Gut Histomorphometric and blood profile of broiler chicks fed varying levels of protein

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Abstract: The gut plays an active role in screening what goes into the bloodstream and blood is a very important parameter to access for us to establish the health status of animals to any dietary variation, this will help the producer's ability to guaranty the consumers' health. Therefore this research was designed to determine gut histomorphometry and blood profile of broiler starter fed varying levels of protein. A total of 144 days-old-broiler chicks were used for the study. Broiler standard starter mash with varied protein percentages of 20, 23, and 26%, respectively for treatments 1, 2, and 3 were used. Data were analyzed using ANOVA at α0.05. Results: Except for crude protein and ash contents, other nutrient digestibilities were not affected by the dietary treatments. Birds fed lower crude protein had the lowest crude protein digestibility while birds fed a standard diet (23% CP) had the best and highest crude protein digestibility. White blood cells decrease with decrease dietary protein, this might have a serious effect on the immunity level of the broilers. Also, globulin increases while albumin decreases with an increase in dietary protein levels. Serum total protein increases with an increase in the dietary protein levels and diet with 26% CP recorded the highest value (5.82 g/dL). Serum cholesterol values were significantly higher at lower protein levels unlike serum protein while different gut parts response significantly different to dietary treatments. Conclusion: At the early stage of broiler growth, diet needs to have higher CP to improve the immunity and health growth of gut.

Introduction:  
Protein nutrition has great economic importance since protein is an expensive item in almost all feed rations. This may be particularly so in areas of the world known to be protein deficient, such as many tropical and subtropical areas (Poultry World, 2016). Wasting or supplying in excess of what the broiler need may cause a lot of economic loss, so no farmer will encourage such. According to Poultry World, (2016) rationing of feed for protein should therefore be manipulated in such a way that optimal rather than maximum performance is achieved without too much feed expenditure on broiler production. Broilers according to Tom Kruchten, (2002) are

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chicken (*Gallus domesticus*) bred and raised specifically for meat production. Typical broilers have white feathers and yellow skin and most commercial broilers bred for meat reach slaughter weight between 5 and 6 weeks of age. According to Amber-Jean Nash, (2012), there are only two original breeds of broiler which are Cornish and white rocks. Given broilers access to a diet of high protein combined with artificial lightning, conditions stimulate growth, thus the desired body weight is achieved and Poultry World (2016) established that protein is a vital nutrient for poultry growth and development. The gut must also play a serious role before this expensive nutrient will be available to the broiler.

Digestion of feed to make the various nutrients available can only be achieved when the gut is sound and able to perform the digestive roles. A different part of the gut performs a different digestive role that makes the ingested feed to become readily available in the gut for microvilli to absorb into the bloodstream. Blood is a vital circulatory fluid that circulates through heart, arteries, capillaries and veins; it is composed of cells suspended in a fluid intercellular substance (plasma) with the major function of maintaining homeostasis (Isaac *et al.*, 2013). Haematological components, which consists of red blood cells, white blood cells or leucocytes, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration is valuable in monitoring feed toxicity especially with feed constituent that affects the blood as well as the health status of farm animals (Oyawoye and Ogunkunle., 2004). Red blood cells (erythrocytes) serve as a carrier of haemoglobin. It is the haemoglobin that reacts with oxygen carried in the blood to form oxyhaemoglobin which transports oxygen to the cells of the body during respiration (Chineke *et al.*, 2006). According to Isaac (2013), red blood cells is involved in the transport of oxygen and carbon dioxide in the body. Thus, a reduced red blood cell count implies a reduction in the level of oxygen that would be carried to the tissues as well as the level of carbon returned to the lungs (Soetan *et al.* 2013). Gut make the nutrient available to the blood and blood provides useful information on nutritional status and clinical investigation on the animal, hence, World Health Organization (WHO) recommend the use of blood parameters for medical and nutritional assessment (Egbunike *et al.*, 2009). Therefore, this research work was designed to determine the effect of different dietary protein levels on the gut histomorphometry, haematological and serum biochemistry of broiler chicks.

