). Dietary macronutrients, endocrine functioning and intermediary metabolism in broiler chickens. Pair wise substitutions between protein, fat and carbohydrate. *Nutr. Res*. **23,** 567-578.

Mitruka B.M. and Rawnsley H.M. (1977). Clinical Biochemical and Haematological Reference Values in Normal Experiment Animals. Masson Publishing USA Inc., New York.

Moran E.T. and Bushong R.D. (1992). Effects of reducing dietary crude protein to relieve litter nitrogen on broiler performance and processing yields. Pp. 466-470 in Proc. 19th World Poult. Sci. Assoc. Meet. Amsterdam, Netherlands.

Moran E.T. (1994). Significance of dietary crude protein to broiler carcass quality. Pp. 1-11 in Proc. Maryland Nutr. Conf. Feed Manufact. University of Maryland, USA.

Moran E.T. and Stilborn B. (1996). Effect of glutamic acid on broiler given sub-marginal crude protein with adequate essential amino acids using feeds high and low in potassium. *Poult. Sci*. **75,** 120-129.

Namroud N.F., Shivazad M. and Zaghari M. (2008). Effect of fortifying low crude protein diet with crystalline amino acids on performance, blood ammonia level and excreta characteristics of broiler chicks. *Poult. Sci*. **87,** 2250-2258.

Neto M.G., Pesti G.M. and Bakalli R.I. (2000). Influence of dietary protein level on the broiler chicken's response to methionine and betaine supplements. *Poult. Sci*. **79,** 1478-1484.

NRC. (1994). Nutrient Requirements of Poultry, 9th Rev. Ed. National Academy Press, Washington, DC., USA.

Oladele S.B. and Ayo J.O. (1999). Compaarative studies on the haematocrit, haemoglobin and total protein values of apparent healthy and clinically sick indigenous chicks in Zaria, Nigerian. Bull. Anim. Health Prod. **47,** 163-167.

Pesti G.M. (2009). Impact of dietary amino acid and crude protein levels in broiler feeds on biological performance. *J. Appl. Poult. Res*. **18(3),** 477-486.

Peters T., Biamonte G.T. and Doumas B.T. (1982). Protein (total protein) in serum. Pp. 100-115 in Selected Methods of Clinical Chemistry. G.W.R. Faulkner and S. Mcites, Eds. Amercian Association of Clinical Chemistry, USA.

Ratriyanto A., Indreswari R., Sudiyono S. and Sofyan A. (2012). Potential use of betaine to substitute methionine in broiler diets. Pp. 135-138 in Proc. Natl Semin. Zootec. Indigen. Res. Dev. Diponegoro University, Indonesia.

Rej R. and Holder M. (1983). Aspartate aminotransferase. Pp. 416-433 in Methods of Enzymatic Analysis. H.U. Bergmeyer, J. Bergmeyer and M. Grass, Eds. Velag Chemie, Weinheim, Germany.

Roschlan P., Bernet E. and Guber W. (1974). Enzymatische bestimmung des gesanty cholesterium in serum. *J. Clin. Biochem*. **12,** 403-407.

SAS Institute. (2001). SAS®/STAT Software, Release 6.11. SAS Institute, Inc., Cary, NC. USA.

Schalm J.W., Jain N.C. and Carol E.J. (1975). Veterinary Haematology. Lea and Febriger Publisher, Philadelphia, USA.

Si J., Fritts C.A., Burnham D.J. and Waldroup P.W. (2001). Relationship of dietary lysine level to the concentration of all essential amino acids in broiler diets. *Poult. Sci*. **80,** 1472-1479.

Si J., Fritts C.A., Waldroup P.W. and Burnham D.J. (2004). Effects of tryptophan to large neutral amino acid ratios and overall amino acid levels on utilization of diets low in crude protein by broilers. *J. Appl. Poult. Res*. **13,** 570-578.

Sterling K.G., Costa E.F., Henry M.H., Pesti G.M. and Bakalli R.I. (2002). Responses of broiler chickens to cottonseed and soybean meal-based diets at several protein levels. *Poult. Sci*. **81,** 217-226.

Suchy I.K. (2000). Haematological studies in adolescent breeding cocks. *Act. Vet. Brno*. **69,** 189-194.

Swennen Q., Janssens G.P.J., Millet S., Vansant G., Decuypere E. and Buyse J. (2005). Effects of substitution between fat and protein on feed intake and its regulatory mechanisms in broiler chickens: endocrine functioning and intermediary metabolism. *Poult. Sci*. **84,** 1051-1057.

Swennen Q., Janssens G.P.J., Collin A., Bihan-Duval E.L., Verbeke K., Decuypere E. and Buyse J. (2006). Diet-induced thermogenesis and glucose oxidation in broiler chickens: influence of genotype and diet composition. *Poult. Sci*. **85,** 731-742.

Waldroup P.W., Jiang Q. and Fritts C.A. (2005). Effects of glycine and threonine supplementation on performance of broiler chicks fed diets low in crude protein. *Int. J. Poult. Sci*. **4,** 250-257.

Yamazaki M., Murakami H., Nakashima K., Abe H. and Takemasa M. (2006). Effect of excess essential amino acids in low protein diet on abdominal fat deposition and nitrogen excretion of the broiler chicks. *Japanese Poult. Sci*. **43,** 150-155.