HAEMATOLOGICAL AND SERUM BIOCHEMICAL INDICES OF BROILER CHICKENS ADMINISTERED NEEM (*Azadirachta indica*) LEAF INFUSION AS A REPLACEMENT FOR ANTIBIOTICS

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ABSTRACT

Ninety-day-old broiler chicks were used in a six-week trial to evaluate the effect of neem (Azadirachta indica) leaf infusion on haematological and serum biochemical profiles of broilers. The birds were randomly assigned into five (5) groups in a completely randomized design. Each group was sub-divided into three (3) replicates of six (6) birds each. The neem leaf infusion (NLI) was included at 0g/L,10g/L, 20g/L, 30g/L and 40g/L as a source of antibiotics. Blood samples were collected from three birds in each treatment and evaluated for packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC) and white blood cell differentials at the end of the trial. Total protein, albumin, globulin, Aspartate aminotransferase enzyme (AST), Alanine transaminase (ALT) and Alanine phosphatase were evaluated for biochemical profile. Data collected were evaluated using one-way analysis of variance. Packed cell volume was significantly ($p<0.05$) affected in favour of birds on 40g/L NLI (33.00%), haemoglobin and red blood cell were not significantly ($p>0.05$) influenced by NLI. White blood cell was significantly ($p<0.05$) different across the treatments in favour of birds on 10g/L NLI (25.88 $X10^9$ /ml). Total protein, albumin and globulin were not significantly ($p<0.05$) affected. Aspartate aminotransferase, ALT and ALP were statistically ($p<0.05$) different across the treatments with birds on 40g/L NLI having the highest values (212.33, 36.30 and 239.35ul, respectively). The current findings suggest that NLI up to 40g/L has no deleterious effect on haematological profile of broilers; nevertheless, biochemical indices revealed that NLI should not be used above 20g/L to avert liver damage.

Keywords: Neem leaf infusion, Antibiotics, Haematological profile, Biochemical profile

INTRODUCTION

The innovation of antibiotics was an accomplishment in controlling infectious pathologies and enhancing feed utilization. Antibiotics, both of natural and synthetic sources are used to both prevent multiplication and destroy bacteria. They are regularly used to treat and avert infections in humans and animals. The poultry business uses antibiotics to improve meat production through improved feed conversion, growth rate advancement and disease prevention. Antibiotics can be used efficaciously at sub-therapeutic dosages in poultry production to support growth (Chattopadhyay, 2014); and safeguard health of birds by transforming the immune status of broiler chicken (Lee *et al*., 2012). Nevertheless, scientific proof submits that the enormous use of antibiotics has headed to amplified problem of antibiotic resistance and occurrence of antibiotics residue in feed and environment (Carvalho and Santos, 2016). In addition to bio-resistance, antibiotics misuse has led to drug residue in animal products (Gonzalez and Angeles, 2017). Numerous antibiotics like penicillin, tetracycline, macrolide, aminoglycoside and amphenicol have been identified in foods (Diarra and Malouin, 2014). Residues in livestock production can truly have adverse effect on human health; this is the case for tetracyclines, which impede teeth growth in young children (Kummerer, 2009). This is similarly the situation with beta-agonists, like clenbuterol, leading at times to food poisoning and muscle tremors, palpations and tachycardia (Chan, 1999).

Consumers' pressure and concerns towards unsafe effects of antibiotic usage and the prohibition of antibiotics in European Union have provoked scholars to contemplate about replacements for antibiotics (Diarra and Malouin, 2014). Much investigation has been carried out to look for natural agents with comparable favourable properties of growth promoters. There are undeniably a number of non-therapeutic alternatives that can replace antibiotics usage. Amongst these, the most prevalent are probiotics, prebiotics, bacteriocins, organic acids, immunostimulants, bacteriophages, enzymes, phytocides, essential oils nanoparticles and phytogenic feed additives. Phytogenic feed additive (PFA) derivative from plants, herbs and spices are used to expand animal performance. They have been very effective because of their encouraging effects on growth, enhanced immune system and decreased stress response. Current outcomes indicated that PFA were good substitutes for antibiotics (Ghasemi *et al.*, 2014). One of such plants is neem. Neem (*Azadirachta indica*) is an indigenous plant of Asian

subcontinent recognised for its valuable medicinal assets since ancient times. Neem has attracted world-wide importance due to its massive array of medicinal properties like antibacterial, antiviral, antifungal, antiprotozoal, hepato-protective and numerous other properties without showing any antagonistic effects (Kale ***et al.,*** 2003). This study was, therefore, targeted at observing the effect of neem (*A. indica*) as an alternative to antibiotics on the organs and gut morphology of broiler chickens.

