Effects of Varying Levels of Neem (Azadirachta indica) Leaf Meal in Layer Diets on the Haematological and Serological Indices, and Faecal Bacterial Counts of Layers

Linus Ifeanyi Odoh  Lawrence Bratte*  
Department of Animal Science, Delta State University, Asaba Campus, Asaba, Nigeria  
* E-mail of the corresponding author: lbratte@yahoo.com

Abstract  
An experiment was conducted to investigate the effects of varying levels of Neem (Azadirachta indica) leaf meal in the diets of laying hens on the haematology, serology and faecal bacterial counts of laying pullets. Five layer diets formulated to contain 0%, 2.5%, 5.0%, 7.5% and 10.0% Neem Leaf Meal (NLM) were fed to a total of one hundred and fifty (150) 20-week old Shikka Brown layers. The birds were randomly assigned to 5 treatment groups of 30 birds each made up of 3 replicates of 10 birds per treatment. The diets were formulated to contain approximately 2500kcal/kg ME and 17% crude protein. NLM had no significant (P>0.05) effect on packed cell volume (PCV), red blood cell count (RBC), white blood cell counts (WBC), Haemoglobin, MCV, MCH, MCHC and plasma protein of the layers. NLM produced significant (P<0.05) differences between treatment means in the neutrophils, lymphocytes, basophils and monocytes counts but not in eosinophils. The neutrophil counts of layers fed diets containing 7.5% NLM were significantly (P<0.05) higher than those of the control, 2.5% NLM and 5.0% NLM treatment groups. Lymphocyte counts obtained from layers fed 5.0% NLM were significantly (P<0.05) higher than those of the control and other NLM treatment groups. Inclusion of NLM in the diets of the layers beyond 5% significantly (P<0.05) lowered basophil counts, while monocyte counts increased with increase in dietary NLM up to 5%, and declined thereafter. Serum albumins, cholesterol and total proteins were similar (P>0.05) between treatments, while total bacterial counts from cultures of faecal samples of the layers showed highly significant (P<0.01) progressive decline with increase in dietary level of NLM. It was concluded that as much as 10% dried Neem Leaf Meal could be included in the diets of laying chickens without deleterious effects on their blood constituents, serum biochemistry, and with the benefit of reducing possible risks of infection from pathogenic bacteria.  
Keywords: Neem Leaf Meal, Azadirachta indica, haematology, serology, layers, bacterial counts

1. Introduction
The worldwide rise in the population of pathogenic microorganisms of livestock in general and poultry in particular has led to shortfalls in animal protein production and availability, particularly in the developing countries in Africa. This has necessitated investigations into several non-conventional plant extracts with antimicrobial properties for possible incorporation into animal feeds for reducing microbial loads of animals, and as replacements for synthetic antibiotic growth promoters.

In the course of producing livestock intensively in Nigeria, it has been estimated that feed costs constitute over 70% of total livestock production costs (Olayemi & Robert 1988; Alu 2010). Current studies on Neem (Azadirachta indica) leaf extracts indicate an increasing interest in the plant owing to its versatile application and promising uses.

Neem (Azadirachta indica), popularly known as Indian neem (margosa) or Indian lilac of the family Malvaceae, is one of such non-conventional and available ingredients sources in the tropics with great potential in the 21st century. It is a tropical tree plant which is widely distributed in Africa, and available all year round (Ganguli 2002; Kabelh & Jalingo 2007; Ogbuewu et al. 2011; Koona & Budida 2011). The tree is well adapted to the climatic and soil conditions in the tropical rainforest regions, all the way to the Sahel savannah part of Nigeria. The leaves are very bitter to taste, and possess a garlic-like smell. The plant is popular because it is readily available, cheap, non-toxic to animals and humans, and efficacious against malaria.

