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Effect of Supplementing *Lawsonia* Inermis on Chicken Ovary and Egg Quality Parameters

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ABSTRACT

The effects of Lawsonia inermis (henna) on the egg quality parameters of White Leghorn chickens over a 4-week period. A total of 25 chickens were housed individually, with free access to feed and water. The results showed notable variations in egg quality attributes due to the supplementation of L. inermis. In terms of albumen height, the control group had the highest measurement (8.01 cm), while Group C recorded the lowest (7.46 cm). The albumen index was also highest in the control group (2.43) and lowest in Group B (2.29). Group D had the longest albumen length (92.86 mm), while Group B had the shortest (89.09 mm). Egg weight was heaviest in the control group (67.78 g) and lightest in Group B (58.21 g). The Haugh unit was highest in Group B (87.63 HU) and lowest in Group C (85.73 HU), while shell thickness was thickest in the control group (2.66 mm) and thinnest in Group E (0.42 mm). Yolk measurements showed Group D had the largest yolk diameter (43.14 mm), while Group B had the smallest (41.11 mm). Yolk height was tallest in Group B (17.58 mm) and shortest in the control group (17.28 mm). Reproductive parameters, including follicle weights, were significantly affected, with Group A showing the highest weights across several follicle categories, while Group E recorded the lowest. The length of reproductive organs varied, with the control group having the longest shell gland (9.75 cm) and reproductive tract length (70.85 cm), and Group E showing the shortest. Lawsonia inermis supplementation affected several egg quality parameters, including shell strength and yolk color. Although these changes were consistent with prior studies, the results did not indicate substantial alterations in egg quality due to L. inermis.

INTRODUCTION

Lawsonia inermis Linn, also referred to as henna or mehndi, is widely found in tropical and subtropical regions. India's ancient history reveals its numerous uses and indicates that it is important for Ayurvedic, or natural herbal, remedies (Supian et al., 2018). Lawsonia inermis Linn, a perennial plant and member of the loosestrife family, or Lythraceae. Many farmers grow henna for cosmetic and therapeutic uses. It is one of the common plants found in nature, and all

components of the plant—root, stem, leaf, flower pod, and seed—have significant medicinal value. This plant is indigenous to North Africa and Southeast Asia, and it is widely grown as an ornamental in India, Persia, and along the African coast of the Mediterranean Sea (Elmanama et al., 2011). Henna leaves are a widely used natural dye for coloring hair, nails, and hands. One of the main components of the plant is the dye molecule, lawsone, which is found in the highest quantity in the petioles (0.5–1.5%) (Singh et al., 2005).

The primary purpose of the ovary is to generate ovarian follicles. Ovarian stromal cells and primordial germ cells work together to generate primordial follicles (Ayers et al., 2013). Most follicles undergo atresia during development and are reabsorbed by the (Okumura, 2017). The initial recruitment of primordial follicles is also known as follicle activation (Zhang & Liu, 2015). Although there isn't much data directly examining how L.inermis leaf meal affects chicken ovary and egg production, it makes sense to speculate that better nutrition and general health may help increase egg production. For instance, it has been discovered that several plant-based feed additives increase hens' ability to produce eggs (Gheisar et al., 2015).

Eggs are an affordable and comprehensive source of proteins, water-soluble vitamins (vitamin B12, riboflavin, and folate), fat-soluble vitamins (A, D, and E), and a number of micronutrients (e.g., iodine, iron, phosphorus, and selenium) (Kumar et al., 2023).Eggs contain about 40 different proteins—including bactericidal and antihypertensive proteins—as well as 18 different amino acids—nine of which are essential—a stable amino acid composition, the ideal ratio of saturated to unsaturated fatty acids, and no trans or carbohydrates, they are referred to as a "miracle food." Eggs therefore have the same biological value as breast milk and are acknowledged as a reference protein for humans (Molnár & Szőllősi, 2020). Calcite columnar crystal units, or palisades, cover the thickness of the eggshell (approximately 330 µm in chickens) and are around 70 to 80 µm broad. Despite the eggshell's thin thickness, the mineral portion contains a large quantity of occluded organic materials that strengthened the ordinarily brittle calcite crystals. Conversely, the eggshell is covered in a very thin layer of organic material called the cuticle, which closes the holes and stops microorganisms from penetrating the shell (Bain et al., 2013).Egg weight, eggshell thickness, egg albumen height, and yolk index are the basic characteristics of eggs (Benavides-Reyes at al., 2021).