**Materials and Methods:**

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm, LAUTECH, Ogbomosho, Oyo State, Nigeria, Ogbomoso is in the derived savannah zone of Nigeria.

**Pre-operational management:**

Preparation before the arrival of the birds include general cleaning of the pen which involved sweeping, washing, scrubbing, fumigation of the pen, removal of unused wood, repair and partitioning of damaged nets and also washing and disinfecting the feeder and watering trough, all these were done before the wood shavings were sourced for at Ogbomoso sawmill. Materials for brooding were made available while alternative provisions were put in place in case of electricity fluctuation.
Animal Handling:

Total of one hundred and forty-four days old broiler chicks (Arbor acre strain) was used for the study and broiler starter mash containing 3000 Metabolisable energy Kcal/kg with a varied protein percentage of 20, 23, and 26%, respectively for treatments 1, 2 and 3, tagged Diet 1, diets 2 and diet 3, respectively, were fed to the broiler chicks (Table 1). The chicks were weighed and randomly allotted to the three dietary treatments in triplicate of 16 chicks each in a completely randomized design.

Table 1: Composition of Starter Diets (g/100g DM)

| Ingredients         | Diet 1 | Diet 2 | Diet 3 |
|---------------------|--------|--------|--------|
| Maize               | 58.25  | 52.05  | 41.95  |
| Soybeans            | 30.20  | 34.20  | 44.30  |
| Fish meal           | 2.00   | 2.00   | 2.00   |
| Brewer’s dried grain| 4.20   | 4.20   | 4.20   |
| Vegetable Oil       | 3.00   | 3.00   | 3.00   |
| Di Calcium Phosphate| 1.50   | 1.50   | 1.50   |
| Oystershell         | 1.25   | 1.25   | 1.25   |
| Salt                | 0.25   | 0.25   | 0.25   |
| Methionine          | 0.15   | 0.15   | 0.15   |
| Lysine              | 0.15   | 1.15   | 1.15   |
| **Premix**          | 0.25   | 0.25   | 0.25   |
| **Total**           | 100.00 | 100.00 | 100.00 |
| Crude protein (%)   | 20.02  | 23.05  | 26.00  |
| Metabolisable Energy (kcal/g) | 3164.45 | 3131.02 | 3056.88 |
| Crude fibre (%)     | 3.32   | 3.68   | 3.75   |

Diet 1: 20%CP, Diet 2: 23%CP, Diet 3: 26%CP, **Premix composition: Premix composition (per kg of diet) Vit (Vitamin) A=1000 IU; Vitamin D₃=2000 IU; Vit E=4000 mg; Vit K₃=900 mg; Vit B₁=500 mg; Vit B₂=2200 mg; Vit B₃=5500 mg; Vit B₁₂=4 mg; pp=18000; Folic acid=400 mg; Choline chloride=150000 mg; Antioxidant BHT=0.05%; Iron=1.80%; Copper=0.20%; Mn=2.40%; Cobalt=0.04%; Zn=2.80%; Iodine=0.04%; Selenium=0.016%; Ca=12.8570% in 2.5kg.

Digestibility trial:

At 28 days, four (4) birds were randomly selected per treatment and transferred to the metabolic cage for digestibility trial. The birds were acclimatized for three days, data on daily feed intake were collected. On the fourth day, the dropping of the birds was collected on aluminum foil paper and weighed at the same time using a sensitive scale. The daily droppings from each treatment were dried, weighed before being ground using a manual grinder, and a sub-sample was taken for proximate composition.

Blood Analysis:

Blood samples were obtained from the jugular vein of two birds per replicate on the 28day into tubes containing EDTA (an anticoagulant) for haematology, while those for serum were without EDTA. The blood analysis was determined as reported by Shittu et al. (2017).
Gut histomorphometric analysis:

The samples were handled as described (Onderci et al., 2008). The samples were placed in a 10% buffered neutral formaldehyde solution (pH 7.2 - 7.4) and were gradually dehydrated with increasing concentrations of ethyl alcohol (50 - 100%). The dehydrated specimens were embedded in paraffin, sectioned at 5µm, and stained with haematoxylin and eosin (Gracia et al., 2009). The sections were analyzed under a light microscope (Nikon Eclipse E200) and the height and width of the villus were measured using computer-assisted image analysis. A total of 15 intact well oriented crypt-villus units were selected randomly for each sample. The mean values attributed to individual birds were used in the statistical analysis. Villus height was measured from the tip of the villus to the crypt-villus junction, whereas crypt depth was defined as the depth of the invagination between adjacent villi (Hu and Guo, 2006). The villus width was defined as the distance from the outside epithelial edge along a line passing through the vertical midpoint of the villus. Data analysis: Data generated were subjected to ANOVA using SPSS version 12 at α = 0.05.

