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Research Article

Digestive Enzyme Kinetic, Health Status and Gut Morphology of Boiler Finisher Fed Varied Dietary Levels of Protein

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Abstract

In formulating a broiler's diet, importance attention is given to protein utilization, because it's one of the major cost components of the broiler diets second to energy and has major effect on growth performance. Information on effect of varied dietary protein levels on nutrient digestibility, blood profile and enzyme activities-(EA) of broiler chickens-(BC) is limited. Therefore this research was designed to evaluate the health status and EA of BC fed different dietary protein levels. One-hundred and eighty (180), one-day-old strain of Arbor Acre were used for the research using a completely randomised design. Broiler starter diets of 3164, 3131.02 and 3056.88ME (Kcal/kg) and finisher diets of 3229.40, 3218.36 and 3121.47ME (Kcal/kg), respectively for treatments 1–3 were used with different protein levels of 20%, 23% and 26% for starter and 17%, 20% and 23% for finisher, respectively for treatments 1, 2 and 3. Each treatment was replicated thrice with 20 birds/replicate. Twenty-four birds were sacrificed from each treatment and crude fibre digestibility, digestive lipase, amylase activity-AA and protease activity-PA were determined using standard procedures. Data were subjected to ANOVA at α0.05. The result shows increased in average villus surface area-(AVSA) from $2.3x10^4$±882.9 µm2 (T1) to $2.8x10^4$±728.36µm2 (T3), this shows the important of protein the on activity of gut enterocyte. The AA in birds fed T2 (46.6±21.2 nm/min) was significantly higher than 25.4±19.5 nm/min (T1) and 23.32±2.9 nm/min (T3), meaning that saturation has been reach where additional increase did not yield any positive EA. Protease activity was significantly higher in T2–fed broilers (4.4±0.7 Unit/mL) compared with 4.3±1.0 Unit/mL and 3.1±1.8 Unit/mL in T1 and T3–fed birds, respectively. Dietary crude protein at 20% fed to broilers finisher improved amylase, PA and AVSA of the gut increased with increase in the dietary crude protein inclusion. Dietary crude protein is not only having effect on growth but also determines the activity of digestive enzyme and gut morphology.

Keywords: Enzyme activity, Digestive enzyme, Protease, Amylase, Lipase.

Introduction

Nutrient management is one of the major practices in animal science to supply the required nutrient for growth and development of animal without wastage since everything given to the animal is a product of cost. According to [1] nutrient management is of major concern for today's modern poultry enterprise because feed represents the greatest single expenditure associated with poultry production. Greater important emphases is given to accuracy in feeding which helps the farmer to reduce cost of feeding, reduce or eliminate wastage and improve economic efficiency of poultry enterprises. In formulating a broiler's diet, major attention is given to balanced dietary protein and amino acids (AA) because it is one

of the major cost components of the poultry diets second to energy and has major effect on growth performance [1] and gut activity [2]. Therefore with improvement in the breeding and genetics to obtain broiler of table size within the shortest time possible, there is need for continuous research on the crude protein requirement of broiler chickens to maximize the nutrient utilization.

Formulating feeding formulae that will promote adequate required growth rate become necessary. Long ago [3] submitted the need for developing feeding programs that utilize concepts such as ideal protein, formulation programs that calculate the ingredient combinations that will closely meet the bird's nutritional requirements at the least possible cost. This will enable cost effectiveness and assurance of reducing or eliminating the wastage of essential nutritional component in broiler production. Gut has a lot to do with the utilization of well formulated feed and performs greatly in converting feed ingredients to the nutrients the animal needs.

An understanding of the gastrointestinal tract (GIT) or gut as it is commonly known is imperative for the nutritionist [4]. Maintaining a healthy GI is one of the core issues in broiler nutrition as it impacts on bird health, field performance (nutrient utilization), bird welfare and ultimately profitability. It also has to do with secretion of enzymes that help to digest the feed ingredients such as protease, amylase, and lipase. Enzymes are macromolecular biological catalysts. Enzyme action is the moles of substrate converted per unit time and is equal to rate multiply by reaction volume. Enzyme activity is a measurement of the quantity of active enzyme present and is thus dependent on condition. According to [5] the major fuel of the body are carbohydrate, fat and protein. These are obtained from the diets and stored in the body's fuel deport. Not only is the gut responsible for assimilating the various dietary components and ensuring their absorption, it is also the most important route of entry for antigens such as food proteins, natural toxins and invading pathogens.

