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Haematology and Serum Biochemistry of Cockerels Fed Diets Containing Neem Leaf Meal

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ABSTRACT

This study was carried out to investigate the effect of neem leaf meal (NLM) on haematology and serum biochemistry of cockerels. A total of 224 grower cockerels were randomly allocated to 7 dietary treatments with 8 birds per replicate and 4 replicates per treatment. The experiment lasted for 7 weeks. A completely randomized design was adopted in which there were control with no NLM, 2.5% NLM, 5.0% NLM, 7.5% NLM, 10.0% NLM, 12.5% NLM and 15.0% NLM inclusions. Water and feed were given ad-libitum. At the end of the trial, eight birds were selected per treatment and bled. Blood was collected for haematology and serum bioassay. The results showed that neem leaf meal contained 24.06% crude protein. Cholesterol, aspartate transaminase and urea were significantly ($p<0.05$) different among the seven treatments. Serum cholesterol level decreased with increase in the level of inclusion of NLM. The haematological indices of the birds fed NLM were not significantly influenced ($p>0.05$) by NLM addition. It was concluded that neem leaf meal could be included in cockerel diets up to 15% without any significant adverse effects on the parameters studied.

Key words: Alternative feed ingredients, Animal protein, Blood count, Poultry production

INTRODUCTION

Poultry production is an important sector in agriculture which provides food in form of meat and other commodities for humans (Esonu et al., 2006). The challenges of acute protein shortage in the diets of most Nigerians and scarcity of conventional ingredients such as soyabean and fish meal have led to high cost of poultry meat due to high cost of production (Egbenwade and Olorede, 2003). These challenges led to different researches on feedstuffs which are not in competition with humans and are suitable for use in poultry production. The use of these local, cheap and readily available materials has received particular attention as the only viable alternative to the use of conventional feedstuffs (Ekenyem, 2006).

Leaf meals are known to contain protein, they are readily available and cheap, and can also be used as medicine for animals. They not only serve as protein source but also provide some necessary vitamins, minerals and also oxycarotenoids which give yellow colour to broiler's skin, shanks and egg yolk (Nwakpu et al., 2000). Thus, leaf meals are able to improve the growth and performance of animals with little or no side effects on humans when consumed (Odunsi, 2003). Although, the major constraints of leaf meal utilization in non-ruminant animal nutrition are their relatively high fibre, low metabolizable energy, antinutritional factors and reduced feed intake. In broilers, leaf meal creates a harmonious gut environment suitable for the release and assimilation of digestive nutrients necessary to enhance growth. In layers, egg yolk colour is positively influenced. It enhances palatability in goat and sheep and help digestibility in rabbit (Suchitra and Wanapat, 2008).

Neem popularly known as Indian neem (margosa) or Dogoyaro is a source of readily available leaf meals in the tropics with great potential. Neem is a tropical tree plant which is widely distributed in Africa and is available all year round. Neem tree is a potential source of animal feed (Kabeh and Jalingo, 2007; Koona and Budida, 2011; Ogbuewu et al., 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 plant is popular because it is readily available, non-toxic to animals and humans, and effective against malaria (Ganguli, 2002). Work has been done on the use of neem leaf meal in broilers (Onyimonyi et al., 2009) and layers (Olabode et al., 2013) nutrition but there is paucity of information on its use in cockerel production. Cockerels are birds known to be hardy, active in behaviour and also very

cheap. They are a good source of poultry meat mostly to small scale farmers (Chukwuemeka, 2017) and rural dwellers. Haematological parameters are used a measure of good health or otherwise in poultry raised intensively and in addition to biochemical parameters, they may be used as physiological indicators (Hrabčáková et al., 2014).

This study was undertaken to investigate the effects of including varying levels of neem leaf meal (NLM) in cockerel diets on their haematological and serum profiles.

MATERIALS AND METHODS

The feeding trial was conducted at the Poultry Unit of the Teaching and Research Farm of the Federal University of Technology, Akure while laboratory analyses were conducted at the Department of Animal Production and Health, Federal University of Technology Akure, Ondo State, Nigeria. The neem leaves used for the feeding trial were harvested fresh, sun-dried for few days to allow the leaves attain a crispy touch in order to facilitate easy grinding and preservation of its quality. The green crispy leaves were then ground with the hammer mill and stored in polythene bags.

A total of 224 (two hundred and twenty-four) eight-week-old cockerels were randomly allotted to 7 treatments with 4 replicates per treatment and 8 birds per replicate in a completely randomized design. Treatment 1 served as the control diet with 0% neem leaf meal (NLM), treatments 2, 3, 4, 5, 6 and 7 had NLM included at 2.5, 5, 7.5, 10, 12.5 and 15% respectively. Table 1 shows the gross composition of the experimental diets. The feeding trial lasted for 7 weeks. The birds were managed intensively on deep litter and given clean water. All management and drug administration were carried out as necessary.