Results and Discussion:

The digestibility of proximate constituents by broiler starter fed varying levels of protein is shown in Table 2. Except for crude protein and ash contents, other nutrient digestibilities were not affected by the dietary treatments. Birds fed lower crude protein had the lowest crude protein digestibility while birds fed a standard diet (23% CP) had the best and highest crude protein digestibility. Table 2 shows that broiler chick crude protein digestibility has to be met and adequate to avoid wastage and unavailable in the broiler diet. Lohakare et al. (2006) suggested that increase dietary crude protein might lead to a possible increase in the endogenous nitrogen losses due to an increase in dietary protein levels and this suggests that the intake was in excess of the animals' capacity to utilize nitrogen efficiently. The result of this study contradicts the report of Youssef et al. (2017), who observed an increase in the crude protein digestibility as the dietary crude protein decreases. This may be as a result of the class and age of the chickens. The fact was supported by Youssef et al. (2017) that different environmental, nutritional, and hygienic conditions can affect the growth of broiler chickens.

Table 3 shows the haematological parameters of the broiler fed varying levels of protein at the starter phase. White blood cells decrease with decrease dietary protein, this might have a serious effect on the immunity level of the broilers. Birds on a lower crude protein diet had the highest blood monocytes (3.56%) level compare to other treatments. Also, globulin increases while albumin decreases with an increase in protein. Platelet activity processes were similar irrespective of the dietary protein level. Packed cell volume (PCV), MCV, MCH, Hb, monocytes of blood is a means of assessing the clinical and nutritional health status of animals in feeding trial (Adeyemi et al., 2000). The protein requirements by animals depend on many factors such as species, breed, age, physiological status, and environmental factors (Abdelrahman and Aljumaah, 2014). White blood cells decrease with decrease dietary protein, this might have a serious effect on the immunity level of the broilers. This agrees with the report of Carломagno et al. (1980) that protein deficiency inhibits antibodies production cells in response to T-dependent antigens. Huwaida et al. (2013) reported variation of different strain to different protein levels eg Cobb fed low protein had higher growth compare to Ross, Habbard, etc. The authors reported variation in antibody titers as affected by strain, Ross fed high crude protein has higher antibodies titers.
Platelet activity processes were similar irrespective of the dietary protein level. This shows that a 20% protein level still enables the normal activity of the thrombocytes of broiler at the starter phase. Platelets help to stop the bleeding at any site of endothelium interruption, this research indicates that protein variation cannot interrupt platelet activities. According to Ijioma et al. (2015) and Van der Bom et al. (2009) fall in platelet count has been associated with high values of bleeding and clotting times with bleeding problems as consequences. Low numbers of circulating platelets, as well as platelet dysfunction, increase the risk of bleeding (Van der Bom et al., 2009). In animals with myeloproliferative disorders such as essential thrombocythemia, high levels of platelets may be associated with thrombotic and, to a lesser extent, with hemorrhagic complications, mostly at platelet counts exceeding normal range.

Table 4 shows the serum parameters of broiler starter fed varying levels of protein at the starter phase. Except for serum proteins and cholesterol, other serum parameters were not affected by varying dietary protein levels. Globulin was steady at lower and recommended levels but the value increased significantly at higher dietary protein level above recommendation (4.38g/dL). Also, serum total protein increases with an increase in the dietary protein levels and diet with 26% CP recorded the highest value (5.82g/dL). Serum cholesterol values were significantly higher at the lower protein level, unlike serum protein.

