Dietary effect of *Moringa oleifera* on native laying hens’ egg quality, cholesterol and fatty-acid profile

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**ABSTRACT**

This study examined the effects of dietary incorporation of *Moringa oleifera* leaf (MOL) meal in the diet of native laying chickens on the quality, yolk cholesterol and fatty-acid profile of eggs. One hundred twenty-eight birds were split into four dietary groups, with 32 birds in each group, comprising four replications with eight birds per replication. The experiment lasted 16 weeks and was conducted at the open houses shed in the Bangladesh Livestock Research Institute. The four dietary treatments were formulated using basal feed as follows: control (T\(_1\)), MOL 0.5% (T\(_2\)), MOL 1.0% (T\(_3\)) and MOL 1.5% (T\(_4\)). The quality, weight, length and width of eggs were not altered by the addition of MOL to the diet. Yolk colour and eggshell breaking strength were significantly higher in the additive groups. The results showed that dietary addition of MOL meal significantly reduced serum, total cholesterol and triglyceride content compared to the control. Feeding MOL at 1 and 1.5% increased \(\omega-3\) fatty-acid levels by 1.35 and 1.46%, respectively. Overall, the results indicate that the addition of 1.5% MOL to layer feed could be an effective way to improve egg quality and fatty-acid profile and reduce cholesterol in egg yolk.

**HIGHLIGHTS**

- Addition of *Moringa oleifera* leaves (MOL) in native laying hens basal diet improves egg quality.
- \(\omega-3\) fatty-acid composition in yolk was enriched.
- Serum and yolk cholesterol levels in hens were significantly reduced \((p < .05)\) in all additive groups.

**Introduction**

Over the last decades, people have become highly aware of the connection between food and health. Food can only be considered functional if, together with its basic nutritional impact, it has beneficial effects on human health and decreases the risk of disease (Clare 2002). Eggs are highly favoured in the food industry as an excellent source of nutrients and for their valuable functional properties (Cook and Briggs 1977). Functional properties of egg yolk are manufacture and stabilisation of emulsions, foaming stability and thermal gelation in the food industry for producing several food products (Rossi et al. 2010). Egg production in Bangladesh depends on commercial hybrids of laying chickens, which are selected for their high production performance. Ajayi (2010) reported that Native chickens are suitable in terms of their resistance, adaptability to the environment and potential genetic resources for producing commercial strains. Moreover, the demand for native chicken eggs and meat market is increasing day by day. Therefore, it is necessary to assess the quality of eggs from native chicken breeding stocks in Bangladesh.

Fatty acids, especially \(\omega-3\) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are known to prevent many diseases caused by cholesterol and saturated fat (DiMarco et al. 2017). Abhishek and Biswadeep (2014) reported that \(\omega-3\) fatty acid has decreased the serum triglycerides and increased the high-density lipoprotein cholesterol levels in human volunteers. Dietary recommendations have been made for \(\omega-3\) fatty acids up to 650 mg of both EPA and DHA daily.
DHA by The International Society for the Study of Fatty Acids and Lipids. These PUFAs are also known to have important roles in the brain, retinal and neural tissues of humans (Simopoulos et al. 1999). Many studies have concluded that the fatty-acid composition of eggs is dependent on the fatty-acid composition of the laying hen feed, as a source of ω-3 PUFAs that are incorporated into eggs (Dai et al. 2016; Hammershøj and Johansen 2016). More than 80 years ago, Cruickshank (1934) first reported that the fatty-acid composition of egg yolk could be modified by dietary manipulation. Subsequently, Sim et al. (1980) adopted Cruickshank’s idea and developed a designer egg, rich in n–3 fatty acids and antioxidants. It has been reported that consumers are willing to pay extra for such n–3 PUFA-enriched eggs (Marshall and Van Elswyk 1994). Cherian and Sim (1993), Galobart et al. (2001) and Narahari (2003; Narahari et al. 2009) enriched eggs with ω–3 fatty acids and antioxidants severalfold. They added flax seeds, fish oil, garlic pearls, fenugreek seeds, basil leaves, bay leaves and spirulina to basal layer feed at different concentrations. Hens readily absorb and transfer omega-3 fatty acids from dietary sources into the yolk (Cherian and Sim 1993). Furthermore, several studies have been conducted to enrich the content of PUFA in eggs using dietary fat sources, such as natural oil containing PUFA (Kim et al. 2007).

