IJAAR PUBLISHING®

African Journal of Sustainable Agricultural Development | ISSN: 2714-4402
Vol. 6, Number 1 (2025) | www.ijaar.org/ajsad

HAEMATOLOGY AND SERUM BIOCHEMICAL INDICES OF LAYER CHICKEN FED NEEM LEAF MEAL AS A PROTEIN SOURCE

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ABSTRACTS

A study was conducted to investigate the inclusion of neem leaf (Azadirachta indica) as a protein source in the diet of layers. A total of one hundred and fifty pullets were assigned to five dietary treatments in a completely randomized design. Dietary treatments were formulated with varying levels of Neem Leaf Meal (NLM) inclusion. D1 (0% NLM), D2 (5% NLM), D3 (10% NLM), D4 (15% NLM) and D5 (20% NLM) with 30 birds per treatment, 10 birds per replicate and replicated thrice. Packed cell volume (PCV), Haemoglobin (Hb), Red blood cell (RBC), White blood cell (WBC), Platelets (PLT), Lymphocytes (LYM), Heterophils (HET) and Eosinophils (EOS) were significantly (P<0.05) influenced by the inclusion of neem leaf. Total protein (TP), Albumin (ALB), Aspartate Aminotransferase (AST), Alanine aminotransferase (ALT), Uric Acid (Uric), Creatinine (CREAT), Glucose (Gluc) and cholesterol (Chol) were significantly (P<0.05) influenced by the treatments in layers diet. Therefore, 20% NLM inclusion is recommended to boost the blood parameters of laying hens' optimal performance.

Keywords: Layers, Neem leaf, Haematology, Serum biochemical, indices

Adebomi T. I., Olorede B. E., & Akinduro V.O. (2025). Haematology and Serum Biochemical Indices of Layer Chicken Fed Neem Leaf Meal as a Protein Source. *African Journal of Sustainable Agricultural Development. 6*(1), 35-43. DOI: 10.5281/zenodo.15423065

INTRODUCTION

Poultry production ranks among the highest sources of animal protein, and the increase in the size of the poultry industry has been faster than that of other food-producing animal industries (Ohimain and Ofongo, 2012). The popularity of poultry production can be explained by the fact that poultry has many advantages over other livestock, and it has been described as the fastest means of solving the problem of protein deficiency in Nigeria (Akpabio *et al.,* 2007). Poultry birds are good converters of feed into useable protein in meat and eggs. Therefore, poultry birds are one of the major sources of animal protein in Nigeria today. The significance of animal protein remains undisputed; animal protein supplies man with quality nourishment that aids growth, development, and tissue replacement. Chickens raised for eggs are usually called layers, while chickens raised for meat are often called broilers. Planning isrequired for egg production to be constant so as to meet market demand (Bozkurt *et al.,* 2009). The appropriate nutrition provision to layers is a prerequisite for optimal egg production. Layers ration should be designed to meet the requirements for laying hens' energy, protein, minerals, and vitamins determined by maintenance, body weight, and level of egg production. Ingredient selection is determined by availability, price, and, if available, nutrient biological availability estimates to minimize the cost of the ration. Specific fats and cereal grains provide energy, content and digestibility of essential amino acids that determine the protein source; a variety of vitamins and trace minerals are included in supplements given to egg-laying hens. The laying hen requires higher levels of calcium and vitamins A, D and choline than other chicken. Hens can be sustained on diets with limited nutrients; however, production levels will be reduced accordingly (Khalid, 2015).

Haematology refers to the study of the numbers and morphology of the cellular elements of the blood: the red cells (erythrocytes), white cells (leucocytes), and the platelets (thrombocytes) and the use of these results in the diagnosis and monitoring of disease (Merck Manual, 2012). Haematological studies are useful in the diagnosis of many diseases as well as the investigation of the extent of damage to blood (Onyeyili *et al*., 1992; Togun *et al.*, 2007). Haematological studies are of ecological and physiological interest in helping to understand the relationship of blood characteristics to the environment (Ovuru and Ekweozor, 2004) and could also be useful in the selection of animals that are genetically resistant to certain diseases and environmental conditions (Mmereole *et al.,* 2013). Haematological parameters are good indicators of the physiological status of animals (Khan and Zafar, 2005). Haematological parameters are those parameters that are related to the blood and blood-forming organs (Waugh *et al*., 2001; Bamishaiye *et al*., 2009). Blood acts as a pathological reflector of the status of exposed animals to a toxicant and other conditions (Olafedehan *et al*., 2010; Isaac *et al*., 2013) (Salami *et al.,* 2021).

