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EFFECT OF DIETS FORMULATED ON THE BASIS OF FOUR CRITICAL ESSENTIAL AMINO ACIDS ON THE CRUDE PROTEIN REQUIREMENT, NUTRIENT DIGESTIBILITY AND HAEMATOLOGICAL PARAMETERS OF BROILER CHICKS REARED UNDER TROPICAL ENVIRONMENT

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ABSTRACT

In commercial poultry production, formulating diets to adequate amino acids and crude protein are critical to maximize performance and meat yield of broiler chickens. This study was conducted to evaluate the effect of diets formulated on the basis of four critical essential amino acids (lysine, methionine, threonine and tryptophan) on the crude protein requirement, performance, nutrient digestibility, haematological and serum indices of broiler chicks reared under tropical environment. One hundred and ninety two, mixed sex Arbor Acre day- old chicks were used in this experiment. The experiment was a completely randomized design, consisting of four treatments with three replicates (16 birds per replicate). Four isocaloric (3101 metabolizable energy) starter (1-28 days) experimental diets were formulated in a gradual crude protein decline from 23 to 20% by 1% interval. All the diets met or exceeded the National Research Council recommendations except for the crude protein which was in graded levels. Final weight, body weight gain, feed intake and feed conversion ratio were significantly ($P<0.05$) affected by dietary treatments. Chicks fed 21%CP with essential amino acids had the best result in terms of the weight gain and feed conversion ratio, although their performance was similar to chicks fed 22%, and 23% crude protein. The values obtained for all the haematological and serum indices fell within the normal range for healthy birds. Dietary treatment had significant ($P<0.05$) effects on apparent crude protein, ether extract, ash and nitrogen free extract. Chicks fed 21%CP with essential amino acids had better apparent nutrient digestibility values. In summary, crude protein requirement for broiler starter chicks (1-28d) reared under the tropical environment can be reduced to 21% with essential amino acids supplementation without having any adverse effects on performance, apparent nutrient digestibility, haematological and blood serum indices.

Key words: Broiler chicks, essential amino acids, crude protein, performance and tropical environment.

INTRODUCTION

It is well known that the nutritional requirements of birds constitute about 75 percent of the cost of production (Bawa *et al*., 2003; Ogundipe *et al*., 2003), and this is becoming an important issue especially in the tropical regions as the prices of feed ingredients continue to rise. Since the large portion of cost associated with poultry production involves meeting the protein or amino acids requirements of the birds (Corzo *et al*., 2005: Eit *et al*., 2005; Onimisi *et al*., 2008), providing balanced dietary crude protein according to the requirement is important. Dietary protein are

essential for the normal growth and reproduction of animals therefore decreasing the crude protein content of broiler chicks below biological limits may adversely affect growth performance, carcass composition and blood profile of broiler chicks. Several theories for why performance is negatively affected have been proposed including amino acid imbalance, increase in blood ammonia level and change in the ratio of net energy to metabolizable energy (Aftab *et al*., 2006), although there have been some reports that broiler chicks fed low protein diets supplemented with essential amino acids performed equivalently with those fed higher protein diets containing only intact protein. Experiment conducted by (Elmutaz *et al*., 2014) observed that the chicks fed 19.2% CP supplemented with all essential amino acids had the best weight gain and feed efficiency. Essentially, dietary requirements for protein are requirements for amino acids. Therefore, dietary formulations based on the amino acid requirements of birds rather than CP can minimize N excretion by simply reducing total dietary N intake (NRC, 1994). It was also reported by (Dean *et al*., 2006) that standard dietary recommendations can be met by supplementing the lower protein diets with essential amino acids to meet the exact requirement. This will allow a relative feed cost reduction together with lower nitrogen excretion as a rising global concern (Kidds *et al*., 2001; Namround *et al*., 2008). Most of these studies conducted were mostly based on the nutrient requirements for chickens reared in the temperate regions (NRC 1994). Hence it is still unclear as to what extent the amino acid supplementation can replace the crude protein without having any adverse effect on performance, nutrient digestibility and blood haematological indices of chicks reared in the tropical regions. Thus, this study was conducted to evaluate the effect of diets formulated on the basis of four critical essential amino acids on the crude protein requirement, performance, nutrient digestibility, haematological and blood serum indices of broiler chicks reared under tropical environment.

