HAEMATOLOGY AND SERUM BIOCHEMICAL INDICES OF BROILER CHICKENS FED NEEM LEAF POWDER AS FEED ADDITIVES.

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ABSTRACT

One hundred and twenty (120) day old Amour breed of broiler chicks were randomly assigned to four dietary treatments replicated thrice with (10) birds per replicate in a completely randomized design. Four diets were formulated to contain neem leaf powder (NLP) at 0, 2, 4, and 6g/kg of the diet designated as T_1 (control), T_2, T_3 and T_4 respectively. Blood samples were collected from two birds per replicate and processed to determine effect of NLP supplementation on haematology and serum biochemical indices of broiler chickens. The results for hematological indices revealed significant (P<0.05) effect of NLP on Packed Cell Volume, Hemoglobin, White Blood Corpuscles, Mean corpuscular volume,Mean corpuscular hemoglobin and lymphocytes Serum biochemistry revealed significant(P<0.05) difference among the treatment group in Total protein, Albumin, Urea, Creatinine, Globulin, Alkaline phosphate, Alanine amino transfarase and Aspartate amino transfarase. It is concluded that NLP did not adversely influence haematology and serum biochemical indices of broiler chickens.

Key Words: Broiler Chickens, Neem Leaf, Blood, Serum Biochemical Characteristics

INTRODUCTION

Feed additives such as enzymes, antibiotics, coccidiostats, prebiotics, organic acids, probiotics, etc are being used to improve growth rate, feed efficiency and control disease. The use of anti- biotics for growth promotion, has received criticism over years. It has been blamed as a factor compromising the effectiveness of antibiotics for treating human infections and posing a serious threat to public health (Wegener, 2003). The situation has put tremendous pressure on the poultry industry to find alternatives. Products of plant origin like spices, herbs and many plant extract have been recently considered (Windisch et al., 2008). Neem (Azadirachta indica) is an indigenous tropical plant, which predominates in Nigeria. It contains several biologically active constituents such as azadirachtin, meliacin, gedunin, salanin, nimbin, valassin and many other derivatives of these principles, (NRC, 1992). The Objectives of this study is to evaluate the effect of Neem leaf power as feed additive on haematology and serum biochemical indices of broiler chickens.

Materials and Methods

Experimental Site

The study was conducted at the poultry unit of the Teaching and Research Farm, Department of Animal Science, University of Maiduguri. The area, falls between Latitude 11^{o} 05' and 12^{o} North and Longitude13^{o} 05' and 14^{o} East. It is characterized by short rain fall (3-4 months) which varies from 50 mm to 500 mm, with long dry cold season (8-9 months). The ambient temperature could be as low as 20^{o}C during the dry hot (February-May). (Raji et al., 2009).

Experimental Diets and Processing of Neem Leaves Powder

The Neem leaves used for the experiment were obtained within the University of Maiduguri environment. They were air-dried in a ventilated room for one week. The leaves were there after reduced to small particles with mortar and pestle to produce Neem leaf powder (NLP). Four experimental starter and finisher diets were formulated. The NLP was added as additive to the diets at 0, 2, 4, and 6g/kg designated as T_1 as control, T_2 (2g/kg feed), T_3 (4g/kg feed) and T_4 (6g/kg feed). Composition and calculated analysis of the experimental starter and finisher diets are presented in tables 1.

Experimental Stock and Design

One hundred and twenty mixed-sex Amour broiler chicks were randomly allocated into four treatment groups T_1, T_2,T_3 and T_4. Each treatment consists of 30 birds and were replicated three times with 10 birds per replicate in a complete randomized design. Feed and water were given ad- libitum, the experiment lasted eight weeks.

Data Collection and Analysis

At the end of the experiment, blood samples were collected from two birds per replicate via punctured wing in well labelled bottles (plain and EDTA). The blood samples in the EDTA bottles were processed for analyzing haematological parameters. The samples in the plain test tubes without anti-coagulant were centrifuged for five minutes to separate the serum from the blood for serum biochemical indices. All data collected were subjected to analysis of variance

Table 1. Composition and Calculated Analysis of Broiler Starter and Finisher Diets

