EFFECT OF GRADED LAWSONIA INERMIS LEVELS ON EGG QUALITY OF ISA BROWN CHICKENS

ADEDEJI, O. S.[1*] – OJO, T. Y.[2] – OLAIYA, I. A.[3] – ARASI, K. K.[1]

[1] *Department of Animal Nutrition and Biotechnology, Ladoke Akintola University of Technology, Oyo State, Nigeria.*

[2] *Department of Animal Production and Health, Ladoke Akintola University of Technology, Oyo State, Nigeria.*

[3] *Department of Pure and Applied Chemistry, Ladoke Akintola University of Technology, Oyo State, Nigeria.*

**Corresponding author*
e-mail: kolapoarasi16[at]gmail.com

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Abstract. Henna (Lawsonia inermis) is recognized for its medicinal properties and potential benefits in animal nutrition. This study assessed the effects of feeding graded levels of Lawsonia inermis on external and internal egg parameters of ISA brown chickens. One hundred and fifty birds (150) point of lay birds at 18-weeks old were randomly divided into 5 treatments of 30 bird per treatment comprising 10 birds per replicates in a completely randomized design at P<0.050. Lawsonia inermis was added to layer mash at inclusion levels of 10g, 20g, 30g and 40g/kg feed. Laying hens fed 40g of Lawsonia inermis had heaviest (53.76g) egg weight. The thickest shell was observed from eggs of laying hens fed 30g of Lawsonia inermis while hens fed diet supplemented with 40g Lawsonia inermis had lighter shell. Egg mass (46.17 and 45.37g) of hens fed 30 and 40g of Lawsonia inermis were higher than those fed 10, 20g Lawsonia inermis and the control. The albumen weight and yolk weight of eggs of laying hens fed 40g Lawsonia inermis were higher compare to other treatment groups. It can be concluded that supplementation of 40g Lawsonia inermis to laying hens diet yielded heaviest egg weight and egg mass.
Keywords: *Lawsonia inermis, layers, graded levels, egg quality, external quality, internal quality*

Introduction

Chicken egg contains all the essential amino acid for human and provide significant amount of several vitamins and minerals including vitamin A, riboflavin, folic acid, vitamin B6, vitamin B12, choline, iron, calcium, phosphorus and potassium along with 163 calories/100g of energy (Réhault-Godbert et al., 2019). Egg quality is the characteristics of an egg that affect its acceptability to the consumer as the appearance of an egg builds consumer confidence in the product and its consumption. Egg quality in general defines both internal and external quality of egg (Hussain et al., 2013). External quality is focused on shell cleanliness, soundness of shell, texture, color and shape. These features are important to the processor as superior quality eggs arrive in a better condition for the consumer. The internal quality refers to egg white (albumen), relative viscosity of albumen, shape and firmness of yolk, strength of yolk, size of air cell and presence or absence of blood or meat spots. Yolk of a fresh lay egg is round and firm (Kumar et al., 2005). As the yolk ages it losses quality by absorbing water and increasing in size and sometime rupturing occur. This absorption of water occurs from thin albumin surrounding the yolk, while the loss of carbon dioxide through egg shell causes thick albumin to be transparent and watery the yolk integrity also depends on the strength of vitelline membrane which is inversely proportional to the duration of storage (Kumar et al., 2005).

The antibiotics used as feed additives in the animal industry have contributed to the intensification of modern animal production. Starting with the intensification of animal husbandry, there is a constant concern regarding the large-scale use of food antibiotics that can lead to the development of the phenomenon of antimicrobial resistance. This represents a potential threat to human health (Gadde et al., 2017). Due to the emergence of the phenomenon of antimicrobial resistance, the World Health Organization (WHO) established guidelines and recommendations to stop the use of antibiotics as growth promoters in 1997. One year later, in 1998, the EU banned the first phase for poultry, and the use of antibiotics as additives in their feed later in 2006, establishing a complete ban on the use of prophylactic antibiotics in the feed of all animals (Al-Mnaser et al., 2022). Consequently, various alternatives were sought to reduce the use of antibiotics in animal production, in order to maintain their health and performance. The types of additives available to increase animal productivity while maintaining the health of the human population include probiotics and prebiotics, plant extracts, essential oils, dietary fiber and enzymes, antimicrobial peptides, functional amino acids, hyperimmune antibodies from eggs, clays, and/or metals (Lillehoj et al., 2018; Gadde et al., 2017). The optimal combinations of different compounds, together with good management and breeding practices, can be the key to intensifying the performance and productivity of animals with the aim of reducing and/or replacing antibiotics in the animal industry (Lillehoj et al., 2018). Henna (Lawsonia inermis Linn) is one of such plants that has attracted the attention of researchers worldwide due to its pharmacological activities ranging from anti-inflammatory to anticancer activities (Gupta, 2003).

