Akintomide Aanuoluwapo Adeyemi et al. / Afr.Res.J.Bio.Sc. 3(1) (2026) 60-68
https://doi.org/10.62587/AFRJBS.3.1.2026.60-68 ISSN: 3006-7464

African Research Journal of Biosciences

Journal homepage: http://www.afrjbs.com

Research Paper Open Access

Production performance and egg quality of isa-brown layers fed neem leaf meal-based diets

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Article Info

Volume 3, Issue 1, January 2026
Received : 22 April 2025
Accepted : 28 October 2025
Published : 25 January 2026
doi: 10.62587/AFRJBS.3.1.2026.60-68

Abstract

This study evaluated the effects of dietary inclusion of NLM on the production performance and egg quality traits of laying hens. Four experimental diets were formulated to contain 0, 5, 10, and 15% NLM, designated as Diets A, B, C, and D respectively. A total of 144 point-of-lay Isa-Brown pullets (18 weeks old) were randomly allocated to the four dietary treatments, with 36 birds per treatment and three replicates of 12 birds each, following a completely randomized design. The feeding trial lasted for 16 weeks, during which data were collected on growth performance, and external and internal egg quality traits. Data were subjected to appropriate statistical analysis. Results showed no significant differences (P>0.05) in growth performance. The initial and final weights ranged from 1,409.17 ± 9.92 to 1,413.02 ± 9.83 g and 1,553.64 ± 26.84 to 1,619.04 ± 18.53 g, respectively. Laying hens fed Diet C had the highest weight gain (208.65 ± 11.94 g), while those on Diet A had the highest daily feed intake (119.86 ± 0.14 g). There were no significant (P>0.05) differences in external and internal egg qualities except the egg yolk color score. Egg weight ranged from 57.79 ± 0.26 to 58.52 ± 0.75 g while yolk color score was highest (7.59) in eggs of birds fed Diet D. In conclusion, neem leaf meal can be incorporated into layer diets up to 15% without adverse effects on growth performance, external and internal egg qualities. Additionally, it enhanced yolk pigmentation, making it a promising feed ingredient for improving yolk quality in poultry production, and could be adopted particularly in tropical regions where neem tree is abundantly available.

Key words: *Alternative feed, Chicken eggs, Internal egg quality, Yolk color*

1. Introduction

Table eggs from poultry have been considered the cheapest and most effective strategy to improve the nutritional

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standards of the populace in developing nations in the fight against protein-calorie malnutrition (Agbede, 2019). Eggs are highly valued for their nutritional content, supplying essential amino acids needed by humans for body growth, along with a variety of vitamins such as vitamin A, B_2, B_9, B_{12}, and minerals including iron, calcium, phosphorus, and potassium (Oso *et al.*, 2023).

However, increased competition with human nutrition, scarcity of conventional feed ingredients, and the rising cost of grain importation have led to a significant increase in the cost of feeding layer birds which has cascade to increased cost of table eggs in a typical sub-Saharan nation such as Nigeria (Akinwale, 2024). These challenges have forced many poultry farmers out of business, leading to a decline in the overall production and availability of eggs for human consumption, and increased pressure on the poultry industry in demand for table eggs (Edeh, 2013). These have led to growing interest in exploring non-conventional feed ingredients, such as leaf and seed meals from various tropical plants, which could serve as cheap alternative sources.

One of such plant is neem (*Azadirachta indica*), a tropical evergreen tree belonging to the *Meliaceae* family. It is known locally to possess antioxidant, antibacterial, antiviral, and hypocholesterolemic properties (Oniyimonyi *et al.*, 2009; Heyman *et al.*, 2017), with a distinctive composition of alkaloids like quercetin, nimbosterol, azadirachtin, and carotenoids (Singh *et al.*, 2017; Osho *et al.*, 2021). Beyond its functional properties, it has a rich nutrient profile containing crude protein (20.68%), ether extract (4.13%), ash (7.10%), and nitrogen-free extract (43.91%) (Esonu *et al.*, 2006). Its successful application as a feed ingredient in various experimental feed trials has revealed that it can improve growth performance, feed conversion ratio, carcass yield, haematological and serum biochemical profiles, and reduce cholesterol (Bonsu *et al.*, 2012; Nayaka *et al.*, 2013; Akintomide *et al.*, 2018). However, there is a paucity of information on its use as major feed ingredient rather than feed additive or extract.

