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BLOOD PROFILE OF FINISHER BROILER CHICKENS FED DIET CONTAINING LEAF MEAL COMPOSITE AS ALTERNATIVE TO COMMERCIAL BROILER PREMIX

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Abstract
The need to explore and harness the potentials of green vegetable plants as part replacements for the more expensive conventional vitamin-mineral premix is of great importance. One hundred and eighty (180) starter broiler chickens were used in a four weeks experiment to determine the effect of varying levels of Leaf Meal Composite (LMC) as an alternative to vitamin-mineral premix using *Telfairia occidentalis* (fluted pumpkin), *Vernonia amygdalina,* (Bitter leaf), *Piper guinenses* (*Uziza*) and *Ipomea batata* (Sweet patato) on the haematology and serum biochemical profile of Starter broiler chickens. The chicks were allocated to six dietary treatments, each having thirty birds, replicated thrice with ten birds per replicate in a Completely Randomized Design. The birds were fed formulated diet, the test materials were ground into meal and was introduced from the first day, feed and water were provided *ad libitum* The composite leaf meal inclusion level was at 0.00 (0.25% premix), 0.125 (0.125% premix), 0.63 (0.0% premix), 0.125 (0.0% premix), 0.188 (0.0% premix) and 0.25% (0.0% premix) at the expense of a commercial premix and designated diets T_1, T_2, T_3, T_4, T_5 and T_6 respectively, with T_1 as the control. Data was obtained for analysis. The result reveals that the LMC was not toxic to the birds, had superior disease fighting ability and not anaemic. All the treatment levels were significant for mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Aspartate amino transferase **(**AST) and Alkaline phosphatase ALP reduced with increasing concentration of LMC, the test material was not toxic to the liver. Total Globulin and Glucose level reduced with increasing concentration of LMC. Leaf meal is effective in reducing fat deposition. Therefore, LMC had no detrimental effect on the haematology and serum biochemical profile of starter broiler chicken and can be used to replace commercial vitamin-mineral premix.

Keywords: Leaf Meal Composite, Vitamin-Mineral Premix, Hamatological indices, Serum biochemical

Introduction

In Nigeria, the rising prices of food items has also affected the cost of animal feeds and feed ingredients; which have resulted in low profit for the farmer because of the ever-increasing cost of feeds and feed ingredient do not commensurate with the market price for livestock products such as live broiler and eggs. This calls for an urgent need to explore a feed formulation that will sustain the poultry enterprises at reduced cost of production. Thus, researchers have made assiduous efforts to source for better means (Nworgu *et al.,* 2007) to reduce the cost of feeds which is 70 – 80% of the total cost of production in poultry enterprise (Nworgu *et al.,* 1999; Alimi *et al*., 2006 Onunkwo *et al*., 2018). Consequently, the use of alternative feed ingredients that would ensure high productivity of the birds without necessarily compromising their health and consumers' interest (Ziggers, 2011) has been recommended. The use of local alternative which is cheaper and readily available without creating any competition between livestock and humans has been considered. This includes agro-industrial by-products and non-conventional feed resources (Ojewola *et al.,* 2003; Gardzielewska *et al*., 2003; Bairagi *et al*., 2004., Cardozo *et al*., 2004; Nworgu *et al.,* 2007; Adewolu, 2008). Vegetables serve as an indispensable constituent of the human diet (Czarral and Fred, 2009) supplying the body with minerals, vitamins and certain hormone precursors, in addition to protein and energy (Abu *et al.,* 2015) support efficient digestion of nutrients (Denli *et al*., 2003) Windisch *et al*., 2007) and has attracted attention in the livestock sector (Kamel, 2001). Leaf meal of some indigenous plants has been be used as feed ingredient to reduce feed cost, while supplying the needed nutrients from natural source to improve growth performance (Kamel, *ibid*; Cardosso *et al*., 2004;

Carrijo *et al*., 2005; Tian, 2008; Odoemelam *et al*., 2012; Sugiharto *et al*., 2019). Leafy vegetables such as pumpkin leaf, bitter leaf, potato leaf and *uziza* leaf have been used in broiler production to test its effect in performance and different results have been obtained with different inclusion margins. These leaves contain minerals, vitamins, amino acid, energy, antioxidants, essential oils, anti-nutritional factors, etc. (Abu *et al.,* 2015).

