Physiological response of laying birds to Neem (*Azadirachta indica*) leaf meal-based diets: body weight organ characteristics and haematology

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Abstract

A 12-weeks feeding trial was conducted to evaluate the effects of Neem (*Azadirachta indica*) leaf meal (NLM) on body weight gain, carcass and organ characteristics and haematological values of laying hens. The leaves were harvested, chopped to facilitate drying in the sun until they became crispy but still greenish in coloration. The sun-dried leaves were milled using a hammer mill to produce the leaf meal. Four layers diets were formulated to contain the NLM at 0%, 5%, 10% and 15% dietary levels respectively and were used to feed 120 Shikka brown layers already 10 months in lay. The birds were divided into 4 groups of 30 each and randomly assigned to the 4 treatment diets in a completely randomized design (CRD). NLM did not show any appreciable difference in weight gain between the birds at 0% and those at 5%, 10% dietary levels. Carcass weight, dressed weight, liver, heart and gizzard weights were significantly ($P < 0.05$) increased at 5% dietary level of NLM. There were no significant difference in Hb and PCV between birds on 0% and 5% treatment diets. However, these differed significantly ($P < 0.05\%$) from those of birds on 10% and 15% treatment diets. There were variations in the differential WBC count; marked lymphocytopenia adversely affected the total leucocyte counts in the birds on 5%, 10% and 15% treatment diets. The results of this study suggest that laying birds could tolerate 5% – 15% dietary levels of NLM without deleterious effects. [Life Science Journal. 2007; 4(2): 37 – 41] (ISSN: 1097 – 8135).

Keywords: physiological responses; laying birds; Neem leaf meal; diets; body weights; organ; haematology

1 Introduction

Nutrition is the most important consideration in any livestock enterprise. Its survival is dependent on the availability of feedstuffs, which are mainly components of human food.

The unavailability of grains and the high cost of imported ingredients have made the price of commercial animal feed to increase over 300%[1]. These problems remain the most important constraints to the expansion of commercial livestock production in Nigeria. The need to exploit other available but neglected cheaper novel feed resources, especially those indigenous to our environment and inedible to man are urgently necessary.

Neem (*Azadirachta indica*) is an indigenous tropical plant, which predominate in Nigeria. It is known by names such as “Ogwu-Iba” in Ibo land and “Dogonyaro” in Hausa. *Azadirachta indica* is medicinal and it is used as insecticide and pesticide. The tree is relevant in organic farming. The leaf meal has a proximate composition of 92.42 dry matter; 7.58% moisture; 20.68% crude protein; 16.60% crude fibre; 4.13% ether extract; 7.10% ash and 43.91% nitrogen free extract. The nutritive value of Neem leaf meal(NLM) had earlier been reported[2]. However, factors such as nutrient imbalance, improper metabolism, presence of anti-nutritional factors and toxic elements in such novel feed ingredient have been implicated in similar products. Research has continued in our laboratory to identify the anti-nutritional factors and toxic elements contained in *Azadirachta indica* leaf. Again, understanding the haematological constituents of birds feeding on some dietary inclusions of the leaf is important, since such data indirectly reflect in the physiological responsiveness of

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the animals[3–11]. The objective of the present study therefore is to determine the effect of NLM on the physiological parameters such as weight gain, internal organ characteristics and haematology of laying birds in a humid tropical environment.

2 Material and Methods

The leaves of Neem (*Azadirachta indica*) were collected from and around the Federal University of Technology, Owerri environs. The leaves were spread evenly and sun-dried for four days until they become crispy while still retaining the greenish coloration. The leaves thereafter were milled using a hammer mill to produce a leaf meal. Sample of the leaf meal was then analyzed to determine the proximate composition[12]. Four experimental layers diets were formulated such that they contained NLM at 0.0%, 5.0%, 10.0% and 15.0% inclusion levels respectively.

One hundred and twenty Shikka brown layers already 10 months in lay were divided into 4 groups and randomly assigned to the four treatment diets in a completely randomized design (CRD). Each treatment was further divided into three replicates of 10 birds. Feed and water were given ad-libitum. The birds were weighed at the beginning and end of the trial. Feed intake was recorded daily. At the end of the 12th week, 3 birds were randomly selected from each of the treatment groups, deprived of feed but not water for 24 hours, slaughtered, eviscerated and the dressed carcass weighed. In addition, 5 ml of blood was collected from each of the slaughtered birds and discarded into EDTA treated bottles for haematological assay. Haematological measurements were determined[13,14]. Data collected were subjected to analysis of variance[15]. Where analysis of variance (ANOVA) indicated significant treatment effect, the means were compared using Duncans New Multiple Range Test (DNMRT)[16].

3 Results

The result of the effect of different inclusion levels of NLM on the mean weight gain of laying hens after 12 weeks of treatment is shown in Table 1. There was no difference in weight gain between the birds in the control group and those fed diet containing 5% inclusion level of NLM. The layers placed on diet 10% inclusion of NLM had 4.6% mean weight gain. The ones fed diet containing 15% of the test material gained 5.1% mean weight after the 12 weeks period of treatment.