Studies have confirmed that nutrition, breed, and age of hens significantly affect both internal and external egg quality traits. Thus, there is a need to examine these, especially when a non-conventional feed material is used. Egg quality traits, such as egg weight, width, length, shell quality, albumen and yolk characteristics, are crucial not only for consumer acceptance but also for the egg processing industry (Song *et al.*, 2000). This study seeks to harness the potential of neem leaf meal on growth performance, internal and external egg quality traits in laying hens, this in an attempt to enhance the availability and affordability of animal protein (eggs) in sub-Saharan nations, thereby contributing significantly to food and nutritional security.

2. Material and methods

2.1. Experimental site

The feeding trial was conducted at the Poultry unit of the Teaching and Research Farm, Department of Animal Production and Health, Federal University of Technology, Akure (FUTA). The farm is located at latitude 7°18′00′′N and longitude 5°08′39′′E (Google Earth, 2025), with an annual temperature range of 27 °C to 38 °C and annual precipitation between 1300 and 1650 mm (Daniel, 2015). Laboratory analyses were carried out at the Nutrition Laboratory of the same Department.

2.2. Collection and processing of neem leaves

Fresh neem (*Azadirachta indica*) leaves were harvested from neem trees within Akure Township, Ondo State. The leaves were identified and authenticated by the curator at the FUTA Herbarium Centre. The harvested leaves were sun-dried until a crispy texture was achieved, then milled to produce Neem Leaf Meal (NLM) and stored in air tight containers prior to its use.

2.3. Diets preparation

Feed ingredients were sourced from a reputable feed mill in Akure, Ondo State. The neem leaf meal was incorporated at 0, 5, 10, and 15% and designated as Diet A, B, C, and D respectively. The composition of the experimental diets is presented in Table 1.

2.4. Experimental birds and management

One hundred and forty-four (144) point-of-lay Isa-Brown pullets (18 weeks old), were purchased from a reputable farm and allowed to acclimatize for two weeks. All routine management practices, including

Table 1: Gross and chemical composition of experimental diets				
Ingredient (%)	**A**	**B**	**C**	**D**
Maize	51.00	48.50	45.00	43.50
Neem leaf meal	0.00	5.00	10.00	15.00
Wheat offal	15.20	14.20	14.90	12.30
Soyabean meal	13.70	12.20	10.00	9.70
Groundnut cake	9.00	9.00	9.00	8.40
Limestone	7.00	7.00	7.00	7.00
Bone meal	3.20	3.20	3.20	3.20
Lysine	0.20	0.20	0.20	0.20
Methionine	0.20	0.20	0.20	0.20
Premix*	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**
Analysis nutrient composition (%)				
Moisture content	11.62	12.94	12.27	14.39
Crude protein	17.44	18.81	18.88	19.36
Crude fibre	4.00	3.78	4.16	4.67
Ether extract	3.56	4.61	4.21	4.37
Ash	4.05	5.12	7.24	7.32
Nitrogen free extract	59.33	54.74	53.24	49.89
Metabolizable energy (Kcal/Kg)	3,042.70	3,016.34	2,932.96	2,844.88

Note: *Premix provided per Kg diet: Vitamin A 8,500 iu, Vitamin D_3 1,500 iu, Vitamin $_E$ 10,000 mg, Vitamin K_3 1,500 mg, Vitamin B_1 1,600 mg, Vitamin B_2 4,000 mg, Niocin 20,000 mg, Pantothenic Acid 5000 mg, Vitamin B_6 1,500 mg, Vitamin B_{12} 1000 mg, Folic Acid 500 mg, Biotin H_2 750 mg, Choline Cloride 175,000 mg, Cobalt 200 mg, Copper 3000 mg, Iodine 1000 mg, Iron 20,000 mg, Manganese 40,000 mg, Selenium 200 mg, Zinc 30,000 mg, Antioxidant 1,250 mg; A = 0% neem leaf meal; B = 5% neem leaf meal; C= 10% neem leaf meal; D = 15% neem leaf meal.