An increase in serum total protein with an increase in the dietary protein levels (table 4), although serum creatinine was not affected establishes that serum total protein is sensitive to dietary protein variation than creatinine. This agreed with the report of Awosanya et al. (2000), which reported that blood protein and creatinine levels were dependent on the quality of dietary protein. Birds on lower crude protein diet had the highest blood monocytes level (3.56%) compare to other treatments, its means irrespective of the protein levels bird were still able to produce sufficient monocytes. It has been established earlier that excessive consumption of protein decreased the availability of protein (Jeong et al., 2010) and increased the excretion of the nitrogen as urea form (Han et al., 2001). The provision of insufficient nutrients limits the potential growth and production of animals while feeding excessive nutrients reduces the economic profitability and causes environmental pollution (Jongbloed and Lenis, 1992 and Paik, 1996).

An increase of globulin and a decrease in albumin are tied to an improved immune system function on broiler (Bovera et al. 2015, 2016) and can positively affect the health status of birds. Aspartate aminotransferase (AST) and alanine aminotransferase are enzymes released into the blood when certain organs or tissues, particularly the liver (disruption of the hepatic cell as a result of necrosis) and heart are injured or damaged. AST and ALT were not affected as a result of varying dietary levels of protein, aspartate aminotransferase (AST) test are not liver-specific, and measuring it alone is not sufficient for liver functionality. Therefore the result of this study indicates that the reduction of protein level up to 20%CP did not affect liver and heart functionality.

Table 5 shows the gut histomorphometric characteristics of broiler fed varied protein level (starter). Except for inter villus and crypt depth, all the gut histomorphometric characterizes measured were significantly affected by the protein levels. Different parts’ response is significantly different from dietary treatments. Lower protein did not significantly affect the crypt depth of the broiler chickens except when compared with the day-old record. Twenty percent protein level had the widest surface area.

Histomorphometric analysis (table 5) was discovered to be the main parameter when studying gastrointestinal pathophysiology (Gulbinowicz et al., 2004). Twenty percent protein level had the widest surface area, this may be because the birds were able to improve the absorptive surface area to maximize the limited available protein in the diet. This might be complementary
atrophy, to enable the animal's ability to obtain the needed nutrient. It seems to be a complex relationship between the small intestine and nutritional factors. According to Caruso et al. (2012) and Thomson et al. (1996), nutrients may directly cause changes or adaptations in the intestinal mucosa. Since all the absorptive and also a part of the digestive capacity of the small intestine occur around and near villi and crypts (Kitt et al., 2001). The similarity between the crypt depths is an indication of sufficient transportation of enterocytes to the tip of the villi. It was reported by Thomson and Keelan, (1986) that various substances and also different physiological or pathological conditions may change the proliferation rate of enterocytes in the crypt and affect their migration rate to the top of the villi.

Table 2: Apparent Nutrient Digestibility of Broiler starterFed Varied Levels of Protein

| Parameters (%) | 20% CP | 23% CP | 26% CP | SEM | SD± |
|----------------|--------|--------|--------|-----|-----|
| Crude protein  | 41.26<sup>c</sup> | 76.21<sup>a</sup> | 54.26<sup>b</sup> | 3.02 | 15.73 |
| Ether Extract  | 84.21  | 83.06  | 82.90  | 0.59 | 0.06 |
| Crude Fibre    | 25.65  | 35.40  | 33.22  | 2.11 | 10.94 |
| Ash            | 50.42<sup>b</sup> | 63.79<sup>a</sup> | 64.19<sup>b</sup> | 1.61 | 8.40 |
| Dry matter     | 69.96  | 73.89  | 71.71  | 0.98 | 5.10 |
| NFE            | 77.63  | 75.87  | 74.79  | 0.72 | 3.76 |