MATERIALS AND METHODS

Experimental design and bird's management

A total of 192 day-old mixed sex broiler chicks (Arbor X Acre) were obtained from a commercial hatchery. On day 1, chicks were randomly assigned to four dietary groups in a completely randomized design. Each group had three replicates with 16 chicks per replicate. The broilers were housed in pens under the deep litter system of (45 cm × 36 cm × 36 cm). The room temperature was maintained at 30 to 33°C from d 1-7, and then gradually reduced 2°C per week until a final temperature of 25°C was reached. Relative humidity was maintained at 55% to 60%. Chicks were exposed to 22 h of light and 2 h of darkness from d 1-8 and 16 h of light and 8 h darkness from d 9-28. The experiment was conducted during the hot season between March and April (2015). The maximum temperatures varied from 29^0C to 43^0C. The litter was made from wood shavings and the amount placed in each pen was 2.2 kg of dry matter/bird housed, so that all treatments had the same initial amount of material used as litter, at a height of 6 cm. The chicks were vaccinated against Marek and Gumboro diseases, 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 isocaloric (3101 metabolisable energy starter (1-28 days) experimental diets were formulated in a gradual decline from 23 to 20% by 1% interval. All the diets met or exceeded

NRC (1994) recommendations except the CP (Table1). All the diets were chemically analyzed according to the standard of AOAC (1990) methods for their proximate composition (Table 2).

Data collection and sampling

The final body weight and feed intake were measured weekly. From the primary data collected for feed intake and weight gain, data for feed conversion ratio was generated. Mortality record was recorded as it occurred. On day 28, three birds per replicate were randomly selected on the basis of equal weight for haematological and blood serum analyses. At the end of the starter phase trial, 2ml of blood samples was collected from three birds per replicate via the wing veins into sterile tubes containing an anticoagulant (ethylene diamine tetra acetic acid, EDTA) for the determining haematological parameters like Packed Cell Volume (PVC) which was determined by the microhaematocrit method, haemoglobin concentration (HB) which was determined photometrically at the wavelength of 540nm, the erythrocytes (RBC) and leucocytes (WBC) were done using the improved Neubauer haemocytometer, Differential leucocytes counts were determined by the thin slide method. Mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) were derived by calculation using the following formulae:

$$MCHC = \frac{Hb}{PCV} \times 100$$

$$MCH = \frac{Hb}{RBC} \times 100$$

$$MCV = \frac{PCV}{RBC} \times 100$$

2mls of blood from three birds per replicate also was allowed to cloth 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) at the Haematology Laboratory Unit, Veterinary Teaching Hospital, Ahmadu Bello University, Zaria, according to the methods described by Lamb (1991).

Nutrient Digestibility Trial

A nutrient digestibility trial was carried out using metabolic cages. This was done by randomly selecting 3 birds of approximately equal weight from each replicate. The birds were placed in alternated cages, with polythene bag attached to the beneath of the cage. The birds were fed with the normal diet for 7 days adjustment period and fasted for 24 hours with only water given to them. Equal quantity of experimental diets was allocated to each bird by 8:00am daily. Faecal samples were collected daily, separated from feeds and other extraneous materials, weighed, bulk together and kept in a deep freezer. At the end of the 6^{th} day the birds were not fed. The remaining faeces were then collected on the 7^{th} day. The total sample was thawed and weighed after it has been thoroughly mixed together and oven dried for 72 hours at 65^0C. The dried samples were then weighed and ground after which samples was taken for proximate analysis along with the sample of the feed at the Department of Animal Science Laboratory using the procedure by AOAC (1990). Nutrient retention was determined for crude protein, crude fibre, ether extract, ash, and nitrogen free extract:

$$\text{Nutrient Retention} = \frac{\text{Nutrient intake} - \text{Nutrient output}}{\text{Nutrient intake}} \times 100$$

Statistical analysis

All data obtained were statistically analyzed using the General Linear Models (GLM) procedure of SAS software, (2001) for the analysis of variance. Duncan's (1955) multiple range tests were used to determine differences among treatment means. Means were considered different at $P<0.05$.