MATERIALS AND METHODS

The experiment was carried out at the Teaching and Research Farm of the Oke-Ogun Polytechnic, Saki. Ninety (90) unsexed one-day-old Cobb broiler chicks were obtained from a reputable hatchery in Ibadan, Nigeria, for the study. The birds were randomly distributed into five treatments (0g/L, 10g/L, 20g/L, 30g/L and 40g/L neem leaf infusion), each consisting of three (3) replicates, with six (6) birds per replicate, in a completely randomized design. The study lasted for six weeks (42 days). Commercial diet containing 23.00% crude protein (CP) and 16.47 MJ/kg was fed during the starter phase and a commercial diet having 20.10% CP and 11.62 MJ/kg was fed for the finisher phase.

Preparation of neem leaf infusion

Neem leaves were harvested within the research farm premises after which the leaves were separated from the stalks. The leaves were weighed (0g, 10g, 20g, 30g and 40g), crushed into paste with mortar and pestle. Each portion was soaked in 1litre of clean water and was filtered using cheese cloth. The resulting filtrate was the neem leaf infusion.

Blood Collection

At 42 days of drenching the birds with the neem leaf infusion (NLI), blood samples (2.0 ml) were collected with needle and syringe through the brachial wing vein of three cocks per replicate into ethylene diamine tetra acetic acid (EDTA) bottles and plain bottles (2mls per cock) directly for the determination of haematological and serum biochemical indices using standard procedures, as described by Weiss and Wardrop (2010). Haematological indices such as Red Blood Cell Count (RBC), Packed Cell Volume (PCV), White Blood Cell Count (WBC) and Haemoglobin concentration were evaluated. Haemoglobin concentration was determined photo-metrically using cyano-haemoglobin method (Elarabany, 2018), and PCV using Hacksley hematocrit centrifuge (UK) according to the procedure by Morris (2001). The PCV values were determined using a micro haematocrit reader. The WBC count was determined using the Neubaer count chamber following procedure described in a previous study (Fudge, 2000). The serum metabolites determined were Total protein, Albumin, Globulin, Aspartate aminotransferase enzyme (AST), Alanine transaminase (ALT) and Alkaline Phosphatase.

Statistical Analysis

Data collected were evaluated using one-way analysis of variance (ANOVA) in a completely randomized design (CRD), employing SAS (2012). Means were separated using Duncan's Multiple Range Test of the same software.

RESULTS AND DISCUSSION

Table 1: Haematological indices of broilers administered varying levels of neem leaf infusion.

Treatment	0g/L NLI	10g/L NLI	20g/L NLI	30g/L NLI	40g/L NLI	SEM
PCV %	28.66^{ab}	32.00^{a}	26.33^{b}	30.33^{ab}	33.00^{a}	1.63
Hb (g/dl)	9.30	10.56	8.36	9.73	10.60	0.65
RBC ($X10^{6}$ /ml)	3.00	3.09	2.25	3.00	3.27	0.22
WBC ($X10^{9}$ /ml)	15.65^{b}	25.88^{a}	14.23^{b}	16.90^{b}	17.46^{b}	59.42
Lymphocytes (%)	59.00^{bc}	64.33^{ab}	58.00^{c}	66.00^{a}	65.33^{a}	1.87
Neutrophills (%)	35.00^{b}	29.33^{ab}	34.33^{a}	26.33^{b}	26.66^{b}	1.77
Monocytes (%)	3.00	2.33	4.00	4.00	3.66	0.71
Eosinophils (%)	3.00	3.66	3.00	3.00	3.61	0.48

a, b, c means with different superscripts within a row are significantly ($p<0.05$) different

Haematological profile of broiler birds placed on varying levels of neem leaf infusion was as presented in Table 1. Values for packed cell volume (PCV) was significantly ($p<0.05$) different across the treatments, its values ranged from 26.33% (20g/L NLI) to 33.00% (40g/L NLI). These values were below the physiological value of 35.00 - 55.00% reported by Kokore, (2021). The highest PCV value was obtained in 40g/L NLI; this implies that neem leaf infusion does not hinder blood volume in the experimental birds. The present result showed no traces of anaemia, as obtained values were within standard range reported by Haemoglobin (Hb) was not significantly ($p>0.05$) different across the treatments. Its values were between 8.36g/dl (20g/L NLI) and 10.60g/dl (40g/L NLI).

