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RESEARCH ARTICLE

THE EFFECT OF NEEM AND PAWPAW LEAVES SUPPLEMENTATION ON BLOOD PROFILE OF BROILERS

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ABSTRACT

A total of 300 fourteen day old arbor acre broilers were randomly divided into 4 treatments and replicated 5 times under completely randomized design to make 75 birds per treatment and 15 birds per replicate. Neem leaf meal (NLM) and pawpaw leaf meal (PLM) were prepared by air drying for 10 days. The birds were assigned to different treatment diets supplemented with the leaf meals and prepared as follows; T_1 (0% NLM+PLM), T_2 (0.5% NLM+PLM), T_3 (1% NLM+PLM) and T_4 (2% NLM+PLM). Except for packed cell volume (PCV), Monophils, eosinophils and platelets, there were significant ($P<0.05$) differences in white blood cell (WBC), lymphocyte, haemoglobin (Hb), heterophils of birds on the treatment diets compared with the control. While total serum protein (TSP) and globulin in the control recorded significant ($P<0.05$) lower values compared with treatments 2, 3 and 4. Albumin, cholesterol, Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alanine phosphatase (ALP) showed no significant ($P>0.05$) differences.

INTRODUCTION

There has been a developing controversy surrounding the use of antibiotics as growth promoters for food animals, these drugs are used at low doses in animal feed to control zoonotic pathogens and improve the quality of the products. The ultimate aim of broiler farmers is to produce table size chicken in the shortest possible time due to a continuous increase in human population. Phytogenic feed additives are good alternatives used in animal feeding to improve performance and boost immune responses of poultry. The neem tree *Azadirachta indica* contains azadirachtin, a biologically active compound which is responsible for its varied medicinal uses (William *et al.*, 2000). Different authors have reported the use of neem dry leaf as protein source and for controlling infections (Onyimonyi *et al.*, 2009; Tollba *et al.*, 2009; Obikaonu *et al.*, 2012; Wankar *et al.*, 2009) while Biu *et al.* (2006) used aqueous extract of neem to control poultry coccidiosis. Pawpaw tree *Carica papaya* is the most abundant natural source of papain. Pawpaw latex contains four identified proteolytic enzymes (Papain, Chymopapain A and B and Papaya peptidase A (Yadava *et al.*, 1990). Papain is an effective natural digestive aid which breaks down protein and cleanses the digestive tract (Poulter *et al.*, 1985). The leaf contains many biochemically active compounds and has some antibacterial effects (Bolu *et al.*, 2009). The tree is a rich source of antioxidants nutrients like carotene, vitamin C, vitamin B, pantothenic acids and some minerals. Pawpaw leaf has also been incorporated to broilers diet successfully (Onyimonyi *et al.*, 2009). The mode of operation of antibiotic growth promoters is to promote growth by enhancing feed utilization and inhibiting the activities of pathogenic microbes.

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In view of this *Carica papaya* and *Azadirachta indica* leaves have been tested and found to contain all the essential ingredients that can enhance feed intake, feed utilization and suppress the growth of pathogenic organisms. This study hereby tested the effect of the combined inclusion of these two plant leaves in the diets of broilers from the starter to the finisher phases on haematology and serum biochemical indices of broilers.

MATERIALS AND METHODS

Birds and Housing: Three hundred healthy one day old Arbor Acre broiler chicks were purchased from a commercial hatchery. The chicks were transferred to the experimental site, Teaching and Research farm, University of Ibadan, Ibadan. They were reared together under the same feeding, drinking, and brooding conditions. After 14 days of rearing on a commercial broiler starter feed, the birds were randomly divided into 4 treatments of 75 birds, and each group further sub divided into 5 replicates of 15 birds. The leaf meal were prepared by harvesting the leaves green from the mother plant within the University of Ibadan environment, they were air dried for 10 days and milled separately to produce neem leaf meal (NLM) and pawpaw leaf meal (PLM). Equal weights of NLM and PLM (1:1) were measured and mix properly to produce the leaf meal combination. They were supplemented into the diets at 0%, 0.5%, 1% and 2% for treatment diets 1, 2, 3 and 4 respectively. The diets were fed in 2 phases, the starter phase (2–4 weeks) and the finisher phase (4-7weeks) as presented in Table 1, they were formulated to meet the nutrient requirements of the chicks according to the strain catalogue recommendations. Samples of NLM and PLM were subjected to proximate analysis using the procedure of A.O.A.C (1990).

