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HAEMATOLOGICAL INDICES OF BROILER CHICKENS ADMINISTERED WATER CONTAINING MEDICINAL PLANT LEAF METHANOL EXTRACT

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Abstract
The discovery that the use of antibiotics in animal production is fueling the increasing problem of transmitting resistance bacteria from food animals to man has led to the search for an alternative to the use of antibiotics. Therefore, the response of broiler chickens fed various medicinal plants methanol extract as a replacement for antibiotics was investigated. The plant extracts encompass four available leaves; *Gercinia kola* (Bitter Kola), *Alchornea cordifolia* (Christmas bush), *Pterocarpus santalinoides* (Red scandal wood) and *Chromolera Odorata* (Hagony or Siam weed). A total of 180 unsexed Ross strain broiler chickens were randomly assigned to these dietary treatments which had 30 birds each. The treatments were replicated thrice with 10 birds per replicate in a Completely Randomized Design (CRD). Feed and water were provided *ad libitum* throughout the experiment which lasted for 56 days. Haematological indices of broiler chickens were evaluated. Significant differences ($p<0.05$) were observed in the mean values of all the hematological parameters measured across the treatment groups, except Packed cell volume and White blood cell. The result values obtained in this present study were within the standard range of PCV of normal birds with the reference range of 25.0 - 45.0%. However, the values obtained did not reveal any health problem. The findings of this study conclude that the medicinal plant methanol extracts have considerable potentials as component of broiler chicken diet. *Alchornea cordifolia* plant methanol extract can successfully be used to replace antibiotics at 1g/litre of drinking water for broiler production. Further research should be carried out on *Alchornea cordifolia* and other medicinal plants to examine their potentials and inhibitory characteristics.

Keywords: **Haematological indices, antibiotics, medicinal plant, methanol extract, *Gercinia kola, Alcorneacordifolia, Pterocarpus santalinoides, Chromolena odorata***

Introduction

The importance of poultry industry to the socioeconomic development of any country cannot be overemphasized as a result of its ability to provide animal protein at a relatively shorter duration and at reasonable cost to the consumer (Bonsu *et al.,* 2012). Therefore, broiler farming seems to be a considerable part of meat production and consumption in Nigeria. In the past two decades, broiler production has grown dramatically; these improvements are largely due to numerous researches and breeding programs which further enhanced feed utilization, growth rate and low levels of activity. Consequently, livestock represents an important source of high quality animal protein, providing about 36.5% of the total protein intake of Nigerians. It is one of the highest investments in agriculture with a net worth of N250 billion (Adejuwon *et al.*, 2014). Poultry farming is the process of raising domesticated birds such as chickens, ducks, turkeys, and geese for the purpose of farming, meat or eggs for food. Poultry mostly chickens are farmed in great numbers. Farmers raise more than 50 billion chickens annually as a source of food, both for their meat and for their eggs (Onunkwo, 2018). Many of the medicinal plants commonly available have not been scientifically studied to validate the efficacy and to identify the phytochemical constituents that may be responsible for their medicinal values. Research on such plants could result in discovery of compounds that could be developed into useful agents for replacement of antibiotics in poultry production. Medicinal Plant methanol extracts also have an appetizing, digestion stimulation properties and antimicrobial effect. According to Stepp and John (2004) leaf vegetables supply minerals, proteins and vitamins which could compliment the inadequacy of most feed stuffs. Sumner (2000) reported that leaf protein has the potential for supplying good quality food protein that would be obtained with cereals, legumes and oil seeds. They are also rich in potassium, calcium and magnesium (Mills, 2000). This study was aimed to determine the haematological indices of broiler chickens administered

four medicinal plant leaves (*Gercinia kola, Alcornea cordifolia*, *Pterocarpus santalinoides* and *Chromolera odorata*) methanol extract.

Materials and Methods

Location of the study

This study was carried out in the Poultry unit of the Teaching and Research farm, Michael Okpara university of Agriculture, Umudike, Abia State. Umudike is located on latitude 05° 21' N and longitude 07° 33'E, with an elevation of about 112m above sea level. The location has an annual rainfall of 177 - 2,000mm per annum, (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°C – 36°C (NRCRI, 2019).