	Starter				Finisher			
	Inclusion Level of Neem							
Ingredients (%)	T_1 (Control)	T_2 (2g/kg)	T_3 (4g/kg)	T_4 (6g/kg)	T_1 (Control)	T_2 (2g/kg)	T_3 (4g/kg)	T_4 (6g/kg)
Yellow Maize	46.00	46.00	46.00	46.00	56.00	56.00	56.00	56.00
Groundnut cake	15.50	15.50	15.50	15.50	12.50	12.50	12.50	12.50
Soyabean meal	15.50	15.50	15.50	15.50	12.50	12.50	12.50	12.50
Wheat offal	12.00	12.00	12.00	12.00	10.00	10.00	10.00	10.00
Fish meal	7.00	7.00	7.00	7.00	5.00	5.00	5.00	5.00
Bonemeal	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Limestone	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Salt	0.25	0.25	0.25	0.25	0.20	0.20	0.20	0.20
Premix *	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Lysine	0.10	0.10	0.10	0.10	0.15	0.15	0.15	0.15
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis								
Crude protein	23.34	23.34	23.34	23.34	20.46	20.46	20.46	20.46
Crude fibre	3.99	3.99	3.99	3.99	4.50	4.50	4.50	4.50
Calcium	1.57	1.57	1.57	1.57	1.68	1.68	1.68	1.68
Phosphorus	1.01	1.01	1.01	1.01	1.07	1.07	1.07	1.07
Lysine	1.15	1.15	1.15	1.15	1.03	1.03	1.03	1.03
Methionine	0.49	0.49	0.49	0.49	0.46	0.46	0.46	0.46
ME (kcal/kg)	2840.36	2840.36	2840.36	2840.36	2920.94	2920.94	2920.94	2920.94

*****Starter **p**remix supplied per kg of feed: Vitamin A =12,000 IU, Vitamins D_3 =2500 IU, Vitamin E =15000mg, Panthotenic acid 15000mg, Vitamin B_{12} =15000mg, Vitamin B_6 =2500mg, Vitamin K=2000mg, Chlorine = 5000mg, Manganase = 10000mg, Nicotinic acid = 40000mg, Vitamin B_1= 2000mg, Vitamin B_2 =6000mg, Biotin = 6000mg, Vitamin C = 3000mg, Copper = 15000mg, Colbalt = 250mg and Selenium =1000mg.

*****Premix finisher supplied per kg of feed: Vitamin A =12,000 IU, Vitamins D_3 =2500 IU, Vitamin E =15000mg, Panthotenic acid 15000mg, Vitamin B_{12} =15000mg, Vitamin B_6 =2500mg, Vitamin K=2000mg, Chlorine = 5000mg, Manganase = 10000mg, Nicotinic acid = 40000mg, Vitamin B_1= 2000mg, Vitamin B_2 =6000mg, Biotin = 6000mg, Vitamin C = 3000mg, Copper = 15000mg, Colbalt = 250mg and Selenium =1000mg.
*ME=Metabolisable energy= 432 + 27.91 (CP + EE x 2.25 + NFE) (Ichaponami, 1980).

(ANOVA) using a Complete Randomized Design (CRD). Significant differences between treatments were compared using least Significant Differences (LSD).

Result and Discussion

Heamatological Parameters

The results for the haematological parameters are presented in Table 2. Neem leaf powder (NLP) supplementation had significant ($P<0.05$) effect on blood parameters of broiler chickens. The result showed that birds on NLP groups recorded higher packed cell volume (PCV) values (26.67- 31.33%) than the control (24.00). However, all values were within normal range of (25 – 45%) for healthy chickens as was reported by others (Mitruka and Rawnsley, 1977; Anon, 1980). The result of this study contradict the reports of Obun et al. (2013) who noted a significant decline in PCV values of broiler finishers supplemented with NLM. It is however, similar to the reports of Esonu et al. (2006) who showed significant increase in PCV values of laying hens fed up to 10% NLM. Haemoglobin (Hb) showed an increasing trend as NLP increases (7.96 – 10.30g/dl). However, all values were within normal range (7-13 g/dl) reported by Jain (1993). The findings of this study are supported by the reports of Esonu et al. (2006), Obikaonu et al. (2012) and Beg et al. (2018). On the contrary, Bonsu et al. (2012) and Odo and Bratte (2015) found no significant effect of NLM on hemoglobin in broiler and layer chickens respectively.