MATERIALS AND METHODS

Experimental Site

The experiment was carried out at the Poultry section of the Department of Animal Science, Osun State University, Ejigbo campus. The area is located within the forest zone of Nigeria on latitude 7^0 54' 0 N and longitude 4^0 18' 54^0 E. The average elevation is 426 meters (1,398ft) (Monga Bay, 2011). It usually has an average annual rainfall of 1,330mm, and the rainy season lasts from April to October.

Housing

The birds were raised in colony cages for twelve weeks. They were groomed for some weeks, during which time their basal diet was offered. Daily routine operations such as washing drinkers, changing water, offering feed, pen cleaning, and maintenance were observed.

Experimental, Animals, Diets and Design

A total of hundred and fifty (150) points of lay pullets were used for this study. The birds were randomly assigned to five (5) dietary treatments in a completely randomized design (CRD) at 30 birds per treatment, 10 birds per replicate and each treatment was replicated thrice. Five dietary treatments were formulated at varying inclusion levels of neem leaf meal (NLM). Diet 1 (control) without NLM, diet 2 contains 5% (NLM), diet 3 contains 10% (NLM), diet 4 contains 15% (NLM), diet 5 contains 20% (NLM) (Table 1). The birds were fed their dietary treatments for twelve (12) weeks.

Table 1: Gross composition of experimental diets for layers

	Treatments				
Ingredients	**D1**	**D2**	**D3**	**D4**	**D5**
Maize	50.00	50.00	50.00	50.00	50.00
Soya Beans Meal	19.00	16.50	14.00	11.50	9.00
Neem Leaf Meal	0.00	2.50	5.00	7.50	10.00
Wheat Bran	15.00	15.00	15.00	15.00	15.00
Palm Kernel Meal	5.00	5.00	5.00	5.00	5.00
Fish Meal	2.00	2.00	2.00	2.00	2.00
Bone Meal	2.00	2.00	2.00	2.00	2.00
Oyster Shell	6.00	6.00	6.00	6.00	6.00
Premix	0.30	0.30	0.30	0.30	0.30
Salt	0.30	0.30	0.30	0.30	0.30
Lysine	0.20	0.20	0.20	0.20	0.20
Methionine	0.20	0.20	0.20	0.20	0.20
Total (%)	100	100	100	100	100
M E (kcal/kg)	2676.45	2676.45	2676.45	2676.45	2676.45
Crude Protein	17.79	17.79	17.79	17.79	17.79
Lysine	1.09	1.09	1.09	1.09	1.09
Methionine	0.49	0.49	0.49	0.49	0.49
Calcium	3.21	3.21	3.21	3.21	3.21
Phosphorous	0.51	0.51	0.51	0.51	0.51

Premix provided to chicks with vitamin A: 1000 IU; vitamin D_3: 500 IU; vitamin E: 5.75 IU; vitamin K_3: 0.5 mg; vitamin B_1: 0.45 mg; vitamin B_2: 1.25 mg; vitamin B_6: 0.75 mg; vitamin B_{12}: 0.00375 mg; Niacin: 6.875 mg; Pantothenic acid: 1.875 mg; Folic acid 0.1875 mg; Biotin H_2: 0.015 mg; C Choline chloride: 75 mg; Cobalt: 0.05 mg; Copper: 0.75 mg; Iodine: 0.25 mg; Iron: 5 mg; Manganese: 10 mg; Selenium: 0.05 mg; Zinc: 7.5 mg and Antioxidant: 0.3125mg

Table 2: Proximate composition of the experimental diets

Parameters (%)	D1	D2	D3	D4	D5	SEM
Crude protein	18.25	16.88	17.82	17.41	17.38	0.060
Ash content	9.33	8.68	10.13	9.08	8.79	0.116
Crude fibre	5.57	5.97	5.60	5.59	6.02	0.300
Ether extract	7.58	7.18	7.40	7.44	6.95	0.160
Dry matter	93.20	93.25	93.61	93.02	93.34	0.251
Moiture content	7.29	7.24	6.88	7.22	7.15	0.051
Nitrogen free extract	52.32	54.29	52.28	52.48	53.95	0.543

SEM = Standard Error of Mean

Hematological and Serum Parameters

In the eleventh week, 3.0 ml blood samples were collected from the wing veins of birds into labelled sterile universal bottles containing Ethylene Diamine Tetra Acetate (EDTA) as an anticoagulant. These were used to determine the haematological components (Packed Cell Volume, Red Blood Cell, Haemoglobin, White Blood Cell and platelet).