This GIT plays significant role in making nutrient available to the animal via digestive process which includes prehension, feed digestion, enzyme activities, nutrient absorptions and usage. The intestine is the body's most crucial immune system because, roughly sixty percent defence of the body cells are exhibited via the enteric mucosa [6]. Only healthy digestive tract enhances genetic potential of the birds [2]. Despite the fact that the GIT is the source of nutrient across all part of the body, it is still the main user of nutrient especially when the gut is challenged with gastrointestinal diseases which can leads to indigestion of feed ingredient under severe condition.

The occurrence of undigested feed particle may not be easily noticed in broiler chickens because they are most of the time raised on deep litter but a careful look at their dropping can clearly show the presence of undigested feed. It is a sign of intestinal problem [7] and faulty enzymes' activities which need to be addressed by the farmer to eliminate wastage as a result of indigestion. Indigestion is a dangerous threat to feed efficiency. Farmers are encouraged to guide against the occurrence since it affected the feed conversion ratio of the animal. Some of the reasons for these are diseased proventriculus, it has been documented according to [8] that disease of proventriculus and ventriculus can slow crop emptying, lead to weight loss and passage of undigested faeces in broiler chickens. Fibre assists in increasing intestinal movement by enhancing the bulkiness of faeces by this indirectly speed up the digesta out of the GIT. Also, Celiac disease, occurs when the animal cannot digest the protein known as gluten found in some grains such as wheat, barley while pancreatitis is the inflammation of an organ in between the duodenum of the broiler chickens. Pancreas is known to secret digestive juices which help the animal to break down the feed, inflammation of such organ will affect the secretion of natural products and the feed digestion will be affected greatly. Trypsin inhibitor, trypsin inhibitors are secondary metabolite. Many are found in the feed ingredients such as soybeans meal. According to [9] safe levels is less than 3mg/g of the feed. Therefore this research work was designed to evaluate growth performance, health status and enzyme activities of boiler finisher fed varied dietary levels of proteins.

Materials and Methods

Experimental Site: The research was conducted at the Poultry Unit of Ladoke Akintola University, Ogbomosho. Ogbomoso is on longitude 4o5' East of the Greenwich Meridian and latitude 8o7 North of the Equator in the derived savannah zone of Nigeria [10].

Pre-experimental operations: The general preparation before the arrival of the broiler chicks were pen cleaning, this cleaning process involved sweeping, washing, fumigant application, removal of unused wood attached to the pen, fixing and partitioning of broken and rusted nets respectively. The feeders and drinkers were also washed and disinfected while wood shaving used as litter was purchased for brooding.

Animals Managements: Broiler chicks totalling one-hundred and eighty, one-day-old strain of Arbor Acre obtained from a reputable hatchery were used for the research using a completely randomised design. Broiler starter diets of 3164, 3131.02 and 3056.88ME (Kcal/kg) and finisher diets of 3229.40, 3218.36 and 3121.47ME (Kcal/kg), respectively for treatments 1 – 3 were used with different protein levels of twenty, twenty-three and twenty-six% for starter and 17%, 20% and 23% for finisher, respectively for treatments 1, 2 and 3.

Routine Animal Handling: Routine management practices during the research period include the daily supply of fresh feed and water to ensure ad libitum feeding. Vaccines were administered at prescribed intervals based on the prevailing schedule of vaccination in the study area.

Collection of Data: The following data were collected during

the research periods:

Weekly weight assessments: This was done on every week over the periods of the study. At the beginning of the experiment, record of initial weights was recorded for each chick. Weekly growth was calculated as the weight differences between present week and previous week weight using sensitive digital scale. This was mathematically evinced as follows: Weekly weight differences = (Current week weight – Previous week weighs) g.