Azadirachta indica leaves also contain compounds with proven antimicrobial activity (Makri et al. 2007; Wikipedia 2007; Valarmathy et al. 2010). The antimicrobial activity of extracts of neem leaves against such micro-organisms as Staphylococcus spp, Streptococcus spp, Pseudomonas spp and Escherichia coli, and some fungal strains have been reported (Tuhin et al. 2007; Valarmathy et al. 2010; Koona & Budida 2011). Studies on the effects of neem on poultry production especially of broilers and laying hens also exist (Koona et al. 2013). Antimicrobial studies on the effects of neem leaves and their extracts on cultured micro-organisms in vitro have also been carried out (Koona & Budida 2011).

Although the beneficial effects of incorporating neem leaf meal into livestock feeds in terms of increased feed value, protein utilization and antimicrobial activity have been reported, data on the effects of dried neem leaf meal on the health status of layers are still scanty. An understanding of the antimicrobial effects
of neem as reflected in the health status and growth performance of birds fed neem leaf meal is important (Esonu et al. 2007).

The present study was therefore undertaken to investigate the effects of varying levels of dried neem (Azadirachta indica) leaf meal (NLM) in layers diets on the hematological and serological indices, and faecal microbial counts of layers.

2. Materials and Methods

2.1 Location

This study was carried out at the Poultry Unit of the Teaching and Research farm of the Faculty of Agriculture, Delta State University, Asaba Campus, Asaba, Nigeria. Asaba is situated in south-western Nigeria, and has an equatorial rain forest vegetation type, and coordinates of 6° 12’N and 6° 45’E. The area experiences a hot dry season (November - March) and a wet season (April – October). Maximum day temperatures range from 27°C to 35°C, while an average annual rainfall of 1800 to 3000mm is prevalent.

2.2 Processing of the Leaves, and Formulation of the Experimental Diets

Fresh leaves of Azadirachta indica were harvested from mature neem plants in and around the Delta State University, Asaba Campus, Asaba, Nigeria, and spread out evenly to dry under sunlight for five (5) days until they became crispy to touch.

Table 1: Percent Composition of the Experimental Diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>NLM0</th>
<th>NLM2.5</th>
<th>NLM5.0</th>
<th>NLM7.5</th>
<th>NLM10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>57.98</td>
<td>56.37</td>
<td>54.75</td>
<td>53.14</td>
<td>51.50</td>
</tr>
<tr>
<td>Neem Leaf Meal</td>
<td>-</td>
<td>2.50</td>
<td>5.00</td>
<td>7.50</td>
<td>10.00</td>
</tr>
<tr>
<td>Soya bean meal</td>
<td>16.92</td>
<td>16.03</td>
<td>15.15</td>
<td>14.23</td>
<td>13.40</td>
</tr>
<tr>
<td>Fish meal</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Wheat offal</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
</tr>
<tr>
<td>Rice offal</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Oyster shell</td>
<td>7.50</td>
<td>7.50</td>
<td>7.50</td>
<td>7.50</td>
<td>7.50</td>
</tr>
<tr>
<td>Bone meal</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Common salt</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>17.06</td>
<td>16.99</td>
<td>16.93</td>
<td>16.97</td>
<td>16.90</td>
</tr>
<tr>
<td>ME (kcal/kg)</td>
<td>2581.23</td>
<td>2545.09</td>
<td>2509.16</td>
<td>2520.46</td>
<td>2503.60</td>
</tr>
</tbody>
</table>

The dry leaves were milled to a fine-powdered state using a hammer mill to produce the leaf meal. Samples of the leaf meal were subjected to laboratory analysis to determine their proximate composition according to the methods of the Association of Official Analytical Chemists (AOAC, 1990). Thereafter, five experimental layers diets were formulated such that they contained milled Neem Leaf Meal (NLM) at 0%, 2.5%, 5%, 7.5%, and 10% inclusion levels, and were designated as NLM0, NLM2.5, NLM5.0, NLM7.5 and NLM10.0 respectively (Table 1). NLM0 had no NLM, and served as the control. The five diets were formulated to be as isocaloric and isonitrogenous as possible, and to contain approximately 2500 kcal/kg Metabolizable Energy (ME) and 17% Crude Protein (CP).