Experimental procedure and design

The fresh leaves of the medicinal plants investigated (*A. cordifolia*, *C. odorata*, *P. Santalinoides* and *G. kola*) were collected within Michael Okpara University of Agriculture, Umudike and air dried for two weeks under shade. The dried parts were pulverized to fine powder using a mechanical grinder, sieved and weighed. A total of 500g of each of the powdered plant materials was soaked in 1500ml of methanol for 24h at room temperature. The extracts were filtered using non-adsorbent muslin cloth into a clean beaker. The filtrate was dried by evaporating off the solvent at 50°C in a hot air oven over a period of one to two days. The experimental design was Completely Randomized Design (CRD) with medicinal plant methanol extract as the only factor of interest. The study lasted for 56 days. A total of 180 day-old Ross strained unsexed chicks were weighed and randomly allotted to six equal treatment groups (T1, T2, T3, T4, T5 and T6); each having 30 chicks. Each treatment was replicated three times with 10 chicks per replicate. T1 was the positive control, T2 (Negative control), T3 (*Chromolaena odorata)*, T4 (*Pterocarpus santalinoides)*, T5 (*Alchornea cordifolia* and T6 (*Garcinia kola*). Administration of medicinal plant methanol extract in their drinking water (1g/l of water) commenced the first day of the experiment. Feed and water were given *ad libitum* throughout the experiment. Other management practices including prophylactic medications and vaccinations were carried out for positive control treatment only. The gross composition of the experimental diet is presented in Table 1. Blood samples were collected from one bird randomly selected from each replicate per treatment for the evaluation of haematological 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.

Data collection and analysis

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 Cole (1986). 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, 2012).

Table 1: Percent ingredients and nutrient composition of experimental diet

Parameter	Starter	Finisher
Maize	48.00	57.00
Soyabean meal	31.00	23.00
Fishmeal	3.00	3.00
Palm kernel meal	10.20	9.30
Wheat offal	4.00	4.00
Bone meal	3.00	3.00
Salt	0.25	0.25
Lysine	0.20	0.10
Methionine	0.10	0.10
Vit/Min premix	0.25	0.25
Total	100	100
Nutrient composition		
Crude protein (CP) (%)	22.98	19.63
Metabolizable energy (ME)	2883.22	2940.9

Results and Discussion

The result of the haematological indices of broiler chickens fed various medicinal plant methanol extracts are presented in Table 2. The results obtained in this study showed significant difference ($P< 0.05$) in packed cell volume, Total white blood cell and monocyte but no significant difference ($P> 0.05$) exist in hemaglobin (HB), Red blood cell and Heterophil. There was no significant ($p>0.05$) difference found in HB, however, treatment 3 gave the highest value, while T2 gave the least value (7.73g/dl and 6.2g/dl) respectively. Apart from T3 and T4, those values were within the normal range for healthy birds (7.0 – 13.0g/dl as reported by Mmereole (2008), the rest of the treatments (1, 2, 5 and 6) fall below the normal range of healthy birds. Robert *et al*. (2003) reported that low level of hemoglobin could imply that dietary protein was not of high quality as poor protein diets would usually result in poor transportation of oxygen from the respiratory organs to the peripheral tissues.

For Packed Cell Volume, T6 have the highest average concentration value of 37.17% which was not significantly different (P>0.05) from T5 which gave the least value of PCV. The result values obtained in this present study were within the standard range of PCV of normal birds with the reference range of 25.0-45.0% (Mitruka and Rawnsley, 1997). Furthermore, no significant difference (P> 0.05) exist in Red blood cell, however treatment I (positive control) gave the highest average value of 2.96 x 106/mm3, while treatment 2 (negative control) gave the least average value of 1.98 x 106/mm3. From this present result, it was observed that with the exception of treatment 2, the rest of the treatments have values within the standard range of Red Blood Cell (2.0 – 4.0 x 106/mm3) (Mitruka and Rawnsley 1997). For treatment 2 that has a value of 1.98 x 106/mm3 slightly below the normal / standard average value could be attributed to a reduction in the level of oxygen that would be cairned to the tissues and the level of carbondioxide returned to the lungs (Soetan *et al.,* 2013)

The range of values for Total white blood cell is 7.19 – 22.32 x 103/mm3 which are treatments 5 and 2 respectively. However, treatment 2 which gave the highest significant value was not significantly different (P > 0.05) from treatment 1, but significantly different (P > 0.05) from treatments 3, 4, 5 and 5. Treatment 5 which gave the least value was not significantly different (P>0.05) from treatments 3, 4, and 6 which were all fed medicinal plant methanol extracts. The values obtained were within the normal range of white blood cell which ranged from 9 - 31 x 103/mm3, except for treatment 5 which has a value slightly lower than the normal value. The low counts in white blood cell recorded in treatment 5 could be as a result of no disease condition, low production from bone marrow or anaphylactic shock (Ganong, 2005).