vaccination and medication schedules, were strictly adhered to. The pullets were housed intensively in a battery cage. The experimental birds were fed twice daily (at 8:00 a.m. and 3:00 p.m.), and potable water was provided *ad libitum.*

2.5. Experimental design

A Completely Randomized Design (CRD) was employed for the study. The experimental birds were randomly allocated into four dietary treatments, with each treatment consisting of 36 birds. Each treatment was further divided into three replicates of 12 birds each. Three (3) birds were housed per cage unit (a cell).

2.6. Data collection

2.6.1. Evaluation of performance characteristics

The live weights of the birds were recorded at both the beginning and end of the 116-day feeding trial (16 weeks). Throughout the trial, daily feed intake and egg production were monitored and recorded. These data were used to calculate the Feed Conversion Ratio (FCR) (see formula below). Eggs were collected thrice daily at 8:00 am, 2:00 pm. and 5:00 pm and recorded.

$$\text{Feed conversion ratio} = \frac{\text{Feed consumed (g)}}{\text{Weight of egg (g)}}$$

2.6.2. Measurement of egg qualities

For egg quality assessment, nine (9) eggs were randomly collected weekly from each treatment group for both internal and external egg quality evaluations.

The following parameters were determined as outlined below:

Egg weight: The whole egg was weighed using a sensitive scale with an accuracy of 0.01 g.

Egg width: The whole egg width was measured using a Vernier caliper.

Egg length: The whole egg length was measured using a Vernier caliper.

Shell thickness: This was determined using a micrometer screw gauge.

Shell weight: This was measured with a sensitive scale after breaking the egg and separating it contents.

Shell ratio: This was calculated by dividing the shell weight by the total weight of the whole egg.

Albumin weight: The albumin was weighed using a sensitive scale with an accuracy of 0.01 g.

Albumin width and height: The width and height of the albumin were measured using a Vernier caliper.

Albumin ratio: The albumin ratio was calculated by dividing the albumin weight by the total weight of the egg.

Yolk weight: The yolk was separated and weighed using a sensitive scale with an accuracy of 0.01 g.

Yolk width and height: These were measured using a Vernier caliper.

Yolk ratio: Calculated by dividing the yolk weight by the total weight of the egg.

Yolk color: The yolk color was evaluated visually using the La Roche Scale (Yolk Color Fan) as described by Bovskova *et al*. (2014).

2.7. Statistical model and data analysis

Statistical model employed is presented below:

Yij = μ + ai + eij

where:

Yij : Represents any of the response variables,

μ : The overall mean,

ai : The effect of neem leaf meal in the i-th treatment (where i corresponds to dietary treatments A, B, C, and D),

eij : Is the random error due to experimental variation.

The data were analyzed using one-way analysis of variance (ANOVA) with SPSS version 26.0. Differences between treatment means were assessed using Duncan's Multiple Range Test, and the significance level was set at a 95% confidence interval.

3. Results

Table 2 presents the growth performance of Isa-Brown laying birds fed diets containing with varying levels of Neem Leaf Meal (NLM). There were no significant differences (P > 0.05) in all the parameters.

The initial body weight of the birds ranged from 1409.17 ± 9.92 to 1411.94 ± 4.92 g/bird, while the final body weight ranged from 1553.64 ± 26.84 to 1619.04 ± 18.53 g/bird. Birds fed Diet A recorded the highest total and daily feed intake (13424.83 ± 15.17 g/day and 119.86 ± 0.14 g/bird/day, respectively), whereas the lowest values (13205.30 ± 13.62 g/bird and 117.90 ± 0.21 g/bird/day) were observed in birds fed Diet D. The Feed Conversion Ratio (FCR) across the treatments ranged from 2.01 ± 0.02 to 2.07 ± 0.04.

