Effects on egg quality attributes of RIR hens fed with *Moringa oleifera* leaf meal-containing diet

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Abstract

This study examined the effects of *Moringa oleifera* leaves supplementation on egg quality characteristics of Rhode Island Red (RIR) hens. One hundred and eighty, 24-week-old Rhode Island Red layer birds were randomly allotted to four dietary treatments having replicates with 15 birds in each. The treatments were T_1 (Control: Basal diet); T_2: 1% MOLM in basal diet; T_3: 3% MOLM in basal diet; T_4: 5% MOLM in basal diet. The results revealed that average daily feed intake per bird (g/day) was lower ($P<0.05$) for hens supplemented with MOLM. Feed conversion ratio (FCR) showed no statistical difference between groups. In T3 and T4, the average HDEP and HHEP percentages were significantly ($P<0.05$) higher than in T1. Overall, MOLM supplementation significantly increased albumen height, width, shell thickness, index, yolk width, weight, Haugh unit, and yolk colour score ($P<0.05$) compared to the control group. MOLM feeding did not affect egg length, width, form index, shell weight, yolk height, or yolk index. All dose levels of MOLM showed significant improvements in egg composition, including increased beta carotene, omega 3 and omega 6 fatty acids, yolk and shell calcium, and decreased yolk cholesterol and overall fat weight ($P<0.05$). MOLM supplementation didn't affect proximate composition and carbohydrate content of egg. MOLM in diet prolongs egg shelf life by slowing albumen and yolk pH shift, increasing yolk and albumen height, and lowering TBARS. According to current studies, adding up to 5% *Moringa oleifera* to RIR laying hens' diets improves production performance, egg quality, and shelf life.

Key words: Egg production; Moringa leaf meal; Rhode Island Red; Shelf life

Introduction

Poultry is one of the agricultural sectors in India that is expanding at the fastest rate. After China and USA, India is the third-largest producer of eggs worldwide. The poultry industry, one of the best sources of animal protein, is essential to social and economic security in both developed and developing countries. Poultry is source of living both as industrial enterprise (Temiraev et al 2020) and small poutry farmers (Kandpal & Kumar 2023) in plains and highlands across the world. Decrease in profitability is a major concern of Poultry farmers (Baruwa & Idowu, 2021) According to Adenjimi *et al.* (2011), accessibility of high-quality feed in adequate amounts for both producers and consumers, at prices they can afford, is crucial to the growth of the chicken industry. To increase production performance and profitability, poultry producers frequently attempt to modify the quality and formulation of chicken feed. Since the use of antibiotics as growth promoters was prohibited, probiotics, prebiotics, enzymes, organic acids, herbs and other substances have been used to replace antibiotics in chicken production.

Phytobiotics, as plant-derived compounds added to feed to improve farm animal performance, are one such option Windisch *et al.* (2008). Additional aspects that encourage the use of tree leaves in chicken feeding include the improvement of egg quality and their high macronutrient content, which is the component of the protein source diversification program (FAO, 2004). The greatest alternative protein source in this situation might be *Moringa oleifera* leaves.

Moringa leaves can be utilized as a feed ingredient and as phytogenic feed additive due to the presence of bioactive compounds and critical nutrients in them (Borah & Haloi, 2024). This helps to improve production performance and dense egg yolks with carotenoids, flavonoids and selenium. The quality of eggs, can quickly deteriorate while being collected and stored until consumption. For this reason, farmers place a high priority on preserving and extending the shelf life of eggs. Numerous experiments have been attempted to thicken eggshells and reduce egg cholesterol levels. The poultry business and general public health will benefit from reducing cholesterol in egg yolks and improving yolk colour. More attempts are required in identify the optimum levels of *Moringa oleifera* as feed additive to improve laying performance, egg quality and shelf life of laying hens. Therefore, this experiment was intended to assess the dietary impact of *Moringa oleifera* leaves addition on laying and egg quality characteristics of Rhode Island Red (RIR) hens.

Materials and Methods

The study was conducted at the Livestock Farm Complex, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh Gujarat (latitude 21°29' N, longitude 70°26' E and altitude 60 meters above the mean sea level). Laboratory work was carried out in the Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh. The climate in Junagadh is tropical and arid.