Lawsonia inermisis a perennial plant commonly called as henna, native to North Africa and South East Asia, and often cultivated as an ornamental plant throughout India, Persia, and along the African coast of the Mediterranean sea (Malekzadeh, 1968). The Chemical names Lawsone, 2-Hydroxy-1,4-naphthoquinone are the chemical names, and C10H6O3 is the molecular formula. Lawsonia inermis has been shown to possess a number of advantageous qualities, including antibacterial and anti-inflammatory effects. The plant powder and its extract are responsible for these effects. Additional products that demonstrated exceptional efficacy against both Gram-positive and Gram-negative bacteria included ethanol extracts from the whole plant, fruit, and flowers, as well as aqueous, chloroform, and petroleum ether extracts (Semwal et al., 2014). Henna grows better in tropical savannah and tropical arid zones, in latitude between 15° and 25° N and S, produces highest dye content in between temperature 35 to 45°C. The optimal soil temperature ranges for germination are 25 to 30°C (Singh et al., 2021). Henna leaves are very popular natural dye to color hand, finger, nails and hair. The dye molecule, Lawson is the chief constituents of the plant; its highest concentration is detected in the petioles (0.5-1.5%). In folk medicines, henna has been used as astringent, antihemorrhagic, intestinal antineoplastic, cardio-inhibitory, hypotensive, sedative and also as therapeutic against amoebiasis, headache, jaundice and leprosy. It has been claimed to have immunomodulatory, antiviral, antibacterial, antifungal, no tropic, antifertility, hepatoprotective, antmitotic, analgesic and anti-inflammatory properties (González-Lamothe et al., 2009). According to González-Lamothe et al. (2009), henna is a significant source of phytochemicals with enormous medical and pharmaceutical significance, including mannitol, gallic acid, hennotannic acid, coumarins, xanthones, triterpenes, sterols, and derivatives of naphthoquinone that are useful as allied agents and immune modulators. Depending on the origin and whether the leaves are fresh or dried, henna's chemical composition can vary greatly. Moreover, lawsone, or 2-hydroxy-1:4 napthaquinone (C10H6O3, m.p. 190º decomp.), is the primary coloring material that gives henna its dyeing properties. Its output ranges from 1% to 20%.

Nonetheless, a lot of research is done in the area of plant phytochemical analysis. Napthoquinone derivatives, phenolic compounds, terpenoids, sterols, aliphatic derivatives, xanthones, coumarin, fatty acids, amino acids, and other elements are among the chemical components that have been isolated from L. inermis. Phytochemical analysis of henna leaf, however, reveals total ash (14.60%), acid insoluble ash (4.50%), and water soluble ash (4.0%) in another study article. It was discovered that the drying loss was 4.5% w/w. The extractive values for aqueous and alcohol-soluble substances were 3.8% and 5.0% w/w, respectively. Alcohol and aqueous extract were found to have practical yields of 13.34 percent and 15.50 percent, respectively (Hema et al., 2010). In contrast, aqueous and alcoholic extracts included significant amounts of gums, mucilage, carbohydrates, glycosides, tannins, and phenolic compounds, but saponins, alkaloids, phytosterols, fixed oils, lipids, proteins, amino acids, and volatile oils were lacking (Jain et al., 2010). According to Audu et al. (2018), the plant extract exhibited a moisture content of 33.2%, crude protein content of 3.38%, ash (29.9%), fiber (21%) and crude lipid (12%) proximate value of the aqueous crude leaf extract of L. inermis. Meanwhile, the calculated value of the nitrogen free extract was found to be 0.52%. The present study therefore wish to explore the potential of Lawsonia inermis leaf on egg quality parameters of ISA Brown chickens supplemented at varying levels.