_Moringa oleifera_, a plant from the family Moringaceae, is a major crop in Asia and Africa. For centuries, people in many countries have used moringa leaves as traditional medicine for common ailments. The most commonly used parts of the plant are the leaves, which are rich in vitamins, carotenoids, alkaloids, glucosinolates, isothiocyanates, tannins and saponins (Leone et al. 2015). These bioactive compounds might explain the antiseptic and antimicrobial properties of the leaves (Collin 2006). In addition, _M. oleifera_ leaves (MOL) are a rich source of natural antioxidants, as they contain various types of antioxidant compounds, such as ascorbic acid, flavonoids, phenolics, and carotenoids (Makkar and Becker 1997; Anwar et al. 2007). _M. oleifera_ leaf has been used to enhance growth performance, feed efficiency and meat quality in broiler chickens (Sarker et al. 2010; Sharmin et al. 2020a). However, the leaves of _M. oleifera_ have yet to be used to improve native laying hens’ egg quality with respect to cholesterol and fatty-acid profile. Therefore, we hypothesised that the addition of MOL to the basal diet of native laying hens would improve egg quality and yolk fatty-acid composition and reduce the cholesterol content of eggs.

**Materials and methods**

**Materials**

Fresh MOL were collected and air-dried during the daytime. After 4–5 days of drying, the leaves were ground to a fine powder to pass through a 0.15 mm sieve. The leaf meal was tightly packaged in polythene plastic bags and kept at room temperature until required. The dried MOL was analysed in triplicate for crude protein (CP), ether extract (EE), moisture and ash, as described by the Association of Official Analytical Chemists (AOAC 2000). The proximate composition data is presented as Supplementary Table S1.

**Experimental design, dietary treatments and management**

One hundred and twenty-eight (128) native laying chickens at age 26 weeks were selected for this study, and the experiment continued until they were age 42 weeks. Birds were distributed according to a completely randomised design (CRD) of experiment. Hens of this age were selected for the trial because they were already reaching peak egg production (>85% hen-day egg production). The cage unit was equipped with nipple waterers and feed troughs in a room with an ambient temperature of around 20°C and a photoperiod of 16 h light: 8 h darkness. Water was provided ad libitum intake throughout the trial period. Hens had access to 467 cm²/hen, and cages were blocked by side (north and south), with 24 cages on each side. The birds were split into four dietary groups of 32 birds each, comprising four replications with eight birds per replication and were fed ad libitum. The management practices were standard with dietary requirements. Four dietary treatment groups were produced from the basal feed as follows: control (T1), MOL 0.5% (T2), MOL 1% (T3), and MOL 1.5% (T4). The MOL was weighed, added to the basal diet, and mixed for 20 min. Diets were formulated to meet nutrient requirements, as recommended by the National Research Council (NRC) (1980). The eggs laid between age 36 weeks and age 41 weeks were pooled per hen and used for analysis. Diet samples were collected from each batch, mixed and pooled for analysis. Moisture (930.15), total ash (942.05), CP (990.03), and EE (991.36) content of the feed samples were analysed in triplicate, according to the method described by the AOAC (2000). The feed ingredients and chemical composition of the basal diets are shown in Table 1.

Animal care for this experiment complied with procedures approved by the Bangladesh Livestock Research
Table 1. Feed ingredients and chemical compositions of the layer basal diets.

| Ingredients (%) | T1  | T2  | T3  | T4  |
|-----------------|-----|-----|-----|-----|
| Maize           | 48.00 | 48.00 | 48.00 | 48.00 |
| Rice polish     | 17.00 | 17.00 | 17.00 | 17.00 |
| Soybean meal    | 17.00 | 17.00 | 17.00 | 17.00 |
| Soybean oil     | 2.00  | 2.00  | 2.00  | 2.00  |
| Protein concentration | 5.00  | 4.50  | 4.00  | 3.50  |
| Fish meal       | 3.00  | 3.00  | 3.00  | 3.00  |
| Oyster shell    | 7.25  | 7.25  | 7.25  | 7.25  |
| Salt            | 0.30  | 0.30  | 0.30  | 0.30  |
| Vitamin-mineral premix<sup>a</sup> | 0.25 | 0.25 | 0.25 | 0.25 |
| L-Lysin         | 0.10  | 0.10  | 0.10  | 0.10  |
| DL-Methionine   | 0.10  | 0.10  | 0.10  | 0.10  |
| Moringa oleifera | -    | 0.50  | 1.00  | 1.50  |
| Total           | 100.00 | 100.00 | 100.00 | 100.00 |

