American Journal of Botany and Bioengineering
https://biojournals.us/index.php/AJBB
ISSN: 2997-9331

Effect of Adding Different Levels of Powdered Leaves of the Moringa Tree on Reproductive Performance and Egg Traits of Female Japanese Quails

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Received: 2026 19, Jan
Accepted: 2026 28, Feb
Published: 2026 05, Mar

Abstract: In the current experiment, we investigated the impact of dietary supplementation of Moringa oleifera leaf powder (MOLP) on the egg production rate and quality traits of Japanese quails (Coturnix japonica) reared. The experiment was carried out between the 27 October 2023 and 26 November 2023 at the Poultry Farm in the Department of animal production, Kirkuk University College of Agriculture. A completely randomized study was used to randomly distribute 57 healthy female quails to four dietary treatments: T1 (control 0% MOLP), T2 (5%), T3 (7.5%), and T4 (10%), which were further subdivided into 6 replications each. Birds were kept in normal conditions of management and fed and watered at will during the course of the experiment. The external egg traits (egg length, egg breadth, index of shape, egg volume, surface area, and shell thickness), internal weight traits (egg weight, yolk weight, albumen weight, shell weight, yolk index, albumen index, and shell index) and dimensional traits (albumen height and diameter, yolk height and diameter) as well as percentage based indices (yolk, albumen, shell percentages, yolk index, albumen index, and shell index) were measured on a daily basis. The results showed that the MOLP supplement made a significant impact on all the parameters of egg length, breadth, volume, and surface area ($p \leq 0.001$), but the egg shape index was not affected. Higher levels of inclusion (T3 and T4), eggshell thickness and indices of shell improved significantly, with T4 recording the highest improvement ($p \leq 0.01$). Internal features (egg, yolk, albumen) weightings reduced significantly in the supplemented groups relative to the control ones (p 0.001) but indices of yolk and albumen were not significantly altered. It did not show any age difference between treatments at first oviposition. Overall, the MOLP supplementation had a balanced effect, whereby the greater the inclusion level, the more adverse effects came with respect to the egg size and internal quality parameters, but the greater the effect the supplement had in enhancing the shell thickness, weight and structural indices. Supplementation (5-7.5% is adequate) could enhance the quality of the shells, without decreasing egg mass, even at high supplementation levels, which would have implications on quail egg production.

Keywords: Egg quality, Japanese quails female, Moringa oleifera , Reproductive performances

Introduction

Moringa oleifera, often known as the miracle tree, is one of the promising future sources of bioactive compounds and nutrients that can be used by humans as well as animals [1]. Its leaves have been described as having anti-inflammatory, anti-oxidant and anti-microbial effects, which makes it a good dietary supplement [2, 3, 4]. The various factors influencing poultry reproductive performance are genetic background, environmental context, nutritional status, management practices, and interactions [5, 6, 7]. The role of nutritional input especially is critical towards the regulation of hormonal pathways, egg quality, and overall reproductive performance [8]. Since the Moringa leaves are rich in protein, vitamins, minerals, and phytochemicals, they can be considered an effective alternative to the common feed additives, and can possibly improve the reproductive performance of Japanese quails and poultry in general [1, 2]. In the study of the impact of dietary supplementation on reproductive parameters in the agricultural setting, an increased number of studies utilize Japanese quails due to their high reproductive efficiency and short breeding cycle. [9]. The high nutrient requirements in these birds highlight the fact that an ideal dietive setup may have positive impacts on the qualities of the egg weight, shells and fertility [10]. There has been many poultry studies that have documented that plant-based supplements such as Moringa can increase reproductive performance, egg weights, and shell quality [11, 12, 13]. Other poultry species have reported similar positive results with regard to feed conversion ratio, egg weight and egg shell strength [11, 12]. There is scarcity of literature, however, that has specifically explored the reproductive performance of Japanese quails and egg characteristics in Japan under the influence of Moringa leaf powder [13, 14].

The current study aimed at evaluating the external and internal egg characteristics and reproductive efficiency of Japanese quails fed with different concentrations of powdered Moringa leaves, hence making a contribution to the book of knowledge of alternative feed additives in poultry diet and fostering the usage of the sustainable, high-efficiency feed additives in poultry management.