RESULTS AND DISCUSSION

Analyses of the mixed diets yielded results in close agreement to the calculated composition (Tables 1 and 2). Performance of broiler starter chicks fed diets formulated on the basis of four critical essential amino acids (0-4 weeks) is shown in Table 3. It was observed that dietary treatment had significant ($P<0.05$) effects on the final weight, weight gain, feed intake and feed conversion ratio. Chicks fed 21% crude protein with the inclusion of the four most critical amino acids performed better in terms of the weight gain than other treatment groups, although their performance was similar to chicks fed 22%, and 23% crude protein (Figure 1). It was not surprising that chicks fed 21%, 22% and 23% CP diet had body weight similar, since this level of CP reduction results in amino acid levels being relatively close to current recommendations by NRC 1994. This result is similar to the reports of Temim *et al*., (2000), Zarate *et al*., (2003) who reported that reducing dietary crude protein by 2% did not affect body weight gain. It was also reported by Fatufe and Rodehutscord, (2005), Dean *et al*., (2006) that broiler performance was not affected even when crude protein of amino acid supplemented diet was decreased by 20 - 25% of positive control. The increased weight gain observed may be due to the fact that the balanced dietary crude protein according to the requirement was attained therefore giving higher that requirement is unnecessary. As dietary levels of CP are further reduced other essential amino acids and nonessential amino acids may become limiting and result in reduced performance which was clearly observed with chicks fed the lowest crude protein (20%). This result is similar to the findings of Bregendahl *et al*., 2002, Aletor*et al*., (2000) who reported reduced growth performance with low crude protein diets. These great discrepancies, in result of feeding broiler chicks, low protein diets supplemented with essential amino acid may be due to among other factors, the extent of reduction of crude protein contents, the inclusion of crude protein and metabolizable energy contributions of the amino acid supplements, the class and age of the chickens and whether or not the intact protein sources are kept at constant ratios to minimize amino acid imbalances. In this study dietary treatment had effect on feed intake as chicks fed 22% crude protein supplemented with the four essential amino acids was observed to have the highest feed intake. This result disagreed with the findings of (Bregendahl *et al*., 2002; Araujo *et al*., 2004; Fatufe and Rodehutscord, 2005) who reported no effect in feed intake. Although the feed intake (1620.80g) for chicks fed the lowest crude level (20%) was similar to the feed intake (1579.20g- 18.18.20g) for chicks fed high crude protein level. This result is similar to the result of (Hussein *et al*., 2001) who reported equal feed intake when comparing high crude protein versus low crude protein diets supplemented with amino acid during 7 to 21 days period. The reason for the similarities in the feed intake may be due to the fact that feed intake depends on the calorific value of the feed because birds primarily eat to maintain their energy intake. Some of the reason for low feed intake observed for chicks fed low crude protein (20% and 21%) or in excess (23%) may be due to inadequate dietary electrolyte balance, changes

in the amino acid content of low crude protein diets, ingestion of diet containing free amino acid may cause a rapid flux of amino acid into the blood stream, which may reduce feed intake by unbalancing plasma amino acid content. Dietary treatment had effect on feed conversion ratio as chicks fed 22% crude protein supplemented with amino acid had the highest value. This result disagreed with the findings of (Araujo *et al*., 2004; Razaei *et al*., 2004) who reported no differences in feed conversion ratio of the birds fed low protein diets supplemented with amino acid according to an ideal amino acid ratio. Chicks fed 23% crude protein had the highest feed conversion ratio (FCR). This result varies with the finding of Moran *et al*., (1992) who reported an increase in feed conversion ratio with decrease in crude protein (23 to 20% and 20 to 17% in starter and grower periods, respectively). Although, chicks fed low crude protein levels of 20 and 21% was observed to have high feed conversion ratio. The increased FCR might be the result of the chicks consuming more feed and growing more slowly as evident in the body weight gain observed for chicks fed 20% crude protein. Packed cell volume, haemoglobin count, red blood cell, white blood cells, uric acid, glucose, ALT, ALP, and cholesterol were significantly ($P<0.05$) affected by dietary treatments (Table 4). Dietary treatments had no significant effects on MCH, MCV, MCHC, urea, creatine, AST, triglycerides, albumin and total protein, though all the values obtained fell within the values for normal healthy chickens as reported by (Oladele and Ayo 1999). The mean values of PCV 26.00-29.67% obtained fell within the normal value (22-35%) for healthy chickens reported by (Jain 1993), these value were also similar to the values reported by (Oladaele and Ayo 1999; Mitruka and Rawnsley 1977). This implies that the chickens were not anaemic and were immunologically balanced. The haemoglobin count for all the dietary treatments (8.73-9.85g/dl) were slightly lower than the value for normal healthy chickens reported by (Oladele and Ayo 1999). This discordant submission could be attributed to environmental and seasonal factors. Their own experiment was conducted in the temperate environment. Chickens fed 0.19% dietary tryptophan had the highest values of red blood cells (4.70) and white blood cells (15.70). The values of RBC were in accordance with the values reported by Mitruka and Rawnsley (1977) for healthy chickens while the mean values of WBC obtained in this present study were above the values for normal healthy chickens as reported by Mitruka and Rawnsley (1977). This implies that the birds were slightly polycythemic although these values did not adversely affect the chickens. This discordant submission could be attributed to environmental and seasonal factors. The mean values (73-246.70mg/dl) of uric acid in this experiment was not in accordance with the report of Victoria *et al*., (1989) who reported high mean values 245±49 - 639 ±175 of uric acid. However the lower mean values of uric acid in this experiment shows that there was no incidence of kidney damage contrary to the values reported by Victoria *et al.,* (1998) which was attributed to kidney damage. Chickens fed 22% CP had the highest mean value of cholesterol (3.73), but generally the mean values of cholesterol in this experiment were similar to the values (2.93±0.51 – 4.23 ±0.39) reported by (Jain, 1993). The mean values of AST and ALT obtained in this present study are in accordance with the values for normal healthy chickens as reported by Jain (1993). This implies that the livers were not adversely affected by levels of crude protein supplemented with essential amino acids.

