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Effects of Graded Levels of Neem (Azadirachta indica) Leaf Meal on Egg Production, and Quality of Eggs of Shika Brown Layers

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Abstract
The effects of feeding graded levels of neem leaf meal (NLM) on egg production and egg quality of Shika Brown layers was investigated. Fresh neem leaves harvested, sun-dried under moderate sunlight for 5 days and milled to obtain neem leave meal (NLM) were incorporated into five experimental diets (T_1-T_5) containing 0%, 2.5%, 5.0%, 7.5%, and 10.0% NLM respectively, with T_1 (0% NLM) as control. One hundred and fifty (150) Shika Brown pullets aged 20 weeks were allotted, on equal weight basis, to the five dietary treatments of 30 birds each, made up of 3 replicates of10 birds per replicate in a Completely Randomized Design. At the end of 10 weeks of feeding, there were no significant ($P>0.05$) differences among the treatment means in total egg production, hen-day egg production (HDP), hen-housed egg production (HHP), and total egg mass. Variation in dietary contents of NLM had no significant ($P>0.05$) effect on the weight, specific gravity, shape index and shell weight of eggs produced. Eggs from layers fed diets containing 7.5% NLM were significantly ($P<0.05$) longer, wider and had more volume than those of the control and 2.5% NLM treatment groups but were not different in length and width from eggs of pullets fed 5.0% and 10.0% NLM diets. In this study, 7.5% dietary NLM significantly improved egg size when compared with the control and 2.5% NLM diet groups. All eggs produced were normal in shape. The shell quality characteristics, egg specific gravity and shell weight, were unaffected ($P>0.05$) by dietary NLM levels. However, shell thickness was numerically higher in the NLM compared to the control group, although the differences were not significant except between the control and 5.0% NLM groups. Albumen weight was significantly ($P<0.05$) higher in eggs from pullets fed 7.5% NLM than in eggs from the control birds. Values for albumen height and Haugh unit were also numerically, though non-significantly ($P>0.05$), higher with 7.5% NLM than in the control (0% NLM) group. Yolk attributes (height, weight and yolk index) for the neem-fed hens were not significantly ($P>0.05$) different from those of the control group. Yolk index values obtained for all treatments in this study ranged from 0.41 to 0.44). It was concluded that incorporation of up to 10% NLM in layer diets does not significantly reduce egg production and the external and internal quality attributes of eggs produced. 7.5% dietary NLM inclusion tended to enhance egg production and quality than in the control, and other NLM groups.
Keywords: Neem Leaf Meal, Shika Brown, layers, egg production, egg quality

1. Introduction

The increasing interest in the possible use of neem leaf meal (NLM) in poultry diets has arisen, in part, from reports of its impressive content of essential nutrients such crude protein (20.68%), ether extract (4.13%), ash (7.10%) and nitrogen-free extract (43.91%) (Esonu, 2006), its successful use as a feed additive or ingredient in several experimental feed trials (Durrani *et* al., 2008; Onyimonyi *et al*.,, 2009; Wankar *et al*., 2009; Zanu *et al*., 2012), and its supposed anti-microbial properties (Durrani *et al*., 2008; Jawad *et al*., 2013; Odoh & Bratte, 2015) for which it may find further use in the future as a replacement for antibiotic growth promoters in livestock diets.

In less developed countries where resources for food production are limited, and feed costs arising from livestock production activities are as high as 60-80% of total production costs due to high costs of conventional feed ingredients (Esonu *et al*., 2006; Ogbuewu *et al.,* 2011), the need to try out non-conventional alternatives such as neem leaf meal becomes imperative.

The chicken egg is a cheap and important source of high quality proteins especially in the less developed countries of the world such as Nigeria. It is known to be a rich source of good quality protein, unsaturated fatty acids, vitamins (A, K and B), and several minerals of nutritional importance to man (Watkins, 1995; Amefule *et al*. 2006). Not only is Nigeria blessed with a climate that supports the use of local and exotic chicken breeds for egg and meat production, the neem plant (*Azadirachta indica*) thrives well in virtually all parts of the country all the year round.