In terms of the effects on the chicken ovary, there is limited direct research available, but studies suggest that some plant-based feed additives may influence egg production, potentially by affecting the health and functioning of the hen's ovary. Therefore, it could be hypothesized that *L.inermis*leaf meal might exert similar effects.This work has been planned to examine the Effect of supplementing *Lawsonia inermis* on egg quality and reproductive organs of hen.

MATERIALS AND METHODS

A total of 25 White Leghorn Chickens were purchased from local market of Hyderabad and bought to the Department of Poultry Husbandry, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam. The chicken was kept in cages individually throughout the entire experimentperiod of 4 weeks. The feed and water was given ad libitum Table 1.

Leaves *of LasowniaInnermis* (Henna) was collected from taluka Mehar district Dadu Sindh. leaves were dried and powdered using an electric grinder device, powdered *L. innermis* leaves were stored in refrigerator for latter characterization.

Table 1

Experimental Design

Groups	Group A	Group B	GroupC	Group D	GroupE
Dosage	Control	*L.inermis* 1g/kg feed	*L. inermis* 2g/kg feed	*L. inermis* 4g/kg feed	*L. inermis* 8g/kg feed
Number of Layers	05	05	05	05	05

Shell thickness is an important measure of egg quality, as it reflects how strong the egg is and the level of protection it provides. It was measured using a digital micrometer screw gauge. The Haugh

Unit measures the quality and freshness of the albumen (egg white), with higher values indicating better egg quality. Egg weight is a key factor in grading eggs and can be influenced by the hen's breed, age, and diet. Albumen height is another indicator of egg freshness and quality, as it decreases over time. It is measured in millimeters and is used to calculate the Haugh unit, which is a common standard for assessing the quality of egg protein. The yolk index measures the shape and quality of the yolk, with a higher index suggesting a rounder, firmer, and fresher yolk. It is calculated by comparing the yolk's height to its diameter.Egg production refers to the number of eggs a hen lays within a given time period. This can be influenced by diet, breed, and environmental conditions. Evaluating egg production is important when studying the effects of dietary additives, such as Lawsonia inermis, on poultry health.The length and weight of a hen's reproductive organs are also important factors, as these can be affected by nutrition and may influence fertility and egg-laying capacity. The number of follicles in the ovary is another key indicator of reproductive health and egg-laying potential. A higher follicle count is desirable in egg-laying breeds.

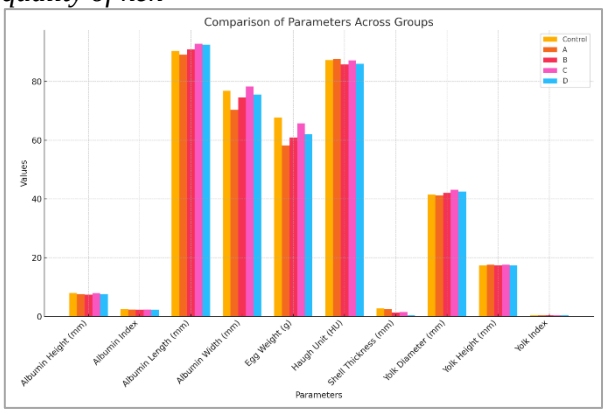
These factors can be influenced by the hen's diet, age, and health, and they are crucial for improving breeding and egg production. The data was analyzed using the statistical tool ANOVA at a significance level of $\alpha = 0.05$. If significant differences were found among the means, a least significant difference (LSD) test was performed using Statistix 8.1.

RESULTS

Albumen height, as shown in Figure 1, indicates a statistically significant difference. The maximum albumen height was observed in the control Group A (8.01 cm), while the minimum was recorded in Group C (7.46 cm). This suggests that Lawsonia inermis affects egg quality. The highest albumen index was found in control Group A (2.43), and the lowest in Group B (2.29). This further supports the influence of L. inermis on egg quality. For albumen length, there was a statistically significant difference as well. The maximum length was noted in Group D (92.86 mm), and the minimum in Group B (89.09 mm), indicating that L. inermis has an effect on egg quality. Albumen width also exhibited significant variation. The largest width was measured in Group D (78.27 mm), while the smallest was observed in Group B (70.24 mm), again pointing to L. inermis influencing egg quality. In terms of egg weight, a statistically significant difference was observed. The heaviest eggs were from control Group A (67.78 g), and the lightest from Group B (58.21 g), suggesting that L. inermis plays a role in egg quality. the Haugh unit, the results revealed significant differences, with the highest value in L. inermis-treated Group B (87.63 HU) and the lowest in Group C (85.73 HU). This indicates that L. inermis affects egg quality. statistically significant difference was found. The thickest shell was measured in control Group A (2.66 mm), while the thinnest was recorded in Group E (0.42 mm). This suggests that L. inermis influences egg quality. yolk diameter also showed significant variation, with the largest diameter in L. inermis-treated Group D (43.14 mm) and the smallest in Group B (41.11 mm). Thus, L. inermis impacts egg quality. Yolk height also exhibited significant differences. The highest yolk height was observed in L. inermis-treated Group B (17.58 mm), and the lowest in control Group A (17.28 mm), further indicating the effect of L. inermis on egg quality. yolk index displayed a statistically significant difference. The highest index was recorded in L. inermis-treated Group B (0.43%), and the lowest in Group D (0.40%), confirming that L. inermis influences egg quality.