Materials and Methods

Study area

The experiment was carried out at the Poultry Production Unit of Teaching and Research Farm, Ladoke Akintola University of Technology Ogbomoso, Oyo State, Nigeria the area is located in the derived savannah zone of Nigeria and lies on Longitude 4°151 East at Greenwich meridians and Latitude 8°151 North at equator. The attitude is between 380 and 600mm above sea level while the mean annual temperature and rainfall are 27°C and 1247mm respectively.

Experimental diets

Lawsonia inermis plants was purchased from local market, at Otte in Kwara State. The leaves was separated from the stems and air dried. The air dried leaves were ground to powder using electric blender to obtain a fine powdery sample. The basal experimental diet was formulated to contain 16.50% CP and 2453.020 Kcal/Kg metabolizable energy to meet the nutritional requirement of laying birds. The basal diet being the control diet (T1), Lawsonia inermis was included in the basal experimental diet at four levels at 10 (T2), 20 (T3), 30 (T4) and T5 40 (g/Kg feed). The ingredient composition of the basal diet is shown in *Table 1*.

Table 1. *Ingredient composition of layers diet*.

Ingredients	Quantity (in percetage, %)

Maize	35.00
Groundnut cake	11.00
Soybean meal	9.00
Palm kernel cake	15.00
Corn bran	15.00
Oyster shell	8.00
Bone meal	4.00
Lysine	0.15
Methionine	0.20
Premix	0.25
Salt	0.20
Total	100
Crude Protein	17.90
Metabolisable Energy	2497.27

Experimental birds and management

One hundred and fifty birds (150) point of laying birds of (ISA Brown) strain at 18 weeks of age was used for the experiment. The birds were randomly divided into 5 treatment groups, each treatment group comprised of 30 birds and 3 replicate with 10 birds per replicate. Regular management practices were observed throughout the experiment. Feed and water were supplied to the birds on an ad libitum basis.

Data collection and data analysis

Data were collected on egg quality traits such as external and internal traits on weekly basis for eight (8) weeks. The egg weight (g) was determined by weighing eggs obtained from each pullets using digital sensitive weighing balance. The egg width (mm) measurement was taken at three points viz; broad end, middle region and narrow end of the egg using Vernier Caliper. The mean of the three points was taken to obtain value for the egg width. Egg length (mm) was determined by measuring the distance between the broad end and the narrow end of theegg using vernier caliper. Shell thickness (mm) was determined by measuring small bits of each portion of the shell (narrow, middle andbroad portions) by the use of micrometer screw gauge. The mean of this readings was taken to obtain the thickness of the shell. Shell weight (mm) was obtained by subtracting the albumen and yolk from the whole egg. Albumen heights (mm) of freshly laid eggs were broken and content was poured into a flat table glass to measure albumen height using a tripod spherometer after the yolk has been removed. Albumen and yolk weight (g) were measured by placing the albumen and yolk on the sensitive scale. Albumen weight (g) was measured using digital sensitive weighing balance after albumen has been carefully separated from the yolk or vice versa. Yolk length (mm) was measured using vernier caliper used. Yolk height (mm) was determined by using a tripod spherometer on a table glass. Yolk weight (g) was obtained by measuring the yolk on a sensitive scale. Yolk colour was determined using the yolk fan. Data on egg quality traits were subjected to One-Way Analysis of Variance in a completely Randomized Design using the Statistical Analysis System (SAS). Significant means were separated by the Duncan's multiple options.

Results and Discussion

The external egg quality of hens fed varying levels of Lawsonia inermis is presented in *Table 2*. The results revealed that dietary treatment significantly (P<0.05) influenced measured parameters except for egg breadth and length. The heaviest (53.76g) egg weight was observed from hens fed diet supplemented with 40g of Lawsonia inermis, while those fed 10g, 20g and 30g Lawsonia inermis had comparative lower egg weight. The highest shell weight was observed from eggs of laying hens fed 10g, 20g, 30g and 40g Lawsonia inermis. The thickest shell was observed from eggs of laying hens fed 30g of Lawsonia inermis while hens fed diet supplemented with 40g Lawsonia inermis had lighter shell. The internal egg quality of laying hens fed different inclusion levels of Lawsonia inermis is presented in *Table 3*. Dietary treatment significantly (P< 0.05) influenced internal egg quality except for albumen height and yolk colour. The egg mass (46.17g and 45.37g) of hens fed 30g and 40g of Lawsonia inermis were higher than those fed 10, 20g Lawsonia inermis and the control. The albumen weight and yolk weight of eggs of laying hens fed 40g Lawsonia inermis were higher compare to other treatment groups. The highest yolk length and yolk height were observed from hens fed diet containing 30g Lawsonia inermis while laying hens fed 20g Lawsonia inermis had lowest yolk length and yolk height.