Experimental details

Rhode Island Red layer birds (n=180), aged 24 weeks, were randomly selected and allocated into four equal groups of 45 birds each, with three sets of 15 birds per group, following a completely randomised design. The birds were reared in cages with standardised management and provided with specific foods from the 24th to the 40th week of age. The animal ethics committee approved the research protocol vide reference number: KU-JVC-IAEC-SA-96-2022. *Moringa oleifera* leaf meal (MOLM) was procured from the local vendor and the quality of MOLM was checked at the Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh. Four experimental diets were prepared with MOLM as T_1 (Control: Basal diet); T_2: 1% MOLM in basal diet; T_3: 3% MOLM in basal diet; T_4: 5% MOLM in basal diet (Table 1). Feed was offered ad libitum in weighed quantity twice a day at 9:00 AM and 5:00 PM. Manual turning and mixing of feeds in the feeder were done frequently at least twice daily. Clean wholesome drinking water was provided to experimental birds *ad libitum*. Light bulbs were provided for the lighting system to increase the lighting period.

Feed intake, feed conversion ratio and egg production

The daily feed intake of each replicate was recorded. The feed consumption per bird was determined by subtracting the leftover feed from the given feed to analyse weekly/daily intake (g). FCR was assessed weekly as the ratio of feed consumed per unit egg weight (Abou-Elezz *et al.,* 2011).

Eggs were collected twice daily, in the morning and evening. The aggregate of the two collections, along with the daily bird count, was recorded and summarised at the end of the period. Hen-day egg production (HDEP) and hen housed egg production (HHEP) were quantified as percentages. The weekly egg weight was documented immediately upon collection, and the average weight was determined by dividing the total weight by the quantity of eggs.

Measurement of egg quality

Egg quality parameters were assessed monthly, focussing on egg weight and shape index externally. Internal quality traits were analysed by breaking the eggs on a flat surface and isolating each component, including shell weight, shell thickness, yolk colour, yolk weight, yolk length, yolk height, yolk index, albumen weight, albumen height, and Haugh unit.

The proximate composition of the egg, encompassing moisture content, crude protein, crude fat, total ash, and carbohydrates, was determined at the end of the experiment utilising the techniques specified in AOAC (2023). The carbohydrate content was assessed according to the method established by Lilla et al. (2005).

$$\text{Total carbohydrates} = 100\ (\text{Protein} + \text{Fat} + \text{Moisture} + \text{Ash})$$

Cholesterol, n-3 and n-6 fatty acid from egg yolk were analyzed using gas chromatography (GC). Egg yolk β-carotene was estimated by high-performance liquid chromatography (HPLC) and calcium from egg yolk and egg shell was measured by inductively coupled plasma mass spectrometry (ICPMS) (Analyzed at TUV India Pvt. Ltd., Mumbai). Eggs were kept at room temperature for 1, 2, 3 and 4 weeks in order to determine their shelf life. At the end of the experiment, 24 eggs from each treatment were randomly selected and measurements of the albumen and yolk as well as their pH were taken using a pH meter. The method mentioned by Witte *et al.* (1970) was used to measure the amount of malonaldehyde (MDA) in the yolk.

Statistical analysis

The data generated during the experiment were collected and statistically analyzed by Chi-square test for frequency data and analysis of variance (ANOVA) for comparison of mean as per procedures suggested by Snedecor and Cochran (1994). Pairwise mean differences among groups were analysed using the Tukey test.

Results and Discussion

Results of productive performance as impacted by the inclusion of MOLP presents in Table 2. From the present results, it is observed that the Overall means of daily feed intake per experimental bird were 116.16± 0.44, 114.2± 0.36, 112.88± 0.55 and 111.3± 0.85 g in T_1, T_2, T_3 and T_4 groups, respectively.

In the current study, it was found that overall average feed intake per bird (g/day) was higher ($P<0.05$) for hens in T_1 than hens in T_2, T_3 and T_4. Hens in T_2 had higher ($P<0.05$) average feed intake per bird (g/day) than hens in T_4, however, T_3 did not significantly differ ($P>0.05$) with T_2 and T_4. This was alike to the findings of Raphael *et al.* (2015) noted that adding of 5% and 10% MOLM to the laying hen's diet reduced feed consumption. They attributed the significant decline in feed intake with increasing amount of leaf meal due to its high fibre content as well bitter taste. In contrast, Sharmin *et al.* (2021) observed that average daily feed consumption of laying hens fed diets containing 1.5% MOLM was significantly higher than that of the hens fed diet with 0%, 0.5% and 1% MOLM.

Overall average FCR were 2.94±0.03, 2.86±0.03, 2.93±0.01 and 2.91±0.05 in the T_1, T_2, T_3 and T_4 groups, respectively. In the current study, no statistical difference was observed for FCR between groups or within group. Overall average FCR was lowest for hens in T_2. But, there was no statistical differences ($P>0.05$) among the groups. The findings are similar to those of Olugbemi *et al.* (2010), who observed that the feed conversion ratio was unaffected by the addition of 5% and 10% MOLM to the laying hen diet. However, Raphael *et al.* (2015) gradually replaced soybean with MOLM at 0, 5 and 10% and observed that with 5% MOLM, the FCR was lower; however, with 10% MOLM, the FCR was higher.