<sup>a</sup>Provided the following nutrients per kg of diet: Vitamin A, 12,000 IU; Vitamin D3, 5000 IU; Vitamin E, 50 mg; Vitamin K3, 3 mg; Vitamin B1, 2 mg; Vitamin B2, 6 mg; Vitamin B6, 4 mg; Vitamin B12, 25 mg; biotin, 0.15 mg; pantothenic acid, 20 mg; folic acid, 2 mg; nicotinic acid, 70 mg; Fe, 66.72 mg; Cu, 41.70 mg; Mn, 83.40 mg; Zn, 66.72 mg; I, 0.834 mg; Se, 0.25 mg. T1: Control; T2: MOL 0.5%; T3: MOL 1%; and T4: MOL 1.5%.

### Determination of cholesterol

At the end of the experimental period, three birds were randomly selected from each replicated pen for a lipid profile analysis to be performed. Blood samples were carefully collected from the selected birds’ brachial vein and quickly transferred to centrifuge tubes. Care was taken to avoid any contamination or disturbance. The blood samples were then centrifuged for 15 min at 1500 g in a cold chamber at a temperature of 4 °C. The sera were then carefully removed, transferred to plastic vials and stored at −20 °C until such time as they were analysed for total cholesterol, total triglyceride, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C). The lipid profiles were carried out using a Snibe Automated Biochemical Analyser Biossays BC1200.

Yolk cholesterol was extracted according to the method described by Sharmin et al. (2017) with minor modifications. Five grams of boiled yolk sample (yolk was separated from albumen and fridge) was weighed in extracting tubes to which a solution of 10 ml of pyrogallol (6% in ethanol, w/v) and 1.0 ml of 5α-cholestanol (Sigma Aldrich, MO, USA) was added as an internal standard (1 mg/ml). After sonication for 10 min, 5.0 ml of 60% potassium hydroxide in deionised water was added. The extracting tube was heated at 80 °C for 1 h in a shaking water bath at 90 rpm (ST30, Nuve, Turkey). After cooling, 15 ml of 2% sodium chloride in deionised water and 15 ml of extracting solvent (hexane:ethylacetate = 85:15, v/v) containing 0.01% BHT (butylated hydroxytoluene) were added and mixed vigorously for approximately 2 min. The tubes were left undisturbed for 10 min, after which the upper layer was collected into a 50-ml volumetric flask. This extraction step was repeated two more times; extracts were combined and passed through anhydrous sodium sulphate, which acted as a filter to remove water. The volume of the extracts was made up to 50 ml with extracting solvent. A 10 ml aliquot of the extract was dried under N<sub>2</sub> gas and redissolved in 1.0 ml n-hexane (purity 95%). Extracts were filtered through a 0.25 μm membrane filter (Advantec, PTFE, Duran Germany) and analysed using gas chromatography (GC). Cholesterol separation was conducted using an Agilent 8890 Series (Agilent technology, USA) GC system equipped with a HP-5 capillary column (30 mm × 0.32 mm, 0.25 μm, Agilent Technologies, Santa Clara, CA, USA) and a flame ionisation detector (FID) (H<sub>2</sub>: 30 ml/min, air: 300 ml/min). The flow rate of the carrier gas (N<sub>2</sub>) was 3 ml/min. Injection and detection temperatures were both 260 °C. Cholesterol was separated on a column set at

### Performance parameters

Body weight (g/hen), feed intake (g/day/hen), laying percentage (%) and mortality were monitored throughout the experimental period. Body weight was measured at the beginning and the end of the experimental period. Feed intake and egg production were recorded daily. Eggs were collected and weighed every week, and egg production was expressed as average hen-day production, calculated as total eggs divided by the total number of hen-days.