Blood sample collected into non-EDTA bottles was used to determine serum biochemical components (Total Protein (TP), Cholesterol, Globulin, Albumin, Urea, Glucose, AST, and ALT). Birds were fasted overnight but provided with water prior to blood collection. Serum biochemical indices and haematological parameters were analyzed using the methods described by Bush (1991) as cited by (Diarra*et al*., 2012; Adeyemo & Sani, 2013).

Statistical Analysis

Data obtained from this study were subjected to a one-way analysis of variance (ANOVA). Significant means were separated using the Duncan New Multiple Range Test as contained in the SAS (2020) package.

RESULTS AND DISCUSSION

In Table 3, haematological parameters of layer chickens fed varying levels of neem leaf meal (NLM) are shown. Packed cell volume (PCV), Haemoglobin (Hb), Red blood cell (RBC), White blood cell (WBC), Platelets (PLT), Lymphocytes (LYM), Heterophils (HET) and Eosinophils (EOS) were significantly ($P<0.05$) influenced by the inclusion of neem leaf in the layers diet. The rest of the haematological parameters were not significantly ($P>0.05$) influenced by the inclusion levels. A significantly ($P<0.05$) elevated PCV, Hb, RBC, WBC, PLT and lymphocytes were observed at D5 compared to the rest of the treatments.

Monocytes and Basophils did not elicit a significant (P>0.05) variation across the treatment groups.

The assertion that neem leaf meal helps in blood formation and purification could be attributed to the availability of adequate nutrients, minerals and phytochemical compounds for the birds since their feed intake increases numerically with the inclusion of neem leaf meal in their diets. The increase in the values of PCV with the use of neem leaf meal indicates that absorption of nutrients and transportation of oxygen increases the use of neem leaf meal, also indicating the hematopoietic effect of neem leaf meal, thus increased primary and secondary polycythemia (Isaac *et al*., 2013) and strongly suggest that neem leaf meal could be useful in hemodialysis (Olufunmilayo *et al*., 2015).

A high level of PCV reading indicated either an increase in the number of (RBCs) or a reduction in circulating plasma volume (Chineke *et al*., 2006), and this is confirmed by the current results Peters *et al*. (2011) posited that PCV and Hb are major indices for evaluating circulatory erythrocytes, diagnosis of anaemia and also serve as useful indices of the bone marrow capacity to produce red blood cells. The highest value of WBC and Lymphocytes observed at 20.00% neem leaf meal is an indication that the birds gained immunity against infections, indicating that neem leaf meal was able to support the transportation of antibodies as indicated by (Soetan *et al*., 2013).

Table 4 showsthe serum biochemical parameters of layer chickens as affected by the dietary treatments. Total protein (TP), Albumin (ALB), Aspartate Aminotransferase (AST), Alanine aminotransferase (ALT), Uric Acid (Uric), Creatinine (CREAT), Glucose (Gluc) and Cholesterol (Chol) were significantly (P<0.05) influenced by the treatments. The TP, ALB, AST, ALT, Uric acid, Creatine and glucose were significantly (P<0.05) elevated in diet 1when compared to the other dietary treatments.

The increase in the values of serum total protein with the use of neem leaf meal without medication, in association with a reduction in creatinine, suggested good health status for the birds. The inclusion of the neem leaf meal without medication could neither impair renal function nor expose the birds to potential kidney damage, specifically by renal filtration mechanism (Wasan *et al*., 2001). The liver and the kidney play a significant role in metabolic processes; while the liver detoxifies substances that are detrimental to the body, the kidney helps in the maintenance of homeostasis by reabsorbing vital substances and excretion of waste products (Greaves, 2007). The positive effect of neem leaf meal on serum metabolites could be attributed to the appreciable amount of alkaloids (Olufunmilayo *et al*., 2015).

The significant increase observed in Albumin of the layers fed neem leaf meal without medication resulted in a significant positive influence on Serum Total Protein in the birds, thereby supporting the report of Mousa *et al*. (2017), who documented that the addition of *Moringa oleifera* in diets of broiler chickens had a significant effect on Serum Total Protein and Albumin. Albumin and globulin are the major proteins found in the blood, and their concentration can drop in cases of malnutrition and infection. Globulin proteins consist of antibodies, enzymes and other types of proteins that help in fighting infections, enhancing the blood clotting process, serving as hormone carriers and transporting the hormones to different parts of the body (Oleforuh-Okoleh *et al*., 2015).

Urea is the first acute renal marker of renal injury, while creatinine is the most reliable renal indicator, and it increases only when the majority of renal functions are lost (Borges *et al*., 2005). Creatinine level decreased with the use of neem leaf meal. The reduction was more

significant with 10% neem leaf meal, indicating that the use of neem leaf meal maintained the liver. The current study supports the findings of Ghazalah and Ali (2008) and Oleforuh-Okoleh *et al.* (2015) that Rosemary and banana leaves significantly reduced the creatinine level of the blood.