Table 1: Composition of Broiler Starter Diets Formulated on the Basis of Four Critical Essential Amino Acids on Crude Protein Requirement (0- 4 Weeks)

	Treatments			
Ingredient (kg)	**1 20%CP**	**2 21%CP**	**3 22%CP**	**4 23%CP**
Maize	61.41	57.46	53.47	49.57
Groundnut cake	15.70	18.98	22.36	25.60
Soya cake	10.00	10.00	10.00	10.00
Fish meal	4.00	4.00	4.00	4.00
Palm oil	3.30	4.07	4.85	5.56
Limestone	0.80	0.80	0.80	0.80
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	0.51	0.46	0.41	0.37
Methionine	0.32	0.29	0.27	0.24
Tryptophan	0.12	0.11	0.09	0.08
Threonine	0.35	0.33	0.31	0.28
Total	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Analysis				
ME (Kcal/kg)	3101	3102	3103	3101
Crude protein (%)	20.00	21.00	22.00	23.00
Ether extract (%)	7.48	8.26	9.10	9.84
Crude fibre (%)	3.18	3.08	3.12	3.25
Calcium (%)	1.31	1.32	1.33	1.33
Lysine (%)	1.27	1.27	1.27	1.27
Methionine (%)	0.64	0.63	0.65	0.65
Available P (%)	0.60	0.60	0.60	0.60
TSAA (%)	0.92	0.92	0.92	0.92
Tryptophan (%)	0.23	0.23	0.23	0.23
Threonine (%)	0.91	0.91	0.91	0.91
Glycine (%)	1.31	1.44	1.57	1.59
Arginine (%)	1.31	1.43	1.44	1.48
Phenylalanine (%)	0.89	0.94	0.99	1.05
Leucine (%)	1.62	1.68	1.72	1.80
Isoleucine (%)	0.84	0.88	0.91	0.95
Valine (%)	0.84	0.93	0.98	1.02
Feed cost/kg (₦)	104.15	105.71	105.87	104.36

Total sulfur amino acid =TSSA, Try = Tryptophan; P=Phosphorus; ME=Metabolizable energy;**Biomix Premix Supplied per kg of diet: Vit. A, 10,000iu; Vit.D_3, 2000iu; Vit E, 23mg;Vit. K, 2mg: Vit.B1,1.8; Vit. B_2, 5.5mg; Niacin, 27.5mg; Pantothenic acid, 7.5mg; Vit. B_{12}, 0.015mg: Folic acid, 0.75mg; Biotin, 0.06mg; Choline Chloride, 300mg; Cobalt, 0.2mg; Copper, 3mg; Iodine, 1mg; Iron, 2 mg; Manganese, 40mg; Selenium, 0.2mg; Zinc, 30mg; Antioxidant, 1.25mg.