2.3 Experimental Design, and Management of Experimental Birds

One hundred and fifty (150) Shikka Brown layers, aged 20 weeks, were used for the experiment. The birds were weighed individually on arrival at the farm, and at weekly intervals thereafter. The birds were randomly assigned to the five treatment dietary groups (NLM0, NLM2.5, NLM5.0, NLM7.5 and NLM10.0) of 30 birds each. Each treatment was further divided into three (3) replicates of ten (10) birds each, and placed in pens in line with the design of the experiment. The experimental design was a one-way classification in a Completely Randomized Design (CRD) with the model in Equation (1):

\[ X_{ij} = \mu + a_i + e_{ij} \]  

(1)

where \( X_{ij} \) = the observed value of each of the response variable, 
\( \mu \) = the overall population mean, 
\( a_i \) = observed effect of the \( i \)th dietary treatment, and 
\( e_{ij} \) = random residual error.

The birds were managed on deep litter, and provided feed and water ad-libitum. Good sanitation practices and
routine vaccinations were carried out.

2.4 Data Collection:
Data were collected and recorded on replicate basis for a period of ten (10) weeks of the experiment. The parameters measured or determined were as follows:

2.4.1 Haematological Parameters:
At the end of 10 weeks of feeding the experimental diets, one bird with similar body weight to the average body weight from each replicate was selected for bleeding. With a 5mL syringe fitted with a 24-gauge sterile hypodermic needle, 5mL of blood was carefully drawn from the left wing at the point of bifurcation of the vein. 3mL of the blood was put into a vacutainer containing EDTA as anticoagulant, and shaken gently to prevent coagulation, while the rest of the blood was put into a vacutainer which had no anticoagulants in it for serum biochemical analysis. All blood samples were immediately sent to the Physiology Laboratory of the Department of Animal Science, Delta State University, Asaba Campus for analysis using routine laboratory procedures as described by Schalm et al. (1975), Saror & Schillhorn van Veen (1977), Friedman & Young (1997), Iyayi & Tewe (1998). The heamaglobin (Hb) content was determined with a digital photo colorimeter (Model 312E by Digital Photo Instruments, Germany) at a wavelength of 625nm and expressed in gramme (g) units. Packed cell volume (PCV) was determined through the Winthrose microhaematocrit technique, and expressed in percentages (%). Red blood cell (RBC) counts were obtained with a Couter Electronic counter (Model ZF by Couter Electronic Ltd. London), and the values expressed in millions per microlitre (µL) of blood (x10^6/µL). The total leucocytes or white blood cells (WBC) were counted with an improved Neubauer haemocytometer and expressed in thousands per microlitre (µL). Plasma protein was obtained with a hand refractometer in gram per litre (g/L) of blood. Other haematological parameters computed or evaluated included mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), neutrophil, lymphocyte, oesinophil, basophil, and monocyte counts.

2.4.2 Serological Parameters
Total protein was determined by the Biuret method, albumins by the bromcresol green method, and serum cholesterol by the enzymatic colorimetric technique.

2.4.3 Antimicrobial Activity:
At the end of experimentation, faecal samples were collected from each replicate group by placing dropping trays covered with aluminum foil under their cages, and processed for antimicrobial activity evaluation. Sterile forceps were used to pick up the faeces from the dropping tray into clean, labeled petri-dishes, and sent to the Animal Science Laboratory of Delta State University Asaba Campus, for analysis. The samples were oven-dried at 60°C – 80°C, transferred into labeled sterilized bottles and stored in a refrigerator (at 5°C) until required for further analysis. Microbial identification and population were done using routine laboratory procedures outlined by John (1984), Collins et al. (1995), Prescott et al. (1999), Baker et al. (2001), Duraipandiyan et al. (2006), Tuhin et al. (2007), and Valarmathy et al. (2010). The analysis included preparation of culture media, microscopic and macroscopic examination of the faecal samples, and growth/population counts. Total Bacteria Counts were then determined. All glassware used were thoroughly washed with detergent, rinsed with distilled water, wrapped in aluminum foil and sterilized in a hot air oven at 170°C for 1 hour.