Materials and Methods

The research was carried out in the poultry farm located in the Animal Production Department of College of agriculture at Kirkuk University between 27 October 2023 and 26 November 2023.Fifty seven female Japanese quails (Coturnix japonica) were randomly assigned to four diet treatments with six replicates in a totally randomized assignment. Standard conditions were used whereby birds were kept in a wire cage with a 16L:8D photoperiod and ad libitum access to both food and water.

They were as follows: T1 (control, 0% Moringa leaf powder), T2 (5% Moringa leaf powder), T3 (7.5% Moringa leaf powder) and T4 (10% Moringa leaf powder). The leaves of the Moringa oleifera were dried in the shade, milled and sieved. There was a 2-week adaptation period followed by an 8-week recording period. The mortality and daily egg production were noted on a per replicate basis. The age of first oviposition was counted separately (days). The replicate of eggs was the collection of the three days eggs a week to determine the quality of eggs; this was done both externally and internally, and the results were compared as the average per week over the trial.

The weight of the egg (EW, g) was weighed on a 0.01 g balance. Digital caliper (0.01mm) was used to measure egg length (EL) and breadth (EB). The egg shape index (ESI) was computed as (EB/EL) 100 [15]. The volume of the eggs (EV, mm 3) was calculated by the formula 0.6057 x EL x EB 2 [15]. The estimate of egg surface area (ESA, mm 2) was 4.835. × EW 2(2/3) [15]. Five readings (membrane removed) (Eggshell thickness (ESTh) 0.01 mm micrometer) were taken at 3 points (air-cell, equator, pointed end), and averaged. The weight of the dried shells (ESW) was obtained after the drying period of 24 h at room temperature. The Eggshell index (EShI) was determined as ESW/EV. Measurements of albumen height (AlH) were done using a tripod micrometer on a level glass plate, albumen diameter (AlD) and yolk diameter (YD) made using a caliper of the yolk carefully separated. The height of yolk (YH) was measured using the micrometer. Weight (g) of albumin was determined as = egg weight(g)- yolk weight (g) and shell weight (g). Relative percentages were presented in the following way: Yolk index (YI) = Yolk height / Yolk diameter; albumen index (AlI) = albumin height / albumin diameter.

One-way ANOVA has been conducted to analyze the data by use of SPSS (v.19) [16]. In the event that any substantial differences were found, the means were separated using the Duncan multiple range test [17]. The level of significance was proclaimed as $p \leq 0.05$.

Result and discussion

In Table 1, the impact of dietary supplementation using Moringa oleifera leaf powder on different external egg characteristics of laying hen is shown. The traits that were under analysis were egg length (EL), egg breadth (EB), egg shape index (ESI), egg volume (EV), egg surface area (ESA), and eggshell thickness (ESTh). Moringa supplementation had significant effects ($p \leq 0.001$) on EL, EB, EV, and ESA. T1 treatment had eggs with greater values on these parameters than the other treatments did. Particularly, EL was much higher in T1 (30.21 mm), and T2, T3, and T4 had similar but less values (28.32 28.90 mm). The same trend was noted in EB, EV and ESA, indicating that the size-related aspects of eggs can be reduced with the rise in the concentration of the Moringa leaf powder.

Furthermore, there was no significant difference in egg shape index between the treatments which means that the shape of the egg is not affected by the addition of the Moringa leaves in the diet; an observation also corroborated by [18] who reported no significant difference in the egg shape index of the egg with the introduction of Moringa in the diet.

Eggshell thickness showed a statistically significant difference (p 0.01) of T4 (0.30 mm) compared to the other treatments meaning that increased concentration of Moringa has the potential of increasing eggshell thickness. This finding is also in line with the scientific study conducted by [19], which revealed that Moringa plant enhances shell qualities owing to the high concentration of calcium and antioxidants. The causes of the identified effect of egg size and volume on increasing Moringa concentrations can be explained by the anti-nutritional effects of tannins and phytates, which may interfere with nutrient uptake at high levels [20]. However, the increase in the thickness of the eggshells indicates a positive impact on the mineral use, especially calcium and phosphorus which are necessary in the eggshell formation [21].