In addition, Ndelekwute *et al.* (2017) advocated that leaf meal should be used in broiler feed as sources of utilizable fibre, starch, vitamins and minerals and in turn, reduces total cost of production. However, these alternatives, such as, plant leaves must provide all nutrient requirements particularly micro nutrients to meet the physiological need of the animals. Proper utilization of this local material in poultry feeds; require adequate knowledge of poultry nutrition such as micro-nutrients; vitamins and mineral requirements (Adegbenro *et al*., 2012 and Onunkwo *et al*., 2018). Among the factors that ensure economic success in broiler production, nutrition plays a very important role as all other factors are built on it. In other words, nutrition has been a major determinant of profit making in livestock business as feeding accounts for 70-80% of the costs of producing livestock, most especially poultry (Onunkwo *et al*., *ibid*). A standard quantity and combination of composite leaf meal has not yet been accepted as an alternative to premix, information on it is scanty and still in the infancy stage, and hence researchers are still searching for the right combination of leaves that could serve this purpose. On this note, *Telfairia occidentalis*, *Vernonia amygdalina, Piper quinenses* and *Ipomea batata* were employed in this study to test the blood and serum biochemical profile, when used as an alternative to vitamin-mineral premix in different proportions.

Materials and Methods

Location of study

The study was carried out at the Poultry unit of Teaching and Research Farm, Michael Okpara University of Agriculture, Umudike, Abia State, situated on latitude 05° 21' N and longitude 07° 33'E, with an elevation of about 112m above sea level, annual rainfall of 177 - 2,000mm (April to October), short period of dry season (November to March), relative humidity of about 50-90%, and monthly temperature range of 17°C -36°C (NRCRI, 2020).

Experimental procedure and design

Pumpkin leaf was sourced from the Cross River Basin Abak, Akwa Ibom State, while Sweet potato, Moringa and Bitter leaves were obtained within Michael Okpara University of Agriculture, Umudike premises. Each leaf was harvested fresh, sliced to reduce the particle size and increase the surface area for quick drying, then air dried at room temperature for four days. Thereafter, it was ground to fine powder and stored in an air-tight container. For compounding the feed, equal quantity of each ground leaf was measured out and mixed thoroughly using an improvised mixer; this made up the Leaf Meal Composite (LMC) used to replace commercial premix in the diet at different levels. The CLM sample were mixed along with other feed ingredients used to formulate the experimental diets (Table 1). The experiment lasted for four weeks. At the 8th week of the experiment, blood samples were collected from one bird randomly selected from each replicate per treatment for the evaluation of haematological and serum parameters. Blood collection was carried out by using a sterile needle to puncture the right jugular vein, and blood drawn into the syringe.

Data collection and analysis

The blood samples were collected into labeled sterile bottles containing EDTA (Ethyl diamine tetra acetic acid) powder as anti-coagulant. These samples were used in the laboratory to determine haematological parameters such as: Red Blood Cell (RBC), White Blood Cell (WBC), Packed Cell Volume (PCV) and Haemoglobin Count (Hb), according to Coles (1986). Values for Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were determined. Also, blood samples were collected into another labeled sterile bottle without anti-coagulant to analyze serum biochemical constituents such as total protein, albumin, globulin, creatinine, triacylglycerols, alkaline phosphatase and aspartate amino transferase. All data generated were subjected to analysis of variance (ANOVA) and treatment means that were significantly different separated using Duncan's Multiple Range Test (Duncan, 1955), according to Steel and Torrie (1980) using computer software IBM SPSS Statistic version 20 (SPSS, 2009).

Results and Discussion

Haematology Profile of finisher Broiler Chicken

The result of the haematological indices of starter broiler chicken fed diet containing leaf meal composite is shown in Table 2. Packed Cell Volume (PCV) is the percentage of Red Blood Cell (RBC) in the blood. It is involved in the transport of oxygen and absorbed nutrients round the body delivering it to target cells or tissues. PCV values in this study ranged between 22.0 - 29.7%, which falls within the normal range for healthy chicken (Mitrula and Rawnsley, 1977) except at T4 and T5 which were lower; this could be due to the sex of the selected animal as the female chick tends to have a lower value than the male as estrogen level impairs erythrocyte synthesis (Fair *et al*., 2007) or due to dehydration, since PCV represents an increase in the number of RBCs, or a reduction in the circulatory plasma volume (Chineke *et al*., 2006) Dehydration results in a low fluid in the blood and therefore, a high RBC and its associated parameters. It is indicative that the experimental birds were not anaemic since Hb and RBC values were within the normal range for healthy broiler chicken (Mitrula and Rawnsley, 1977), but there could have been dehydrated; this condition will be reversed when the water intake improves. RBC transports oxygen to animal tissues for the oxidation process to release energy and transport carbon-dioxide

