APRW -57

Haematological and Serum Biochemical Indices of Layer Chickens Fed Cassava Pulp Based Diets Supplemented with Morinda (*Morinda lucida)* and Neem (*Azadirachta indica*) Leaf Meals

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Abstract
There is dearth of information on the effects of varying levels of inclusion of morinda and neem leaf meals on the blood profile of layer chickens fed cassava pulp based diets. A total of ninety (90) 73-weeks-old Nera black layers were allotted to five treatments containing 18 birds each with six replicates per treatment and fed for 8 weeks with diets containing no leaf meals, 2.5% and 5.0% morinda leaf meal (MLM), and 2.5% and 5.0% neem leaf meal (NLM). At the end of 8 weeks, blood samples were collected and analysed for haematological and serum biochemical indices. Data were analysed using 2x3 factorial arrangement of variance and means separated by Duncan Multiple Range Test of the same software package at $p<0.05$. MLM and NLM produced significant ($p>0.05$) interaction effects on packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell (RBC), white blood cell (WBC), platelet count, lymphocytes, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) values. NLM favoured more platelet count at 5.0% level of inclusion than MLM. PCV, RBC, lymphocyte and MCHC were higher in birds fed MLM than NLM at corresponding levels of inclusion. There were significant interaction effects of leaf meals and their level of inclusions on aspartate aminotransaminase, serum glucose, urea and total protein. Serum glucose was significantly higher ($p<0.05$) and total protein and albumins were significantly reduced ($p<0.05$). However, both MLM and NLM did not affect physiological health and thus, were not hepatotoxic.

Keywords: Blood, layers, neem, morinda, cassava pulp

Introduction

Plants play significant roles on the welfare of both humans and animals; from providing basic needs such as food, shelter and clothing to being used as herbal medications. Medicinal plants contain many useful substances someof which are biologically active and are known as secondary metabolites. These substances can be used for therapeutic purposes and applied for several decades to serve as precursors for the synthesis of new drugs (Evans, 2002).The bewildering increase in the population of micro-organisms that are pathogenic in nature has brought imperative attentions to several non-conventional plant extracts with antimicrobial properties for possible incorporation into animal feeds to reduce microbial loads in animals and serve as a replacement for synthetic antibiotic growth promoters, all culminating to reducing not only the cost of production but also the potential health risk. Such plant parts or their extracts can be easily obtained from most tropical plants of which morinda and neem are of interest.

Morinda lucida has been used for several decades by rural communities across the tropical region for its medicinal potentials. Adeyemi*et al.* (2014) reported that Morinda contains alkaloids, tannins, anthraquinones and steroids. The availability of anthraquinone in Morinda leaves makes it a potential laxative; while the presence of steroid, alkaloid and tannins explains its ability to treat heart ailments, malaria and diarrhea respectively among other ailments.Neem (*Azadirachta indica*) popularly known as Indian neem is a tropical tree plant that is predominantly distributed in Africa and virtually available all the time (Koona and Budida, 2011). The leaves are very bitter to taste, and possess a garlic-like smell. The plant is popularly known because it is readily available, economical, non-toxic to animals and humans, and efficacious against malaria. It has proven anti-microbial activity and anti-oxidative properties (Valarmathy *et al.,* 2010).

Blood is one of the indicators of animal welfare since its composition depicts the quality of feed ingested by the animal. Blood is susceptible to internal and external changes occasioned by nutrition, pests, diseases, metabolism, season and any other stress factors that the animal may respond to (Ladokun *et al.,* 2008). Hence, blood is a major index or reflector of physiological, pathological and nutritional status of an animal because of its dynamic nature. For egg-producing animal like layer chickens, nutritional studies should imperatively include haematological and serum biochemical indices.

This research therefore, aimed at exploiting and comparing morinda and neem leaf meal on the blood profile of layer chickens fed cassava pulp based diets.

Materials and Methods

The study was carried out at the Poultry Research Unit of Ladoke Akintola University of Technology Teaching and Research Farm, Ogbomoso, Oyo State, Nigeria. Cassava pulp was purchased from a reputable commercial starch-producing firm in Ado-Awaye, Oyo State, while morinda and neem leaves were harvested from Ladoke Akintola University of Technology, Ogbomoso Teaching and Research Farm plots. The Cassava pulp was pressed to reduce the water content (using a jack) for a day, sun-dried by spreading it on sack and then grinded using a clean grinding machine, model GX300. The leaves were detached from the stalk, air-dried at room temperature for two weeks and then grinded using a clean Century blender, model 8231D to form powder.