Birds on 2g/kg NLP recorded highest White blood cells (WBC) value ($14.86\times10^3/mm^3$) while the least ($10.53\times10^3/mm^3$) was recorded for T3 (4g/kg). However, these values were within normal range of 9- $31\times10^3/mm^3$ reported by Banerjee (2006). This result contradicts the report of Bonsu et al. (2012) who observed higher WBC in the control compared with those birds fed the NLM diets. Mean Corpuscular Volume (14.64 - 17.55 μ^3) and Mean Corpuscular Haemoglobin (5.01 - 5.82) values recorded for birds on NLP groups were significantly ($P<0.05$) higher than the control (12.61 and 4.16 respectively). On the other hand, the control had the highest (75.00) lymphocytes values than NLP groups, however, all the values obtained in this study were within the range of 45-70 reported by Barnajee (2006). The superiority in haematological indices of the chickens on NLP group could be attributed to high protein (20.68%) and organic matter (12%) content of neem leaf which are required for blood formation (Ubua et al., 2018).

Serum Biochemical Indices

Neem leaves supplementation had no significant ($P>0.05$) effect on serum glucose, cholesterol, direct and conjugate bilirubin. It however, had significant ($P<0.05$) effect on the other serum biochemical indices measured in this study (Table 3).

Total Protein, Albumin and Globulin

The values for total protein for birds on all neem leaf groups (53.33-61.66 g/L) were significantly higher than the control value (45.33 g/L). These might be as a result of high protein content (20.68%) of neem leaf. However, all the values are within normal range (40-62 g/L) reported for broiler chickens by Nanbol et al. (2016).

Table 2:Heamatological Parameters of Broiler Chickens Fed Neem Leaf Powder

Parameters	(Inclusion level of neem)				
	T_1 (Control)	T_2 (2g/kg)	T_3 (4g/kg)	T_4 (6g/kg)	SEM
PCV %	24.00^{b}	28.67^{ab}	26.67^{ab}	31.33^{a}	0.025 *
HBg/DL	7.96^{a}	9.53^{ab}	8.86^{ab}	10.40^{a}	0.803 *
WBC×10^3	12.66^{b}	14.86^{a}	10.53^{c}	13.06^{b}	0.665 *
RBC×10^6	19.00	19.00	18.00	17.93	1.296^{NS}
MCV (μ^3)	12.61^{b}	15.07^{ab}	14.64^{ab}	17.55^{a}	1.357 *
MCH	4.16^{b}	5.01^{ab}	5.40^{ab}	5.82^{a}	0.695 *
MCHC	33.19	33.26	33.24	33.19	0.080^{NS}
Neutrophils	22.66	29.66	35.33	23.00	6.403^{NS}
Lymphocytes	75.00^{a}	69.33^{ab}	62.33^{b}	74.66^{ab}	5.416*
Monocytes	0	0	0	0.6	0.235^{NS}
Eosinophils	2.33	0.33	1.66	1.66	1.795^{NS}

a, b, c = Mean within the same row bearing different superscript differ significantly (P<0.05)
NS = Not significantly different (P>0.05)
* = Significant (P<0.05)
SEM = Standard Error Mean.

Table 3: Serum Biochemical Indices of Broiler Chickens Fed with Neem Leaf Powder

	(Inclusion level of Neem leaf powder)				
Parameters	T_1 (0g/kg)	T_2 (2g/kg)	T_3 (4g/kg)	T_4 (6g/kg)	SEM
Total Protein. g/L	45.33^{c}	53.33^{b}	61.66^{a}	54.33^{b}	2.981 *
Albumin.g/L	28.00^{c}	33.33^{b}	40.00^{a}	33.33^{b}	1.900 *
Urea.mmol/l	3.90^{b}	4.96^{ab}	5.93^{ab}	6.46^{a}	0.995 *
Creatinine.mmol/L	47.66^{b}	75.00^{a}	72.66^{a}	70.33^{a}	9.210 *
Globulin.g/dl	17.33^{a}	20.00^{ab}	21.00^{ab}	21.00^{ab}	9.210 *
Cholestrol.mmol/L	2.90	4.5	4.6	4.2	0.796
Alkaline Phosphate.IU	40.00^{d}	73.66^{b}	86.00^{a}	57.66^{c}	3.018 *
Conjugate bilirubinIU	3.60	4.26	4.06	2.66	1.101
Direct Bilirubin.mmol/L	3.06	3.00	3.50	1.60	0.925
Glucose.mmol/L	3.43	3.90	3.26	3.63	1.001
ASAT (IU/L)	25.00^{d}	37.33^{c}	52.33^{b}	72.00^{a}	3.778 *
ALAT (IU/L)	27.00^{c}	67.33^{a}	46.66^{b}	30.00^{c}	5.190 *

ALAT = Alanine amino transferase
ASAT = Aspartate amino transferase

a, b, c = means within the same row bearing different superscript differ significantly (P<0.05)
* = Significant (P<0.05)
SEM = Standard Error Mean.