### Measurements of egg traits

Sixty-four eggs (four eggs/replication) were collected randomly from each group and weighed individually to determine the external and internal egg quality. Egg weight was recorded first. Before the eggs were broken, eggshell breaking strength was measured using the Egg Force Reader. The components of the eggs (albumen, yolk, shell) were then manually separated and a tripod micrometer was used to determine the height of the albumen that immediately surrounds the yolk. Eggshell thickness was determined using a Vernier calliper. Yolk colour was measured using a Roche yolk colour fan. Yolk chemical composition, CP, moisture, EE and ash content were determined on the experimental last period.
250°C for 5 min and elevated to 260°C at a rate of 5°C/min. The column temperature was reduced to 250°C at a rate of 5°C/min and maintained for 2 min before the next injection. Cholesterol data processing was carried out with Open Lab CDS software, and peaks were identified by comparison with retention times of standards. The cholesterol standard was purchased from TCI (Tokyo, Japan) and used for cholesterol quantification. A 1 μl aliquot was injected into the GC column. Concentrations were calculated by comparing peak areas with those obtained from the standard solutions and were expressed as mg/100 g cholesterol in egg yolk.

Determination of fatty acid profile

Ether extraction of yolk was performed according to the AOAC method (991.36) at the Poultry Nutrition and Feed Analysis Laboratory of BLRI, Savar, Dhaka. The sample was then sent to the Institute of Food Science and Technology at Bangladesh Council of Scientific and Industrial Research, Dhaka, Bangladesh, for analysis of the fatty-acid profile of egg yolk. The fatty-acid profile of yolk samples (EE) was determined using a gas chromatograph (Shimadzu GC-14B, Japan) according to AOAC method 940.28 (1995). The gas chromatograph was equipped with a fused silica capillary column (15 mm × 0.25 mm × 0.25 μm, FAMEWAX, Crossbond® polyethylene glycol, Restek, Pennsylvania, USA) and an FID. The results were expressed in g/100 g total fatty-acid methyl esters.

Statistical analysis

The analytical measurements were performed in triplicates, and the results were presented as the average of the three analyses ± standard error mean. Statistical analysis was conducted using one-way ANOVA with the SPSS statistical package (IBM Corp., IBM SPSS Statistics for Windows, Version 16.0, Armork, NY, USA). Each pen was considered an experimental unit. A value of p < .05 was taken as statistically significant based on Duncan’s tests.

Results

Composition of M. oleifera and egg

As shown in Table 2, the moisture content of albumen was slightly higher in the T3 group of laying hens, which were fed a diet with 1.0% MOL, than in the 0%, 0.5% and 1.5% addition groups. Furthermore, birds fed diets with 0.5 and 1.5% MOL had a higher CP content in than those of the control group. The CF contents of albumen and yolk were higher in the eggs of the birds fed with lower doses of MOL (0.5% to 1.5%). However, no change was observed in the crude ash content of yolk.

Performance parameters

The final body weights for the control, T3 and T4 groups were not statistically different (p < .05). Surprisingly, the final body weight of the T2 group was significantly reduced. There was no difference (p < .05) in the egg production or the total number of eggs/periods among the experimental groups except Haugh unit (Table 3). Laying hens fed diets with 1.5% MOL had a significantly higher average daily feed intake than the hens fed on the other three diets. Hen’s fed moringa leaf meal showed similar egg mass but significantly lower egg production in 1.5% (T4 group).

External and internal egg quality parameters

It was observed that the egg weight, egg length, egg width, shape index and shell thickness of the eggs laid by hens fed diets with added MOL were similar during the experimental period (Table 4). These results indicate that feeding with moringa leaf meal up to 1.5% had no adverse effects on the external or internal qualities of eggs. Regarding the effect of time on eggshell thickness, a slightly higher value was recorded in the T4 group than in the other groups. However, albumen width and albumen index increased significantly after the addition of 0.5–1.5% MOL meal. Egg yolk height and yolk index significantly improved at the level of 0.5% MOL meal compared to

| Parameter (%) | T1 | T2 | T3 | T4 | SEM | p-Value |
|---------------|----|----|----|----|-----|---------|
| Moisture      | 51.57 | 53.06 | 52.20 | 51.37 | 0.23 | .35     |
| Crude protein | 14.90 | 15.63 | 16.59 | 15.71 | 0.38 | .51     |
| Ether extract | 8.45 | 8.83 | 8.91 | 8.88 | 0.18 | .68     |
| Crude fibre   | 0.70d | 0.85c | 0.90ab | 0.95a | 0.05 | .47     |
| Crude ash     | 2.50 | 2.70 | 2.10 | 2.32 | 0.08 | .01     |