Figure 1

Effect of supplementing Lawsoniainermis on egg quality of hen



Effect of *Lawsonia inermis* Reproductive Performance

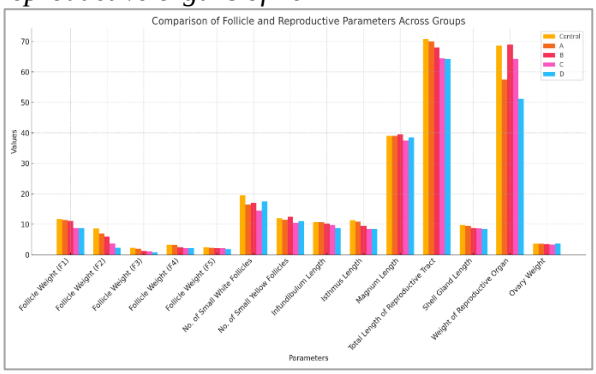
The study revealed a statistically significant variation in follicle weights and reproductive organ measurements across different groups treated with Lawsonia inermis Figure 2. Group A had the highest follicle weight for F1 (11.70 g), while the

lowest was recorded in Groups D and E (8.85 g). For F2, the highest weight was also in Group A (8.60 g), and the lowest in Group E (2.30 g). In the case of F3, Group A had the maximum weight (2.35 g), while Group E had the lowest (0.75 g). Similarly, for F4, Group A showed the highest weight (3.30 g), and Group E the lowest (2.15 g). For F5, the maximum weight was in Group A (2.50 g), with Group E having the lowest (1.80 g). The number of small white follicles showed significant variation, with Group A (control) recording the highest number (19.50), and Group D the lowest (14.50). Small yellow follicle counts were highest in Lawsonia-treated Group C (12.50), and lowest in Group D (10.50). The infundibulum length varied significantly, with the longest being in Group A (10.75 cm) and the shortest in Group E (8.85 cm). Similarly, the isthmus length was highest in Group A (11.35 cm) and lowest in Group D (8.50 cm).

Magnum length showed variation, with Group C (Lawsonia-treated) recording the longest (39.50 cm) and Group D the shortest (37.50 cm). The length of the reproductive tract was highest in Group A (70.85 cm) and lowest in Group E (64.35 cm). Shell gland length varied, with Group A showing the longest (9.75 cm) and Group E the shortest (8.50 cm). The weight of the reproductive organs also showed significant differences. Group C (Lawsonia-treated) had the heaviest organ weight (69 g), while Group E had the lightest (51.15 g). Ovary weight varied, with the highest recorded in Group E (3.75 g) and the lowest in Group D (3.40 g). Overall, the findings indicate that Lawsonia inermis has a significant impact on follicle weight, reproductive organ size, and related parameters.

Figure 2

Effect of supplementing Lawsonia inermis on reproductive organs of hen



DISCUSSION

Numerous bioactive substances have been found in *Lawsoniainermis*, such as fatty acids, alkaloids, quinones, tannins, steroids, xanthones, polyphenols, leucocyanidin, epicatechin, catechin, and quercetin (Oda et al., 2018). Approximately 80% of people on the planet utilise plants as medicine because, in contrast to modern medication, they have no negative side effects and function in harmony (Sharma et al., 2016).

The current research result indicated that *Lawsonia inermis* significantly effect on all parameters of egg quality including (albumin height, Albumin index, Albumin length, Albumin width, egg weight and Haugh unit). This study confirmed previous results by confirming lycopene's proven ability to improve yolk colour, which is ascribed to its direct incorporation into the egg yolk (Sun et al., 2014). In a previous investigation, fennel was shown to considerably improve the egg shape index in laying hen diets throughout weeks 4 and 8 of the trial. Shell and albumen weight percentages were considerably higher ($P < 0.05$) at 40 weeks of age when 0.5% fennel was added to the diet. There was not a noticeable effect on the proportion of yolk weight (Abou-Elkhair et al., 2018). Gharaghani et al., (2015) noted that in comparison to control and other treatment groups, the Haugh unit percentage was highest when fennel was incorporated in the diets of laying hens.Furthermore, it has been demonstrated that the bioactive components of herbal plants protect the uterus and magnum and promote albumen production in laying birds (Radwan Nadia et al., 2008).