<sup>a,b,c</sup> connote means in the same row with dissimilar superscripts are significant (p<0.05). CP= Crude protein, SEM = standard error of mean, SD = standard deviation, NFE = Nitrogen free extract
### Table 3: Haematology and serum parameters of the broiler starterfed varied levels of protein

| Parameters     | 20% CP | 23% CP | 26% CP | SEM  | SD±  |
|----------------|--------|--------|--------|------|------|
| PCV (%)        | 26.67a | 22.22b | 20.56b | 0.50 | 3.68 |
| Hb (g/dL)      | 8.93   | 8.72   | 9.16   | 0.21 | 1.11 |
| MCHC (%)       | 43.31b | 46.59b | 54.24a | 1.27 | 9.40 |
| MCV (µL)       | 78.01a | 66.65b | 61.51c | 1.38 | 10.17|
| MCH (µg)       | 33.74a | 30.98b | 31.82b | 0.27 | 1.96 |
| Hb (g/dL)      | 11.52a | 10.33b | 10.62b | 0.11 | 0.74 |
| RBC (x10⁹/L)   | 3.42a  | 3.33b  | 3.34b  | 0.01 | 0.07 |
| WBC (x10³/L)   | 8.04a  | 11.04b | 13.95a | 0.43 | 3.16 |
| Lymphocytes (%)| 63.89  | 64.00  | 64.00  | 0.02 | 0.19 |
| Heterophils (%)| 26.77  | 24.33  | 26.56  | 0.23 | 38.49|
| Monocytes (%)  | 3.56a  | 2.79b  | 2.89b  | 0.13 | 0.94 |
| Eosinophils (%)| 3.33b  | 5.33a  | 2.22c  | 0.24 | 1.78 |
| Basophils (%)  | 0.33   | 0.67   | 0.44   | 0.07 | 0.05 |
| Platelet (x10⁴/L) | 13.32 | 14.32  | 13.69  | 2.47 | 18.14|

a,b,c : connote means in the same row with dissimilar superscripts are significant (p<0.05). CP= Crude protein, SEM = standard error of mean, SD = standard deviation

### Table 4: Serum parameters of the broiler fed varied levels of protein (Starter phase)

| Parameters     | 20% CP | 23% CP | 26% CP | SEM  | SD±  |
|----------------|--------|--------|--------|------|------|
| AST (IU/L)     | 159.54 | 157.25 | 160.23 | 3.04 | 22.33|
| ALT (IU/L)     | 2.99   | 2.92   | 3.00   | 0.09 | 0.69 |
| Albumin (g/dL) | 1.47   | 1.35   | 1.26   | 0.03 | 0.24 |
| Globulin (g/dL)| 2.83b  | 2.83b  | 4.38a  | 0.16 | 1.19 |
| Total protein (g/dL) | 4.31b | 4.42b | 5.82a | 0.16 | 1.19 |
| Creatinine (mg/dL) | 1.85  | 2.89   | 1.58   | 0.19 | 1.44 |
| Cholesterol (mg/dL) | 129.60b | 134.46a | 81.78b | 4.59 | 33.76|

a,b: connote means in the same row with dissimilar superscripts are significant (p<0.05). CP= Crude protein, SEM = standard error of mean, AST = Aspartate transaminase, ALT = Alanine transaminase
Table 5: Gut Histomorphometric Characteristics of Broiler fed Varied Protein Level (Starter)

| Parameters          | 20% CP | 23% CP | 26% CP | Day-old | SEM | SD± |
|---------------------|--------|--------|--------|---------|-----|-----|
| Villus Height (µm)  | 203.58 | 151.92 | 190.74 | 35.43   | 9.01| 69.86|
| Villus width (µm)   | 51.76  | 51.49  | 47.47  | 6.06    | 2.57| 19.97|
| Crypt Depth (µm)    | 38.32  | 40.43  | 38.40  | 7.16    | 1.89| 14.67|
| MT (µm)             | 19.83  | 18.10  | 26.65  | 12.26   | 1.03| 8.01 |
| Inter Villus (µm)   | 7.94   | 9.45   | 8.19   | 2.47    | 0.45| 3.50 |
| VH:CD               | 4.01   | 2.96   | 3.88   | 5.93    | 0.17| 1.32 |
| AVSA (µm)           | 34944.28 | 26731.61 | 31618.08 | 699.64 | 87.56 | 223.41 |

MT = Muscular Thickness, AVSA = Average Villus Surface Area, SEM = standard error of mean, SD = standard deviation, BDG = Brewery dry grain, SSG = Sorghum spent grain.

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