Values of average monthly hen day egg production (HDEP) % of experiment were 69.08±1.88, 73.76±1.50, 75.67±1.71 and 79.17±1.15 in the T_1, T_2, T_3 and T_4 groups, respectively. The values were significantly ($P<0.05$) higher in T_3 and T_4 groups as compared to T_1 group and numerically higher than T_2 group. Average values of monthly HHEP% of experiment were 65.96±2.11, 72.13±1.79, 74.44±1.91 and 78.74±1.28 in the T_1, T_2, T_3 and T_4 groups, respectively. The values were gradually increased from T_1 to T_4 and have significantly higher values in T_3 and T_4 as compared to control. The T_3 was non-significant different from T_2 and T_4. T_4 also showed significantly higher value than T_2. In the present investigation, an increase in laying percentages with advancement of age was noted in all dietary treatments. The current investigation was consistent with Ebenebe *et al.* (2013), who found that different levels of MOLM (0%, 2.5%, 5.0%, and 7.5%) in laying chicken diets had similar outcomes. Raphael *et al.* (2015) also noted significant effect on egg production when fed 5% MOLM as compared to 0 and 10% MOLM in Kabir strain chickens. On the other hand, Olugbemi *et al.* (2010) observed a non-significant effect on laying % for hens added MOLM at 0, 5, and 10% of the diet. Abu and Akangbe (2017) also found that addition of 0, 1 and 5% MOLM Japanese quails' diet had no significant effect on HDEP % when compared to a diet free of MOLM. Improved balanced nutritional supply provided by MOLM in the diet may be the cause of the greater egg production in layers given the diet containing MOLM. Lysine, methionine, and a variety of other amino acids are present in MOLM, which may provide the necessary quantity of nutrients for improved production.

Table 1. Ingredient and nutrient composition of the experimental diets

Ingredients (%)	T 1	T 2	T 3	T 4
Ingredient composition (% DM basis)				
Maize	53.40	53.40	50.00	45.00
Soyabean DOC	27.70	26.70	24.70	22.70
Deoiled Rice Bran	6.41	6.41	9.81	14.80
Moringa oleifera leaf meal (MOLM)	0	1.00	3.00	5.00
Calcite Powder	3.00	3.00	3.00	3.00
Limestone	6.90	6.90	6.90	6.90
DCP	1.63	1.63	1.63	1.63
Salt	0.30	0.30	0.30	0.30
Premix (Vitamins, Enzymes etc.)	0.66	0.66	0.66	0.66
Total %	100	100	100	100
Calculated nutrient composition				
ME (Kcal/kg)	2613	2616	2622	2629
DM %	89.50	89.30	89.30	89.80
OM %	93.00	93.00	92.90	92.80
CP %	18.00	18.40	18.60	18.30
CF %	8.17	8.01	7.92	7.94
EE %	3.45	3.51	3.67	3.80
NFE %	63.40	63.00	62.60	62.70
Total Ash %	6.92	6.95	7.10	7.20
Silica %	1.11	1.22	1.16	1.19
Calcium %	3.05	3.21	3.28	3.36
Phosphorus %	0.38	0.39	0.39	0.40

Table 2. Effects of dietary treatment on production parameters of RIR laying hen

Parameter	T_1	T_2	T_3	T_4	SEM	P value
Feed Intake, g/day	116 ± 0.44^{A}	114 ± 0.36^{B}	112 ± 0.55^{BC}	111 ± 0.85^{C}	3.10	0.001
Feed Conversion Ratio(FCR)	2.94±0.03	2.86±0.03	2.93±0.01	2.91±0.05	0.25	0.960
Hen Day Egg Production %	69.08 ± 1.88^{B}	73.76 ± 1.50^{AB}	75.67 ± 1.71^{A}	79.17 ± 1.15^{A}	4.97	0.002
Hen House Egg Production %	65.96 ± 2.11^{C}	72.13 ± 1.79^{BC}	74.44 ± 1.91^{AB}	78.74 ± 1.28^{A}	5.83	0.003

^{ABC} Means with different superscripts in a row differ significantly (P<0.05)

Table 3. Effects of dietary treatment on egg quality parameters of RIR laying hen