Calculation of Feed Intake: The feed intake was calculated by measurement of the quantity of feed supplied and the leftover feed. Mathematically as: Total feed Intake = (Feed supply – leftover feed). Feed to Gain Ratio: Computed as total feed intake divided by the total weight gained i.e. Feed conversion ratio = $\frac{\text{Sum of Feed Intake}}{\text{Total Weight Gain}}$

Blood Collection and Evaluation: Bloods used were obtained from the vena jugularis of six broilers per group at the end of the research into bottles containing EDTA for haematological parameters assessment and that of serum bottles were without EDTA. Packed cell volume (PCV) and haemoglobin (Hb) were obtained using Micro-Haematocrit method and Cyan Meth-Haemoglobin methods respectively as described by [11]. Total serum protein was obtained using Biuret method accounted by [12]. Albumin was analyzed using Bromocresol green method as described by [13]. RBC and WBC were determined using the method accounted by [14]. Cholesterol in the serum was determined as reported by [15] while aspartate amino acid transaminase (AST), Alanine amino acid transaminase (ALT) and Alanine phosphatase activities were determined using the method of [16].

Enzymes Activities Procedure:

Amylase Activity

The method of [17,18] was used in the determination of serum amylase activity based on the enzymatic hydrolysis of starch into maltose and other reducing sugars by the amylase enzyme present in the blood serum, after which the remaining unhydrolyzed starch was allow to react with iodine to form a blue-colored complex measurable spectrophotometrically.

The assay utilized soluble starch substrate (soluble starch substrate: prepared by dissolving 1 g soluble starch in 100 ml distilled water, heating gently with continuous stirring until complete dissolution and allowing the solution to cool to room temperature) and phosphate buffer (phosphate buffer solution pH 6.9: prepared by dissolving 6.8 g potassium dihydrogen phosphate and adjusting the pH with 0.1 N sodium hydroxide before making the final volume to 1 L with distilled water).

The color reagent was iodine reagent (iodine reagent: prepared by dissolving 0.5 g iodine crystals and 5 g potassium iodide in distilled water and diluting to 100 ml). During the assay, 0.5 ml of soluble starch substrate was mixed with 0.5 ml phosphate buffer in a clean test tube and the mixture pre-incubated at 37 °C in a water bath for approximately 5 minutes. Subsequently, 0.1 ml of the serum sample was added to initiate the enzymatic reaction and the mixture was incubated at 37 °C for 10 minutes to allow starch hydrolysis. At the end of the incubation period, 1 ml iodine reagent was added to terminate the reaction and to react with the residual starch to produce a colored complex.

The absorbance of the reaction mixture was then measured at 620 nm using a visible spectrophotometer, with distilled water serving as the blank, and the decrease in color intensity was taken to be proportional to the activity of amylase present in the serum sample.

Lipase Activity

The determination of serum lipase activity was based on the enzymatic hydrolysis of triglycerides into glycerol and free fatty acids catalyzed by lipase present in the serum sample, after which the liberated fatty acids was quantified through neutralization with sodium hydroxide according to [19]. The assay employ an olive oil triglyceride substrate (olive oil substrate emulsion: prepared by mixing 10 ml olive oil with 90 ml distilled water containing 2 g gum arabic and homogenizing thoroughly to produce a stable emulsion) and phosphate buffer solution (phosphate buffer pH 7.5: prepared by dissolving 8.5 g disodium hydrogen phosphate and 6.8 g potassium dihydrogen phosphate in distilled water and making the final volume up to 1 L). Phenolphthalein indicator solution (phenolphthalein indicator: prepared by dissolving 0.1 g phenolphthalein in 100 ml of 95 % ethanol) and sodium hydroxide titrant (0.05 N sodium hydroxide solution: prepared by dissolving 2 g sodium hydroxide pellets in distilled water and diluting to 1 L) was used for titrimetric determination.

In the assay procedure, 5 ml of the olive oil substrate emulsion were mixed with 2 ml phosphate buffer in a conical flask and equilibrated at 37 °C for 5 minutes in a water bath. Thereafter, 0.5 ml serum sample was added and the mixture incubated at 37 °C for 30 minutes to allow lipase-mediated hydrolysis of triglycerides. After incubation, three drops of phenolphthalein indicator were added and the liberated fatty acids were titrated with 0.05 N sodium hydroxide solution until a faint pink color persists for approximately 30 seconds. The volume of sodium hydroxide consumed was assumed to correspond to the amounts of fatty acids released and was used to calculate lipase activity expressed in enzyme units per liter of serum.