Table 1: Effect of adding different levels of Moringa powdered leaves of on the egg external traits

Traits	T1	T2	T3	T4	Sig.
EL	30.21±0.19 a	28.90±0.32 b	28.32±0.25 b	28.84±0.22 b	***
EB	24.74±0.20 a	22.88±0.20 b	23.20±0.21 b	23.15±0.15 b	***
ESI	81.90±0.52 a	79.44±1.03 a	82.00±0.83 a	80.36±0.72 a	N.S.
EV	5323.67±143.68 a	4013.43±137.75 b	3827.19±152.97 b	3969.13±102.24 b	***
ESA	1070.84±24.42 a	839.92±21.86 b	816.58±27.17 b	835.69±16.48 b	***
ESTh	0.25±0.01 b	0.26±0.01 b	0.27±0.01 b	0.30±0.01 a	**

EL= Egg Length, EB= Egg Breadth, ESI=Egg Shape Index, EV=Egg Volume, ESA= Egg Surface area, ESTh=Eggshell Thickness. T1 (control, 0% Moringa leaf powder), T2 (5%), T3 (7.5%), and T4 (10%).

Table 2 shows the impact of the addition of different amount of supplements of supplementing laying-hen diets with *Moringa oleifere leaf powder on internal egg weight characteristics such as egg weight (EW), yolk weight (YW), albumin weight (AlW) and eggshell weight (ESW). The mean difference (EW) in all treatments (T2 and T4) was found to be significantly lower (p 0.001) compared to the control group (T1). The highest EW was observed in the control (10.53 g) and significantly lesser in the treatment groups (8.55 g -8.58 g). These results were in agreement with the ones reported by [22], who pointed out that the high levels of the nutrient in the Moringa can reduce the nutrient levels in the egg due to the anti-nutritive characteristics of the tannin in this element.

Yolk weight also reduced significantly ($p \leq 0.001$) in all treatments compared to that of the control group. The T1 had the greatest YW (3.27 3.27 g), with T2 to T4 having a range of 2.48 to 2.60 g. The low yolk mass is presumably an indication of low deposition of dietary lipid or essential micronutrients needed to form yolk. In the same way, [23] reported a decrease in YW with heavy supplementation of the diet with Moringa which is explained by the reduction in the density of the diet.

The weight of albumin showed that there is a significant decrease ($p \leq 0.001$) in all treatments as compared to T1. The AlW value of the control was 6.18 g, which was significantly high as compared to that of T2 and T3/T4 of 4.83 g and 5.12 g respectively. Protein intake and amino-acid balance are very sensitive to the content of albumin; [24] emphasized that despite a high level of protein in the egg, overconsumption of the protein can affect the general absorption of nutrients, thus reducing the formation and deposition of albumin in the egg.

There was a significant difference ($p \leq 0.05$) in the eggshell weight between the treatments. T3 generated the highest ESW (1.12 g), which was well above values acquired in T2 and T4 (0.98 0.99 g), and T1 obtained a medium ESW of 1.08Mb. This trend indicates that the middle inclusion level (T3) can be beneficial to shell mass, which can be explained by better calcium exploitation. This observation is reinforced by [21] who detected enhanced shell deposition with optimal levels of inclusion of the Moringa because it contained high levels of calcium and magnesium.

Table 2: Effect of adding different levels of Moringa powdered leaves on Internal egg traits weight

Traits	T1	T2	T3	T4	Sig.
EW	10.53±0.20 a	8.58±0.14 b	8.55±0.23 b	8.58±0.12 b	***
YW	3.27±0.12 a	2.48±0.08 b	2.60±0.12 b	2.51±0.08 b	***
AlW	6.18±0.14 a	5.12±0.12 b	4.83±0.23 b	5.09±0.13 b	***
ESW	1.08±0.04 ab	0.98±0.04 b	1.12±0.05 a	0.99±0.03 b	*

EW=Egg weight, YW=Yolk weight, AW=Albumin weight, ESW=Eggshell weight. T1 (control, 0% Moringa leaf powder), T2 (5%), T3 (7.5%), and T4 (10%).

The table (3) shows the effect of different amounts of the Moringa oleifera leaf powder on the dimensional internal characteristics of eggs in laying hens. The traits that have been studied include albumen height (AlH), yolk height (YH), yolk diameter (YD) and albumen diameter (AlD).

The findings indicate a very significant ($p \leq 0.001$) difference between groups in regard to albumen height. The albumen height was greatest in the control (T1) group and all supplemented groups (T2 and T4) recorded significantly lower albumen height (between 5.20 and 5.89mm). The height of albumin is a vital parameter of the freshness and internal quality of eggs and its decline under the influence of Moringa supplementation could be explained by the decrease in protein bioavailability or the disproportionate intake of amino acids, especially at the high-inclusion levels [25]. The pattern is similar to the previous results which indicated a reduction in the weight of albumen and indicative of the hypothesis that the excessive addition of Moringa can affect protein metabolism. Treatment differences (p �iques=.05) in yolk height did not exist statistically and all the groups had a range of similar values at 9.0 ish mm.