Experimental diets: Five dietary treatments were compounded such that control diet (Treatment 1) contained maize and cassava pulp at equal proportions supplying energy to the diet. Morinda leaf meals (MLM) were included in Treatments 2 and 3 at 2.5% and 5.0% respectively while Treatments 4 and 5 contained 2.5% and 5.0% Neem leaf meals (NLM) respectively all at the expense of wheat offal. Ninety (90) Nera black layers (73 weeks old) were procured from a reputable commercial farm in Ogbomoso. The birds were housed in a two tier-cage compartment in open-sided pen. The birds were randomly divided into the five (5) dietary treatments with six (6) replicates per treatment. Each replicate contained three (3) birds, which were subjected to normal management practices.

Data collection: Three ml of blood samples were collected from twenty (20) randomly selected layers at four (4) birds per treatment at the end of the experiment (8 weeks) through the jugular vein into both plain blood sample bottles and that containing ethylene diaminetetracetic acid (EDTA), an anti-coagulant. The samples were subjected to haematological analysis packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC), white blood cell count (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)}.Serum indices measured were total protein (Kaneko, 1989), serum albumin, serum glucose (Barham and Trinder, 1972), serum creatinine (Roschlau *et al.,* 1974), Alanine aminotransaminase (ALT) and Aspartate aminotransaminase (AST).

Data collected were analysed in a 2 X 3 factorial design using the General Linear Model procedure of SAS (2012) to determine treatment effects. Significant mean differences were determined using Duncan Multiple Range Test of the same package at $p<0.05$.

Result and Discussion

Packed cell volume (PCV) and other haematological parameters are measurable indicators of physiological state of animal in terms of blood cells formation and functions. Table 1 shows that interaction effects of morinda leaf meal (MLM) and neem leaf meal (NLM) were significant ($p<0.05$) on all the haematological parameters of layer chickens. PCV, RBC, Hb, WBC, MCV and MCH were significantly ($p<0.05$) increased with increasing level of MLM, however, at 2.5%NLM, PCV, RBC, Hb, MCV MCHC and MCH were significantly ($p<0.05$) higher compared to other levels of NLM. PCV, WBC, MCV and MCH were ($p<0.05$) highest for layers fed 5%MLM while PLT and HET were ($p<0.05$) highest in layers on 2.5%MLM.

Table 1: Interaction effects of inclusion level and leaf meals on haematology of layer chickens

Ingredients	MLM			NLM			SEM
Parameters/Levels	0%	2.5%	5%	0%	2.5%	5%	
PCV	27.75^{c}	28.69^{bc}	32.14^{a}	27.75^{c}	31.03^{ab}	29.10^{bc}	0.49
HB	9.16^{c}	9.64^{bc}	10.30^{ab}	9.16^{c}	10.75^{a}	9.73^{bc}	0.17
RBC	3.42^{b}	3.37^{b}	3.39^{b}	3.42^{b}	3.55^{a}	3.43^{b}	0.02
WBC	13950^{c}	16212.50^{b}	18850^{a}	13950^{c}	10387.50^{d}	18600^{a}	640.90
LT	128750^{b}	153125^{a}	146750^{a}	128750^{b}	124500^{b}	151250^{a}	2634.9
LYM	61.00^{bc}	57.75^{c}	60.00^{bc}	61.00^{bc}	66.75^{bc}	72.5^{a}	1.38
HET	33.50^{a}	35.25^{a}	32.0^{a}	33.50^{a}	25.75^{ab}	20.0^{b}	1.59
MCV	81.20^{b}	85.17^{b}	94.8^{a}	81.20^{b}	87.33^{ab}	84.96^{b}	1.35
MCHC	32.99^{ab}	33.62^{ab}	32.08^{b}	32.99^{ab}	34.66^{a}	33.46^{ab}	0.27
MCH	267.87^{b}	286.31^{ab}	303.77^{a}	267.87^{b}	302.60^{a}	284.08^{ab}	4.41

SEM: Means with different superscripts along the same row were significantly (p<0.05) different.