out of the tissues (Omiyale *et al*., 2012) and the manufacture of haemoglobin. RBC values in this study increased numerically with increased inclusion of LMC. The control had the least value, while, the highest was observed at T6 group. The observed values range between 2.53 - 3.3 x 10^6/mm^3 which is within the normal range for healthy broiler chicken (Mitrula and Rawnsley, 1977), T2, T3, T6 were significantly different ($P<0.05$) from the control. This shows that the LMC enabled proper functioning of the RBC in respiration; expiration supply of nutrients and manufacture of haemoglobin which indicates a better health status. LMC resulted in an elevation RBC value; this could be due to the high nutrient in LMC, such as, iron in *Telfera occidentalis* and high protein quality in potato. Iron is essential in body functions, such as, formation of haemoglobin and myoglobin (Ekenyem and Madubuike, 2006). These rich nutrients have blood boosting ability and a rich blend of amino acids. White Blood Cell (WBC) defends the body against invasion by foreign organisms and to supply antibodies for immune response. There is significant difference among T_2, T_3, T_4 and T_1 (control). The WBC values obtained in this study ranged between 10.7-18.4 x 10^6/mm^3, which is not in agreement with Mitrula and Rawnsley (1977). All the inclusion levels were higher than the normal range, indicating that the LMC influenced the birds' immune status; being an intrinsic body defence system (Ganong, 1991), and will optimise performance under stressful condition. Animals with high WBC values are capable of generating antibodies and a high degree of disease resistance (Soetan *et al.,* 2013). The increase in WBC could also be attributed to the presence of some Phytochemicals in bitter leaf, which induces the animal to respond as if it had an infection (Aregheore *et al*., 1998) or due to the presence of residual anti-nutrients, which induces production of more antibodies that stimulates more WBC production to fight the infection being a defence system. Birds with low WBC are exposed to high risk of disease infection, while an increase are capable of producing antibodies in the process of phagocytosis and have a higher degree of disease resistance (Soetan *et al*., 2013). The increase in WBC indicates a superior disease fighting ability, showing that the LMC did not compromise the bird's immunity (Eroschenko, 2000). Haemoglobin (Hb) values of the experimental broiler chickens were 7.0-8.8, which are within the normal range. T6 had the highest value and significantly different from the control, while the least was observed in T4. Haemoglobin is the oxygen carrying protein in the RBC. Hb levels are a direct reflection of the amount of oxygen in the blood. Increased Hb is seen in dehydration, chronic obstructive pulmonary disease etc. while a decrease results in anaemia, blood loss, liver disease etc. MCV, MCH, MCHC were significantly different and numerically lower than the control. The significant difference recorded in this study maybe correlated with the quality of protein in the experimental diet since haemocrit and haemoglobin are influenced by the quality of protein. MCH indicates the blood carrying ability of RBC; the study reveals that the LMC reduced the blood carrying ability of the RBC.

Serum biochemical indices of starter broiler chickens fed diet containing Composite Leaf Meal

The result of the serum biochemical indices of broiler chicken fed diet containing leaf meal composite is shown in Table 3. Serum protein shows that only T6 was similar to control, while other treatment groups were statistically different ($P<0.05$), and numerically lower than the control. Serum proteins are synthesized in the liver to maintain blood volume through the colloidal osmotic effect, buffer blood pH, transport hormones and drugs, cell coagulation, catalyze enzymatic reactions, regulate hormones, and defend body against foreign materials (Melillo, 2013). The high value in T6 was due to high values of globulin and albumin which sum up to give the total protein. ALT and ALT are enzymes in the liver and are released when the liver is damaged, although there was a decrease in both with increasing concentration of CLM than the control, the levels of both in this study reflects normal liver function and shows that the liver was not damaged by the CLM, but the reduction in CLM levels can be due to hepato protective effect of CLM which improves liver health. Serum cholesterol (triglycerides) is synthesized in the intestinal mucosa and in the liver from the digestion of dietary components and the absorption of fatty acids. The result shows that the leaf meal significantly ($P<0.05$) influenced the triglyceride level as it is observed that there is a decreasing value of triglyceride with increasing inclusion of composite leaf meal, glucose also follows this trend. This shows that the leaf meal is effective in reducing abdominal fat, hence it can be used in fattening broilers to prevent excessive deposition of fat and in breeding animals to prevent excess fat build up that could interfere with reproductive ability. This report supports Nwanjo (2005) and Ndelekwute *et al.* (2017) who observed that bitter leaf reduces body fat.