Heterophils are commonly involved in fighting infection and healing process and they function more effectively in combination with lymphocytes. However, there was no significant difference (P>0.05) among the treatment groups. Treatment 3 recorded the highest value of 22.67%, while treatments 4 and 6 which gave the same value of 9.33% was the least value. The lack of information in the available literature on heterophil counts in poultry did not warrant any comparison with this result. Thus, further studies are required in this direction.

Lymphocyte is a type of white blood cell or leukocyte which produces antibodies in the humeral immune response. There was no significant difference (P>0.05) recorded in lymphocyte counts, however treatment 6 gave the highest value of 81.33%, while treatment 5 gave the least value count of 64.67%. The values obtained in this study were higher than range values of 50 – 62% recorded in broiler chickens in Nigeria (Adeyemo and Sani, 2013). Though, this result agreed with the report by Onibi *et al*. (2011) who observes a high Lymphocyte counts that ranged from 78 – 90%.

The range of values of monocyte obtained in this present study ranged from 4.00 to 16.00% for treatments 3 and 5 respectively. Though significant difference exists among the treatments (P<0.05), Nevertheless treatments 1, 2, 3 and 6 have no significant difference (P>0.05), but significantly different from treatments 4 and 5 that in the same way have no significant difference (P>0.05) among them. Basophil did not have any significant difference among the treatment groups (P> 0.05). There were no Basophil counts recorded for treatments 2, 4 and 6 (0.00%), however, treatment 1 recorded 1.00% and treatment 3 (2 %), while treatment 5 was 0.67%. Eosinophil did not show any significant difference among the treatment groups (P<0.05). However, the values ranged from 0.00-2.67%. A 0.00% was observed in treatments 2 and 6, while treatment 3 gave the highest value. This present result is in line with Onibi *et al*. (2011) that reported range values of 0-2%, but contradicts the report of Adeyemo and Sam (2013) who reported a range value of 3-5%.

Table 2: Hematological indices of broiler chickens fed medicinal plants methanol extract

Parameters	Levels of Inclusion of Leaf Meal Composite						
	T_1	T_2	T_3	T_4	T_5	T_6	SEM
Hb (g/dl)	6.80	6.20	7.73	7.90	6.47	6.33	0.25
PCV (%)	29.23^{b}	31.33^{ab}	32.63^{ab}	32.90^{ab}	27.13^{b}	37.17^{a}	1.05
RBC ($x10^6/mm^3$)	2.96	1.98	2.15	2.30	2.13	2.64	0.14
WBC ($x10^3/mm^3$)	18.30^{ab}	22.32^{a}	10.52^{b}	10.31^{b}	7.19^{b}	9.21^{b}	0.36
Heterophil	10.67	14.67	22.67	9.33	16.67	9.33	1.85
Lymphocyte	80.00	80.67	71.33	78.67	64.67	81.33	2.24
Monocyte	6.00^{bc}	4.67^{bc}	1.33^{c}	10.67^{ab}	16.00^{a}	9.33^{ab}	1.47
Eosinophil	2.33	0.00	2.67	1.33	2.00	0.00	0.55
Basophil	1.00	0.00	2.00	0.00	0.67	0.00	0.24

$^{a-b-c}$:Means along the same row with different superscripts are significantly (p< .) different. S.E.M= Standard Error of Mean. Hb=Haemoglobin, RBC=Red Blood Cell, PCV=Packed Cell Volume, MCV=Mean Corpuscular Volume, MCH= Mean Corpuscular Haemoglobin, MCHC= Mean Corpuscular Haemoglobin Concentration

Conclusion

The findings of this study conclude that the medicinal plant methanol extracts have considerable potentials as component of broiler chicken diet. *Alcornea cordifolia* plant methanol extract can successfully be used to replace antibiotics both for starter and finisher phases of broiler production. Further research should be carried out on *Alcornea cordifolia* and other medicinal plants to examine their potentials and inhibitory characteristics.

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