Table 2: Proximate Composition of Broiler Chicks Fed Diets Formulated on the Basis of Four Critical Essential Amino Acids on Crude Protein Requirement (0 – 4 weeks)

	Level of Crude Protein (%)			
Parameters	20	21	22	23
Dry matter	90.81	92.19	91.76	90.48
Crude protein	18.99	20.98	21.01	21.95
Crude fibre	2.98	3.14	3.20	3.98
Ether extract	7.01	7.89	8.70	8.10
Ash	4.10	5.58	4.32	3.18
Nitrogen free extract	66.92	62.41	62.77	62.79

Table 3: Crude Protein Requirements and Performance of Broiler Chicks fed Diets Formulated on the Basis of Four Critical Essential Amino Acids (0- 4 Weeks)

	Crude protein level (%)				
Parameters	20	21	22	23	SEM
Initial weight (g)	51.45	51.43	51.39	51.29	0.07
Final weight(g)	1061.88^{b}	1085.17^{a}	1079.17^{a}	1083.33^{a}	10.62
Weight Gain (g)	1010.43^{b}	1033.73^{a}	1027.78^{a}	1032.04^{a}	10.56
Ave daily gain (g)	36.09^{b}	36.92^{a}	36.71^{a}	36.92^{a}	0.02
Feed Intake (g)	1620.80^{ab}	1579.20^{ab}	1818.80^{a}	1500.00^{b}	76.73
Feed Intake (g/b/d)	57.88^{ab}	56.40^{ab}	64.96^{a}	53.57^{b}	2.74
FCR	1.60^{a}	1.52^{a}	1.77^{b}	1.45^{a}	0.05
Feed cost/kg Gain(₦)	103.19	102.27	103.22	101.26	0.15
Mortality (%)	0.00	0.00	0.00	0.00	0.00

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

Table 4: Haematological Parameters and Serum Biochemical Indices of Broiler Chicks fed Diets Formulated on the Basis of Four Critical Essential Amino Acids at Each Protein Level (0 – 4 Weeks)

Parameters	Crude Protein Levels (%)				SEM
	20	21	22	23	
PCV (%)	28.33[a]	26.00[b]	29.67[a]	26.33[b]	1.80
Hb (g/dl)	9.43[ab]	8.04[b]	9.87[a]	8.73[b]	0.29
RBC ($x10^6$/l)	4.77[a]	3.90[b]	4.70[a]	4.03[b]	0.31
WBC ($x10^6$/l)	15.70[a]	12.93[ab]	10.43[b]	14.00[a]	0.83
MCH(Pg)	19.81	23.53	21.07	21.66	2.53
MCV(fl)	59.50	70.90	63.35	65.30	8.59
MCHC(g/dl)	33.27	33.25	33.26	34.27	1.08
Urea(mmol/l)	3.83	3.73	3.17	3.54	4.51
Uric acid(mg/dl)	246.70[a]	73.30[c]	80.00[c]	146.70[b]	71.50
Creatine(mg/dl)	51.00	51.00	47.67	47.33	11.90
Glucose(mg/dl)	4.83[b]	4.43[b]	5.00[b]	7.03[a]	0.90
AST(u.i/l)	32.33	28.77	21.00	22.00	9.83
ALT(u.i/l)	19.00[b]	20.33[b]	32.00[a]	22.33[b]	6.16
ALP(u.i/l)	43.33[c]	55.33[b]	60.33[b]	75.00[a]	9.33
Cholesterol(mg/dl)	2.90[b]	2.93[b]	3.73[a]	3.09[ab]	0.24
Triglycerides(mg/dl)	1.63	1.27	1.07	1.13	0.27
Albumin(g/dl)	4.23	4.10	4.40	3.70	3.20
Total protein(g/dl)	2.80	2.53	2.73	2.73	0.29

a, b,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

Table 5: Nutrient Digestibility of Broiler Chicks fed Diets Formulated on the Basis of Four Critical Essential Amino acids at Each Protein Level (0 - 4 Weeks)

