MON -52

Crude Protein Levels versus Diets Formulated on The Basis of Critical Essential Amino Acids on Haematological Indices and Serum Biochemicals of Broiler Chicks

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Abstract
An experiment was conducted in order to evaluate the effect of diets formulated on the basis of essential amino acid at varying levels of crude protein on blood parameters and blood serum biochemical indices of broiler chicks. Four broiler starter diets containing different levels of crude protein (20, 21, 22 and 23%) were formulated. The diets were formulated to be isocaloric and met or exceeded the NRC (1994) nutrient requirements for all the essential amino acids. One hundred and ninety-two day old unsexed Arbor Acres broiler chicks were assigned to the four dietary treatments in a completely randomized design of three replicates each and 16 chicks per replicate. Haematological parameters (PCV, Hb, RBC, WBC, MCH, MCV and MCHC) and blood serum (albumin, total protein, glucose, cholesterol, triglyceride, urea, uric acid, aspartate aminotransferase, alanine amino-transferase, alkaline phosphatase and creatine kinase) indices were measured at 28d. PCV, Hb, RBC, WBC, uric acid, glucose, ALT, ALP and cholesterol were significantly ($p<0.05$) affected by dietary treatments. It was generally observed that most of the values for the haematological and blood serum parameters fell within the normal values except for WBC which was extremely high. It can be concluded from this study that even up to the lowest level of crude protein tested the haematological parameters and blood serum indices werenot adversely affected.

Keywords: Essential amino acids, crude protein, haematological, serum biochemicals, broiler chicks

Introduction
Recently research among poultry nutritionist now tends to explore the possibilities of reducing the crude protein level for broiler chickens by feeding rations balanced in critical essential amino acids thereby reducing the cost of poultry feed. In the tropical environment, so many factors have been reported to be responsible for the increased economic losses of broiler chicks. Some of these factorsinclude; unsuitable genotypes and unfavourable weather conditions. Importation of temperate-zone high - performance stocks to hot regions is on the rise. However, the use these stocks in hot regions may result in large economic losses due to depression in general performances and higher mortality (Mohamed *et al*, 2012).

In the tropical regions, it is still unclear as to what extent the crude protein should be reduced without having any adverse effect on the general performance of broiler chickens. However, the knowledge of haematological parameters/ serum biochemical indices can be used to assess the health status of broiler chicks (Abdalla and Nawal 2009). Normally, the values of blood constituents are affected by many factors such as genotype, age, physiological condition, gender, diet, micro- and macro-climatic conditions, the method of rearing, season and pathological factors(Mohamed *et al*, 2012).

This research was conducted to evaluate the effect of diets formulated on the basis of four critical essential amino acids at each protein level on the haematological and serum biochemical indices of broiler chicks reared during the hot season under tropical condition.

Materials and Methods
The experiment was carried out in the premise of poultry research unit, livestock Unit, Kabba College of Agriculture, Ahmadu Bello University using one hundred and ninety- two (192), day- old unsexed broiler chicks of strains Arbor Acres. There were four treatment groups each containing three replicates. Each replicate had 16 birds in a completely randomized design. The birds were reared on a deep litter system. All medication and vaccination were administered as at when due as describe by Veterinary Teaching Hospital, Ahmadu Bello University, Zaria. Diets, 1, 2, 3, and 4 contained 20, 21, 22 and 23% crude protein, respectively (Table 1). The diets were isocaloric and were also formulated to meet the NRC (1994) nutrient requirements for the first four critical essential amino acids which were lysine, methionine, tryptophan and threonine. The chicks were fed the experimental diets from 0-4 weeks.

At the end of the starter phase, 2ml of blood samples were collected from each of three birds per replicate via the wing veins into sterile tubes containing an anticoagulant (ethylene diaminetetraacetic acid,

EDTA) for the determination of haematological parameters. Packed Cell Volume (PVC) was determined by the microhaematocrit method, haemoglobin concentration (HB) by photometrically at the wavelength of 540nm, the erthroocyte (RBC) and leucocytes (WBC) were done using the improved Neubauer haemocytometer.2ml of blood was also allowed to clot and then centrifuged. The 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 1975) at the Haematology Laboratory, Veterinary Teaching Hospital, Ahmadu Bello University, Zaria. Each sample was analyzed in triplicates. The laboratory analyses were carried out at the Department of Physiology, Faculty of Veterinary Medicine, Ahmadu Bello University.

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

Table 1: Composition of broiler starter diet formulated on the basis of four critical essential amino acids on crude protein requirement (0- 4 Weeks)

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.

Results and Discussion

Packed cell volume, haemoglobin count, red blood cells, white blood cells, uric acid, glucose, ALT, ALP, and cholesterol were significantly ($p<0.05$) affected by dietary treatments (Table 2). 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 Suchy (2000). 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 values were also similar to the values reported by Oladele and Ayo (1999). 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. Chickens fed 20% CP fortified with balanced essential amino acids had the highest values of red blood cells (4.70) and white blood cells (15.70). The values obtained for red blood cells were in accordance with the values reported by Jain (2000) and Suchy (2000) 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 Jain (2000). This implies that the birds were slightly polycythemic, although the chicks were not adversely affected as no sign of illness was observed. This discordant submission could be attributed to environmental and seasonal factors, age, genotype and physiological status of the chicks.

Table 2: Hematologicalparameters 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
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

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

Table 3 shows the effect of diets formulated on thebasis of four critical essential amino acids on the blood biochemical indices.

Table 3: 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)

	Crude Protein Levels (%)				
Parameters	20	21	22	23	SEM
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; AST=Aspartate Amino-Transferase; ALT=Alanine Amino-Transferase; ALP=Alkaline Phosphatase

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 245±49 - 639 ±175, however the lower mean values of uric acid in this experiment showed that there was no incidence of kidney damage as the high mean values reported by Suchy (2000) 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 Ayo *et al.* (1996). The mean values for AST and ALT obtained in this present study are in accordance with the values for normal healthy chickens as reported by Jain (2000).

Conclusion

This present study therefore, showed that feeding broiler chicks with 20% CP supplemented with critical essential amino acids may not really affect their health status.

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