Original Contribution

BLOOD PARAMETERS, CARCASS YIELD, ORGAN WEIGHT AND VILLI MORPHOMETRICS OF BROILERS FED LOW PROTEIN DIET IN EXCESS OF DIETARY LYSINE

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ABSTRACT

One hundred and fifty day-old Abor-acre strain broilers chicks were used to evaluate the blood parameters, carcass yield, organ weight and villi morphometrics of broilers fed low protein diet in excess of dietary lysine. The chicks were weighed on the eight day and then randomly assigned to five experimental diets in such a way that each dietary treatment had 30 birds replicated thrice. Diet 1 was the control with 22.22% crude protein (CP) while diets 2, 3, 4 and 5 contained 16.6% CP each at both starter and finisher phases with varying levels of lysine ranging from 0.50 to 0.90%. They all had the same level of methionine. Results showed that the birds fed diet 4 (16.6% CP and 0.8% lysine inclusion level) had a significantly ($p < 0.05$) higher breast, villi height, crypt depth and muscle thickness while the lymphocyte count, total protein, abdominal fat and back size increased linearly with lysine inclusion unlike decreases in heterophil among birds fed LCP diets. It can be concluded that increased villi morphology enhanced breast yield due to enhanced absorption with increased abdominal fat as observed in birds fed 16.6% CP and 0.8% lysine inclusion level.

Key words: amino acid, haematology, jejunum, meat, organ, serum

INTRODUCTION

The advent of commercial available synthetic amino acids has made it possible to decrease crude protein to a degree, but has also furthered the need for the assessment of the limiting amino acids in low protein diets. Amino acids have been known to play an important role in animal performance. (1) reported that animals with good blood composition are likely to show good performance. According to (2) and (3), the examination of blood gives the opportunity to investigate the presence of several metabolites and other constituents in the body of animals which (4) stated could serve as baseline information for comparison as it plays a vital role in the physiological, nutritional and pathological status of an organism while (5) buttress the fact that haematological parameters are good indicators of the physiological status of animals. Meanwhile, serum parameters indicate the status and

integrity of vital organs in the body which (6) observed to contribute to the viscosity and maintenance of normal blood pressure and pH.

Carcass yield and quality is important in the poultry industry, due to increasing consumer interest in lean meat and low cholesterol diet (7) which (8) noted that can be influenced by the crude protein and amino acid status of a diet. Although, (9) had earlier stated that different nutritional component, protein and the amino acid type in the diet are fundamentals, because they are related with synthesis of structural tissues. (10) reported that diets formulated with high lysine level thus promoted a better conversion of the diet into efficient carcass. The concentration of dietary lysine significantly influenced breast meat according to (11) because the breast meat contains a high concentration of lysine and it represent a large portion of carcass meat, (12) observed an increase in thigh weight with increasing lysine in a low crude protein diet contrary to the observation of (13) that increasing lysine concentration in broiler diets from 21 to 42 days of age did not affect breast meat and thigh meat, but significantly decrease excess abdominal fat.

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A protein deficiency, caused by either one or more limiting amino acids or an overall inadequate consumption of protein, resulted in decreases in parameters such as nitrogen retention and feed utilization (14), while an over-consumption of protein according to (15) results in the catabolism of amino acids through deamination and excretion as uric acid which is both energetically and economically inefficient, or in severe cases, ammonia toxicity (16). It is essential to try to meet the requirement of the bird as closely as possible in order to maximize production and profitability.

Thus, combining available feed ingredients in the best possible way becomes paramount because feeding birds with poor quality feed due to high cost of feeding will result in physiological imbalance and yield poor carcass (17).

MATERIALS AND METHOD

Experimental site, feedstuffs and duration

The experiment was carried at the Teaching and Research Farm of the Ladoke Akintola University of Technology, Ogbomoso, Nigeria located in the derived savannah zone on

latitude 18° 15' north of the equator and longitude 4° 5' east of the Greenwich meridian (18). Maize and cassava pulp flour were the major energy sources while soybean was the major protein source. The experiment lasted for six weeks.

Experimental Birds

One hundred and fifty day old broilers of Abor-acre strain bought from a reputable farm at Ibadan were used for this feeding trial. The broilers were fed on conventional feed during the first week to allow for acclimatization on a deep litter. The chicks were weighed on the eight day and then randomly assigned to the experimental diets (**Table 1**) in such a way that each dietary treatment had 30 birds replicated thrice. The experimental feed and water were supplied *ad libitum*. Routine vaccination and necessary medications were administered. Records of feed intake were taken daily, body weight gain on weekly basis and mortality records were also taken. At the last week of the experiment an indigestible marker, Titanium dioxide (TiO₂) was included in the diets to monitor digestibility of the feed.