In a recent study, Odoh and Bratte (2015), working with Shika Brown layers, reported that incorporation of up to 10% NLM in layer diets did not adversely affect the health status of the birds as adjudged from their haematological and serological profiles. Reports about the response of Shika Brown pullets (developed in Nigeria) fed graded levels of NLM in terms of egg production, and the external and internal quality of eggs obtained therefrom are scanty.

This present study was therefore designed to investigate the effects of feeding graded levels of neem leaf meal on egg production and quality of eggs of Shika Brown hens.

2. Materials and Methods

2.1. *The Experimental Location*

This study was performed at the Poultry Unit of the Department of Animal Science, Delta State University, Asaba Campus, Asaba, Nigeria, with latitude 6^0 12' N and longitude 6^0 45' E as coordinates. It is in the tropical rainforest agro-climatic zone southwest of Nigeria, and has an annual rainfall range of 1800-3000mm and maximum day temperatures of 20-35^{0}C.

2.2 *Processing of the Test Ingredient, and Formulation of the Experimental Diets*

Azadirachta indica leaves harvested fresh from neem plants in and around the Delta State University, Asaba Campus, Asaba, Nigeria, were spread out evenly to dry under moderate sunlight for five (5) days until they became crispy, and ground with a hammer mill to obtain Neem Leaf Meal (NML). Sample of the meal were then analyzed for their proximate composition using the methods of AOAC (1990), and the information (Table 1) applied to formulate five layer diets (T_1-T_5) which contained 0% (control), 2.5%, 5.0%, 7.5% and 10.0% NLM respectively (Table 2). The experimental diets contained approximately 17% crude protein (CP) and 2500kcal. kg^{-1} metabolizable energy (ME).

Table 1: Proximate composition of Neem Leaf Meal (NLM)

Proximate Fraction	%
Moisture	8.33
Crude protein	21.87
Crude fibre	14.33
Ether extract	6.33
Ash	9.16
Nitrogen-free extract	36.98

Table 2: Composition of the experimental diets

	Dietary Treatments				
	T_1	T_2	T_3	T_4	T_5
Ingredients	**0% NLM**	**2.5% NLM**	**5.0% NLM**	**7.5% NLM**	**10.0% NLM**
Maize	57.98	56.37	54.75	53.17	51.50
Neem Leaf Meal	-	2.50	5.00	7.50	10.00
Soybean meal	16.92	16.03	15.15	14.23	13.40
Fishmeal	3.00	3.00	3.00	3.00	3.00
Wheat offal	10.00	10.00	10.00	10.00	10.00
Rice offal	1.00	1.00	1.00	1.00	1.00
Oyster shell	7.50	7.50	7.50	7.50	7.50
Bone meal	3.00	3.00	3.00	3.00	3.00
Lysine	0.20	0.20	0.20	0.20	0.20
Methionine	0.20	0.20	0.20	0.20	0.20
Common salt	0.20	0.20	0.20	0.20	0.20
Total	**100**	**100**	**100**	**100**	**100**
Calculated					
Crude protein	17.06	16.99	16.93	16.97	16.90
Energy	2581.23	2545.09	2509.16	2520.46	2503.65

NLM = Neem Leaf Meal

2.3 The Experimental Birds and Experimental Design

One hundred and fifty (150) Shikka Brown pullets aged 20 weeks, obtained from the National Animal Production Research Institute (NAPRI) in Shika, Zaria, Nigeria were weighed individually on arrival at the farm, and allotted on equal weight basis to the five treatments of 30 birds each, made up of 3 replicates of10 birds per replicate in a Completely Randomized Design (CRD).

The birds were kept on deep litter, and provided unrestricted access to feed and drinking water. All routine vaccinations were done.

2.4 Measurements

Data on egg production and on the external and internal characteristics of randomly-selected samples of eggs produced were recorded on replicate basis every 2 weeks for a period of ten (10) weeks of the experiment. Hen-Day egg production (HDP) and Hen-Housed egg production (HHP) were computed as shown in Equations 1 and 2 respectively.

$$HDP = \frac{100 \times (\text{Total number of eggs laid in 10 weeks})}{(\text{Average number of hens housed per day of laying period})} \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$HHP = \frac{100 \times (\text{Total number of eggs laid in 10 weeks})}{(\text{Total number of hens housed at the beginning of lay})} \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Egg weight was measured with a top-loading electronic scale to the nearest 0.01g. Egg length and diameter were determined with a Vernier caliper, and egg volume by simple water displacement. Other parameters determined included the shell weight (with membranes), shell thickness (with a micrometer screw gauge), albumen and yolk heights (with a tripod micrometer), albumen and yolk weights (after carefully separating both with a yolk separator), and yolk diameter with a Vernier caliper.