2.5% NLM favoured RBC and haemoglobin formation compared to other dietary treatments. LYM was significantly (p<0.05) influenced at 5%NLM compared to the corresponding inclusion level of MLM. Even though, there were significant (p<0.05) interaction effects of MLM and NLM and their inclusion levels on all haematological indices of layer chickens, but the reported values for PCV, Hb, RBC, WBC and LYM were within range of reference values for clinically normal chickens (24.9 - 40.7% PCV; 1.58 - 3.82 x 10^6/µl RBC; 9.2 - 28.6 x 10^3/mm^3 WBC; 7.4 - 12.2g/dl Hb) by Mitruka and Rawnsley (1981) and 47.2-81.2% LYM by Mitruka and Rawnsley (1977). The presence of significant (p<0.05) interaction effects on PCV, Hb, MCH, MCV and MCHC were in accordance with the findings by Esonu *et al*. (2007) who reported that PCV and Hb were significantly (p<0.05) influenced with high levels of neem leaf meal in diets. The implication of normal levels of the haemoglobin indices (Hb, MCH and MCHC) is that incorporation of MLM and NLM as much as 5% did not cause iron deficiency anaemia in layer chickens (Linus *et al.,* 2015). Both leaf meals had significant (p<0.05) interaction effects on WBC and PLT and were highest at 5% inclusion level. This negated the findings of Linus *et al*. (2015) when they found that NLM had no significant (p>0.05) effects on WBC. LYM was significantly (p<0.05) highest at 5%NLM than MLM at all inclusion levels. This was in agreement with the findings of Linus *et al*. (2015), but on the contrast, increased with increasing levels of NLM.

Table 2 indicated that there were significant (p<0.05) interaction effects of leaf meals and inclusion levels on aspartate aminotransaminase (AST), serum glucose, urea and total protein. Increased inclusion levels of NLM elevated AST but was significantly (p<0.05) highest at 2.5% NLM. Similar but lower significant AST level was obtained in birds fed control diet and MLM at all inclusion levels compared to NLM fed birds. Glucose was statistically similar across both leaf meals but significantly (p<0.05) highest at 5% MLM compared to NLM. Total protein increased with increasing level of inclusion of MLM but decreased with corresponding increased inclusion level of NLM.

Table 2: Interaction effects of inclusion level and leaf meals on serum biochemical indices of layer chickens

Ingredients	MLM			NLM			SEM
Parameters/Levels	0%	2.5%	5%	0%	2.5%	5%	
AST, IUL	45.93^{b}	47.13^{b}	45.38^{b}	45.93^{b}	68.27^{a}	64.16^{ab}	2.93
ALTIUL	8.39	5.69	8.61	8.39	7.51	5.57	0.61
Glucose	190.10^{b}	188.64^{b}	232.20^{a}	190.10^{b}	201.18^{b}	196.36^{b}	4.41
Urea	17.31^{a}	17.17^{a}	12.85^{b}	17.31^{a}	13.41^{b}	15.40^{ab}	0.58
Total protein	2.94^{a}	2.40^{bc}	2.51^{b}	2.94^{a}	2.72^{ab}	2.10^{c}	0.08
Albumin	1.81	1.86	1.76	1.81	2.00	1.82	0.04
Creatinine	0.87	0.73	0.74	0.87	0.83	1.07	0.05
Globulin	1.13^{a}	0.54^{bc}	0.75^{b}	1.13^{a}	0.72^{b}	0.28^{c}	0.08

SEM: Means with different superscripts along the same row were significantly (p<0.05) different.

Serological indices are reflection of the effects of dietary treatments on animals in terms of the quality of feed ingested and nutrients available to an animal to meet its physiological requirements (Gbore and Akele, 2010). Olajide *et al.* (2010) submitted that methanol extracts of the leaves of *Morinda lucida* have a strong glucose lowering property when administered to streptozotocin-treated rats. The absence of significant interaction differences of both leaf meals on serum albumin (ALB) and alanine aminotransaminase indicates that MLM and NLM were not detrimental to the immunity of the birds (Linus *et al*., 2015) and thus were not toxic. In conclusion, the study demonstrated that both Morinda leaf meal and neem leaf meal could be included in cassava pulp based diet without negative effect on blood parameters while physiological health status was maintained.

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