Protease Activity

The determination of protease activity was based on the hydrolysis of protein substrates by proteolytic enzymes present in the serum, resulting in the release of amino acids such as tyrosine which was subsequently react with Folin–Ciocalteu reagent to produce a blue-colored complex measurable spectrophotometrically.

The assay utilize casein substrate (1 % casein reagent: prepared by dissolving 1 g casein powder in 100 ml phosphate buffer and gently heating the solution to approximately 40 °C with continuous stirring until complete dissolution occurs) and Tris buffer solution (Tris buffer pH 7.8: prepared by dissolving 6.05 g Tris-hydroxymethyl aminomethane in distilled water, adjusting the pH with dilute hydrochloric acid and making the volume up to 1 L). Protein precipitation reagent consists of trichloroacetic acid solution (10 % TCA solution: prepared by dissolving 10 g trichloroacetic acid in distilled water and diluting to 100 ml). The colour development reagent was Folin–Ciocalteu reagent (Folin reagent: prepared by diluting commercial Folin–Ciocalteu reagent with distilled water in a ratio of 1:1 before use). In the assay procedure, 1 ml of the 1 % casein reagent was mixed with 1 ml Tris buffer in a test tube and pre-incubated at 37 °C. Subsequently, 0.2 ml serum sample was added and the mixture incubated at 37 °C for 20 minutes to allow enzymatic hydrolysis of casein by serum protease.

The reaction was then terminated by adding 2 ml of 10 % TCA solution which will precipitate unhydrolyzed protein. The mixture was centrifuged at 4000 rpm for 10 minutes and 1 ml of the resulting supernatant transferred into a clean test tube. Thereafter, 5 ml alkaline sodium carbonate solution (alkaline sodium carbonate solution: prepared by dissolving 2 g sodium carbonate in 100 ml distilled water) was added followed by 0.5 ml diluted Folin reagent.

The mixture was allowed to stand for 30 minutes to allow development of a blue coloration and the absorbance measured at 660 nm using a spectrophotometer, where the intensity of the colour correspond to the quantity of tyrosine released and therefore represent protease activity in the serum sample.

Coagulation Time Procedures: 1ml blood was placed in the test tube. Stopwatch was on, immediately as soon as the blood is dropped in the test tube and the test tube place on the stand at 37^0C. At 3 minutes, test tube was tilted at 45^0 angles. This was repeated every 30 seconds until the test tube can be completely inverted without spilling the content [20].

Gut Preparation and Assessment: A total of twenty-four broilers each, five from each treatment on week 8 were selected and subluxated for histological assessment, five-centimetre portion of small intestine (jejunum) was harvested. These 5cm jejunal cuts were preserved in “Bouins fluid” [21] for twenty-four hours, dehydrated in different ascension grade of alcohol concentration (70%, 80%, 90%, 95%, 100%), xylene was used to cleared, infiltrates in paraffin, embedded in paraffin and finally the paraffin-blocks were cut at 5µm thick using sliding microtone as described by [22]. After cuttings, the portions were floated on slide using an egg albumin and dried on slide warmer. The sections were later stained with standard haematoxylin and eosin stain [21] for the overall histological assessment.

Jejuna Mucosal Development Assessment

The jejunal mucosa histological carried out according to [23]. The portions were analysed using a light microscope. The length/height and breadth of the villus were evaluated with the help of computer enabled image analysis. Fifteen intact, properly pointed crypt villus portions were selectively randomly picked for all the samples. Average readings calculated for each chicken were employed in the analysis. Villi heights were determined beginning at the top of the villi down the crypt and villi junction, also the crypt depths were described as the invagination depth between adjacent villi [24]. Villi width of villus was described as the space from the outer epithelial boundary along a line extending via the vertical midpoint of the villi. Villus average areas were estimated from the height of villi and width of it at one-half height.

Effect of Villi on Surface Area of GIT: In this study two dimensional images were used to determine the consequence of villi on the surface area of the intestine. The linear measurements along the edge of two villi were done to compare the length of villi to the length along the wall of intestine. The increase in length of villi along the surface is also an improvement in the surface area of the GIT. The radius and the height of villus of the small intestine were measured along the surface as a linear measurement.