Table 3 presents the external egg quality parameters of Isa-Brown laying birds fed diets containing varying levels of Neem Leaf Meal (NLM). None of the parameters measured were significantly (P > 0.05) influenced by NLM addition.

Egg weight ranged from 57.79 ± 0.26 in birds fed Diet A to 58.52 ± 0.75 g in those fed Diet D. Egg width ranged from 4.39 ± 0.08 to 4.46 ± 0.08 cm, while egg length varied between 5.37 ± 0.06 cm and 5.57 ± 0.07 cm across the treatments. Shell thickness slightly increased from 0.19 ± 0.01 mm in the eggs of birds fed Diet A to 0.20 ± 0.01 mm in those fed Diet D. Shell weight ranged (P > 0.05) from 5.86 ± 0.10 g (eggs of birds fed Diet A) to 5.98 ± 0.38 g (eggs from birds fed Diet C), with no consistent trend observed across treatments. Shell ratio remained constant across treatments with a value of 0.10 ± 0.01.

Table 2: Growth performance of Isa-Brown laying birds fed diets containing varying neem leaf meal

Parameters	Experimental diets				
	A	B	C	D	p-value
Initial live weight (g/bird)	1413.23 ± 9.83	1409.17 ± 9.92	1410.39 ± 10.04	1411.94 ± 4.92	0.99
Final live weight (g/bird)	1553.64 ± 26.84	1559.34 ± 41.50	1619.04 ± 18.53	1573.33 ± 32.53	0.48
Weight gain (g/bird)	139.75 ± 36.54	150.17 ± 47.61	208.65 ± 11.94	161.39 ± 30.85	0.53
Total feed intake (g/bird)	13424.83 ± 15.17	13260.96 ± 21.81	13413.82 ± 13.74	13205.30 ± 13.62	0.09
Daily feed intake (g/bird/day)	119.86 ± 0.14	118.40 ± 1.09	119.76 ± 0.21	117.90 ± 0.21	0.09
Feed conversion ratio	2.07 ± 0.04	2.04 ± 0.03	2.07 ± 0.04	2.01 ± 0.02	0.12

Note: n = 9 values are presented as mean ± Standard Error of Mean; A = 0% neem leaf meal; B = 5% neem leaf meal; C = 10% neem leaf meal; D = 15% neem leaf meal.

Table 3: External egg quality of Isa-Brown laying birds fed diets containing varying levels of neem leaf meal

Parameters	Experimental diets				
	A	B	C	D	p-value
Egg weight (g)	57.79 ± 0.26	57.98 ± 0.97	57.88 ± 0.64	58.52 ± 0.75	0.88
Egg width (cm)	4.39 ± 0.08	4.40 ± 0.04	4.46 ± 0.08	4.37 ± 0.03	0.76
Egg length (cm)	5.37 ± 0.06	5.44 ± 0.11	5.42 ± 0.07	5.57 ± 0.07	0.37
Shell thickness (mm)	0.19 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.90
Shell weight (g)	5.86 ± 0.10	5.87 ± 0.07	5.98 ± 0.38	5.63 ± 0.32	0.56
Shell ratio	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.61

Note: n = 9 values are presented as mean ± SEM; SEM: Standard Error of Mean; A = 0% neem leaf meal; B = 5% neem leaf meal; C = 10% neem leaf meal; D = 15% neem leaf meal.

Shown in Table 4 is the internal egg quality parameters of Isa-Brown laying birds fed diets containing varying levels of Neem Leaf Meal (NLM). No significant (P > 0.05) differences were observed in albumen weight (35.13 ± 0.68 to 35.50 ± 0.49 g), albumen width (6.32 ± 0.13 to 6.44 ± 0.10 mm), albumen height (0.90 ± 0.08 to 1.04 ± 0.05 cm), yolk weight (14.00 ± 0.44 to 14.40 ± 0.49 g), yolk width (4.01 ± 0.07 to 4.16 ± 0.11 cm), or yolk height (1.68 ± 0.13 to 1.94 ± 0.04 cm). However, yolk color significantly (P < 0.05) increased from 4.92 ± 0.08 (Diet A) to 7.59 ± 0.21 in eggs obtained from Diet D.