Table 2 shows the results of dietary levels of NLM on carcass and organ weights of laying hens. The carcass weight of birds fed 10% inclusion level of NLM was significantly different from those on 15% level but similar to the carcass weights of birds on 0% and 5% inclusion levels. Dressed percentages of the birds fed 5% and 10% inclusion levels of NLM were not significantly different, but similar to those on the control (0.0%) diet. However, the dressed percentages of these birds within the 5% and 10% treatment levels differed significantly from those on 15% inclusion level of NLM. Liver weight of birds placed on diet with 5% inclusion levels of NLM increased significantly and differed from the 0% group but similar to the livers of birds fed 10% and 15% levels of NLM. The weight of the hearts of birds on 0%, 5% and 10% treatment levels remained the same but differed significantly from those of the birds in the 15% treatment group. The weight of the gizzards of layers fed 5% inclusion level of NLM is significantly different from the gizzards of the birds fed 0% and 15% inclusion level of NLM.

Table 1. Effect of different inclusion levels of NLM on weight gain of laying hens (after 12 weeks of treatment)

Group (% inclusion)	Pretreatment wt (g)	Post-treatment wt (g)	wt gain (g)(%)
0	1500	1580	80 (5.3)
5	1500	1580	80 (5.3)
10	1510	1580	70 (4.6)
15	1508	1585	77 (5.1)

Figure in parenthesis are percentage weight gain.

The data on the haematological parameters of laying hens analyzed for the treatments are presented on Table 3. There were significant differences ($P < 0.05$) in the haematological parameters of birds fed 10% inclusion levels of NLM, except for the eosinophils and lymphocytes. Birds fed diet with 10% NLM recorded the highest haemoglobin value of 12.0 g/100 ml, but this is significantly different from that of birds on the 15% diet (8.8 g/100 ml) but similar to the control (10.2 g/100 ml) and 5% (10.5 g/100 ml) inclusion levels. Again the packed cell volume (PCV) of layers in the 10% treatment group differed significantly from that of 15% treatment group, but similar to the PCV of the control (0%) and 5.0% treatment levels of NLM. There was a significant difference in the red blood cell (RBC) values of layers fed 0% and 10% inclusion levels of NLM and the RBC values of birds on 15% NLM. There was a significant difference in the total white blood cell (WBC) counts of laying hens fed diets containing 5% inclusion level of NLM, but the value is similar to those in the 0% treatment group. The percentage heterophils of birds on 10% and 15% diet levels were significantly different from that of 0% treatment group, but similar to the heterophil values of birds in the 5% treatment group. The percentage of eosinophils increased significantly among the birds fed ration with 15% inclusion level of NLM, but similar to the value in the birds belonging to the 0% treatment group. The lymphocyte values of the 0% treatment group differed significantly but only similar to those of birds in the 5% group. There was also a significantly difference in the monocyte values of laying hens placed on 10% inclusion level of NLM. This difference was equally similar to the monocyte values of birds on 5% and 15% treatment groups.

The mean corpuscular volumes (MCV) of layers fed diets of NLM at 5%, 10% and 15% differed significantly from the MCV of birds on 0% inclusion levels of the test diet. Again these laying birds had significantly higher but similar mean corpuscle haemoglobin (MCV) values at 5% and 10% inclusion levels of NLM than layers on 0% and 15% treatment levels. The mean corpuscular haemoglobin concentrations (MCHC) of layers on 0%, 5% and 10% inclusion levels of NLM are significantly higher than the MCHC of birds on 15% treatment group.

Table 2. The effect of dietary levels of NLM on carcass and organ weights of laying hens

Parameters	0%	5%	10%	15%	SEM
Live wt (g)	1400	1470	1480	1438	19.24
Whole carcass wt (% live wt)	91.0^{bc}	91.6^{bc}	92.9^{ac}	90.2^{b}	0.66
Dressed wt (% live wt)	62.5^{bc}	62.9^{ac}	62.9^{ac}	62.0^{b}	0.21
Liver wt (% live wt)	1.8^{b}	2.0^{ac}	1.9^{bc}	1.9^{bc}	0.04
Heart wt (% live)	0.4^{a}	0.4^{a}	0.4^{a}	0.3^{b}	0.02
Gizzard wt (% live wt)	3.1^{b}	3.9^{ac}	3.4^{bc}	2.8^{b}	0.24

abc: Means within the same row having different superscripts are significantly different ($P < 0.05$).