Blood collection and Analysis

At 42 days of age, 6 birds from each replicate were randomly selected for haematological test. The sampled birds were bled from the jugular vein to aspire 5mls of blood, which were also collected into the ETDA bottle for haematological assay as described by Bermudez and Stewart-Brown (2003). Haematological parameters such as Red Blood Cells (RBC), White Blood Cells (WBC), Packed Cell Volume (PCV), Haemoglobin (Hb), absolute count of neutrophils and lymphocytes will also be recorded according to Jain, (1986). Blood samples were processed immediately for RBC, WBC and PCV as follows: RBC and WBC + thrombocytes (Natt and Herrick, 1952) were determined using a Neubauer haemocytometer. Haematocrit value was determined by the standard micro-Haematocrit method, and expressed in percentage. Duplicate blood samples were loaded into standard heparinized capillary tubes, spun in a micro-Haematocrit centrifuge at 12,000 rpm for 5 min and measured on a micro-capillary reader. Haemoglobin (Hb) in erythrocytes was determined using the cyanmethaemoglobin method. Prior to reading the absorbance, Haemoglobin test samples were centrifuged to remove dispersed nuclear material. Sera were analyzed for the activities of aspartate aminotransferase (AST) and alkaline phosphatase (ALP) and for the concentration of proteins, albumin, glucose, total cholesterol, using commercial kits (Lamb, 1981).

Data Analysis

Data collected were subjected to one way analysis of variance while significant means were separated using Duncan multiple range test.

RESULTS

Proximate Analysis of Neem leaf and Pawpaw leaf Meals

The proximate composition of Neem leaf meal and pawpaw leaf meal are presented in tables 2 and 3 respectively. While Neem leaf proximate results showed a dry matter, crude protein, crude fibre and ether extract of 92.34, 23.50, 13.40 and 7.1% respectively, Pawpaw leaf gave a lower dry matter content of 86.14%, higher crude protein (27.2%), lower crude fibre (6.0%) and ether extract of 2.4 %.

Haematological Parameters

The results of the haematological parameters of broilers fed diets in treatments 1, 2, 3 and 4 are as shown in Table 4. Packed cell volume analyzed reported values obtained ranged from 23.66 in T_1 to 28.50 in T_3, Haemoglobin records the highest value (9.5) for birds on T_3 which differs significantly from the rest of the group. The RBC counts in treatments 3 was significantly ($p<0.05$) higher than birds on treatments 4. Total white blood cells counts in the control diet (22.91) differs significantly as having the highest value compared with those on experimental diets which have almost same values. Heterophils in treatments 2, 3 and 4 were significant ($P<0.05$) compared with the control. Basophils level was highest in T_3 and is significant ($P<0.05$) compared with the rest of the treatment.

Blood Serum

Total protein and globulin results showed birds on T_1 had the lowest total protein (3.01) which is significant ($p<0.05$) when compared with 4.17, 4.21 and 4.33 for treatments 2, 3 and 4 respectively.