Table 2. Effect of feeds ingredient on proximate composition of yolk and albumen.
the other two additive groups. The results also showed that the addition of MOL meal to the hens’ diet for a longer period of time (16th week) improved the eggshell breaking strength compared to the control (T1) group. Moreover, a significantly higher yolk colour value was observed in the 1.5% MOL group compared to the control group.

**Cholesterol content**

The cholesterol content of serum and egg yolk from laying hens fed with and without MOL were measured at the end of the experimental period (after 16 weeks) and are presented in Table 5. As can be seen, although the egg yolk from the experimental diets had a slightly higher fat content than the control, a significantly (p < .05) reduced cholesterol content was found in the T4 group (126.22 mg/100g yolk) compared to the control (145.91 mg/100g yolk). A significant improvement in the HDL-C status of serum was also observed. By contrast, the LDL-C levels were significantly reduced in all additive groups compared to the control.

**Yolk fatty-acid composition**

The effects of the dietary treatments on fatty-acid composition – saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFAs) – in egg yolk are shown in Table 6. Stearic acid and palmitic acid content were significantly higher (p < .05) in the additive groups than in the control, whereas myristic acid was significantly decreased in the T2 and T3 groups (p < .05). The dietary addition of MOL meal in laying hen diets significantly improved the alpha-linoleic fatty-acid content in the T2 and T4 experimental group, which showed values of 21.31 and 21.74%, respectively, compared to the control and T3 groups, with values of 19.48 and 19.46%, respectively. Significantly higher amounts of Ω3 fatty acid were found in the eggs of T3 and T4 group (with 1% and 1.5% MOL meal in the diet), with

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### Table 3. Performances of laying hens fed with dietary addition on *Moringa oleifera* (average values/group) 26–42 weeks of age.

| Parameter                        | T1     | T2     | T3     | T4     | SEM     | p-Value |
|----------------------------------|--------|--------|--------|--------|---------|---------|
| Initial body weight (g/hen)      | 1248   | 1254   | 1254   | 1252   | 1.31    | .27     |
| Final body weight (g/hen)        | 1278b  | 1270b  | 1278a  | 1280a  | 1.49    | .47     |
| Feed intake (g/day/hen)          | 86.33  | 84.00ab| 83.66ab| 86.02a | 0.44    | .53     |
| Egg production, hen day (%)      | 38.70  | 38.62  | 38.81  | 38.02  | 0.13    | .09     |
| Egg weight (g)                   | 46.56  | 47.86  | 47.37  | 47.33  | 0.26    | .021    |
| Egg mass (g)                     | 18.02  | 18.48  | 18.38  | 18.21  | 0.09    | .12     |
| Egg production (no./year)        | 152.82a| 151.64ab| 153.82a| 150.31b| 0.52    | .09     |
| Mortality (%)                    | 0.84a  | 0.50b  | 0.48b  | 0.55a  | 0.05    | .32     |

T1: Control; T2: *Moringa oleifera* leaf (MOL) 0.5%; T3: MOL 1%; and T4: MOL 1.5%. Values are expressed as mean ± standard error of means. Means represent four replicates, three eggs per replicate.

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### Table 4. External and internal egg quality traits after dietary addition on *Moringa oleifera*.