In contrast Saki et al., (2014) observed that supplementing with phytogenic feed additives greatly increased egg weight while having no effect on laying hen performance. It was also observed that during the six-week period, there was no significant effect of dietary interventions on albumen height, Haugh unit, or shell thickness. Supplementing phytogenic feed additives did not significantly alter the yolk weight, diameter, height, yolk index, or colour. Similarly, Ghasemi et al., (2010) recorded that the yolk color increased by feed additive addition in the other study, but the yolk index, Haugh unit, and eggshell thickness were unaffected by dietary treatment. The dietary

treatment of lycopene did not substantially impact the egg's shell strength, thickness, or Haugh unit, which is consistent with the findings of earlier research (Akdemir et al., 2012). In the previous study, there was no significant impact of papaya seed pytogenic feed additive supplementation on egg shape index, eggshell weight, eggshell thickness, albumin height, or haugh unit (Dissa et al., 2023). Similar findings were reported by Rahmasari et al., (2022), who reported that there was no change in eggshell thickness when 0.3%, 0.6%, and 1.2% of papaya seeds were included in the diet of 75–83-day-old layer quails. Li et al. (2016) found that in a study in which 24-week-old Lohmann Brown laying chickens were fed a 0.5% to 1% Chinese herbal combination (including 70% pine needles and 30% Artemisia annua) in their food, there was no difference in the parameters of egg weight, eggshell thickness, egg white height, HU, and eggshell breaking strength. Although there was a difference in egg weight and yolk colour across the treatments, the addition of turmeric, ginger, or garlic had no effect on feed intake, egg mass and feed conversion, or several measures related to egg quality (such as shape index, Haugh unit, and egg components, egg shell thickness, yolk, and albumen index) (Phuong &Thuy, 2021). Studies with birds showed that egg reproduction rates and hatchability were enhanced when Xinghua breeding chickens were fed 40 mg/kg of lycopene for 35 days or longer Sun et al. (2014).

The results obtained from this study contribute valuable insights into the effects of *lawsoniainermis* supplementation on various aspects of reproductive organ in poultry. Our study has revealed that Lawsoniainermis treated group showed variation in weight and length of reproductive organ in comparison to control group. The herbal feed additive mixture significantly raised the weight of the ovary and the number of small follicles. However, on large follicles (greater than 10 mm), no reflection was seen (Sakiet al., 2014). According to Zhao et al., (2005) oviduct weight increased but neither ovarian weight nor the number of hierarchical follicles was impacted by supplementing with Daidzein, a kind of isoflavone found in many herbs. Ayu Fertin, a bioactive herbal treatment, can be administered starting on day 21 postpartum to encourage follicular development in the buffalo's ovary. Follicles with the capacity to ovulate are produced as a result of this process, and successful ovulation ensues (Ilieva et al., 2021). In their study, Kaludjerovic et al., (2012) did not discover any relationship between dietary daidzein and the weight, quantity, or multiple oocyte follicles of mice's ovaries. Previous studies by Rochester et al., (2009) shown that feeding Japanese quail chicks dietary red clover (Trifoliumpratense) increases oviduct growth and decreases ovary growth.

Ovarian endocrinology, or the amounts of FSH and LH, was shown to be affected by an ethanolic extract of Curcuma longa given orally for 30 days at different dosage levels. A full day following the previous medication dosage, the gonadotropins LH and FSH were both tested. Another observation relates to the weight of the uterus, which is higher in rats treated with Curcuma longa compared to other rats treated with extracts. There was a notable variation in the weight of the uterus and ovaries between the Curcuma longa-treated groups and the control group (Thakur et al., 2009).

CONCLUSIONS

The study's conclusions about *Lawsoniainermis's* effects on egg quality and attributes highlight the plant's major influence on all aspects of eggs, such as the enhancement of yolk color, shell strength, thickness, or Haugh unit. While consistent with certain previous research, notably, this study did not observe notable alterations in these specific egg quality parameters despite *Lawsoniainermis* supplementation. While this study provides valuable insights into the influence of *Lawsoniainermis*on egg quality parameters, additional comprehensive investigations are warranted to elucidate the nuanced impact of *Lawsoniainermis* supplementation on various aspects of egg quality and production in poultry, considering the potential differences across breeds and environmental conditions.

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