	Crude Protein Levels %				
Parameters (%)	20	21	22	23	SEM
Dry matter	85.93	84.53	86.83	86.52	2.95
Crude protein	73.29^{b}	83.60^{ab}	87.50^{a}	84.83^{ab}	2.72
Crude fibre	72.48	71.44	70.11	76.05	4.33
Ether extract	71.47^{b}	85.30^{a}	77.99^{b}	85.47^{ab}	2.12
Ash	73.98^{b}	85.62^{a}	77.78^{ab}	71.17^{b}	3.01
NFE	57.34^{c}	71.22^{a}	69.35^{b}	68.58^{b}	1.05

a, b= Means with different superscript on the same row differ significantly (P<0.05)
SEM = Standard error of means
NFE = Nitrogen free extract

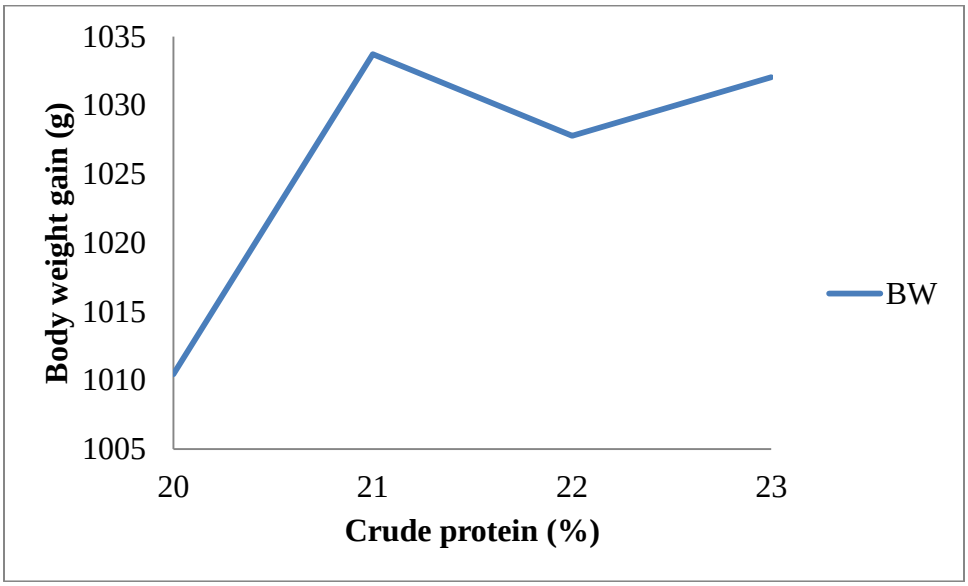


Figure 1: A graph showing the optimum body weight gain and crude protein requirement for chicks fed diets formulated on the basis of four essential amino acids.

Dietary treatment improved the apparent CP, EE, ash and NFE digestibility. Chicks fed 22, 23 and 21% CP supplemented with essential amino acids respectively had the highest values for the digestible CP (Table 5). The result obtained in this study was similar to the result of (FairaFilho *et al*., 2007), who reported that digestibility of CP increased with increase in the levels of crude protein. This indicates more efficient nutrient digestion and absorption. The chicks fed low crude protein supplemented with the essential amino acids recorded the lowest value for digestible CP; this may be due to amino acid imbalance which might be associated with low protein utilization. The high values for digestible EE observed in chicks fed 21 and 23% CP supplemented with essential amino acids confirms the report of (El-husseiny*et al*., 2007) who noted that digestibility of EE was improved with increase levels of dietary CP. This may be due to the ability of ether extract in making the feed to be palatable thereby increasing feed intake and its utilization. Ash digestibility however, was influenced by dietary treatment. It was observed that chicks fed 21 and 22 % CP supplemented with essential amino acids had the highest values for ash digestibility. The elevated high values recorded across the treatment suggest that more minerals were available to the birds as none of the chicks showed any deficiency symptoms throughout the experimental period. Digestibility of NFE was affected by dietary treatment this result disagrees with the report of (FarioFilho*et al*., 2007), who reported no significant difference for chicks fed varying levels of crude proteins supplemented with amino acids, although it was observed that the values recorded were low across the treatment groups. This may be due to amino acid imbalance interfering with metabolic process.

CONCLUSION

Crude protein requirement of broiler starter (1-28 days) diet can be reduced to 21% with essential amino acids supplementation without having any adverse effects on growth performance, nutrient digestibility, haematological and blood serum biochemical indices. A reduction in the dietary crude protein below 21% supplemented with essential amino acids may impair growth performance although adverse effects on blood and serum biochemical may not be observed.

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