Table 1: Gross percent composition of experimental finisher broiler diet

Ingredients	T1	T2	T3	T4	T5	T6
Maize	50.00	50.00	50.00	50.00	50.00	50.00
Wheat offal	1.00	1.00	1.00	1.00	1.00	1.00
PKC	10.00	10.00	10.00	10.00	10.00	10.00
Rice Bran	2.00	2.00	2.00	2.00	2.00	2.00
SBM	30.00	30.00	30.00	30.00	30.00	30.00
Fishmeal 65	3.00	3.00	3.20	3.00	3.00	3.00
Bone meal	3.00	3.00	3.20	3.15	3.10	3.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25	0.25
Vit/Min Premix	0.25	0.125	0.00	0.00	0.00	0.00
CLM	0.00	0.125	0.063	0.125	0.188	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

premix supplied per kg diet: vitamin A 15,000 I.U, vitamin D3 13000 iu, thiamine 2mg, Riboflavin 6mg, pyridoxine 4mg, Niacin 40mg, cobalamine 0.05g, Biotin 0.08mg, choline chloride 0.05g, Manganese 0.096g, Zinc 0.06g, Iron 0.024g, Copper 0.006g, Iodine 0.014g, Selenium 0.24mg, Cobalt 0.024mg and Antioxidant 0.125g, NFE = Nitrogen free extract

Table 2: Haematological indices of finisher broiler chicken fed diet containing Leaf Meal Composite

Variable	1	2	3	4	5	6	S.E.M
HB (g/dl)	8.2^{ab}	8.2^{a}	7.67^{ab}	7.0^{a}	7.8^{ab}	8.87^{c}	0.203
PCV (%)	22.3^{a}	28^{bc}	25.3^{a}	22.0^{a}	24^{a}	29.7^{c}	0.823
RBC($1O^6/mm^3$)	2.53^{a}	3.2^{bc}	2.9^{ab}	2.6^{a}	2.7^{ab}	3.3^{c}	0.089
WBC($1O^6/mm^3$)	15.4^{b}	18.6^{c}	10.7^{a}	11.4^{a}	17.1^{bc}	17.7^{bc}	0.779
MCV (fl)	100.4^{b}	97.7^{a}	96.9^{a}	97.5^{a}	96.9^{a}	97.5^{a}	0.538
MCH (g/dl)	32.5^{b}	25.9^{a}	26.9^{a}	27.2^{a}	28.1^{a}	26.6^{a}	0.642

Means with different super scripts in the same row are significant different (p<0.05), S.E.M: Standard Error of mean

Table 3: Serum biochemical indices of starter broiler chicken fed diet containing Leaf Meal Composite

Parameter	T1	T2	T3	T4	T5	T6	S.E.M
Total Protein(g/dl)	3.07^{b}	2.58^{a}	2.57^{a}	2.53^{a}	2.68^{a}	3.06^{b}	0.058
Globulin (g/dl)	1.63^{c}	1.11^{a}	1.18^{ab}	1.15^{ab}	1.37^{ab}	1.33^{b}	0.048
Albumin (g/dl)	1.44^{a}	1.47^{a}	1.39^{a}	1.38^{a}	1.55^{ab}	1.73^{b}	0.034
Urea (mg/dl)	9.59^{b}	8.99^{ab}	8.91^{ab}	8.31^{a}	8.43^{a}	8.98^{ab}	0.126
Creatinine (g/dl)	0.96^{ab}	0.91^{ab}	1.07^{b}	0.82^{a}	0.92^{ab}	1.01^{ab}	0.031
Glucose (mg/dl)	145.33^{a}	184.0^{ab}	210.33^{b}	319.0^{c}	230.0^{b}	191.33^{ab}	14.100
AST (u/l)	35.45^{b}	33.48^{ab}	32.53^{ab}	33.60^{ab}	31.48^{a}	32.38^{ab}	0.483
ALT (u/l)	122.6^{b}	120.9^{a}	117.23^{a}	120.22^{a}	119.93^{a}	119.01^{a}	0.774
TG (Mg/dl)	1.31^{c}	1.24^{b}	1.26^{b}	1.26^{b}	1.25^{b}	1.21^{a}	0.747

Means with different super scripts in the same row are significant different (p<0.05), S.E.M: Standard Error of mean

Conclusion

Telfairia occidentalis, *Vernonia amygdalina, Piper quinenses* and *Ipomea batata* were employed in this study to test the blood and serum biochemical profile, when used as an alternative to vitamin-mineral premix in different proportions. This combination of leaf meal composite had no detrimental effect on the haematology and serum biochemical profile of broiler chicken and can be used to replace commercial premix. More research is required, since age of leaves before was not considered, and this influences the nutrient content in leaves.

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