The surface area of the villus is equal to $2\pi rh$

The area beneath the villus was calculated using πr^2

The radius of the villus was assumed to be villus width divided by 2 and $\pi = 3.142$.

Digestibility Trial: At 8 weeks, two broilers per replicate were taken to digestibility cage for digestibility study. The broilers were allowed three-day acclimatization period after which their total feed intake and feacal output were collected for three days, oven dried, weighed and stored in plastic bags. The sample were bulked together, ground and sub samples were taken for proximate composition. The formula below was used to calculate the nutrient digestibility:

Apparent Digestibility=

$$\frac{(\%\text{Nutrient in the feed XFI}) - (\%\text{ Nutrient in the faeces XFO}) X\ 100}{\%\text{Nutrient in the feed X Feed Intake}}$$

FI =Feed intake, FO = Faecal output

Proximate Analysis

Proximate analysis was done as described by [25]

Results and Discussion

The table 1 shows the growth parameter of broiler chickens fed varied levels of dietary protein at finisher phase. Except average daily feed consumption and average total feed consumption, other parameters measured were affected ($p<0.005$) and the best FCR (2.14) was obtained under treatment 2 (20%CP). Treatments 1 and 2 (17 and 20% CP) had similar ($p>0.005$) final weight and higher weight com-

pared to the final weight of birds fed on 23% CP. Twenty percent crude protein had numerically higher final weight gain when compared to treatment one (17%CP). Price per kilogram weight gain attained high cost with increase crude protein levels and treatment 3 (23%CP diet at finisher phase) recorded significantly higher (N273.93) cost per kilogram weight gain. Higher protein level above recommended value at finisher phase did not produce additional growth benefit in the birds (Table 1). The result was in conformity with the report of [26] that feed with more than required protein levels always results into additional expenses, increased nitrogenous excretions and at times retarded growth. Feeding different levels of crude protein to broiler finisher did not affect the dry matter intake (g/d) differently, although there were numerical variations and broiler fed 23% CP had the highest (96.43g/day) feed intake. That means that the crude protein might have influenced the additional intake, this was in accordance with the account of [27,28] that crude protein levels of the feed often times had to do with the positive dry matter intake of the animal. The result is contrary to the report of [29] who stated that chicks fed low crude protein diets gained less weight, consumed more feed, and utilized the feed less efficiently compared with chicks fed the control diet.

Parametric quantity	T1(17%CP)	T2 (20%CP)	T3 (23%CP)	SEM
Avg Initial Weight (g)	42.58	42.58	42.33	-
Avg Total Feed Intake (g)	5364.22	5292.44	5400.28	53.53
Avg Daily Feed Intake (g)	95.78	92.72	96.43	0.96
Final Weight Gain (g)	2400.00^{a}	2433.33^{a}	2266.67^{b}	2.73
FCR	2.25^{b}	2.14^{b}	2.39^{a}	0.03
Cost/Kg Feed (N/kg)	99.84^{c}	106.87^{b}	114.84^{a}	1.20
Cost/Kg Weight Gain (N/kg)	224.18^{b}	228.41^{b}	273.93^{a}	5.39

Different superscripts (a,b) connote significant means ($p<0.05$). CP= Raw protein, SEM = standard error of mean, FCR = feed conversion ratio, Avg = Average, CP= crude protein.

Table 1: Growth Parameters of Broiler Chickens raised on different Level of Protein (Finisher phase)

Nutrient Digestibility of Broiler Chickens Fed different Levels of Protein (Finisher Phase)

The table 2 shows the nutrient digestibility of broiler chickens raised on different levels of dietary protein at finisher phase. The nutrient digestibility was significantly affected across the treatments except crude protein and ether extract digestibilities. Treatment 1 (lower CP inclusion: 17%CP) had the highest ash, dry matter and NFE digestibilities while 20% and 23% CP recorded similar nutrient digestibility. Fibre digestibility was high when compared with average prediction of [30]. The authors reported range of fibre digestibility to be between 10.96 and 11.44% in various experiment and reported 11.24% average in all the trials when rice bran was treated differently in broiler feeding trial. High nutrient digestibility under birds fed with lower crude protein may be because the birds attempt to meet up with the nutrients requirement for growth and development. Also digestibility of feed may be as a result of age effect of the chickens because as the bird grows there is an increase in gastro intestinal mucosal nutrient transporters which eases improved nutrient uptake by growing birds [31] and also, the digestibility of the nutrients by the birds may be influenced by the nutritive content of the ingredients. The observed higher digestibility of DM, CF and EE may be because of more dietary feed intake. It has been reported that increase dietary feed intake resulted in a quicker rate of passage of digesta [32]