Table 2*. External egg quality of laying hens fed varying levels of Lawsoniainermis.*

Parameters	T1	T2	T3	T4	T5	SEM
Egg weight	51.83^{b}	51.74^{b}	52.11^{b}	51.83^{b}	53.76^{a}	0.290
Shell index	0.77^{b}	0.77^{b}	0.78^{a}	0.78^{a}	0.79^{a}	0.004
Egg length	4.97	4.86	4.90	4.87	5.01	0.050
Egg breadth	3.91	3.86	3.89	3.89	3.82	0.050
Shell weight	6.68^{b}	6.77^{a}	6.91^{a}	7.08^{a}	7.00^{a}	0.120
Shell thickness	0.33^{b}	0.31^{b}	0.31^{b}	0.33^{a}	0.31^{c}	0.001

Note: ^{abcd}means along the same rows with different superscript are significantly (P<0.05) different; EW=Egg Weight (g); SI=Shape Index; EL=Egg Length (mm); EB=Egg Breadth (mm); SW=Shell Weight (g); ST=Shell Thickness (mm); SEM=Sum of error means.

Table 3*. Internal egg quality of laying hens fed varying levels of Lawsonia inermis.*

Parameters	T1	T2	T3	T4	T5	SEM
Egg mass	44.56^{c}	43.78^{c}	44.19^{c}	46.17^{a}	45.35^{a}	0.36
Albumen weight	32.75^{a}	31.89^{c}	32.16^{c}	31.67^{c}	33.01^{a}	0.24
Yolk weight	12.67^{b}	12.44^{b}	12.73^{b}	13.17^{a}	13.27^{a}	0.15
Albumin height	6.41	6.31	6.43	6.43	6.43	0.13
Yolk length	3.08^{a}	3.06^{b}	3.09^{a}	3.12^{a}	3.08^{a}	0.04
Yolk height	1.80^{b}	1.72^{c}	1.86^{a}	1.85^{a}	1.85^{a}	0.02
Yolk colour	6.76	6.12	6.19	6.36	6.19	0.22

Note: ^{abcd}means along the same rows with different superscripts are significantly (P<0.05) different; EM=Egg mass (g); AW=Albumen Weight (g); YW=Yolk Weight (g); AH=Albumen Height (mm); YL=Yolk length (mm); YH=Yolk Height (mm); YC=Yolk Colour; SEM=Sum of means errors.

A greater economy pricing for table and viable eggs is influenced by the quality of the eggs. According to Kocevski et al. (2011), one element that affects the economy price of viable and table eggs is egg quality. The breed or variety of the observed chicken is one of the main elements influencing the egg quality criteria. There are other considerations as well. From a consumer's perspective, the most significant attribute of quality is egg weight. The acceptance of shell eggs by consumers is influenced by a number of external parameters, such as egg and shell weight, cleanliness, freshness, and weight (Dudusola, 2010). Shell strength is crucial for both consumers and manufacturers. Egg weight or size may be the most significant marketing criterion, aside from egg shell quality (Nikolova, 2014). From the perspective of the consumer, the internal quality of the egg is crucial, yet it cannot be evaluated without cracking the egg. The yolk and white, or albumen, make up an egg laid by a hen. As the market for liquid, frozen, powdered, and yolk oil grows, interior features including yolk index, Haugh unit, and chemical composition become more significant in the egg product sector (Silversides and Scott, 2001). The highest egg weight observed in laying hens fed higher level of Lawsonia inermis corresponded with the observation of Ruelas et al. (2023) who fed moringa leaf meal to Hy-line brown laying hens and also comply with the findings of Kedir et al. (2023) who reported higher egg weight from laying hens fed rosemary leaf meal at higher inclusion level. In Setu et al. (2018) study, they found that a group of birds given papaya leaf suspension had higher egg weights than the group fed basal diet. A research discovered that adding Moringa oleifera leaf meal to laying hens' diets at a 5% level enhanced the weight of their eggs; however, when the inclusion level was increased to 20%, the egg weight fell. Due to the numerous phytochemicals present in Moringa leaves, the authors hypothesized that greater feed intake, FCR with lower egg production percentage, egg mass, and egg weight at a higher dose of supplementation were caused by impaired nutrient digestibility. Research on chickens has shown a great deal of interest in enhancing the anti-oxidative qualities of eggs by adding natural, unconventional resources (Mahfuz et al., 2017).