Table 3. Effect of dietary levels of NLM on haematological values of laying hens, 12 weeks post treatment

Parameters	Dietary 0%	Levels 5%	Of Neem 10%	Leaf meal 15%	SEM
Hemoglobin (g/100ml)	10.2^{bc}	10.5^{bc}	12.0^{ac}	8.8^{b}	0.65
Packed Cell Volume (%)	34.0^{bc}	35.0^{bc}	40.0^{ac}	30.0^{b}	2.06
Red blood cell ($\times 10^6$/L)	6.3^{ac}	6.0^{bc}	6.6^{ac}	5.2^{b}	0.29
MCV (fl)	27.2^{b}	29.2^{a}	30.3^{ac}	28.9^{ac}	0.64
MCH (pg)	8.2^{b}	8.8^{ac}	9.1^{ac}	8.5^{b}	0.19
MCHC (gm%)	30.0^{a}	30.0^{a}	30.0^{a}	29.3^{b}	0.18
White Blood Cell ($\times 10^9$/L)	8.6^{bc}	9.3^{ac}	8.4^{b}	8.0^{b}	0.27
Heterophils (%)	50.0^{b}	52.0^{bc}	54.0^{ac}	53.0^{ac}	0.85
Eobinophils (%)	2.0^{bc}	1.0^{b}	1.0^{b}	3.0^{ac}	0.47
Lymphocytes (%)	46.0^{ac}	44.0^{bc}	41.0^{b}	41.0^{b}	1.22
Monocytes (%)	2.0^{b}	3.0^{bc}	4.0^{ac}	3.0^{bc}	0.41

abc: Means on the same row with different superscripts are significantly different ($P < 0.05$).

4 Discussion

The result for the body weight gain across treatment groups showed that higher dietary inclusion levels resulted in decreased weight gain. This was probably due to the effects of incomplete elimination of toxic factors[1,17]. Other workers[18,19] have also reported the effects of nutrient imbalance and poor metabolism[20] on monogastric animals fed high levels of unconventional feed ingredients.

There was a progressively slight increase in the whole carcass weight, up to 10% inclusion level of NLM. This indicates that it may be the optimal inclusion level of NLM that could result in increase in carcass weight. The highest dressed percentage was obtained from layers receiving 5% and 10% NLM diets, this shows that further increase in the quantity of NLM depleted the energy in the muscle tissues which could have been utilized for egg production[20,21]. The percentage weights of the liver, heart and gizzard obtained at 5% dietary levels of NLM indicate that it is at this level that these organs gain their maximum weights. In addition, the decrease in organ weights observed immediately above 5% NLM inclusion level indicates that there may be certain toxic substances present in the test materials. This is in agreement with earlier reports[18,22]. Future research studies is needed to identify the chemical compound responsible for the slight hepatomegaly and changes in other organ weights observed in these laying hens fed diets containing higher levels of NLM.

The haemoglobin values and PCV of the layers were significantly higher at 10% inclusion level of NLM than other treatment groups. However, the trend observed across these values was a progressive increase with a later decrease among birds placed on diet containing 15% NLM. With the exception of the 15% treatment group, the Hb values at other levels of dietary inclusion of NLM were within the reported range of 9 – 13 g/d[23].Also the PCV of birds across all the treatment levels were within the normal range (30% – 40%)[23]. Although there were significant differences in RBC counts of the laying hens placed on 0%, 5% and 10% dietary levels of NLM, these values were much higher than the reported normal count of 3.0 ×100/L in temperate environment[23]. The observed relative polycythaemia may be due to a reduction in the fluid component of the blood from insufficient fluid intake[23] or due to the slight hepatomegaly[24,25]. NLM has high fibre content which may have resulted in dehydration due to inadequate water intake by the birds for egg production and other normal physiological processes. Observations on the haematological indices revealed that although the MCV of birds on diets containing 5%, 10% and 15% inclusion levels of NML were significantly higher than the control, all the values were below the reported range of 127 (fl) for chickens raised under temperate conditions[24]. The MCHC which is an indication of the average amount and concentration of haemoglobin in the red blood cells is within reported normal range of 29 gm%[26], suggestive of macrocytic normochromic red cells. Again, this may be as a result of the slight hepatomegaly observed with the ingestion of different treatment diets of the NLM.

There was a reduction in the circulating WBCs among birds placed on both control diet and dietary levels of NLM. These values were below the reported range of 9 – 56×10^3/L[23].The variations in the WBCs of the layers on different levels of diet in this experiment could therefore be attributed to nutrient ambiance and/or poor nutrient utilization, thus leading to leucopenia. Although the differential WBC counts exhibited some levels of significance, the heterophils eosinophils and monocytes were within the reported ranges of 3 – 17, 0 – 0.5 and 0 – 5 $\times 10^3$/L respectively for clinically healthy birds[23]. The lymphocyte values on the other hand were generally below reported range of 10 – 30×10^3/L[23]. This may have caused the observed leucopenia, since lymphocyte numbers account for almost half of the WBC population. The decreased lymphocyte numbers here suggest the effect of nutrient imbalance and/or nutrient utilization.

5 Conclusion

We conclude that though up to 15% treatment diet of NLM may have increased both hen-day egg production and egg yolk colour[2], it did not improve many of the physiological and haematological parameters measured. The relatively poor weight gain by laying birds receiving increasing levels of NLM suggests nutrient imbalance and/or poor nutrient utilization. Further detailed research on the pathophysiology of laying birds fed ad-libitum on the same dietary levels of NLM might be fruitful in determining the actual differences in structural, functional and haematological parameters of birds fed NLM.

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