Parametric quantity (%)	17.00%CP	20.00%CP	23.00%CP	SEM	SD±
Raw protein	69.22	71.01	70.34	1.83	1.54
Ether Extract	88.55	83.93	87.73	0.56	2.98
Crude Fibre	57.64^{a}	37.11^{b}	52.36^{a}	2.32	1.06
Ash	70.84^{a}	59.47^{b}	62.23^{b}	1.41	7.37
Dry matter	82.33^{a}	72.90^{b}	76.17^{b}	1.05	5.46
NFE	86.02^{a}	78.98^{b}	77.12^{b}	0.98	5.11

Different superscripts (^{a,b}) connote significant means ($p<0.05$). CP= crude protein, NFE = Nitrogen free extract.

Table 2: Apparent Nutrient Digestibility of Broiler Chickens fed on Different Levels of Protein (Finisher Phase)

Haematological characteristic of the broiler chickens fed different levels of dietary protein (Finisher)

The table 3 shows the haematological responses of broiler fed different levels of protein at finisher stage. Apart from WBC count, monocytes and platelet, other parameters measured were not significantly ($p>0.005$) affected by the different levels of protein in the diet. White blood cells decrease with increase in crude protein levels. It ranges from $15.45x10^4$ /L (23% CP) to $20.28 x10^4$/L (17%CP). Also, platelet ($x10^4L$) record were 26.07, 22.21 and 17.22 respectively for treatments 1, 2 and 3. There was numerical differences in platelet functionality and 17% CP recorded the least time (1.30 minutes) while treatments 2 and 3 had similar ($p>0.05$) clotting time of 2.33 minutes. The Hb and PCV of chickens at finisher phase were within the suggested value range and according to [11] they are indicators of erythrocytic normalcy and general wellbeing of the birds [33]. Non-significant variations in the measured parameters therefore suggest an optimal nutrient availability for the broiler regardless of dietary protein levels. The most important parameter according to [34] is bird's haemoglobin concentration. Variation suggested abnormality in the oxygen-carrying capability of the blood. Concentration of blood total haemoglobin most suitably speculates the potential of birds to fulfil its oxygen requirements. Varied protein levels at finisher phase did not affect the Hb and RBC and this indicated equal and sufficient haematopoiesis. According to [35], the improvement in RBC and haemoglobin concentration indicates quality of the sound haematopoiesis promoting ability. As a result, this increased densities of haemoglobin improve aerophilic capability, on the other hand, reduced values are linked to inadequate states of regenerative/non-regenerative anaemia. In birds, regenerative anaemia is always as a result of haemorrhage and haemolysis, many times ascribed to parasites, wounds [36] and physiological state produced by a poison or other toxic substance [37]. For this type of anaemia, the decreases in blood oxygen-containing potentials generally lead to better erythropoiesis, leading to the fast yield of more amounts of young red blood cells (reticulocytes). In contrast, non-regenerative anaemia (without improve quick response of bone marrow to increase need for erythrocytes) is accelerated by chronic diseases, this can also be as a result of nutritional challenges or starving [38]

Parametric quantity	17.00%CP	20.00%CP	23.00%CP	SEM	SD±
PCV (%)	22.00	22.00	22.22	0.25	1.28
Hb (g/dL)	8.96	8.70	9.36	0.22	1.17
MCV (µ3)	84.65	75.72	73.39	3.49	18.16
MCHC (%)	40.69	39.51	40.28	0.83	4.29
MCH (µµg)	33.99	29.94	29.37	1.30	6.76
RBC (X106L)	3.72	3.01	3.26	9.33	4.50
WBC (X104 /L)	20.28^a	17.41^{ab}	15.45^b	0.71	3.69
Lymphocytes (%)	59.33	59.44	61.56	1.80	9.37
Heterophils (%)	32.11	32.00	32.22	1.62	8.41
Monocytes (%)	3.33^a	2.33^{ab}	2.22^b	0.22	1.15
Eosinophils (%)	3.11	3.67	2.75	0.24	1.20
Basophils (%)	0.00	0.33	0.00	0.08	0.42
Platelet (x104L)	26.07^a	22.21^{ab}	17.22^b	1.25	6.57
*Platelet function (Minute)	1.30	2.33	2.33	0.26	1.14

Different superscripts (a,b) imply significant means ($p<0.05$). *time taking for blood to clot. CP= crude protein.