The improvement in egg weight of laying hens fed Lawsonia inermis could be due to the presence of active bio-ingredients in Lawsonia inermis that could have a beneficial effect on digestion and feed intake by improving the microflora of the gastrointestinal tract. A more resilient shell is one that can absorb and tolerate more impact and other physical forces without cracking. Shell integrity is related to its structure and the pattern in which the calcium minerals are deposited (i.e. crystal size and organization) to form the different layers of the shell (Butcher and Miles, 1990). The heaviest shell and thicker shell observed in laying hens fed 30g Lawsonia inermis agreed with the finding of Kedir et al. (2023) who reported improvement on egg shell weight and shell thickness of laying hens fed dried rosemary leaf. Also, Bölükbaşı et al. (2008) reported that eggshell thickness increased significantly in a group of chickens supplemented with 250 mg/kg rosemary essential oil compared to control groups. Bölükbaşı et al. (2008) attributed this to the fact that essential oils may have a beneficial effect on the rate of mineral absorption, particularly calcium and magnesium ions (Panda et al., 2003). According to Kujero et al. (2021), adding 3% of dietary ginger to laying hens' feed increased their shell weight, shell thickness, and shell percentage. Furthermore, Zomrawi et al. (2014) found that laying hens given different amounts of ginger root powder had greater shell weight and thickness values. The generation of eggs with thicker and heavier shells suggests that the eggs were of greater external quality. However, the addition of neem leaf meal to the diets of laying hens in the study of Kargbo and Kanu (2017) did not significantly (P>0.05) affect the weight and thickness of the shells. The internal qualities parameters of eggs fed diet supplemented with Lawsonia inermis such as egg mass, yolk weight, yolk height, yolk length and yolk height were higher with diet containing 40g Lawsonia inermis when compared to other treatment groups. This observation corresponded with the finding of Lu et al. (2016)

who recorded an increase in egg quality with the addition of 10% of Moringa leaf meal in laying hens. This result suggest that using high level of Lawsonia inermis at high level could result in adequate nutrient availability for the hens, which could be responsible for the positive effect on the egg mass and egg qualities. There is paucity of information on utilization of Lawsonia inermis in poultry birds, however comparing different studies using various phytogenics is very difficult because the response of poultry depends on several factors, such as plant parts, composition, levels, applied method, birds, housing factors and bioactive substances (Attia et al., 2018).

Conclusion

The findings of the study shed light on the significant impact of Lawsonia inermis supplementation on both the internal and external quality of eggs. Notably, the incorporation of 40g of Lawsonia inermis into the hens' diets resulted in remarkable improvements in egg weight and egg mass, indicating a tangible enhancement in overall productivity. Furthermore, the supplementation of 30g of Lawsonia inermis demonstrated a notable benefit in enhancing the thickness of the eggshell, a critical factor in ensuring egg quality. These findings underscore the potential of Lawsonia inermis supplementation as a viable strategy for optimizing laying hen performance. By offering tangible benefits such as increased egg weight, egg mass, and enhanced eggshell thickness, Lawsonia inermis emerges as a promising dietary supplement in poultry nutrition. It is recommended that poultry producers consider integrating Lawsonia inermis supplementation into their hens' diets, particularly at the dosage levels identified in this study. Specifically, supplementation of up to 40g of Lawsonia inermis is suggested for achieving optimal egg weight and egg mass, thereby maximizing productivity. Additionally, incorporating 30g of Lawsonia inermis can be beneficial for improving eggshell thickness, contributing to overall egg quality.

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Conflict of interest

The authors confirm that there is no conflict of interest involve with any parties in this research study.

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