Table 1. Gross Composition of Broiler Basal Diet fed to Experimental Birds

Ingredient	Starter Diet	Finisher Diet
Maize	53	65
Full fat Soya	15	28
Groundnut Cake	23	1
Wheat Offal	3	0
Fishmeal (72%)	2	2
Bonemeal	2.3	2.3
Oyster Shell	1	1
Methionine	0.1	0.1
Lysine	0.1	0.1
Premix	0.25	0.25
Salt	0.25	0.25
Total	100	100
Calculated Values		
Metabolisable Energy (Kcal/kg)	3001	3200
Crude Protein (%)	22.99	20.25
Calcium	1.3	1.2
Phosphorous	0.82	0.38
Methionine	0.52	0.51
Lysine	1.18	1.01
Crude Fibre	3.99	2.99

Table 2. Proximate Composition of *Azadirachta indica* leaf Meal

Nutrients	Composition
Dry matter	92.34
Crude protein	23.5
Crude fibre	13.4
Ether extract	7.1
Ash	7.5
Nitrogen free extract	40.84

Table 3. Proximate Composition of *Carica papaya* leaf Meal

Nutrients	Composition
Dry matter	86.14
Crude protein	27.2
Crude fibre	6
Ether extract	2.4
Ash	9.2
Nitrogen free extract	41.34

Table 4. Haematological analysis of Broilers fed leaf Meal supplements

Treatment/Parameters	1	2	3	4	SEM
PCV (%)	23.66^{a}	24.33^{a}	28.50^{a}	26.00^{a}	2.35
Hb (g/100ml)	7.87^{cd}	8.07^{bcd}	9.50^{a}	7.60^{d}	0.66
RBC (10^{6}/ul)	3.97^{bc}	3.85^{bc}	4.07^{ab}	3.67^{c}	0.16
Heterophils (10^{9}/l)	23.00^{b}	30.33^{a}	30.00^{a}	30.00^{a}	3.33
WBC (x 10^{3}/ul)	22.91^{a}	16.98^{b}	16.67^{b}	18.67^{b}	1419
Lymphocyte (%)	71.67^{a}	63.33^{b}	64.00^{b}	64.50^{b}	3.4
Monocytes (10^{9}/l)	3.33^{a}	2.66^{a}	3.00^{a}	2.50^{a}	0.98
Eosinophils (10^{9}/l)	1.67^{a}	3.33^{a}	2.0^{a}	3.00^{a}	1.48
Basophils (10^{9}/l)	0.33^{ab}	0.33^{ab}	1.00^{a}	0.00^{b}	0.46
Platelet (x 10^{4}/l)	19.56^{a}	16.86^{a}	14.95^{a}	12.75^{a}	45566.88

a,b,c,d means in the same row with different superscript are significantly different

Table 5. Blood Serum Characteristics of Broilers fed leaf Meal supplements

Treatment/Parameters	1	2	3	4	SEM
Total Protein (g/dl)	3.01^{b}	4.17^{a}	4.21^{a}	4.33^{a}	0.45
Albumin (g/dl)	0.95^{a}	1.31^{a}	1.25^{a}	1.19^{a}	0.23
Globulin (g/dl)	2.06^{b}	2.86^{a}	2.96^{a}	3.14^{a}	0.32
AST (U.I/l)	96.59^{a}	93.85^{a}	101.30^{a}	94.69^{a}	5.06
ALT (U.I/l)	17.76^{a}	16.64^{a}	13.85^{a}	18.71^{a}	3.22
ALP (U.I/l)	259.82^{bc}	308.82^{a}	234.05^{c}	274.53^{ab}	18.28
Cholesterol (mg/dl)	90.57^{a}	84.44^{a}	75.79^{a}	102.20^{a}	18.11

a, b, c: mean in the same row with different superscripts are significantly ($P<0.05$) different

There was no significant ($p>0.05$) change for albumin and cholesterol values obtained across the treatments, while AST and ALT also showed no significant ($P>0.05$) difference across the treatments.