At the end of the feeding trial, eight cockerels were randomly selected per treatment, mechanically stunned and slaughtered by severing the jugular veins with a sharp knife. Blood was allowed to flow freely into labeled blood bottles which contained a speck of the anticoagulant, Ethylene diamine tetra-acetic acid (EDTA) powder to prevent clotting. These blood samples were used for the determination of haematological parameters. About 30ml of blood sample was also taken from each cockerel into test tubes without anticoagulant for serum assay. The test tubes were allowed to stand in a test tube rack (slanting position) till the serum was formed. Randox® commercial kits were used for the assay.

Data collected were subjected to one-way Analysis of Variance using Minitab statistical package (v.17) and significant differences were separated using Turkey test of the same package.

RESULTS AND DISCUSSION

The result of proximate analysis of the neem leaf meal (NLM) used is presented in Table 2. It had 24.06% crude protein (CP) and 12.10% crude fibre. This is similar to the result of Onyimonyi *et al.* (2009). The high fibre content of NLM is typical of leaf meals. The NLM based diets had higher crude protein content than the control (Table 1). This can be attributed to the higher crude protein content of NLM when compared to that of maize and wheat offal.

Haematological Indices

Blood plays an important role in the body of the animals hence it is vital in determining the physiological, nutritional and pathological status of animals (Doyle, 2006). Blood transports nutrients and other materials to different parts of the body. Therefore, whatever imparts negatively on the blood, either pathogenic organism or nutrition, will certainly affect the entire chickens' body (Etim, 2010).

Table 3 shows the haematological values of cockerels fed NLM at varying levels. There were no significant ($p>0.05$) differences in all parameters viz: erythrocyte sedimentation rates, packed cell volume, red blood cells count, haemoglobin concentration, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration (MCHC), lymphocytes, heterophils, basophils, and eosinophils.

Most values of the haematological indices obtained in this study fell within the normal range presented by Mitruka and Rownsley (1977). Although all parameters were not significantly different ($P>0.05$), the MCHC used as a marker of anaemia was within the normal range. The red blood cell counts did not follow any particular trend in relation to control but were lower than reference values, cockerels on 7.5% NLM had the highest value. Albokhadaim *et al.* (2012) also reported biochemical values lower than all reference values. The normal ranges of haematological indices reported by researchers are thus not strictly standard due to variations (Etim *et al.,* 2013) in age, breed, strain and sampling techniques of chickens studied. So, in relation to control, it could be deduced that the cockerels tolerated the NLM based diets.

Serum Biochemical Indices

The serum parameters of cockerels fed NLM based diets are presented in Table 4. Total protein, albumin and creatinine were not significantly affected ($P>0.05$) by the dietary treatments while cholesterol, aspartate transaminase (AST) and urea were significantly ($P<0.05$) different.

Table 1: Composition of experimental diets (%)

	1	2	3	4	5	6	7
	Control	**2.5% NLM**	**5% NLM**	**7.5% NLM**	**10% NLM**	**12.5% NLM**	**15% NLM**
Maize	56	55	54	52.5	51	49.5	48
NLM	0	2.5	5	7.5	10	12.5	15
Wheat offal	19	18	17	16.3	15.3	14.3	14.3
Soyabean meal	10	10.5	10	10	10	10	10
Groundnut cake	10	10	10	9.7	9.7	9.7	9.7
Bone meal	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Limestone	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Lysine	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100	100
Calculated analysis							
Crude protein (%)	17.73	17.82	17.9	18.07	18.32	18.58	18.58
Metabolizable energy(kcal/kg)	2838.22	2865.26	2892.3	2913.48	2936.76	2960.14	2975.22
Calcium (%)	0.52	0.56	0.59	0.62	0.66	0.69	0.72
Phosphorus (%)	1.17	1.19	1.21	1.24	1.26	1.28	1.31
Ether extract (%)	3.89	3.92	3.96	3.98	4.01	4.05	4.08
Crude fibre (%)	3.95	4.17	4.39	4.64	4.89	5.13	5.39
Analysed composition (%)							
Moisture	11.82	12.26	11.7	11.75	11.89	11.6	10.67
Crude protein	16.03	17.04	18.4	19.27	20.65	21.7	22.08
Ash	4.23	8.24	5.53	5.24	7.46	6.3	6.34
Ether extract	4.9	5.19	5.15	4.8	5.2	5.35	5.3

NLM: Neem leaf meal

Cholesterol values decreased with increase in dietary levels of NLM and 15% NLM (64.90mg/dl) really differed significantly from control (109.29mg/dl).Values recorded for AST did not follow any particular trend but was highest (48.13µ/l) for cockerels on the 7.5% NLM diet, although the value at 7.5% NLM was not significantly different from the control. Values for urea was also highest at 7.5% NLM, but it decreased progressively from 7.5%-15% NLM.