Parameter	T_1	T_2	T_3	T_4	SEM	P value
Egg weight (g)	54.93 ± 0.59^{A}	54.52 ± 0.40^{AB}	55.71 ± 0.43^{A}	53.13 ± 0.57^{B}	3.70	0.001
Egg length (mm)	56.36±0.31	55.66±0.28	56.31±0.23	55.5±0.30	2.16	0.060
Egg width (mm)	42.13±0.16	42.23±0.48	42.38±0.15	41.70±0.19	2.50	0.370
Shape index (%)	74.83±0.41	75.97±0.16	75.37±0.37	75.26±0.42	4.30	0.320
Shell thickness (mm)	0.31 ± 00^{C}	0.33 ± 0^{B}	0.34 ± 00^{AB}	0.34 ± 00^{A}	0.02	0.001
Egg shell weight (g)	6.98±0.09	7.19±0.10	7.22±0.10	6.95±0.10	0.74	0.090
Albumen height (mm)	8.69 ± 0.19^{B}	9.56 ± 0.15^{A}	9.69 ± 0.20^{A}	9.87 ± 0.19^{A}	1.31	0.001
Albumen width (mm)	72.53 ± 0.70^{A}	68.87 ± 0.53^{B}	69.81 ± 0.56^{B}	68.95 ± 0.66^{B}	4.47	0.001
Albumen index (%)	12.12 ± 0.36^{B}	13.99 ± 0.27^{A}	14 ± 0.35^{A}	14.35 ± 0.33^{A}	2.35	0.001
Yolk height (mm)	19.89±0.16	20.12±0.56	20.23±0.22	20.12±0.14	2.39	0.880
Yolk width (mm)	40.35 ± 0.19^{A}	39.86 ± 0.16^{AB}	39.46 ± 0.22^{B}	39.4 ± 0.19^{B}	1.42	0.001
Yolk index (%)	49.42±0.46	50.53±0.49	51.31±0.60	51.1±0.40	6.16	0.330
Egg yolk weight (g)	13.96 ± 0.25^{B}	15.12 ± 0.16^{A}	15.55 ± 0.20^{A}	14.98 ± 0.14^{A}	1.37	0.001
Egg albumen weight (g)	34 ± 0.42^{A}	32.21 ± 0.35^{BC}	32.94 ± 0.35^{AB}	31.21 ± 0.49^{C}	2.90	0.001
Haugh unit	93.49 ± 0.80^{B}	98.31 ± 0.70^{A}	98.77 ± 0.95^{A}	100 ± 0.87^{A}	5.79	0.001
Yolk color score	5.90 ± 0.13^{B}	7.68 ± 0.10^{A}	7.68 ± 0.14^{A}	7.73 ± 0.16^{A}	0.65	0.001

^{ABC} Means with different superscripts in a row differ significantly (P<0.05)

Table 4. Effects of feeding different levels of MOLM on chemical composition of eggs of Rhode Island Red hens

Parameters	T 1	T 2	T 3	T 4	SEM	P value
Yolk Cholesterol (mg/100g)	893.49 ±0.94^{A}	893.55±1.09^{A}	734.63±2.06^{B}	633.75±1.75^{C}	3.76	0.0001
Total Fat (g/100g)	18.65±00^{A}	12.33±00^{B}	11.51±00^{C}	10.63±00^{D}	0.00	0.0001
Yolk n-3 FA (g/100g)	0.11±00^{B}	0.13±00^{A}	0.12±00^{B}	0.12±00^{AB}	0.006	0.0009
Yolk n-6 FA (g/100g)	1.38±0.01^{B}	1.75±0.04^{A}	1.70±0.06^{A}	1.80±0.04^{A}	0.09	0.0001
Yolk Beta carotene (μg/100g)	430.31±3.70^{D}	768.49±4.98^{C}	837.28±8.21^{B}	920.47±2.54^{A}	12.99	0.0001
Yolk Calcium (mg/100g)	70.48±1.59^{C}	79.07±2.76^{B}	83.86±1.38^{B}	92.40±2.52^{A}	5.26	0.0001
Egg Shell Calcium %	20.75±0.03^{D}	26.22±0.05^{C}	29.68±0.21^{B}	33.04±0.04^{A}	0.27	0.0001
n6:n3	12.15±0.17^{B}	13.48±0.27^{AB}	14.6±0.76^{A}	14.62±0.52^{A}	1.20	0.004

^{ABC} Means with different superscripts in a row differ significantly (P<0.05)

Table 5. Effects of feeding different levels of MOLM on proximate composition of eggs of Rhode Island Red hens