The results showed that Moringa supplement did not cause any adverse effects on the structural integrity of the yolk, even though a decrease of total yolk weight was seen in a prior table [19]. Additionally, it has been indicated in other studies that there were no significant changes in yolk height when Moringa was added in moderate concentrations and hence its neutral effect on yolk density and shape. The greatest Moringa group (T4, 20.85 mm) had a significantly lower yolk diameter than the control (T1, 22.06 mm), which was also significantly lower (p=.05 or lower) when compared to the remaining three Moringa groups (T2-T3, 21.58-22.92 mm). There were intermediate values in groups T2 and T3. This small decrease could be attributed to the changes in lipid deposition and yolk maturation, which was previously noted where anti-nutritional agents like phytates and saponins which are found in Moringa have the capacity to disrupt lipid metabolism [20].

The significant reduction ($p \leq 0.01$) in albumen diameter occurred in all Moringa-treated (T2 through T4) compared to the control. T1 registered the greatest diameter (73.87mm) and T2 and T3 registered the lowest diameters (63.59-63.66mm). The use of albumen spread is typically used to determine egg freshness and protein quality, hence, this loss implies the high concentration of Moringa can disrupt albumen framework and water-binding ability, which concurs with the data obtained by [26].

On the whole, the findings suggest that the addition of Moringa leaf powder in the ration of laying hens can adversely influence the albumen quality factors especially height and diameter with minimal or no effect on yolk height. The maximum level of Moringa found relatively small yolk diameter, which could be related to a poor lipid assimilation process.

Table 3: Effect of adding different levels of Moringa powdered leaves on the internal traits dimensions

Traits	T1	T2	T3	T4	Sig.
Albumin high	6.67±0.28 a	5.65±0.19 b	5.20±0.26 b	5.89±0.17 b	***
Yolk high	9.08±0.11 a	8.94±0.10 a	9.14±0.15 a	9.03±0.11 a	N.S.
Yolk Diameter	22.06±0.32 a	21.24±0.24 ab	21.13±0.52 ab	20.85±0.26 b	*
Albumin Diameter	73.87±2.20 a	63.66±1.49 b	63.59±2.60 b	66.33±2.45 b	**

AlH=Albumin high, YH=Yolk high, YD=Yolk Diameter, AlD=Albumin Diameter. T1 (control, 0% Moringa leaf powder), T2 (5%), T3 (7.5%), and T4 (10%).

The results of the different concentrations of Moringa oleifera leaf powder on percentage-based characters and structural indices of eggs are given in the table (4), which includes yolk percentage (YP), albumin percentage (AlP), eggshell percentage (ESP), yolk index (YI), albumin index (AlI) and eggshell index (EShI). There was no statistically significant difference (p 0.05) between groups in the yolk percentage (28.90 0.30) which was inconsistent in the pattern across treatments. This observation shows that the proportionate deposits of yolk are not affected by addition of Moringa which supports the results of [18] who recorded a consistent percentage of yolk with a maximum of 10 percent addition of Moringa.

Per cent (percent) of albumin showed a wide difference ($p \leq 0.05$), though treatment T3 had minimum percentage (56.05⁻) which was significantly lower than that of treatment T2 (59.60%). Intermediate values were observed in the control group and the T4. These variations indicate that there might be a dose related effect, in which moderate dosages might reduce albumin production or deposition. In line with [26], interaction at moderate Moringa levels may explain such results since there is less protein utilization efficiency. Eggshell percentage rose considerably (p 0.01) especially in group T3 (13.20 per cent), which was higher than T1 (10.33 per cent) and other interventions.

The implications of this observation are that Moringa powder can be used to improve shell formation performance particularly when moderate doses are used. The findings confirm the assumption that the mineral composition of Moringa such as calcium, magnesium, and phosphorus promotes the growth of eggshell [21]. However, an increase in shell percentage may also indicate low interior egg mass as has been recorded in previous tables. There was no significant difference ($p \geq 0.05$) in yolk index and albumin index and this implies that no change in egg structural integrity had occurred despite Moringa supplementation. These indices are commonly used as freshness measures and the current findings are in agreement with [19] who reported that internal structural consistency can be maintained with natural feed additives applied in proportions. The eggshell index showed that the difference was very significant ($p \leq 0.001$), T3 had the highest index (0.14), then T2 and T4 (0.12), with T1 having the lowest index (0.10).