| Traits                        | T1     | T2     | T3     | T4     | SEM     | p-Value |
|-------------------------------|--------|--------|--------|--------|---------|---------|
| External egg quality traits   |        |        |        |        |         |         |
| Egg length (cm)               | 5.04   | 5.45   | 5.30   | 5.51   | 0.02    | .039    |
| Egg width (cm)                | 3.90   | 3.95   | 4.19   | 4.33   | 0.01    | .261    |
| Shape index (%)               | 77.38  | 78.34  | 79.05  | 78.58  | 0.42    | .443    |
| Shell thickness (mm)          | 0.37   | 0.39   | 0.41   | 0.42   | 0.01    | .007    |
| Shell weight (g)              | 5.11b  | 5.77b  | 5.67b  | 5.88a  | 0.09    | .052    |
| Internal egg quality traits   |        |        |        |        |         |         |
| Albumen height (cm)           | 0.53ab | 0.69a  | 0.65a  | 0.68a  | 0.01    | .322    |
| Albumen width (cm)            | 6.94b  | 7.69a  | 8.06a  | 7.69a  | 0.11    | .131    |
| Albumen index (%)             | 7.68b  | 9.01a  | 8.05a  | 8.88a  | 0.17    | .654    |
| Yolk height (cm)              | 1.42b  | 1.71a  | 1.65a  | 1.59ab | 0.03    | .252    |
| Yolk width (cm)               | 3.71b  | 4.01a  | 4.01a  | 4.11a  | 0.03    | .713    |
| Yolk index (%)                | 37.12ab| 42.89a | 41.14ab| 38.50ab| 0.69    | .181    |
| Yolk colour                   | 7.32b  | 8.13ab | 8.66ab | 9.00a  | 0.20    | .001    |
| Egg shell break strength/kg/cm³| 0.29b | 0.36a| 0.34a| 0.33a | 0.30    | .546    |

T1: Control; T2: *Moringa oleifera* leaf (MOL) 0.5%; T3: MOL 1%; and T4: MOL 1.5%.

Values are expressed as mean ± standard error of means. Means represent four replicates, three eggs per replicate. a,b,cMean with different superscripts within same rows are significantly different (p < .05) by Duncan’s multiple range tests (DMRT).
values of 1.35 and 1.46%, respectively, and total n-6 content was significantly higher \((p < .05)\) in the T2 and T4 groups (23.02 and 23.65%, respectively) compared to the control and T3 groups (20.61 and 20.99%, respectively).

### Discussions

This experiment was designed to evaluate the impact of MOL meal on the quality, cholesterol content and fatty-acid profile of native laying hens’ eggs. Moringa leaves contain phenolic and flavonoid compounds, which can affect fatty-acid composition (Sreelatha and Padma 2009). The chemical composition results revealed that the CP content of egg yolk did not differ significantly across the groups. This is consistent with the findings of Naber (1979), who observed that egg protein characteristics are not influenced by diet. Cobos et al. (1995) supplemented layer diets with lipids and also found no differences in egg yolk protein content.

Dietary addition of MOL meal had an effect on performance parameter. The egg production was not significantly different \((p > .05)\) across the groups fed diets with different levels of MOL, which indicates that the addition of MOL up to 1.5% to the diet of native laying hen has no detrimental effect on egg production. Hens fed with MOL 1.5% showed higher egg mass but slightly reduced egg number. At the same time, egg production did not affect between the control and MOL groups. However, Kakengi et al. (2007) observed that substitution of sunflower cake with MOL 5% in layers’ diet improved egg number and egg mass, and Teteh et al. (2013) showed that 2% MOL could affect laying rate and egg weight. Abou-Elezz et al. (2011) reported that egg production decreased with 15% MOL added to diet, possibly due to anti-nutritional factors. The results of the current study suggest that the addition of MOL up to 1.5% had no negative effect on mortality of birds. Several researchers have found that the production performance of poultry varies widely depending on processing procedure, feed composition, as well as the level of additives.

### Table 5. Serum and yolk cholesterol levels in hens fed with *Moringa oleifera* added feeds.

| Traits                  | T1          | T2          | T3          | T4          | SME | p-Value |
|-------------------------|-------------|-------------|-------------|-------------|-----|---------|
| Total cholesterol (mg/dL) | 157.24^a    | 148.98^b   | 145.11^c   | 123.47^d   | 3.74| .17     |
| HDL cholesterol (mg/dL)  | 25.19^f     | 26.23^g    | 29.09^h    | 36.21^i    | 1.34| .48     |
| LDL cholesterol (mg/dL)  | 99.21^a     | 87.22^b    | 88.09^c    | 73.43^d    | 2.87| .87     |
| Triglyceride (mg/dL)     | 775.09^a    | 761.32^b   | 758.42^c   | 683.22^d   | 3.15| .11     |
| Yolk cholesterol (mg/100 g) | 145.91^a   | 136.32^b   | 132.03^b   | 126.22^a   | 2.18| .33     |

T1: Control; T2: *Moringa oleifera* leaf (MOL) 0.5%; T3: MOL 1%; and T4: MOL 1.5%. HDL: high-density lipoprotein; LDL: low-density lipoprotein. Mean with different superscripts are significantly different; \((p < .05)\) by Duncan’s multiple range tests (DMRT).