2.4.4 Serological Parameters
Total protein was determined by the Biuret method, albumins by the bromcresol green method, and serum cholesterol by the enzymatic colorimetric technique.

2.5 Data Analysis
Data collected and observations made were subjected to one-way analysis of variance (ANOVA) according to the procedure of Steel & Torrie (1980). Where ANOVA indicated significantly different treatment means, they were separated using the Duncan’s New Multiple Range Test (DNMRT) at 5% probability (Duncan 1955; Snedecor & Cochran 1978; Obi 1990).

3. Results
Proximate analysis performed on samples of the test ingredient revealed that it contained approximately 8.33% moisture, 21.87% crude protein, 14.33% crude fibre, 6.33% ether extract, 9.16% ash and 39.96% nitrogen-free extract.

Mean (± SE) values of haematological characteristics and plasma proteins of the layers fed varying levels of dietary NLM are presented in Table 2. The result indicate that NLM had no significant (P>0.05) effect on PVC, RBC, WBC, haemoglobin, MCV, MCH, MCHC and plasma proteins.
Table 2. Effects of Feeding Varying Levels of Dried Neem Leaf Meal to Laying Pullets on their Heamatological Characteristics and Plasma Protein (Mean ± SE)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NLM0</th>
<th>NLM2.5</th>
<th>NLM5.0</th>
<th>NLM7.5</th>
<th>NLM10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Packed Cell Volume (%)</td>
<td>27.67±2.67</td>
<td>27.33±1.20</td>
<td>28.67±2.60</td>
<td>27.67±0.88</td>
<td>29.00±1.52</td>
</tr>
<tr>
<td>Red Blood Cells (x10^6/μL)</td>
<td>2.80±0.50</td>
<td>2.53±0.85</td>
<td>4.00±0.20</td>
<td>2.93±0.77</td>
<td>4.13±1.01</td>
</tr>
<tr>
<td>White Blood Cells (x10^9/μL)</td>
<td>14.50±1.93</td>
<td>14.03±3.32</td>
<td>16.22±6.30</td>
<td>10.94±0.81</td>
<td>10.14±2.58</td>
</tr>
<tr>
<td>Haemoglobin (g/L)</td>
<td>60.00±18.87</td>
<td>65.07±16.39</td>
<td>60.00±11.45</td>
<td>69.50±10.10</td>
<td>67.50±1.05</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>108.72±35.77</td>
<td>161.79±56.40</td>
<td>77.00±18.98</td>
<td>144.52±18.98</td>
<td>95.90±29.15</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>26.43±5.28</td>
<td>33.25±10.18</td>
<td>18.03±1.87</td>
<td>29.10±10.40</td>
<td>20.87±6.78</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>31.03±11.03</td>
<td>32.26±9.50</td>
<td>28.05±5.06</td>
<td>33.32±1.08</td>
<td>31.22±1.72</td>
</tr>
<tr>
<td>Plasma protein (g/dL)</td>
<td>8.07±0.94</td>
<td>8.60±0.70</td>
<td>8.13±0.18</td>
<td>8.97±0.33</td>
<td>7.53±0.35</td>
</tr>
</tbody>
</table>

Results of the effects of treatment on the differential white blood cell counts of the layers are shown in Table 3. NLM produced significant (P<0.05) differences between treatment means in the neutrophils, lymphocytes, basophils and monocytes counts but not in eosinophils. The neutrophil counts of layers fed diets containing 7.5% NLM were significantly (P<0.05) higher than those of the control, 2.5% NLM and 5.0% NLM treatment groups. Lymphocyte counts obtained from layers fed 5.0% NLM were significantly (P<0.05) higher than those of the control and other NLM treatment groups. Inclusion of NLM in the diets of the layers beyond 5% significantly (P<0.05) lowered basophil counts, while monocyte counts increased with increase in dietary NLM up to 5%, and declined thereafter.