Parameters	T 1	T 2	T 3	T 4	SEM	P value
Moisture (%)	66.91±0.45	67.91±0.12	68.95±0.38	69.65±0.55	1.44	0.1812
EE (%)	12.45±0.25^{A}	11.26±0.08^{B}	10.55±0.19^{B}	10.86±0.20^{B}	0.33	0.0006
CP (%)	11.00±0.20	11.17±0.12	11.14±0.07	11.32±0.07	0.22	0.447
Ash (%)	1.03±0.04	1.21±0.12	1.37±0.10	1.41±0.05	0.15	0.0591
CF (%)	0.52±0.03^{B}	0.61±0.01^{A}	0.61±0.03^{A}	0.62±0.02^{A}	0.03	0.0142
CHO (%)	8.61±0.55	8.44±0.38	7.99±0.21	6.76±1.73	1.62	0.5297

^{ABC} Means with different superscripts in a row differ significantly (P<0.05)

Table 3 present the egg quality parameters of RIR hens under the experimental groups. Overall average egg weight was lower (P<0.05) in T_4 than T_1, T_2 and T_3. The overall average shell thickness was 0.31±0, 0.33±0, 0.34±0 and 0.34±0 mm in the T_1, T_2, T_3 and T_4 groups, respectively. Significantly higher (P<0.05) shell thickness was observed in T_3 and T_4 than in T_2 and T_1. T_2 also had significantly higher (P<0.05) shell thickness than T_1. However, egg length, egg width, shape index and shell weight were not affected by feeding of MOLM. The overall mean of albumen height, albumen width, albumen index, yolk width, yolk weight, Haugh unit and yolk color score were significantly improved (P<0.05) for MOLM supplemented groups than the control group. However, overall mean of yolk height and yolk index were remain at par for all groups. Albumen weight was higher (P<0.05) for T_1 than T_2 and T_4. Similarly, T_3 had higher (P<0.05) albumen weight than T_4, but was similar (P>0.05) with T_1 and T_2.

According to Swain *et al.* (2017), shell (%), shape index and shell thickness (mm) were not significantly affected by adding MOLM at 0.5, 1, 1.5 and 2% to the basal diet of laying hens. Similar observations were also reported by Shen *et al.* (2021) and Sharmin *et al.* (2021) on external egg quality traits. Similarly, Sheikh *et al.* (2015) found that adding *Moringa oleifera* leaf powder (MOLP) to the diet of Lohmann Brown laying hens at 0, 1, 1.5 or 2 g/kg resulted in enhanced egg yolk color and a significant decrease in yolk weight at all dose levels. However, contrary to the current findings, Swain *et al.* (2017) and Ashour *et al.* (2020) reported that addition of MOLM at varied level in layer birds' diet had non-significant impact on internal egg quality traits.

In commercial egg production, having a thick shell is a crucial bio economic characteristic since it can reduce the number of cracked eggs and the rate of loose eggs that are produced. If the layer diet does not contain sufficient amounts of calcium, phosphorus and vitamin D, shell quality will not be maintained for very long. Current study is supported by the fact that MOLM contains these nutrients, which improved the quality of egg shells. Because MOLM has a high carotene concentration, adding it to the laying hens' diets improved the yolk color, which is a desirable quality traits. Because MOLM includes such vitamins and amino acids and enhances the quality of eggs both inside and outside, the results of the study corroborate the scientific basis for improving albumen and yolk quality.

Data on egg chemical composition of Rhode Island Red hens as affected by dietary level of MOLM are illustrated in Table 4. Results showed that MOLM at all dose levels improved egg composition which is highlighted by a significant increase (P<0.05) in beta carotene, omega 6 fatty acid, yolk and shell calcium, while induced a significant reduce (P<0.05) in yolk cholesterol and total fat weight at all dose levels.

In relation to the current findings, Ahmad *et al.* (2018) also observed that birds fed diet with 1.5% MOLM had significantly (P<0.05) increased levels of β-carotene in the egg yolk. Amount of total cholesterol in the yolk dropped linearly with the MOLM supplementation and was lower at 1.5% MOLM. Similar observation was reported