Table 3: Haematological parametric quantity of the broiler raised on dissimilar levels of protein (Finisher Phase)

Serum responses of the broiler chickens fed different levels of dietary protein (Finisher)

Serum responses of broiler chickens fed different levels of dietary protein at finisher stage. Apart from creatinine other parameters examined were not significantly affected with the different levels of protein in the diet. The lowest crude protein had the least creatinine value (2.44g/dL) compared to others with had statistically similar creatinine values. Cholesterol had numerical decrease with the increase in crude protein levels. Similarity in all the liver enzymes measured indicates the normality of the liver. Liver has potential to purify harmful chemicals and break them down into harmless chemicals. The protein levels did not affect the liver to synthesize the blood protein which includes albumin and coagulating protein. According to [39] normal liver helps to synthesize and excretes bile necessary for digestion and absorption of fats and vitamins, also helps to store sugar, fats and vitamins. The author also established excessive aspartate amino-

transferase (AST) and alanine aminotransferase (ALT) due to faulty liver. The cholesterol data from this study normalise the normal state of the liver in having ability to ameliorate the production of cholesterol. Creatinine is a nitrogenous waste product that is conveyed through the blood to the kidney where it is eliminated through the urine. Variations in creatinine levels in the blood are related to excretion in the blood therefore reflect kidney function. This might be connected to damage kidney as a result of infection, poor circulation, reduced blood flow or dehydration, although [40] has concluded that the effect of protein on creatinine level is uncertain.

Parametric quantity	17.00%CP	20.00%CP	23.00%CP	SEM	SD±
Total protein (g/dL)	2.59	2.36	2.52	0.06	0.34
Globulin (g/dL)	1.45	1.41	1.41	0.05	0.26
Albumin (g/dL)	1.14	0.95	1.11	0.07	0.34
AST (IU/L)	20.82	18.73	18.79	1.11	5.74
ALT (IU/L)	2.61	3.01	2.96	0.37	1.92
Cholesterol (mg/dL)	100.33	99.33	98.72	1.57	3.77
Creatinine (g/dL)	2.44b	4.55a	3.14a[b]	0.39	2.07

Different superscripts (a,b) connote significant means (p<0.05). *time taking for blood to clot, CP= crude protein.
Table 4: Serum Evaluation Results of broiler raised on different levels of protein (Finisher Phase)

Enzyme activity of broiler chickens raised on different levels of protein (Finisher phase)

Table 5 indicates the enzyme activities of broiler chickens raised on different levels of protein at finisher stage. Apart from lipase other two major digestive enzymes evaluated were significantly affected by the protein levels in the diet. The highest dietary protein level recorded the least values for both amylase (23.32nm/min) and protease (3.14unit/mL) compared to 20% CP (standard diet) which recorded the highest enzyme activities (amylase: 46.64 nm/min and protease: 4.39 unit/mL). T2 (20% CP) had 46.64 µ/l and T1 (17% CP) had 25.44 µ/l amylase activity. There was increase in the value of amylase activity recorded at T1 to T2 (25.44 - 46.64 µ/l) and decrease value at T3 (23% CP) with the value of 23.32 µ/l. Enzymes are macromolecular biological catalysts and their action is the moles of substrate converted per unit time and is equal to rate multiply by reaction volume. Enzyme activity is a measurement of the quantity of active enzyme present and is thus dependent on condition. According to [5] the major fuel of the body are carbohydrate, fat and protein. These are obtained from the diets and stored in the body's fuel deport. This result (Table 5) is in agreement with [41] that an increase amount of substrate tends to enhance the rate of reaction with enzymes, nevertheless the moment it passes a certain target, the activities will level out because the number of binding sites useable is fixed. This means that nutrient saturation may reach a stage, which the additional unit of nutrient may not bring or yield additional digestion/benefit to the animal. Indigestible underlying anti-nutritional components, like trypsin inhibitors, glucosinolates and phytic acid substances can enhance gut contents viscosity, affect activities of enzyme of digestion and negatively impair the rate of growth in poultry [42]. The authors described proteases as proteolytic enzymes that do not interfere with protein digestion alone but also improve the digestive ability of other nutrients. Researches in non monogastric animal have also indicated the proteases ability to interrupt cell wall linked proteins, which improves the ability of microbes to utilize substrate [43].