Planned contrasts on the data aimed at comparing the average of the NLM groups with the control group showed that feeding NLM significantly lowered lymphocyte counts, (t = -2.64, df = 10, P < 0.05), compared with the control birds. Eosinophil counts were, however, not significantly (P>0.05) altered by NLM.

Table 3. Effect of Feeding Varying Levels of Dried Neem Leaf Meal to Laying Pullets on their Differential White Blood Cell Counts (Mean ± SE)

<table>
<thead>
<tr>
<th>Parameters (%)</th>
<th>NLM0</th>
<th>NLM2.5</th>
<th>NLM5.0</th>
<th>NLM7.5</th>
<th>NLM10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>47.43±1.38c</td>
<td>49.98±0.58bc</td>
<td>40.54±1.36d</td>
<td>54.98±1.45a</td>
<td>53.20±0.49ab</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>39.50±1.86b</td>
<td>35.45±1.27b</td>
<td>41.02±1.06a</td>
<td>33.41±1.14b</td>
<td>33.57±0.32b</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>9.02±3.27a</td>
<td>9.02±1.77a</td>
<td>11.25±1.92a</td>
<td>9.84±0.72a</td>
<td>10.83±0.61a</td>
</tr>
<tr>
<td>Basophils</td>
<td>3.07±1.06a</td>
<td>3.14±0.34ab</td>
<td>4.50±0.39a</td>
<td>1.45±0.32a</td>
<td>1.08±0.01c</td>
</tr>
<tr>
<td>Monocytes</td>
<td>1.00±1.00c</td>
<td>1.57±0.36ab</td>
<td>2.71±0.04a</td>
<td>1.10±0.03c</td>
<td>1.09±0.01b</td>
</tr>
</tbody>
</table>

Means with similar superscripts within the same row are not significantly (P>0.05) different.

Serum albumins, cholesterol and total proteins were similar (P>0.05) between treatments, while total bacterial counts from cultures of faecal samples of the layers showed highly significant (P<0.01) progressive decline with increase in dietary level of NLM (Table 4). Planned contrasts on the bacterial counts data showed that feeding NLM drastically lowered bacterial populations in the culture media, (t = -57.96, df =10, P < 0.01) compared with the control birds.
Table 4. Effect of Feeding Varying levels of Dried Neem Leaf Meal to Laying Pullets on their Serological Characteristics and Total Bacterial Counts (TBC) (Mean ± SE)

<table>
<thead>
<tr>
<th>Dietary Treatments</th>
<th>NLM0</th>
<th>NLM2.5</th>
<th>NLM5.0</th>
<th>NLM7.5</th>
<th>NLM10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumins (g/dL)</td>
<td>2.81±0.46</td>
<td>2.84±0.10</td>
<td>2.81±0.35</td>
<td>1.97±0.24</td>
<td>2.12±0.17</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>71.85±3.73</td>
<td>70.82±8.35</td>
<td>91.01±13.97</td>
<td>83.24±16.88</td>
<td>78.84±18.46</td>
</tr>
<tr>
<td>Total Protein (g/dL)</td>
<td>4.91±1.48</td>
<td>4.22±0.26</td>
<td>6.05±0.47</td>
<td>3.92±0.64</td>
<td>5.56±0.39</td>
</tr>
<tr>
<td>TBC (x10^6/µL)</td>
<td>206.67±6.11</td>
<td>140.00±11.14</td>
<td>93.33±8.02</td>
<td>50.00±6.25</td>
<td>26.67±2.89</td>
</tr>
</tbody>
</table>