Table 6. Effects of MOLM on egg shelf life parameters of Rhode Island Red hens

EST (Days)	T 1	T 2	T 3	T 4	SEM	P value
Albumen Height (mm)						
7	6.91±0.64	7.45±0.14	7.68±0.41	7.85±0.94	1.50	0.720
14	5.73±0.71	6.78±0.38	7.06±0.27	7.35±0.24	1.08	0.086
21	4.38±0.17^{B}	5.83±0.45^{A}	6.27±0.31^{A}	6.41±0.22^{A}	0.76	0.0006
28	3.27±0.30^{B}	4.32±0.38^{AB}	5.04±0.34^{A}	5.63±0.54^{A}	0.99	0.003
Mean±SE	5.07±0.37^{B}	6.09±0.30^{A}	6.51±0.26^{A}	6.81±0.32^{A}	1.12	0.0001
Yolk Height (mm)						
7	11.88±0.29	13.60±0.48	14.51±0.73	14.69±1.38	2.04	0.100
14	8.71±0.32^{B}	10.46±0.13^{A}	11.03±0.26^{A}	11.59±0.54^{A}	0.85	0.001
21	6.01±0.71^{B}	7.74±0.57^{AB}	8.04±0.33^{A}	8.20±0.25^{A}	1.23	0.022
28	4.96±0.60^{B}	6.45±0.36^{AB}	6.76±0.15^{A}	6.90±0.25^{A}	1.00	0.012
Mean±SE	7.89±0.61^{B}	9.56±0.60^{A}	10.08±0.65^{A}	10.35±0.73^{A}	1.36	0.0001
Yolk pH						
7	6.72±0.11^{A}	6.11±0.03^{B}	6.05±0.03^{B}	6.03±0.04^{B}	0.16	0.001
14	7.38±0.27^{A}	6.56±0.07^{B}	6.44±0.09^{B}	6.37±0.14^{B}	0.41	0.001
21	8.88±0.17^{A}	6.85±0.13^{B}	6.79±0.11^{B}	6.75±0.08^{B}	0.32	0.0001
28	9.09±0.20^{A}	7.13±0.17^{B}	6.99±0.22^{B}	6.84±0.22^{B}	0.51	0.0001
Mean±SE	8.02±0.23^{A}	6.66±0.10^{B}	6.57±0.10^{B}	6.50±0.09^{B}	0.37	0.0001
Albumen pH						
7	9.59±0.07^{A}	9.17±0.07^{AB}	8.89±0.12^{B}	8.77±0.17^{B}	0.29	0.0005
14	10.02±0.19^{A}	9.28±0.23^{B}	9.21±0.15^{B}	9.15±0.04^{B}	0.42	0.0063
21	10.65±0.05^{A}	9.36±0.20^{B}	9.36±0.09^{B}	9.26±0.16^{B}	0.34	0.0001
28	10.95±0.37^{A}	9.53±0.11^{B}	9.45±0.07^{B}	9.44±0.10^{B}	0.50	0.0001
Mean±SE	10.30±0.15^{A}	9.34±0.08^{B}	9.23±0.07^{B}	9.15±0.08^{B}	0.40	0.0001
TBARS Value (mg MDA/kg)						
7	1.91±0.13	1.64±0.15	1.57±0.16	1.45±0.10	0.34	0.150
14	2.24±0.18	1.78±0.24	1.65±0.15	1.53±0.13	0.45	0.060
21	2.33±0.21^{A}	1.82±0.07^{AB}	1.78±0.11^{AB}	1.64±0.10^{B}	0.34	0.012
28	2.62±0.05^{A}	1.89±0.05^{B}	1.88±0.08^{B}	1.78±0.12^{B}	0.21	0.0001
Mean±SE	2.28±0.09^{A}	1.78±0.07^{B}	1.72±0.07^{B}	1.6±0.06^{B}	0.34	0.0001

^{ABC} Means with different superscripts in a row differ significantly (P<0.05)

by Sharmin *et al.* (2021) reported significantly higher amounts of total n-3 and n-6 fatty acids in the eggs of laying chicken groups with 0.5, 1 and 1.5% MOLM in the diet. A significantly (P<0.05) higher calcium contents in the eggshell than control when MOLM was supplemented at 2 and 4% in diet of laying hens.

Researchers are still becoming interested in the concept of using dietary management to enrich eggs with n-3 and n-6 fatty acids. In the present study, the addition of MOLM to the hens' diet improved the fatty-acid level of eggs, especially the n-6 fatty acid content. According to Ghasi *et al.* (2000), antioxidants enhance the synthesis of bile salts, which emulsify fat and reduce its absorption, thus lowering cholesterol levels. Carotenoids and flavonoids are potent natural antioxidants found in abundance in MOLM. High concentration of these chemicals in MOLM may be the reason for enrichment of β-carotene in the egg yolk.

Effect of feeding varies levels of dietary MOLM on proximate composition of Rhode Island Red hen's egg is shown in Table 5. Moisture, CP and total ash content of egg linearly but non-significantly increased with MOLM addition rate, with the highest values observed for the group fed the MOLM-5% diet. Furthermore, birds fed diets with all level MOLM had a significantly (P<0.05) lower EE and higher CF content in than those of the control group. Carbohydrate content linearly decreased with MOLM level in feed. Consistently, Ahmad *et al*. (2018) showed that the addition of MOLM to a ration significantly lowered the fat content and higher the ash content in HyLine W36 layers eggs. Unlike the results of this investigation, Sharmin *et al.* (2021) reported that birds fed diets with MOLM had a higher CP and EE content in eggs than those of the control group.