Table 1. Gross composition of experimental diet

Ingredients (%)	T ₁ (Control)	T ₂	T ₃	T ₄	T ₅
Maize	54.20	54.10	54.00	53.90	53.80
Soybean meal	40.00	26.00	26.00	26.00	26.00
Cassava meal	0.00	14.00	14.00	14.00	14.00
Lysine	0.50	0.60	0.70	0.80	0.90
*Fixed ingredients	5.30	5.30	5.30	5.30	5.30
Total	100.00	100.00	100.00	100.00	100.00
Calculated Nutrient Composition					
Crude Protein (%)	22.22	16.68	16.67	16.66	16.65
ME (Kcal/kg)	2941.23	3047.79	3044.36	3000.93	2997.49
Lysine (%)	1.73	1.44	1.53	1.63	1.72
Methionine (%)	0.58	0.50	0.50	0.50	0.50

*Fixed ingredients are Titanium dioxide, Dicalcium phosphate, Limestone, Salt, #Broiler Premix and Methionine. ME-metabolizable energy

#2.5 kg Premix used supplied vitamin A, 12,500,000iu; vitamin D, 2500,000iu; vitamin E, 40,000mg; vitamin K3, 2000mg; vitamin B1, 3000mg; vitamin B2 5500mg; Niacin, 55000mg; calcium pantothenate, 11500mg; vitamin B6, 5000mg; vitamin B12, 25mg; Folic acid, 1000mg; biotin, 80mg; choline chloride 500,000mg; manganese, 120,000mg; Iron 100,000mg; zinc, 80,000mg; copper 8500mg; iodine 1500mg; cobalt 3000mg; selenium 120mg and anti-oxidant 120,000mg.

Data Collection

Blood Chemistry Analysis

Three birds per treatment were randomly selected and slaughtered by cutting the jugular vein. 5ml of blood was collected into two sets of three sterilized glass bottles/tubes. For haematology, blood samples were collected into two sets of three sterilized bottles containing Ethylene Diamine Tetra-acetic Acid (EDTA). Blood samples for serum biochemical studies were collected into plain bottles (i.e

without anticoagulant) for serum separation. Serum was obtained by centrifugation and serum samples were stored in a deep freezer (at minus 10°C) until required for analysis.

Blood parameters such as packed cell volume (PCV) and haemoglobin (Hb) were determined using the micro haematocrit method and cyanmethemoglobin methods respectively as described by (19). Erythrocyte count (RBC) and Leukocyte count (WBC) were determined using the improved Neubauer haemocytometer

after the appropriate dilution (20). Differential leukocyte counts were determined by scanning Giemsa's stained slides in the classic manner (20).

Mean corpuscular haemoglobin (MCH) was calculated as haemoglobin/RBC x 10, and Mean corpuscular volume (MCV) was also calculated by Haematocrit/RBC x 10 while Mean corpuscular haemoglobin concentration (MCHC) was estimated by Haemoglobin/Haematocrit x 100.

Total cholesterol, triglycerides and high density lipoproteins (HDL) was assayed using the method of (21). Very low density lipoproteins (VLDL) were estimated (triglycerides/5) (22) while low density lipoproteins (LDL) was estimated using the Friedewald equation ($LDL = \text{Total cholesterol} - (HDL + \text{triglycerides}/5)$) Alanine Transaminase (ALT), Aspartate amino Transaminase (AST) and Alanine phosphatase (ALP) was determined manually by spectrophotometric method respectively as described by (23). Total serum protein was determined using the biuret method while albumin was determined using the BCG (Bromocresol green) method as described by (24) with globuline concentration obtained by subtracting albumin from the total protein.

Organs and Carcass Evaluation

At the end of the experimental period, 3 birds per treatment were selected randomly and weighed. After 6 hours of feed withdrawal, the birds were slaughtered by cutting off the jugular vein and allowed to bleed completely. Visceral organs (liver, kidney, pancreas, gizzard, spleen, heart, and lungs were eviscerated before scalding and defeathering) and carcass cuts (wing, thigh, drum stick, breast, and shank) were weighed using a

sensitive weighing balance and expressed as percentage of live weight.