The albumin values were significantly different among the groups. Chickens on 4g/kg NLP recorded the highest (40.00 g/l) albumin value followed by those on 2 and 6g/kg groups while the control recorded the least (28.00 g/l) value. All values were within the range of 20-35g/l reported by Mitruka and Rawnsley (1977). The value for birds on 4g/kg NLP (40 g/l) was higher than the reported range above. This could be attributed to change in protein reserve in animals which is usually associated with alteration in the albumin fraction as reported by (Pirpne et al., 2009). The Globulin values (17 - 21 g/l) obtained in this study were higher than the normal range level of 2.33-3.33g/l reported by Anon (1980). These might be attributed to high level of protein, since globulin is dependent on the availability of protein in the blood.

Urea and Creatinine

The values obtained for serum urea for all the groups (3.90 -6.46 mmol/l) were within normal range of 4.00 - 7.50 mmol/l reported by Anon (1980). However, values recorded for all groups on neem leaf supplementation (4.96-6.46 mmol/l) were higher than the value obtained for the control (3.90 mmol/l). Since serum urea is also an indicator of the quality of dietary protein, it indicates the sufficiency of neem leaf protein. Mean creatinine values obtained in this study showed significant (P<0.05) difference. Chickens on the neem leaf groups recorded higher values where birds on 2g/kg NLP had 75.00 mmol/l, followed by birds on 4g/kg 72.66 and 6g/kg 70.33mmol/L respectively. The values were within the normal range of 65-97mmol/l reported by Anon (1980). However, the control recorded lower than the normal range (47.66 mmol/l). The reports of Nworgu (2004) indicated that the level of creatinine measures the degree of muscles wastage. This suggests that birds on the treatment groups might have utilized their protein reserve for maintenance. Similarly, since blood creatinine levels indicate kidney function, high levels in the NLP group could hint a decline in kidney function.

Alkaline phosphate

Alkaline phosphate revealed Significant (P<0.05) difference among the treatments groups. Values for birds on NLP groups (57.66-86.00 mmol/L) were higher than the control (40.00 mmol/L). However, the range of values recorded (40.00- 86.00 mmol/L) for all the groups were within the normal range of 20-160IU/I reported by Anon (1980).

Aspartate Amino Transferase (ASAT)

The mean values obtained for ASAT were significantly (P<0.05) different among the treatment groups. The values obtained for NLP group (25.00 - 72.00IU/I) were higher than the value recorded for the control. All values were however, lower than the normal level of 102-130IU/l as reported by (Majekodunmi et al., 2013). Since, the amount of ASAT in the blood is directly related to the extent of the tissue damage, elevated levels in the treatment groups suggests

some levels of tissue damage in the liver (Anon, 1980).

Alanine amino transfarase

Alanine amino transfarase values obtained showed significant ($P<0.05$) differences among the treatments groups with increasing trend (30.00 - 67.33 IU/L) as neem leaf supplementation increases. The values obtained for the control and T2 (2g/kg NLP) were within the normal range of 7-35IU/I reported by Majekodunmi et al. (2013). However, values recorded for birds on high (4 and 6 g/kg) NLP were higher (52.33 and 72.00IU/I) than the normal range. This observation suggests some damages within the cells of the animal especially in the liver as reported by Robbin (2011). Elevated level of ALAT is a specific indication of liver damage while low ALAT level results in a normal healthy liver. This observation coupled with decline in the weight of the liver and high ASAT values observed in this study, suggest that NLP might have adversely affected functions of the liver and caused some liver damage in the experimental birds.

CONCLUSION

It can be concluded from this study that neem leaf powder did not adversely influence hematological parameters of broiler chickens. It however, caused some adverse effects on some serum biochemical indices of broiler chickens. It is therefore, recommended that NLP should not exceed 2g/kg feed Inclusion level.

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