Effect of feeding different levels of dietary MOLM on shelf life of eggs of Rhode Island Red hens is shown Table 6. Results revealed that the overall mean of shelf life parameters are affected by MOLM supplementation with storage time. Overall mean of albumen height and yolk height were significantly higher in MOLM fed groups than control group during storage time. Mean values of yolk height and albumen height of all groups were significantly lowered ($P<0.05$) with increasing EST. Mean values of albumen pH and yolk pH of all groups were linearly increased with EST. During storage, the MOLM-fed groups' overall mean albumen and yolk pH values were significantly lower ($P<0.05$) than those of the control group. Lipid oxidation was identified with the thiobarbituric acid reactive substances (TBARS) assay method. Malondialdehyde (MDA), a degradation product derived from the oxidation of unsaturated fatty acids, is quantified using this approach. Results revealed that the TBARS values of all groups linearly increased with EST and decreased with MOLM level. Overall mean of TBARS value was significantly lowered ($P<0.05$) in MOLM fed groups than control group during storage time.

Tesfaye *et al.* (2018) found that internal egg quality had a longer shelf life in relation to storage period. Pappas *et al.* (2005) also described the decrease in albumen as well as yolk pH alteration rate as a function of the antioxidant level of egg components. According to Jung *et al.* (2010), *Moringa oleifera* is one of the most promising species because of its strong antioxidant activity, high micronutrient content and phytochemicals that may contribute to the stability and shelf life of chicken products. Significant effect in egg shelf life in terms of pH of yolk and albumen at various storage times was consistent with the findings of various researchers. Phenolic compounds have a high antioxidant activity may be linked to the improvement in egg shelf life observed in the current study as a result of MOLM inclusion.

Conclusion

The results of this study showed that *Moringa oleifera* leaf meal incorporation in hens' diet significantly improved egg quality parameters like, shell thickness, albumen height and width, albumen index, yolk weight, Haugh unit, yolk color score and shelf life of egg. Beta carotene, n-3, n-6, yolk and egg shell calcium contents were significantly increased with *Moringa oleifera* leaf meal incorporation, whereas, total fat, ether extract and yolk cholesterol were significantly decreased on MOLM incorporation. The inclusion of MOLM in the diet of RIR hen induced significant changes in laying performance, internal and external egg quality, composition and shelf life of egg.

References

1) Abou-Elezz, F. M. K., Sarmiento-Franco, L., Santos-Ricalde, R., & Solorio-Sanchez, F. (2011). Nutritional effects of dietary inclusion of Leucaena leucocephala and Moringa oleifera leaf meal on Rhode Island Red hens' performance. Cuban Journal of Agricultural Science, 45(2), 163-169.
2) Abu, O. A., & Akangbe, E. E. (2017). Effect of Moringa oleifera (Lam) leaf meal on egg production, blood and serum profile of laying Japanese quail (Coturnix coturnix japonica). Nigerian Journal of Animal Science, 2, 165-176.
3) Adejinmi, O. O., Hamzat, R.A., Raji, A.M., & Owosibo, A.O. (2011). Performance, nutrient digestibility and carcass characteristics of broilers fed cocoa pod husks-based diets. Nigerian Journal of Animal Science, 13, 61-68.
4) Ahmad, S., Khalique, A., Pasha, T. N., Mehmood, S., Ahmad, S. S., Khan, A., & Hussain, K. V. (2018). Influence of Moringa oleifera leaf meal used as phytogenic feed additive on the serum metabolites and egg bioactive compounds in commercial layers. Brazilian Journal of Poultry Science, 20(2), 325-332.
5) AOAC (2023). Official Methods of Analysis. 5th edition. Association of Official Analytical Chemists (AOAC), Washington D.C., USA.
6) Ashour, E. A., El-Kholy, M. S., Alagawany, M., El-Hack, M. E., Mohamed, L. A., Taha, A. E., Sheikh, A. I., Laudadio, V., & Tufarelli, V. (2020). Effect of dietary supplementation with Moringa oleifera leaves and/or seeds powder on production, egg characteristics, hatchability and blood chemistry of laying Japanese quails. Sustainability, 12, 2463.
7) Baruwa, O.I., Idowu, Y.O. 2021. Profitability and Constraints of Poultry Egg Enterprise in Ogun State, Nigeria. Journal of Livestock Science 12: 42-49. doi. 10.33259/JLivestSci.2021.42-49
8) Borah L., Haloi S. 2024. Moringa oliefera leaf as potential, alternative protein source f or livestock. Journal of Livestock Science 15: 141-149, doi. 10.33259/JLivestSci.2024.141-149
9) Ebenebe, C. I., Anigbogu, C. C., Anizoba, M. A., & Ufele, A. N. (2013). Effect of various levels of Moringa leaf meal on the egg quality of Isa brown breed of layers. Advances in Life Science and Technology, 14, 45-49.
10) FAO (2004). Proteins sources for the animal feed industry. FAO Animal production and health proceedings. Expert consultation and workshop Bangkok 29 April-3 May 2004.