4. Discussion

4.1 Proximate Composition of the Test Ingredient

The crude protein (CP) content of 21.87% obtained for dried NLM used in this study compares favourably with that reported by Esonu et al. (2007), is higher than those obtained by Sokunbi et al. (2003) (18.90%) and by Kudke et al. (1999) (13.4% and 15.4% in India and Pakistan respectively), and slightly lower than the value of 24.06% reported by Onyimonyi et al. (2009). Its high fibre content (14.33%) may, however, be a limiting factor to its use in high amounts in non-ruminant nutrition.

4.2 Haematological Characteristics

Packed cell volume (PCV) and haematological variables give an insight into the physiological state of animals in terms of blood cells formation and function. The non-significant effect of dietary NLM on the major haematological indices of the layers is an indication that NLM, at the inclusion levels employed in this study, is not detrimental to the formation and function of the blood cells and their constituents, including haemoglobin. Although this is at variance with the findings of a previous study (Esonu et al. 2007) in which PCV and the haemoglobin values of layers were reported to be significantly higher at 10% than at lower levels of NLM inclusion, differences between treatment means, in this study, were not significant (P>0.05), while values obtained fell within the ranges of reference values for clinically normal chickens (24.9-40.7% PCV; 1.58-3.82 x10^6/µL RBC; 9.2-28.6 x10^3/mm³ WBC; 7.4-12.2g/dL Hb; 102-129fL MCV; 31.9-40.7pg; 25.9-33.9%MCHC) by Mitruka & Rawnsley (1981). The implication of normal levels of the haemoglobin indices (Hb, MCH and MCHC) is that feeding as much as 10% neem leaf meal (NLM) did not cause iron deficiency anaemia in the layers. Normal MCV values obtained in this study are an indication of the absence of macrocytic anaemia, which is often caused by dietary deficiencies in vitamin B₁₂ and folate among other causes (Hoffbrand & Provan 2007).

4.3 White Blood Cell Counts (WBC) and Differential WBC Counts

This study found that dietary NLM had no significant (P>0.05) effects on WBC and eosinophil counts, but significantly (P<0.05) affected the neutrophil, lymphocyte, basophil and monocyte counts. An elevated WBC count (leukocytosis) or a depressed WBC count (leukopenia) are indicative of an inflammatory or infectious process, autoimmune disorder, leukemia, allergy, bone marrow depression, malnutrition or stress (Cavanaugh 2003). Mean WBC values observed in this study ranged from 10.14±2.58 to 16.22±6.30 x10^3/µL, and fell within the range of reference values for clinically normal chickens by Mitruka and Rawnsley (1981) (9.20 – 28.60 x10^3/µL).

Eosinophils are commonly believed to play a role destroying invading pathogens, protecting the lungs against asthma and defending the host against helminthic parasites in addition to other important but recently identified immune regulatory functions performed both in health and disease (Jacobsen et al. 2012). Lymphocyte counts recorded in this study declined slightly but significantly (P<0.05) at the 7.5% and 10.0% dietary NLM levels when compared with the control and 5.0% NLM treatment groups, as was also reported in an earlier study by Esonu et al. (2007) in which 10% or 15% NLM significantly decreased the proportion of circulating lymphocytes in layers. The slight lymphopena at the higher levels of NLM feeding may have slightly compromised the affected layers’ ability to ward off infections since values obtained (33.41 and 33.57 % for 7.5 and 10.0% NLM respectively) fell below the range of reference values for normal chickens of 47.2 – 81.2% recommended by Mitruka & Rawnsley (1981). This may explain the slightly elevated neutrophil counts at the 7.5% and 10.0% levels of NLM inclusion, which, according to von Vietinghoff & Ley (2008) may be an indication of infection, inflammation or stress.

