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Blood profile of starter broiler chickens fed diet containing leaf meal composite as alternative to commercial broiler premix

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Target Audience: *Feed producers and poultry farmers.*

Abstract

The need to explore and harness the potentials of green vegetable plants as part replacement for the more expensive conventional vitamin-mineral premix is of great importance. One hundred and eighty starter broiler chickens were used in a four-week experiment to determine the effect of varying levels of Leaf Meal Composite (LMC) as an alternative to vitamin-mineral premix using Telfairia occidentalis, Vernonia amygdalina, Piper quinenses and Ipomea batata on the growth, 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 each in a Completely Randomized Design. The composite leaf meal was blended at the ratio 1:1:1:1 and inclusion level was 0.00 (0.25% premix), 0.125 (0.125% premix), 0.063 (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 1, 2, 3, 4, 5 and 6 respectively.Treatment one (T1) served as the control. The birds were weighed on a weekly basis. At the 4th 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. *Data was obtained for analysis. The growth performance result reveals that the Average Daily Feed Intake (ADFI), Average Daily Weight Gain (ADWG), Total Feed Intake (TFI) and Final Body Weight (FBW) were all significantly (P<0.05) affected by the dietary treatments. Feed Conversion Ratio (FCR) was not significantly (P>0.05) different among all treatment groups including the control, although, the control was numerically lower than T2, T3 and T6 while T4 and T5 were lower than the control. The blood profile result reveals that the LMC was not toxic to the birds, had superior disease fighting ability and were not anaemic. All the treatment levels were significant for mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean cell haemoglobin concentration, (MCHC). aspartate aminotransferase (AST) and alanine transaminase (ALT) 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. It can be concluded conceivably within the limit of this study that this leaf meal composite had no detrimental effect on the growth, haematology and serum biochemical profile of starter broiler chicken and can be used to replace commercial Vitamin-Mineral premix. Hence, LMC could help to stem over dependence of broiler farmers on importation of commercial Vitamin-Mineral premix.*

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

Description of Problem

Vitamin-mineral premixes, an important feed ingredient, which contribute substantially to animal well-being in terms of providing essential micro-nutrients, have however not received the same attention as that of protein

and energy ingredients. Vitamin-mineral premix is included in the animal feeds, although in minute quantity, but it is necessary for optimum productivity and leaving it out would restrict performance with very heavy losses (1; 2).

The commercial vitamin -mineral premix inclusion often comes with a very large cost of the total financial outlay earmarked for the feed component of the livestock enterprise. The reason for this could be attributed to the fact that most of the vitamin-mineral premixes used in the developing country like Nigeria are imported, and hence sold to the local livestock farmers at a cost that is usually not affordable and mostly unbearable to them (3).

Vitamins and minerals are unarguably essential components in animal nutrition, poultry not being an exception. Although required in small quantities, they are crucial for the immune system as defence against unhealthy pathogens, metabolic reactions, skeletal support, nutrient absorption, growth, reproduction, and production.

Broiler chicken being a monogastric do not have the required microbes in its guts to sequester or synthesize enough vitamin and mineral (4) to meet its requirement unlike the ruminant who graze on green pasture where these nutrients abound and the microbes in the guts help to synthesize more. Hence, vitamins and minerals in the form of "premix" are added to broiler chicken feed (some are also administered through drinking water) to meet its requirement for optimal performance. This premix originates from a synthetic source and comes at a premium (5) since it is mostly imported and distributed by a few dealers to the local farmers in developing countries; there is occasional scarcity, adulteration challenge, and high cost (4)

This birth the urge among animal nutritionist to research on indigenous plant sources that can serve as an alternative to synthetic Vitamin-Mineral Premix that will furnish the animal with adequate blend of vitamins and minerals, serve as a source of fibre since it has already been established that vegetables provide fibre to birds. In addition, the quality will be verified, and it will come at a cheap rate to address the economic challenges with the use of synthetic Vitamin-Mineral premix.

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 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

The study was carried out at the Poultry unit of Teaching and Research Farm, Michael Okpara University of Agriculture, Umudike, Abia State. Umudike is situated on latitude 05^{o} $21^{`}$ N and longitude $07^{o}$ $33^{`}$E, with an elevation of about 112m above sea level with an annual rainfall of 177 - 2,000mm (April to October) and a short period of dry season (November to March) with a relative humidity of about 50-90% and monthly temperature range of 17^{o}C - 36^{o}C (6).

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, the leaves were blended at the ratio of 1:1:1:1 and 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 LMC sample were mixed along with other feed ingredients used to formulate the experimental diets (Table 1). The experiment lasted for four weeks.

The birds were weighed on a weekly basis. At the 4th 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. 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 (7). 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 were separated using Duncan's Multiple Range Test (8) according to (9) using computer software IBM SPSS Statistic version 20 (10).

Table 1: Gross percent composition of experimental starter 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	3.00	3.00	3.00	3.00
Bone meal	3.00	3.00	3.20	3.15	3.1	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**	**100.01**	**100.02**	**100.03**	**100.0**

*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.