Aspartate aminotransferase (AST) and alanine transaminase (ALT) are the two main liver functional blood serum tests. AST and ALT are found primarily in the liver, heart, kidney, pancreas, muscles, and red blood cells. They are normally elevated in cases of tissue damage, especially in the heart and liver. The magnitude of elevation of the values varies, depending on the cause of the hepatocellular injury. A very high level of ALT suggests viral or severe drug-induced hepatitis or other hepatic disease with extensive death of liver cells.

The AST level fluctuates depending on the extent of cell death. Serum biochemical parameters related to hepatic functions in the present study indicated that there was no significant influence on AST concentration in the control group when compared with neem leaf meal diets. However, the mean values obtained were within the normal range reported by Fraser (1986) and Anyaehi *et al.* (2015). Thus, the values obtained on AST and ALT levels imply that neem leaf meal could not cause toxic effects on the heart tissue (Crook, 2006).

Table 3: Haematological parameters of layer hens fed the dietary treatments

Parameter	D1	D2	D3	D4	D5	SEM
PCV (%)	20.33^{c}	20.67^{c}	26.67^{b}	24.33^{b}	33.67^{a}	0.76
Hb (g/dl)	6.47^{c}	6.80^{c}	8.63^{b}	7.50^{c}	10.97^{a}	0.32
RBC (x 10µL)	1.55^{c}	1.58^{c}	2.79^{b}	2.72^{b}	3.27^{a}	0.07
WBC (x 10 µL)	11566.67^{c}	11583.33^{c}	14566.67^{b}	13283.33^{b}	16600.00^{a}	460.8
Platelets (µl)	111666.67^{b}	115000.00^{b}	121000.00^{ab}	116666.67^{b}	140000.00^{a}	6091.89
LYM (%)	$53.00b^{c}$	48.33^{c}	55.67^{b}	56.67^{b}	68.00^{a}	1.79
HET (%)	39.67^{ab}	44.00^{a}	38.00^{ab}	35.67^{b}	25.67^{c}	2.17
MON (%)	4	2.67	2	3.33	2	0.62
EOS (%)	2.67^{b}	4.67^{a}	4.00^{ab}	4.00^{ab}	4.00^{ab}	0.49
BAS (%)	0.667	0.333	0.333	0.333	0.333	0.33

abcd: Means on same row with different superscripts differ significantly (P<0.05), SEM = Standard Error of Mean

PCV = packed cell volume, Hb = Haemoglobin, HET = Heterophils, RBC = Red blood cell, MON = Monocytes, WBC = White blood cell, EOS = Eosinophils, LYM = Lymphocytes, BAS = Basophils

Table 4: Serum biochemical indices of layer hens fed the dietary treatments

Parameter	D1	D2	D3	D4	D5	SEM
Total protein (g/dl)	8.17^{a}	6.50^{b}	5.67b^{c}	5.73^{bc}	4.80^{c}	0.35
Albumin (g/dl)	2.57^{a}	1.40^{b}	0.86^{bc}	1.03^{bc}	0.73^{c}	0.2
Aspartate aminotransferase (U/L)	241.67^{a}	201.00^{b}	188.67^{b}	189.00^{b}	178.67^{b}	8.92
Alanine aminotransferase (U/L)	35.67^{a}	26.33^{b}	24.33^{b}	26.00^{b}	19.33^{b}	2.36
Uric acid (mg/dl)	0.33^{a}	0.08^{b}	0.12^{b}	0.09^{b}	0.07^{b}	0.03
Creatine (mg/dl)	0.73^{a}	0.53^{b}	0.53^{b}	0.50^{b}	0.50^{b}	0.03
Glucose (mg/dl)	269.00^{a}	212.00^{b}	183.33^{b}	172.67^{b}	169.67^{b}	12.88
Cholesterol (mg/dl)	177.33^{a}	169.33^{a}	152.33^{b}	118.33^{c}	115.33^{c}	3.32

abcd: Means on the same row with different superscripts differ significantly (P<0.05), SEM = Standard Error of Mean

CONCLUSION

It was concluded that the use of neem leaf meal helps in the stability of health as it induces positive actions on blood haematological parameters such as adequate production of RBC, WBC, PCV and Hb. Therefore, the birds on the experimental diets were not anaemic and had strong immunity to fight infections. The Total protein, Aspartate Aminotransferase, Creatine, Glucose and Cholesterol decrease with an increase in the inclusion level of the neem leaf meal in the laying bird's diets. Therefore, a 10% inclusion level of neem leaf meal is recommended for optimum production.

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