Egg shape index was obtained by expressing the maximum diameter of the egg as a percentage of its length, and yolk index as yolk height divided by the yolk diameter. Haugh Unit (HU) was computed as shown in Equation 3.

$$HU = 100 \times \log_{10}(h - 1.7W^{0.35} + 7.6) \quad \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Where HU = Haugh Unit (%), h = albumen height (mm) and W = egg weight (g).

Total egg mass (per replicate) was taken as the product of the mean egg weight (g) and the total number of eggs laid by birds in that replicate.

2.5 Data Analysis

Treatment means of all data collected were compared by the one-way analysis of variance (ANOVA) procedure using IMB's SPSS (v 20) computer software, while significantly different treatment means were separated by the Duncan's multiple range post-hoc function of the same statistical package.

3. Results and Discussion

The effects of feeding graded levels of Neem Leaf Meal (NLM) on egg production of Shika Brown layers are presented in Table 3.

Table 3: Effect of graded levels of NLM on egg production in Shika Brown layers (Mean±SE)

	Dietary Treatments				
Parameters	**T_1 0% NLM**	**T_2 2.5% NLM**	**T_3 5.0% NLM**	**T_4 7.5% NLM**	**T_5 10.0% NLM**
Total No. of Eggs Produced	641.00±20.30	642.00±19.52	646.67±10.70	651.00±12.53	620.00±20.66
HDP (%)	91.57±2.90	91.39±2.79	92.39±1.53	92.86±1.57	88.97±2.95
HHP (%)	64.10±2.03	64.20±1.95	64.67±1.07	65.10±1.25	62.00±2.07
Egg Mass (kg)	34.29±10.86	35.07±10.64	25.56±18.96	37.35±7.00	34.43±8.28

HDP =Hen-day egg production; HHP = Hen-housed egg production; NLM = Neem Leaf Meal;
SE = Standard error.

There were no significant ($P>0.05$) differences among the treatment means in total egg production, hen-day egg production, hen-housed egg production, and egg mass production thus indicating that up to 10% dietary inclusion of NLM in layer diets is unlikely to have any deleterious effect on egg production. Elangovan *et al.* (2000), working with Japanese quail fed varying levels of neem kernel meal, similarly reported no significantly different treatment means in egg production and quality. Work by Gowda *et al.* (1999) showed significant ($P<0.01$) reductions in feed intake, egg production and egg weights when White Leghorns were fed diets containing 15% or more neem kernel meal.

The external and internal egg quality characteristics of eggs produced by Shika Brown layers fed varying levels of NLM are presented in Tables 4 and 5 respectively. Variation in dietary contents of NLM had no significant ($P>0.05$) effect on the weight, specific gravity, shape index and shell weight of eggs produced. Eggs from layers fed diets containing 7.5% NLM were significantly ($P<0.05$) longer, wider and had more

volume than those of the control and 2.5% NLM treatment groups but were not different in length and width from eggs of pullets fed 5.0% and 10.0% NLM diets. Egg size as perceived by its length, width and overall volume, is an important determinant of consumer preference for table eggs. Ayim-Akonor & Akonor (2014) in a survey of egg consumption patterns and consumer preferences in Accra, Ghana reported that consumers identified egg size as an important factor. In another study in Sokoto, Nigeria, egg size rather than weight ranked highest among consumer preferences for table eggs (Jibir *et al.* 2013). In this study, 7.5% dietary NLM significantly improved egg size when compared with the control and 2.5% NLM diet groups. NLM did not significantly (P>0.05) alter shape index (SI) of the eggs. All eggs produced were normal in shape, as shape index values obtained in this study (72.43±0.43 to 74.90±0.90%) fell within the range of 72-76% for normal eggs (Altuntas & Sekeroglu, 2008). The shell quality characteristics, egg specific gravity and shell weight, were unaffected (P>0.05) by dietary NLM levels. However, shell thickness was numerically higher in the NLM compared to the control group, although the differences were not significant except between the control and 5.0% NLM groups.