Table 4: Internal egg quality of Isa-Brown laying birds fed diets containing varying levels of neem leaf meal

Parameters	Experimental diets				
	A	B	C	D	p-value
Albumen weight (g)	35.13 ± 0.68	35.50 ± 0.49	35.29 ± 0.17	35.24 ± 0.66	0.97
Albumen width (cm)	6.40 ± 0.17	6.44 ± 0.10	6.32 ± 0.13	6.39 ± 0.13	0.93
Albumen height (cm)	0.94 ± 0.07	1.04 ± 0.05	0.93 ± 0.08	0.90 ± 0.08	0.59
Albumen ratio	0.61 ± 0.01	0.62 ± 0.01	0.61 ± 0.01	0.60 ± 0.01	0.55
Yolk weight (g)	14.00 ± 0.44	14.20 ± 0.39	14.40 ± 0.49	14.21 ± 0.35	0.93
Yolk width (cm)	4.01 ± 0.07	4.12 ± 0.03	4.09 ± 0.01	4.16 ± 0.11	0.88
Yolk height (cm)	1.70 ± 0.12	1.94 ± 0.04	1.68 ± 0.13	1.71 ± 0.05	0.20
Yolk ratio	0.25 ± 0.01	0.25 ± 0.01	0.25 ± 0.01	0.25 ± 0.01	0.99
Yolk color	4.92 ± 0.08^{c}	6.08 ± 0.50^{b}	6.25 ± 0.25^{b}	7.59 ± 0.21^{a}	0.01

Note: n = 9 values are presented as mean ± SEM; SEM: Standard Error of Mean; A = 0% neem leaf meal; B = 5% neem leaf meal; C = 10% neem leaf meal; D = 15% neem leaf meal.

4. Discussion

4.1. Growth performance of Isa-Brown laying birds fed diets containing varying levels of neem leaf meal

Body weight (initial weight) of the birds at 18 weeks was comparable to the value (1.36 kg) reported by Musa *et al*. (2023) for a typical light weight layer breed. Studies has revealed a positively correlated relationship between body weight and egg production in pullets, as birds that attain optimal body weight at the onset of lay tend to exhibit earlier onset of laying, higher egg output, and improved egg quality, while both underweight and overweight birds may experience reduced reproductive performance. Similarly, it was observed in the study that the birds gained weight during the experimental period which implies that diet were adequate to support egg production and additional weight gain, thus they did not experience deficiency in nutrient supply.

Feed intake in birds is largely influenced by quality and quantity of feed given. The daily feed intake obtained ranged from 115 to 125 g/bird/day, which aligns with the recommendations of Adegbenro *et al*. (2020) for Isa-Brown laying birds. Intake above this range may not improve production performance, particularly egg production and weight. Conversely, intakes below 115 g/bird/day has shown to reduce egg production and weight (Adegbenro *et al*., 2020).

Feed conversion ratio obtained from birds fed experimental diets were relatively low, birds fed diet D had the least FCR (P>0.05). This implies that the birds consumed less to produce a gram of egg, the lower the FCR value the better the efficiency of the bird. The low conversion ratio could be attributed to the high-quality diet, which is easily digestible and provides readily available nutrients to support growth and egg production. It could be hypothesized that these birds converted their feed better, as egg weight obtained from this group were higher.

4.2. External egg quality of Isa-Brown laying birds fed diets containing varying levels of neem leaf meal

According to Ayim-Akonor and Akonor (2014), one of the major factors that influence consumers' preferential selection for table eggs is egg quality. Egg quality refers to various criteria that define both external and internal quality. Internal quality (albumen and yolk qualities) may be difficult to assess unless the shell is broken, but valuable to confectionery who make use of these parts of egg. However, external traits such as weight, length, width and overall volume are easier to assess and are of most importance to consumers.