Results and Discussion

The growth performance of broiler starter chickens fed diet containing comp9+9`osite leaf meal is presented in Table 2. The result reveals that the Average Daily Feed Intake (ADFI), Average Daily Weight Gain (ADWG), Total Feed Intake (TFI) and Final Body Weight (FBW) were all significantly ($P<0.05$) affected by the dietary treatment. In all the growth parameters evaluated for performance, T4 and T2 were different from the control, though T3, T5 and T6 were not significantly ($p>0.05$) different from the control, there were all numerically higher than the control.

The higher performance in T4 and T2 is indicative of the fact that both treatments had received adequate nutrients including a blend of vitamins and minerals from the composite

leaf meal which influenced feed intake and resulted in higher weight gain than the control, there was a better feed utilization. Minerals and vitamins biologically available in broiler chicken diet improves feed intake, this is in tandem with the report of (11) who found that both micro and macro elements improve feed intake in poultry. The decrease in feed intake in T3 could be due to inadequacy of vitamins, minerals and protein in the leaf meal to meet the requirement for optimal performance. Although T5 and T6 received higher quantity of composite leaf meal, there was reduced feed intake which affected its general performance, this could be as a result the birds not being able to tolerate the bitter taste of the leaf meal at high concentration which made the feed unpalatable. Increasing levels of bitter leaf (12) and moringa (5) decreases feed intake, (13) reported a reduced performance with increasing levels of leaf meal. The leaf meal could also have increased the fibre content of the feed which impairs efficient feed utilization. Birds could not eat enough because of the bitter taste of the feed and the little quantity eaten could not be adequately utilized because of the high fibre. This is in support of (14).

Feed conversion ratio (FCR) in broiler chicken production measures the efficiency of converting feed into muscle. In this study, FCR was not significantly different among all treatment groups including the control, although, the control was numerically lower than T2, T3 and T6 while T4 and T5 were lower than the control. A lower FCR is desirable in broiler production as it indicates efficiency of production. Higher FCR in T6 shows depressed growth, T4 indicates better efficiency of feed utilization having higher live weight.

Table 2: Growth Performance of Starter Broiler Chicken Fed Composite Leaf Meal

Parameter	1	2	3	4	5	6	S.E.M
IBW	39.00	39.00	39.00	39.00	39.00	39.00	0.00
ADFI	22.69^{a}	31.61^{b}	25.16^{a}	30.89^{b}	24.89^{b}	24.56^{a}	24.69
ADWG	15.02^{a}	19.06^{b}	15.17^{a}	20.52^{b}	14.84^{a}	15.05^{a}	0.64
TFI	635.45^{a}	885.20^{b}	704.59^{a}	865.12^{b}	696.80^{a}	687.75^{a}	691.53
FBW	420.60^{a}	533.6^{b}	424.77^{a}	574.57^{b}	415.42^{a}	421.47^{a}	17.98
FCR	1.51	1.66	1.66	1.50	1.50	1.68	1.5

Haematology Profile of Broiler Chicken

The result of the haematological indices of starter broiler chicken fed diet containing leaf meal composite is shown in Table 3. Packed Cell Volume (PCV) 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 (15) except at T4 and T5 which were lower; this could be due to the sex, though sex was not blocked in this study. (16) reported that the female chick tends to have a lower value than the male as estrogen level impairs erythrocyte synthesis (17) or due to dehydration since PCV represents an increase in the number of RBCs or a reduction in the circulatory plasma volume (18). 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 (16), 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 (19) 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 $*10^6/mm^3$ which is within the normal range for healthy broiler chicken (12) 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 (20). 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 T2, T3, T4 and T1 (control). the WBC values obtained in this study range between 10.7-18.4$*10^6/mm^3$ which is not in agreement with (20). 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 (21) and will optimise performance under stressful condition. Animals with high WBC values can generate antibodies and a high degree of disease resistance (22). 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 (5) or due to the presence of residual anti-nutrients which induces production of more antibodies that stimulate 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 can produce antibodies in the process of phagocytosis and have a higher degree of disease resistance (22). The increase in WBC indicates a superior disease fighting ability showing that the LMC did not compromise the bird's immunity (23).

Haemoglobin (Hb) values of the experimental broiler chickens is 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 is 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.

Table 3: Haematological indices of starter 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
MCHC (g/dl)	36.7^{b}	29.3^{a}	30.4^{a}	31.7^{a}	32.5^{a}	29.3^{a}	0.709

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 4. Serum protein shows that only T6 is similar to control while other treatment groups are 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. (24). The high value in T6 is 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) are 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 (25) and (26) who observed that bitter leaf reduces body fat.

Table 4: Serum biochemical indices of starter broiler chicken fed diet containing Leaf Meal Composite.

PARAMETER	1	2	3	4	5	6	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
Creatine(mg/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	122.16^{b}	120.29^{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

Conclusion and Applications

The findings of this study conclude that:

1. Inclusion of leaf meal composite in broiler chicken diet at 0.25kg/100kg (T6) depressed growth but was efficiently utilized at 0.125kg/100kg (T4).
2. This combination of leaf meal composite reduced 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

3. 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.
4. More research is required since age of leaves was not considered and this might influence the nutrient content in leaves.

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