Table 4: Effects of Feeding Varying Levels of Neem Leaf Meal (NLM) on External Egg Characteristics of Shika Brown Layers (Mean±SE)

	Dietary Treatments				
External Egg Characteristics	**T_1 0% NLM**	**T_2 2.5% NLM**	**T_3 5.0% NLM**	**T_4 7.5% NLM**	**T_5 10.0% NLM**
Egg Weight (g)	53.13±0.64	55.27±1.07	57.00±2.29	57.40±5.05	55.50±2.30
Egg Length (cm)	$5.57±0.06^{b}$	$5.60±0.10^{b}$	$5.67±0.05^{ab}$	$5.90±0.20^{a}$	$5.80±0.17^{ab}$
Egg Width (cm)	$4.17±0.06^{b}$	$4.17±0.10^{b}$	$4.20±0.10^{ab}$	$4.40±0.10^{a}$	$4.20±0.17^{ab}$
Egg Volume (ml)	$51.67±1.44^{b}$	$53.33±2.89^{b}$	$53.33±2.87^{b}$	$58.33±2.89^{a}$	$54.33±1.15^{ab}$
Specific Gravity (g/ml)	1.03±0.04	1.04±0.05	1.07±0.02	0.99±0.10	1.02±0.05
Shape Index (%)	74.90±0.90	74.57±1.35	74.10±0.72	74.67±1.25	72.43±0.43
Shell Weight (g)	5.30±0.69	6.17±0.50	5.67±1.15	5.90±0.30	5.60±0.79
Shell Thickness (mm)	$0.37±0.01^{b}$	$0.39±0.01^{ab}$	$0.41±0.02^{a}$	$0.38±0.02^{ab}$	$0.39±0.02^{ab}$

[a, b]: Within each row, means with different superscripts are significantly (P<0.05) different;
SE = Standard error.

Table 5: Effects of Feeding Varying Levels of Neem Leaf Meal (NLM) on Internal Egg Characteristics of Shika Brown Layers (Mean±SE)

	Dietary Treatments				
Internal Egg Characteristics	**T_1 0% NLM**	**T_2 2.5% NLM**	**T_3 5.0% NLM**	**T_4 7.5% NLM**	**T_5 10.0% NLM**
Albumen Weight (g)	$29.17±2.72^{b}$	$32.70±0.87^{ab}$	$31.70±5.86^{ab}$	$36.67±4.10^{a}$	$30.59±0.4.04^{ab}$
Albumen Height (mm)	6.43±0.58	6.30±0.85	7.39±1.17	7.41±1.64	6.07±0.89
Yolk Weight (g)	14.97±1.72	14.30±1.21	13.93±2.97	14.93±0.35	15.33±1.83
Yolk Height (mm)	18.84±0.73	19.05±1.77	18.55±0.91	17.94±0.79	17.49±0.51
Yolk Diameter (mm)	43.87±1.76	43.83±1.44	42.17±1.76	43.83±1.44	43.17±0.29
Yolk Index	0.43±0.01	0.44±0.06	0.42±0.04	0.43±0.04	0.41±0.01
Haugh Unit (%)	82.10±3.89	80.38±5.47	86.53±7.42	86.18±10.35	78.63±6.68

[a, b]: Within each row, means with different superscripts are significantly (P<0.0) different.

Albumen quality parameters were also not significantly diminished by NLM. On the contrary, albumen weight was significantly (P<0.05) higher in eggs from pullets fed 7.5% NLM than in eggs from the control birds (Table 5). Values for albumen height and Haugh unit were also numerically, though non-significantly (P>0.05), higher with 7.5% NLM than in the control (0% NLM) group.

Yolk attributes (height, weight and yolk index) for the neem-fed hens were also not significantly (P>0.05) different from those of the control group. Yolk index values obtained for all treatments in this study (0.41-0.44) were similar to those obtained (0.41-0.48) by different evaluation procedures and at varying temperatures for two strains of laying chickens by Keener *et al*. (2006).

4. Conclusion

From the results obtained in this study, it can be concluded that incorporation of up to 10% NLM in layer diets does not significantly reduce egg production and the external and internal quality attributes of eggs produced. 7.5% dietary NLM inclusion tended to enhance egg production and quality than in the control, and other NLM groups.

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