Villi morphometrics

Jejunum samples from the intestine of slaughtered birds were preserved in 10% formalin on replicate basis in vacuum bottles before being taken to the laboratory for the micrographic evaluation.

Statistics

The experiments employed a complete randomized design, all data generated were subjected to analysis of variance in case of significance the means were separated with Duncan multiple range test using SAS package (25).

RESULTS

Blood parameters

Effect of low crude protein supplemented with varying lysine inclusion level on haematological parameters of broilers chicken is shown in **Table 2**. The Packed cell volume (PCV), Haemoglobin (Hb), Red blood cell (RBC), White blood cell (WBC), Monocytes, Eosinophil, Basophil, Platelet, Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) were not significantly affected ($P > 0.05$) by the dietary treatment, while the lymphocyte and heterophil count significantly varied significant ($p < 0.05$) with lymphocyte counts increasing while the heterophil count decreased linearly with lysine inclusion among the diets from those fed the control diet to those fed LCP diets. The lymphocyte counts was lowest for the control ($54.67 \times 10^6/\mu\text{l}$) and highest at bird fed T5 ($71.67 \times 10^6/\mu\text{l}$) while those fed T2-T4 were comparable while T1 ($37.67 \times 10^6/\mu\text{l}$) had the highest value and T5 ($21.00 \times 10^6/\mu\text{l}$) had the least for heterophil count.

Table 2. Haematological parameters of broilers fed low crude protein diets supplemented with varying levels of lysine

Parameter	T ₁	T ₂	T ₃	T ₄	T ₅	SEM
PCV (%)	30	31.3	27.3	30	30.3	0.62
Haemoglobin (g/dl)	9.50	10	8.8	9.5	10	0.24
RBC ($10^6/\mu\text{l}$)	3.55	3.52	2.83	3.40	3.28	3.12
WBC ($10^3/\mu\text{l}$)	24.7	20.4	20.5	24.8	20.2	1.37
Lymphocyte($\times 10^6/\mu\text{l}$)	54.7 ^b	60.3 ^{ab}	64.7 ^{ab}	65 ^{ab}	71.7 ^a	2.12
Heterophil ($\times 10^6/\mu\text{l}$)	37.7 ^a	33 ^a	28.3 ^{ab}	27.7 ^{ab}	21 ^b	1.98
Monocytes($\times 10^6/\mu\text{l}$)	3.33	3	3.33	2.33	3	0.34
Eosinphils $\times (10^6/\mu\text{l})$	3.67	3.33	3.67	4.67	3.67	0.35
Basophils $\times (10^6/\mu\text{l})$	0.67	0.33	0	0.33	0.67	0.13
Platelet $\times (10^4/\mu\text{l})$	29.5	21	21.5	29.5	22.3	22.3
MCV (fl)	84.8	88.9	103	88.2	92.7	84.1
MCH (Pg)	26.9	28.5	33.2	27.9	30.6	1.19
MCHC(%)	0.32	0.32	0.32	0.32	0.33	0.003

^{a,b} means the superscript differs significantly at ($p < 0.05$) within the same row

SEM; standard error of mean, PCV=Packed Cell Volume, RBC=Red Blood Cell, WBC=White Blood Cell, MCV= Mean corpuscular volume, MCH=Mean corpuscular haemoglobin, MCHC=Mean corpuscular haemoglobin concentration

Table 3 shows the serum parameters of broiler chickens fed with low crude protein supplemented with varying levels of lysine. The total protein and globulin, were significantly ($p<0.05$) influenced by the dietary treatment. Total Protein followed the same trend as Globulin with birds on T₄ having a

higher ($p<0.05$) value from those fed other treatments. Triglyceride, Cholesterol, Albumin, Uric Acid, Urea, Very Low Density Lipoprotein (VLDL), Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL) were not significantly influenced ($p>0.05$) by the dietary treatments.