DISCUSSION

The values obtained for crude protein content of NLM (23.50) were synonymous with those reported by Onyimonyi *et al.* (2009); and Esonu *et al.* (2006). The crude protein value obtained in this study is higher than those reported by Sokunbi *et al.*, (2003), and Obikaonu *et al.*, (2012). Crude fibre reported in this study is similar to those reported by Onyimonyi *et al.*, (2009) and Obikaonu *et al.*, (2012). The difference in the crude protein and crude fibre levels from the results reported by authors could be largely due to the age at which the plants were harvested for use. It has been reported that neem plant is one of the plant species that do not shed leaves all year round, in this study fresh and blooming leaves from young neem plants were harvested and this is evidenced by the high crude protein, low crude fibre and high ether extract recorded in this study. The proximate composition of PLM showed that the crude protein was 27.2%, this was lower than the value reported for PLM by Onyimonyi *et al.*, (2009). The moisture content of the PLM was higher than those reported by Onyimonyi *et al.*, (2009). The moisture was higher because the PLM used for this study were harvested during the raining season and the method used for the processing was air drying which could only evaporate the moisture to a certain level; furthermore the leaves were tender and succulent and contain more moisture than the matured ones. However, the crude fibre, ether extract and ash content obtained from the proximate analysis were similar to those reported Onyimonyi *et al.*, (2009). The high moisture content found in PLM and NLM further suggests their capacity to supplying the necessary active ingredients like papain and chymopapain since they are all found in large quantities in the moisture content. The overall mean packed cell volume (PCV), Haemoglobin (Hb), Red blood cells (RBC) and heterophils as given in table 4 showed that Hb, RBC and Heterophils were reduced significantly in birds on the control diet and treatments 2 and 4 which suggests anaemic changes due to mal-absorption of the nutrients, and this reduction could have led to reduction of the oxygen carrying capacity of the blood (Aduloju, 2000). Birds on treatments 3 fell in the normal range for birds Haemoglobin as published by Mitruka and Rawnsley, (1977); Fraser, (1986); Aiello and Mays, (1998); Okeudo, (2003). Birds on T_3 had higher values for haemoglobin, PCV and RBC with a significance difference in haemoglobin compared with other treatments.

Red blood cells are responsible for the transportation of oxygen and carbon dioxide in the blood as well as manufacture of haemoglobin, hence higher values indicated a greater potential for this function and a better state of health. This may be due to the inclusion of the mixture of neem and pawpaw leaf meal at 1% level which signifies a better utilization of the diet and good health and good respiratory system for the broilers (Schalm *et al.*, 1975), since higher values for RBC were witnessed in treatments supplemented with leaf meals, this also resolve with the view of Penny (1987). Diets have been reported to have significant influence on haematological variables (Veulterinora, 1991). White blood cells are involved in protecting body from infection and consist of lymphocytes, monocytes, eosinophils and basophils. They amongst other functions kill virus-infected cells, enhance the production of

antibodies and engulf foreign materials that enter the body (Olugbemi *et al.*, 2008). The significant higher values of WBC observed in birds on the control diet implied that birds on the treatment diet were better immunized against infection due to the protective nature of the leaf meals than those on the control diet as high WBC is an indication of disease condition. The lymphocytes were significant in the control which indicated that the neem leaf and pawpaw leaf meals did not produce any form of infection since these parameters only observed when there are infection (Frandson, 1974). Serum biochemistry is a labile biochemical system which can reflect the condition of the organism and the changes happening to it under influence of internal and external factors. From the results obtained in this study there were no significance difference in the albumin, Aspartate Transaminase (AST), Alanine Transaminase (ALT) and cholesterol levels in the control and in the treatment diets. AST is found in the liver, heart, skeletal tissues; it is commonly measured as a marker for liver health while ALT measured diagnostic evaluation of hepatocellular injury, to determine liver health. This results indicates that the treatments effect pose no threat to the health of the birds. The total serum protein (TSP) and globulin vary significantly in the control and treatment diets. The TSP and globulin are indirect indices for measuring the nutritional protein adequacy (Eggum, 1987; Tewe. 1985). The similar TSP and globulin values in the experimental diets with other standard values reported in literature (Frendson, 1986; Aletor and Egberongbe, 1992) suggest that the nutritional quality of leaf meals as supplements may be acceptable.

Conclusion

From this study, economic broiler production can be achieved by feeding broiler diets supplemented with a combination of neem and pawpaw leaf meal at 1%, this will boost performance, reduce pathogenic bacteria activity and reduce mortality due to infections.

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