The values of Hb recorded by all groups were, however, within the normal range (7.0g - 18.6g/dl) reported by Mitruka and Rawnsley (1977) in chicken. Red blood cell (RBC) was not different significantly ($p>0.05$) across the treatments. Its values were from 2.25($X10^6$ /ml) in birds administered 20g/L NLI to 3.27 ($X10^6$ /ml) in birds administered 40g/L NLI. The white blood cell (WBC) was significantly ($p<0.05$) reduced as the level of NLI increased. The birds on 10g/L NLI had highest value of WBC (25.88 $X10^9$ /ml) whereas their counterparts in the other groups had similar values as birds on 20g/L NLI had the least value of 14.23 $X10^9$ /ml. Lymphocyte values were significantly different ranging from 58.00% (20g/L NLI) to 66.00% (40g/L NLI), these values were slightly lower to the value (76.64 - 61.64%) reported by (Aiello and Mays, 1998). Neutrophils values were similarly high in 10 and 20g/L NLI (29.33% and 34.33% respectively) and significantly ($p>0.05$) reduced as NLI increased. Though the obtained values were within the physiological standard (37.69 - 22.19%) reported by Aiello and Mays (1998).

Neem leaf infusion had no significant ($p>0.05$) difference on monocytes and eosinophil. Neem leaf infusion had no significant difference on the total protein, albumin and globulin as presented in Table 2. Aspartate aminotransferase enzyme (AST) level was significantly ($p>0.05$) different. Its value ranged from 192.66 ul (20g/L NLI) to 212.33 ul (40g/L NLI). Alanine transaminase (ALT) also revealed significant ($p>0.05$) difference from 21.65 ul (20g/L NLI) to 36.30 ul (40g/L NLI). The liver is reported to contain enzymes like ALT and AST; it releases these enzymes in the blood when damaged (Sherwin, 2003). Elevation of serum AST and ALT can occur with states of altered hepatocellular membrane permeability either due to circulatory hypoxia, exposure to toxins and toxaemia, inflammation, metabolic disorders or proliferation of the hepatocyte. In the stunting syndrome of broiler chickens, increased activities of AST and ALT were also associated with liver and intestinal damage (Rani *et al*., 2011); hence, the increased level of AST above 139.30 – 197.30 ul and ALT above 10.30 and 11.00 ul reported by Hassan *et al*. (2016). The presence of significant differences among treatments in serum AST and ALT in the present study may collectively reflect the abnormal liver and intestinal functions of chickens placed on NLI.

Table 2: Serum biochemical indices of broilers administered varying levels of neem leaf infusion.

Treatment	0g/L NLI	10g/L NLI	20g/L NLI	30g/L NLI	40g/L NLI	SEM
Total Protein (g/dl)	3.73	3.53	3.43	3.60	3.76	0.11
Albumin (g/dl)	0.70	0.56	0.66	0.73	0.70	0.07
Globulin (g/dl)	3.03	2.96	2.86	2.93	2.13	0.42
AST (ul)	198.00^{bc}	204.33^{ab}	192.66^{c}	200.34^{ab}	212.33^{a}	3.28
ALT (ul)	27.00^{ab}	31.00^{ab}	21.65^{b}	29.00^{ab}	36.30^{a}	3.24
ALP (ul)	212.00^{b}	220.66^{b}	203.00^{b}	210.00^{b}	239.35^{a}	5.90

a, b, c means with different superscripts within a row are significantly ($p<0.05$) different

CONCLUSION

It could be concluded that neem leaf infusion had no deleterious effect on the blood function of the experimental birds as it enhanced the defence mechanism of the birds. However, higher inclusion level above 20g/L NLI as a replacement for antibiotic in broilers is detrimental to the liver integrity.

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