Table 2: Proximate composition of neem leaf meal

Components	**%**
Crude protein	24.06
Crude fibre	12.1
Ash	15.74
Moisture	7.29
Ether extract	6

Cholesterol is an essential substance produced by human body and it is also derived from consuming poultry products. Although, total cholesterol values do not necessary indicate risk of being diseased, very high values could mean there is chronic inflammation in the body (Mercola, 2010). And more researches as to the harmfulness or benefits of cholesterol are on. Even at 2.5% NLM inclusion, there was a reduction in the serum cholesterol of the cockerels. This result validates the ability of neem leaves to reduce cholesterol levels in poultry as Dey *et al.* (2011) reported the efficacy of NLM as an hypocholesterolemic agent in laying pullets and Obikaonu *et al.* (2011) reported a reduction in cholesterol values with inclusion of NLM in broiler chicks' diets between 5 and 10%. Serum AST levels are used to measure liver health, other tests should however be performed to rule out interference from other tissues such as the muscles. According to Hochleithner (1994), AST value above 230µ/l is abnormal for poultry. Values recorded in this trial, although highest for cockerels fed 7.5% NLM are thus normal. Urea, which is an end product of protein metabolism, is more of an indicator of dehydration in chickens rather than being used to detect diseases (Hochleithner, 1994). The highest value recorded for cockerels fed 7.5%NLM and subsequent decrease in value from 10% NLM to 15% NLM might mean a higher protein availability/intake for cockerels fed the 7.5% NLM diet.

Table 3: Effects of neem leaf meal on haematological indices of cockerels

Parameters	1	2	3	4	5	6	7	Pooled standard Deviation
	Control	**2.5% NLM**	**5.0% NLM**	**7.5% NLM**	**10.0% NLM**	**12.5% NLM**	**15% NLM**	
ESR (mm/hr)	3.5	4	3.8	3.16	3.5	3.4	3.14	1.23
PCV (%)	22.83	23	24.4	21.67	22.37	22	23.14	4.08
RBC ($x10^6ml^{-1}$)	1.64	1.6	1.86	1.99	1.51	1.47	1.66	0.51
HBC (g/dl)	7.71	7.65	8.1	8.43	7.45	7.32	7.7	0.72
Lymphocytes (%)	60.33	60.83	60	61.5	60.37	60	60.28	1.06
Heterophil (%)	23.67	20	24.4	23.66	22.75	22.2	24.29	2.85
Eosinophils (%)	1.33	1.16	1.2	1.5	1.37	1.4	1	0.36
MCHC (%)	33.86	33.25	33.11	33.93	33.29	33.27	33.27	37.02
MCH (pg)	49.04	43.64	44.57	43.15	49.72	50.32	47.4	5.97
MCV (%)	144.9	146.27	134.27	110.7	149.32	151.2	142.43	24.84

ESR= Erythrocyte sedimentation rate; PCV= Packed cell volume; RBC= Red blood cell; HBC= Haemoglobin concentration; MCHC= Mean cell haemoglobin concentration; MCH= Mean cell haemoglobin; MCV= Mean cell volume

Table 4: Effects of neem leaf meal on serum parameters of cockerels

Parameters	1	2	3	4	5	6	7	Pooled standard deviation
	Control	**2.5% NLM**	**5% NLM**	**7.5% NLM**	**10% NLM**	**12.5% NLM**	**15% NLM**	
Total protein (g/l)	38.01	40.29	33.06	39.57	28.04	39.13	31.26	12.91
Albumin (g/l)	13.38	13.45	12.61	12.81	11.08	11.53	9.79	4.48
Cholesterol (mg/dl)	109.29^{a}	89.39^{ab}	85.20^{ab}	84.87^{ab}	77.00^{ab}	93.15^{ab}	64.90^{b}	39.45
AST (µ/l)	38.69^{ab}	37.41^{b}	38.81^{ab}	48.13^{a}	38.67^{ab}	41.33^{ab}	34.78^{b}	9.45
Creatinine (mg/dl)	2.45	2.99	2.6	3.11	2.93	2.59	2.1	2.4
Urea (mg/dl)	11.04^{ab}	9.18^{ab}	9.25^{ab}	13.05^{a}	9.81^{ab}	8.87^{ab}	7.44^{b}	4.23

$^{a, ab, b}$: Means within rows having different superscripts are significantly different (P<0.05); NLM- Neem leaf meal; AST- Aspartate transaminase

CONCLUSION

Neem leaf meal did not have any adverse effect on the haematological indices of cockerels. The leaf meal was also potent as a cholesterol reducing agent in cockerels. Neem leaf meal could be included in cockerel diets up to 15% based on the parameters studied. There is the necessity for a current research on the blood and biochemical parameters of cockerels and poultry at large available in Nigeria so as to obtain their relevant reference values.

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