Table 3. Serum biochemistry of broilers fed of low crude protein supplemented with varying levels of lysine

Parameter	T ₁	T ₂	T ₃	T ₄	T ₅	SEM
Triglyceride (mg/dl)	50.4	58.3	80.4	54.8	54.8	4.91
Cholesterol (mg/dl)	74.2	73.2	94.8	103	79.3	5.68
Total Protein(g/dl)	2.47 ^b	2.63 ^b	2.63 ^b	3.83 ^a	2.61 ^b	0.17
Albumin (g/dl)	1.44	1.35	1.58	1.37	1.83	0.09
Globulin (mg/dl)	1.03 ^b	1.28 ^b	1.05 ^b	2.45 ^a	0.78 ^b	0.19
Uric Acid (mg/dl)	8.89	20	10.1	28.9	25.6	3.37
Urea (mg/dl)	0	0.74	0	1.28	1.11	0.34
VLDL (mg/dl)	10.1	11.7	16.1	11	11	0.98
HDL (mg/dl)	67.1	87	92.7	92.4	70.3	4.73
LDL mg/dl	17.1	2.14	18.2	21.3	20	4.38

^{a,b} means the superscript differs significantly at ($p<0.05$) within the same row

SEM=standard error of mean, ADG= Average Daily Gain, ADFI=Average Daily Feed Intake, VLDL=Very Low Density Lipoprotein, HDL=High density lipoprotein, LDL=Low Density Lipoprotein

Organs weight

The relative weights of the organs and abdominal fat were expressed as percentage of their live weights is as shown in **Table 4**. There were no significant differences ($P>0.05$)

in the weights of the liver, kidney, lungs, whole gizzard, empty gizzard, pancreas, proventriculus, spleen and gastrointestinal tract (GIT) except for the heart and abdominal fat ($P<0.05$) with no definite pattern.

Table 4. Relative values of organs of broilers fed low crude protein diet supplemented with varying levels of lysine

Parameters (%)	T ₁	T ₂	T ₃	T ₄	T ₅	P.value	SEM	R ² value
Liver	2.50	2.63	2.27	2.30	2.28	0.90	8.12	0.09
Kidney	0.44	0.45	0.35	0.52	0.46	0.53	0.03	0.25
Lungs	0.49	0.56	0.52	0.40	0.55	0.64	0.03	0.25
Heart	0.53 ^a	0.43 ^b	0.37 ^b	0.56 ^a	0.41 ^b	0.01	0.02	0.74
Whole gizzard	2.40	2.74	2.60	2.40	2.40	0.48	0.07	0.27
Empty gizzard	1.57	1.64	1.66	1.48	1.57	0.72	0.04	0.17
Pancreas	0.28	0.25	0.21	0.24	0.23	0.86	0.02	0.11
Proventriculus	0.40	0.39	0.32	0.37	0.41	0.51	0.02	0.26
Spleen	0.13	0.12	0.11	0.10	0.09	0.56	0.01	0.24
Abdominal Fat	1.02 ^b	1.93 ^{ab}	2.42 ^a	2.30 ^a	1.78 ^{ab}	0.08	0.18	0.53
GIT	13.9	16.2	15.1	14.6	15.0	0.58	0.41	0.23

^{a,b} means the superscript differs significantly at ($p<0.05$) within the same row

SEM; standard error of mean, ADG= Average Daily Gain, ADFI=Average Daily Feed Intake, GIT: Gastrointestinal Tract

Carcass yield

Carcass yield of broiler chickens fed low protein diets supplemented with lysine is presented in **Table 5**. The dietary treatments had no significant ($P>0.05$) effect on

drumstick, thigh, wings, neck and shank, while the breast and back were significantly influenced ($P<0.05$). The breast was increasing as the lysine inclusion increases but reached a plateau at T₄ while that of the back kept increasing.

Table 5. Carcass yield of broiler finishers fed low crude protein diets supplemented with lysine

Parameters %	T ₁	T ₂	T ₃	T ₄	T ₅	P.value	SEM	R ² value
Drumstick	10.8	10.7	10.1	9.35	9.34	0.17	0.25	0.45
Thigh	11.4	10.6	11.7	10.5	10.7	0.16	0.18	0.46
Wings	9.13	8.21	9.24	8.93	9.58	0.44	0.23	0.29
Breast	23 ^{ab}	16.5 ^b	19.9 ^{ab}	25.2 ^a	20.6 ^{ab}	0.09	1.08	0.52
Neck	5.41	5.19	5.76	5.03	5.27	0.45	0.12	0.28
Back	11.3 ^b	11.3 ^b	12.0 ^{ab}	12.1 ^{ab}	13.2 ^a	0.16	0.28	0.46
Shank	4.10	4.47	4.04	3.78	3.83	0.70	0.16	0.18

^{a-b}: along the same row with different superscript are significantly different (P<0.05)