11) Ghasi, S., Nwobodo, E., & Ofili, J. O. (2000). Hypo-cholesterolemic effects of crude extract of leaf of Moringa oleifera in high-fat diet fed Wistar rats. Journal of Ethnopharmacology, 69, 21-25.
12) Jung, S., Choe, J. H., Kim, B., Yun, H., & Kruk, Z. A. (2010). Effect of dietary mixture of gallic acid and linoleic acid on antioxidative potential and quality of breast meat from broilers. Meat Science, 86, 520-526.
13) Kandpal A.S., Kumar S. 2023. A study on constraints faced by small poultry farmers in highlands. Journal of Livestock Science 14: 276-282 DOI.10.33259/JLivestSci. 2023.276-282
14) Lilla, Z., Sullivan, D., Ellefson, W., Welton, K., & Crowley, R. (2005). Determination of “net carbohydrates” using high-performance anion exchange chromatography. Journal of AOAC International, 88(3), 714-719.
15) Olugbemi, T. S., Mutayoba, S. K., & Lekule, F. P. (2010). Evaluation of Moringa oleifera leaf meal inclusion in cassava chip based diets fed to laying birds. Livestock Research for Rural Development, 22,118.
16) Pappas, A. C., Karadas, F., Surai, P. F., & Speake, B. K. (2005). The selenium intake of the female chicken influences the selenium status of her progeny. Comparative Biochemistry and Physiology, 142, 465-474.
17) Raphael, K. J., Christian, K. T., Juliano, R. S., Lisita, F., Soultan, M. Y., Herve, M. K., & Alexis, T. (2015). Effects of substituting soybean with Moringa oleifera meal in diets on laying and eggs quality characteristics of Kabir chickens. Journal of Animal Research and Nutrition, 1, 1.
18) Sharmin, F., Sarker, S. K., Sarker, N. R., & Faruque, S. (2021). Dietary effect of Moringa oleifera on native laying hens’ egg quality, cholesterol and fatty-acid profile. Italian Journal of Animal Science, 20(1), 1544-1553.
19) Sheikh, N. I., Shazly, E. S., Abbas, E. A., Ghada, I. A., & Gobary, E. I. (2015). Effect of Moringa leaves on lipid content of table eggs in layer hens. Egyptian Journal of Chemistry and Environmental Health, 1(1), 291-302.
20) Shen, M., Li, T., Qu, L., Wang, K., Hou, Q., Zhao, W., & Wu, P. (2021) Effect of dietary inclusion of Moringa oleifera leaf on productive performance, egg quality, antioxidant capacity and lipid levels in laying chickens. Italian Journal of Animal Science, 20,1.
21) Snedecor, G., & Cochran, W. (1994). Statistical methods. 8th edition. Oxford and IBH. New Delhi (pp. 503).
22) Swain, B. K., Naik, P. K., Chakurkar, E. B., & Singh, N. P. (2017). Effect of supplementation of Moringa oleifera leaf meal (MOLM) on the performance of Vanaraja laying hens. Indian Journal of Animal Sciences, 87(3), 353-355.
23) Temiraev V.H., Baeva A.A., Vityuk L.A., Mamukaev M.N., Yurina N.A., Ktsoeva I.I., Bobyleva L.A., Zagaraeva E.F., Kokov T.N., Vologirova F.A. 2020. Effect of probiotics on digestive metabolism in growing and laying poultry birds. Journal of Livestock Science 11: 33-39. doi. 10.33259/JLivestSci.2020.33-39
24) Tesfaye, E., Alebachew, W., & Tamir, B. (2018). Performance of Koekoek chicken fed with different levels of Moringa oleifera leaf meal. Journal of Animal Science and Research, 2(3), 115.
25) Windisch, W. K., Schedle, C., Plitzner, A., & Kroismayr, A. (2008). Use of phytogenic products as feed additives for Swine and Poultry. Journal of Animal Science, 86, 140-148.
26) Witte, V. C., Krause, G. F., & Bailey, M. E. (1970). A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. Journal of Food Science, 35, 582-585.