The eggshell index refers to the weight of the shell against the volume or surface area of the egg; an index of higher value indicates thicker or denser shells. These results are in line with earlier evidence of increment in eggshell weight and thickness to support the activity of Moringa in enhancing shell mineralization. Moringa supplementation, especially moderate levels in T3 did not affect yolk and albumin indices but increased eggshell weight and index, which is a sign of higher quality of the shell. Nonetheless, moderated supplementation can also decrease albumin ratio at the same time, which highlights the importance of optimal doses.

Table 4: Effect of adding different levels of Moringa powdered leaves on the egg traits percentage

Traits	T1	T2	T3	T4	Sig.
Yolk percentage	30.94±0.85 a	28.90±0.80 a	30.75±1.46 a	29.34±0.90 a	N.S.
Albumin Percentage	58.74±0.84 ab	59.60±0.82 a	56.05±1.64 b	59.13±1.00 ab	*
Eggshell percentage	10.33±0.37 b	11.50±0.45 b	13.20±0.65 a	11.54±0.44 b	**
Yolk Index	0.41±0.009 a	0.42±0.006 a	0.44±0.013 a	0.43±0.008 a	N.S.
Albumin Index	0.09±0.005 a	0.09±0.004 a	0.09±0.007 a	0.09±0.005 a	N.S.
Eggshell Index	0.10±0.004 c	0.12±0.006 b	0.14±0.007 a	0.12±0.005 b	***

YP=Yolk percentage, AlP= Albumin percentage, ESP=Eggshell percentage, YI=Yolk Index, AlI=Albumin Index, EShI=Eggshell Index. T1 (control, 0% Moringa leaf powder), T2 (5%), T3 (7.5%), and T4 (10%).

Table 5 indicates that the oviposition age was not significantly different when the treatment groups of T1 (50.22 days), T2 (50.82 days), T3 (50.74 days) and T4 (51.39 days) were compared. This observation implies that the treatments that were used did not have significant effects on the initiation of egg laying. Aligned with Smith et al. [27], who did not find a significant influence of dietary variation on oviposition age on laying hens, the results are an indication of a larger role in regulating egg production by genetic and hormonal factors.

Previous studies identify with the fact that, though the nutritional interventions could adjust the parameters of the egg production it seldom changed the physiological timing of the initial oviposition unless the nutrient imbalance was extreme [28]. Based on this, the results of the current research confirm the hypothesis that moderate diet changes or environmental differences do not play an important role in determining the age at which a laying is initiated.

Yolk index of the different groups of treatments was in the range of 0.41-0.44; as the numbers differed slightly (T3: 0.44; T4: 0.43), statistical test showed that there was no significant difference ($P \geq 0.05$). This homogeneity means that there was no difference in the yolk shape and firmness which are important measuring sticks of internal egg quality among treatments. According to [29], both breed and age of hens influence yolk index but it does not change much when there is constancy in the rearing and nutritional conditions. In line with [30] that proved that no great variations in yolk quality are observed with moderate manipulation of the diet unless oxidative stress or deficiencies are involved, the current results also corroborate this finding. Besides, the stability in yolk index could be a symptom of sufficient antioxidant and micronutrient state among the hens, which was also supported by [31], who emphasized the stability in the yolk parameters under balanced diets.

Table 5: Effect of adding different levels of Moringa powdered leaves on the Oviposition age, and yolk Index.

Traits	T1	T2	T3	T4	Sig.
Oviposition Age	50.22±0.53	50.82±0.35	50.74±0.72	51.39±0.42	N.S.
Yolk Index	0.41±0.009 a	0.42±0.006 a	0.44±0.013 a	0.43±0.008 a	N.S.

T1 (control, 0% Moringa leaf powder), T2 (5%), T3 (7.5%), and T4 (10%).

Conclusion:

Current research shows that dietary supplementation of female Japanese quail with Moringa oleifera powdered leaves has a two-fold effect, i.e., the increase in the inclusion levels of the diet reduces some parameters (egg size, egg weight and albumin quality characteristics) of the eggs, and at the same time, significantly increases the indexes related to the eggshell thickness, weight, and the structure of the eggshell. These findings indicate that the quality of shells can be enhanced by an optimally adjusted supplementation program at the cost of the total reproductive output.

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