Villi morphometrics

Table 6 shows that lysine supplementation of LCP diets had significant effect (P<0.05) on villi height, villi width, cryptal depth and muscle thickness. Birds fed T₄ had higher values for the observed jejunum characteristics except for villi width which was highest at T₅ (148.08) The villi height of the broiler fed T₄ (1167.87) was from those fed treatments 1, 2, 3

and 5 which had 561.61, 758.53, 614.37 and 659.75 respectively. The villi width of birds fed T₅ (148.04) had a higher value compared to those fed other treatments and notably as the lysine supplementation increases within the LCP diets the villi width increases. For the cryptal depth, birds on T₄ had a higher value than the others and this is similar to what was observed for the muscle thickness.

Table 6. Small intestine characteristics (Villi morphometric) of broilers fed low crude protein diet

Parameters	T ₁	T ₂	T ₃	T ₄	T ₅	SEM
Villi height (µm)	562 ^b	759 ^b	614 ^b	1168 ^a	660 ^b	64.8
Villi width (µm)	126.7 ^{ab}	104 ^b	107 ^b	116 ^{ab}	148 ^a	6.27
Cryptal depth (µm)	230 ^{bc}	153 ^c	392 ^b	598 ^a	177 ^c	50.7
Muscle Thickness (µm)	71.5 ^{ab}	86.0 ^{ab}	68.3 ^{ab}	92.3 ^a	62.5 ^b	4.23

^{a-b}: along the same row with different superscript are significantly different (P<0.05)

SEM: Standard Error of Mean

DISCUSSION

Blood parameters

Blood parameters comprises of the hematological parameters as well as the serum biochemistry of the birds. The serum biochemistry is routinely used for detection of organ diseases in domestic mammals (26). It has been reported that serum biochemical constituents positively correlate with the quality of the diet (27). The non significant difference observed in most blood parameters agrees with the report of (28) and (13) which stated that lysine supplementation in feed of poultry had no effect on hematological parameters. However, hematological parameters obtained from this study suggest that dietary lysine supplementation has no deleterious effect on some physiological parameters of broiler chicken since hematological parameters fell within the normal range as reported by (29 - 30). The significant response in Total protein and Globulin observed in this study are within the normal physiological values of chicken (31). Elevated total protein and globulin in birds fed T₄ could indicate improved AA economy (32). The lower and non-significant cholesterol levels observed in this study are an indicator of health benefit of incorporating lysine to broiler chicken diets.

Organs Weight

The weight of organs in broilers according to (33) reflects the anatomical response of birds to the type of diet consumed. The percentage organs weight (liver, kidney, lungs, gizzard, pancreas, Proventriculus, spleen and GIT) were not significantly different (P>0.05). The non significant weight observed in most of the internal organs measured in this study in response to feeding low crude protein diet supplemented with varying level of lysine to broilers is an indication that such levels are tolerable by the broiler chicks. Liver, kidney, spleen, lungs, gizzard, pancreas, proventriculus and GIT weights were generally similar (P>0.05) for treated and control groups or higher for treatment groups compared to control showing that low crude protein diet supplemented with lysine was not detrimental to the birds. This was in agreement with (34), who reported that dressing percentage, liver, heart, spleen, gizzard, small and large intestine weights were generally similar for treated and control groups or higher for test groups compared to control showing that *Gongronema latifolium* inclusion in the diets was not detrimental to the birds.

The weight of the fat of broilers in the test groups (treatments 2, 3, 4 and 5) had higher

values and were significantly different from the control group. The observed result was in agreement with (35) who stated that decreasing dietary protein increased abdominal fat significantly ($P < 0.05$). The significantly lower heart weight in groups fed treatments 2, 3 and 5 may be as a result of reduced cardiac activities. This result contradicts that of (34), who observed that, there was significantly larger heart associated with test birds that resulted from the inclusion of *G. latifolium* in the diets.

Small Intestine

The villi height, villi width, cryptal depth and muscle thickness of the jejunum were significantly affected by the dietary treatment. This was in consonance with the findings of (36), who reported that villi height and width along with the number of crypts per the microscopic field of broilers fed organic acids or antibiotic growth promoter were differed between the control and the treated group.

CONCLUSION

It can be concluded from the results observed that birds fed 16.6% CP and 0.8% lysine inclusion level had improved feed quality, increased abdominal fat, increased villi morphology and enhanced breast yield due to enhanced absorption.

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