At dietary inclusion levels exceeding 5.0%, NLM significantly reduced the number of circulating...
basophils and monocytes. Basopenia (low basophil count) is an indication of allergies and possible hyperthyroidism. Diet-related causes of low monocyte counts are vitamin B\textsubscript{12} and folate deficiency, and oral ingestion of corticosteroids. It is not unlikely that one or more of the over 300 bioactive compounds which have been isolated from neem (Tiwari \textit{et al.} 2014), and which include several steroids, may have accounted for the low basophils and monocytes.

4.4 Serological Characteristics of Layers Fed the Experimental Diets

The absence of significant differences between treatments in mean serum albumins, serum cholesterol and total proteins (Table 4) was an indication that 5% to 10% dietary levels of NLM had no significant (P>0.05) effect on these parameters. The cholesterol level was lower than the report of Ogbuewu \textit{et al.} (2010). The serum albumins and total proteins are similar to the findings of Ogbuewu \textit{et al.} (2010). The serum albumins level indicates that the representative diets had no detrimental effect on the immunity of the birds and the albumin level and its functions on the birds.

Serum cholesterol in NLM-fed layers was numerically, though non-significantly (P>0.05), higher than those of the control birds contrary to the findings of Ogbuewu \textit{et al.} (2011), Uko \textit{et al.} (2006) and Bonsu \textit{et al.} (2012) who reported hypocholesemic effects of NLM in rabbits and broilers respectively. Values obtained in this study, however, were still within the range of reference values (52.0-148.0mg/dL) for normal chickens (Mitrula & Rawley 1981), and so pose no serious risk of cardiovascular disease to humans at 10% inclusion in layer diets.

The non-significant effects of treatment on total serum proteins including albumins (Table 4) indicate that the inclusion of NLM in the layer diets did not significantly alter dietary protein utilization and synthesis in the liver, or suppress the immune system of the birds. Serum proteins are involved in transport of important body substances, and maintenance of normal distribution of water between blood and tissues through osmotic pressure (Polat \textit{et al.} 2011). Low protein is evidence of malnutrition, impaired protein synthesis in the liver, loss (as by haemorrhage) or excessive protein catabolism while elevated protein levels may result from dehydration.

4.5 Total Bacteria Counts (TBC) of the Faecal Bacterial Culture

Bacterial counts of colonies of faecal samples declined significantly (P<0.01) and progressively with increase in the proportion of dietary NLM (Table 4) thus confirming the findings of a previous study by Divya \textit{et al.} (2013) who demonstrated the antibacterial effect of neem oil on various drug-resistant bacteria isolated from humans. In another study, Abalaka \textit{et al.} (2012) also showed that a minimum inhibitory concentration (MIC) of 5mg, and a minimum bactericidal concentration (MBC) of 50mg were potent against \textit{Pseudomonas aeruginosa}, \textit{Klebsiella ozanae}, \textit{Staphylococcus aureus} and \textit{Escherichia coli}. It appears, therefore that at 10% dietary inclusion, NLM can safely serve as an antimicrobial and natural growth-promoting agent in layer diets.

5. Conclusion

As much as 10% dried Neem Leaf Meal can be included in the diets of laying chickens without deleterious effects on their blood constituents and serum biochemistry, and with the beneficial effect of reducing possible risks of infection from pathogenic bacteria.

References


The IISTE is a pioneer in the Open-Access hosting service and academic event management. The aim of the firm is Accelerating Global Knowledge Sharing.

More information about the firm can be found on the homepage:
http://www.iiste.org

CALL FOR JOURNAL PAPERS

There are more than 30 peer-reviewed academic journals hosted under the hosting platform.

Prospective authors of journals can find the submission instruction on the following page: http://www.iiste.org/journals/ All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Paper version of the journals is also available upon request of readers and authors.

MORE RESOURCES

Book publication information: http://www.iiste.org/book/

Academic conference: http://www.iiste.org/conference/upcoming-conferences-call-for-paper/

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digital Library, NewJour, Google Scholar