Parametric quantity	17%CP	20%CP	23%CP	SEM	SD±
Amylase (nm/min)	25.44[b]	46.64[a]	23.32[b]	3.94	20.49
Lipase (IU/L)	193.33	193.33	241.67	14.13	73.42
Protease (Unit/mL)	4.29[a]	4.39[a]	3.14[b]	0.11	0.66

Different superscripts (a,b) connote significant means (p<0.05).
Table 5: Enzyme activity of broiler Raised on Dissimilar levels of protein (Finisher Phase)

Gut Histomrphometric Responses of Broiler Raised on Different Protein Levels (Finisher)

Table 5 demonstrates the gut tissue morphometric response of broilers fed varied protein levels and all the indices evaluated were significantly (p<0.005) different. Day old readings were smaller when compared to other at finisher phase, this might be as a result of continuous increase in the demand for nutrient as the chicks grows. Average villus surface area

increases numerically as the dietary protein level increases and 23% crude protein had significantly higher average villus surface area. Villus width (µm) decreases with increase dietary crude protein. Tissue morphometric analysis is observed to be a major parametric quality in studies on gastrointestinal patho-physiology [44]. Twenty three per cent protein level induced the broadest gut surface area, this might be as a result of the birds trying to ameliorate the area of absorptive surface, to effectively utilise the available useable protein available in the feed. This is complementary atrophy, to modify the broiler capability to get the required nutrients. It appears to be a composite associative relation between gut intestinal integrity and nutritional factors. Notably higher crude protein recorded highest histomophometry across the treatments except villus width, this may be an indication that the feed has gut histo-modulatory ability on gut. As reported by [45], nutritional contents can directly induce variations or modifications in the mucosa architectural integrity. [46] associated most absorption as well as high ability of the gut to promote digestion to the small intestine. Likewise, within the crypt depths is an indication of adequate transfer of enterocytes to the peak of villus. [47] accounted that diverse materials and also varied physio-pathological factors can alter the growth ranges and/or developmental degree of enterocytes in the crypt and impact their movement speed to the peak of the villi.

Parametric quantity	17%CP	20.00%CP	23.00%CP	One day	SEM	SD±
Villus Height (µm)	96.01^{b}	118.54^{a}	134.12^{a}	35.43^{c}	6.07	47.07
Villus width (µm)	65.71^{a}	65.33^{a}	61.55^{b}	6.06c	3.29	25.48
Crypt Depth (µm)	43.44^{c}	54.15^{b}	72.35^{a}	7.16d	3.19	24.73
MT (µm)	34.65^{b}	36.12^{b}	52.22^{a}	12.26^{c}	2.42	18.72
Inter Villus (µm)	3.71^{b}	4.09^{b}	4.59^{a}	2.47^{c}	0.17	1.32
VH:CD	1.46^{c}	1.18^{c}	2.18^{b}	5.93^{a}	0.25	1.95
AVSA (µm2)	$23,224.81^{b}$	$27,762.85^{a}$	$28,902.09^{a}$	699.63^{c}	63.17	882.86

Different superscripts (a,b,c) connote significant means ($p<0.05$). MT = Muscular thickness, VH:CD = Villus Height : Crypt Depth, AVSA = Average villus surface area

Table 6: Gut Histomrphometric Response of Broiler Raised on different Protein Levels (Finisher phase)

Conclusion

The research establishes 20% crude protein for the broiler finisher because it has highest final weight, encourages higher digestive enzyme activity and recorded moderate average villus surface area of the gut. Also 20% dietary crude protein diet resulted into higher amylase and protease activities of the broiler finisher. Therefore, this research recommends multiple phases feeding of broiler as against the convention two phase feeding of broiler chickens

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