It was observed that the egg weight was relatively below the 60-65 g reported for Isa-Brown layer, a typical light breed layer. This could be as a result of age as birds used in this study were point-of-lay which are expected to produce small (peewees) eggs for a certain period before they advance in size and weight as their age progressed. This was similar to the observation of Musa *et al*. (2023) who reported increase in egg weight and size as the age of Isa-Brown layers progressed.

The egg width and length were relatively higher than the values (3.76 to 3.33cm and 5.13 to 5.16 cm respectively) reported by Adegbenro *et al*. (2023) for Isa-Brown layers but comparable to 4.25 cm and 5.52 cm reported by Abanikannda *et al*. (2007) for eggs from Harco breed layers. There is a direct relationship between egg weight and egg width and length as observed in this study, Aryee *et al*. (2020) opined that increase in egg width and length result to increase in egg weight.

Shell quality is a crucial parameter for table eggs, as a strong eggshell enhances the egg's ability to withstand handling and transportation without breaking or cracking, while also preserving the integrity of the internal contents during storage. The shell characteristics, mainly the weight and thickness were similar to the values reported by Adedeji *et al*. (2024), showing no significance as a result of varying inclusion levels of Neem Leaf Meal (NLM) across treatments.

Though not significantly different, shell weight followed a similar pattern to feed intake, which may suggest that feed intake has direct influence on egg shell weight. It is safe to say that higher feed intake led to more available calcium and phosphorus-minerals that form a large portion of the eggshell. However, the shell thickness recorded in this study was below 0.34 mm reported by Tadesse *et al*. (2015) for Isa-Brown layers and 0.33 mm for White Leghorn hens (Rath *et al*., 2015). This variation could be due to differences in nutrition, age and breed. Additionally, while the ash content in the diet increased with NLM inclusion, this did not appear to have a direct impact on shell thickness and weight, as observed in this study.

4.3. Internal egg quality of Isa-Brown laying birds fed diets containing varying levels of neem leaf meal

Egg internal characteristics showed no significant variations with NLM inclusion, except yolk color. Albumen attributes (height, weight, and width) were within the values reported by Adegbenro *et al*. (2023) except the albumen weight which was higher in this study, this could be attributed to the variation in egg weight as higher egg weight/mass would result in higher albumen weight. Similarly, yolk width and weight were below the values reported by Sam (2023). However, studies revealed that yolk width and weight largely influence egg size, as reported by Etalem *et al*. (2009).

Yolk color is a critical egg quality to many confectionery industries as deeper pigmentation is often preferred and command more market value (Moura *et al*., 2016). The significant variation in yolk color observed in this study can be attributed to the inclusion of NLM in the diet. This could be linked to the presence of natural pigments like carotenoids, xanthophylls, and flavonoids in neem leaves, this contributed to the enhanced yolk coloration as reported by Ahmad *et al*. (2022). The improvement in yolk color, with no adverse effects on other internal quality traits, highlights the potential of neem leaf meal not only as a nutritional feedstuff but also as a natural yolk color enhancer. These findings align with previous studies where plant-based feed additives rich in phytochemicals improved yolk pigmentation without negatively impacting egg quality parameters (Matache *et al*., 2024).

5. Conclusion

The inclusion of neem leaf meal in the diet of Isa-Brown layers had no significant effect on growth performance, internal and external egg characteristics. However, it had the propensity to improve yolk color while maintaining other aspects of egg quality.

Neem leaf meal can therefore be incorporated into the diets of laying hens at levels up to 15% without any deleterious effect on growth performance and egg quality. However, further studies might be necessary to evaluate it effects on haematological profile, organ status of laying birds and the nutrient composition of the eggs.

Acknowledgment

The authors acknowledge the Tertiary Education Trust Fund (TETFund) for funding this research.

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Cite this article as: Akintomide Aanuoluwapo Adeyemi, Oso Teniola Samuel, Osho Innocent Bamidele and Onibi Gbenga Emmanuel (2026). Production performance and egg quality of isa-brown layers fed neem leaf meal-based diets. *African Research Journal of Biosciences.* 3(1), 60-68. doi: 10.62587/AFRJBS.3.1.2026.60-68.