### Table 6. Effect of *Moringa oleifera* as natural feed additives on fatty-acid profile of egg.

| Parameters (%)                  | T1          | T2          | T3          | T4          | SEM | p-Value |
|-------------------------------|-------------|-------------|-------------|-------------|-----|---------|
| Myristic acid (C14:0)         | 0.37^b      | 0.28^b      | 0.27^b      | 0.49^a      | 0.08| .05     |
| Palmitic acid (C16:0)         | 22.29^b     | 23.03^a     | 24.58^a     | 24.29^a     | 1.25| .18     |
| Stearic acid (C18:0)          | 3.56^a      | 5.74^a      | 4.57^b      | 5.51^a      | 0.25| .18     |
| Arachidic acid (C20:0)        | 0.23^a      | 0.22^a      | 0.10^b      | 0.11^b      | 0.19| .05     |
| Myristoleic acid (C14:1)      | 0.03^b      | 0.67^a      | 0.10^b      | 0.20^b      | 0.09| .02     |
| Palmitoleic acid (C16:1)      | 1.25^b      | 2.37^b      | 2.89^a      | 1.08^b      | 0.21| .08     |
| Oleic acid (C18:1)            | 42.09^b     | 43.85^b     | 45.76^a     | 45.36^a     | 0.68| .01     |
| Eicosenoic acid (C20:1)       | 0.00        | 0.76        | 0.67        | 0.00        | 0.13| .38     |
| Eicosatetraenoic acid (C20:3) | 0.64        | 0.73        | 0.83        | 0.88        | 0.42| .54     |
| Linolenic acid (C18:3), ω–3   | 0.22        | 0.22        | 0.28        | 0.28        | 0.18| .48     |
| Eicosapentaenoic acid (C20:5), ω–3 | ND          | ND          | ND          | ND          | ND  | ND      |
| Docosahexaenoic acid (C22:6), ω–3 | 0.18       | 0.21        | 0.24        | 0.30        | 0.41| .46     |
| Linoleic acid (C18:2), ω–6    | 19.48^b     | 21.31^a     | 19.46^b     | 21.74^a     | 1.04| .23     |
| Arachidononic acid (C20:4), ω–6 | 0.08       | 0.45        | 0.18        | 0.15        | 0.78| .13     |
| ΣSFA                          | 26.45^a     | 29.27^a     | 29.52^a     | 30.4^a      | 0.32| .37     |
| ΣMUFA                         | 43.37^a     | 47.65^a     | 49.42^a     | 46.64^a     | 0.69| .32     |
| ΣPUFA                         | 20.61^a     | 23.02^a     | 20.99^a     | 23.65^a     | 0.46| .34     |
| Σω–3                          | 1.04^b      | 1.16^b      | 1.35^a      | 1.46^a      | 0.05| .31     |
| Σω–6                          | 19.57^b     | 21.76^a     | 19.64^b     | 21.89^a     | 0.31| .21     |

T1: Control; T2: *Moringa oleifera* leaf (MOL) 0.5%; T3: MOL 1%; and T4: MOL 1.5%. Results are expressed as a percentage of the total fatty acids. Values are expressed as mean±standard error of means. Means represent four replicates, three eggs per replicate. Mean with different superscripts within the same rows are significantly different \((p < .05)\) by Duncan’s multiple range tests (DMRT). SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; ω–3 = total omega-3 fatty acid; ω–6 = total omega-6 fatty acid.
included in diet and environmental hygiene (Uuganbayar et al. 2005; Windisch et al. 2008; Park et al. 2010).

The egg quality data for the laying hens that fed to the experimental diets are shown in Table 4. Laying hens fed with and without MOL added to diets showed no significant differences in terms of egg weight, egg length, egg width and eggshell thickness. This supports the results of Bhatnagar et al. (1996) and Olugbemi et al. (2010), who found that supplementation of MOL up to 10% did not affect egg weight. Cho et al. (2010) observed no significant difference in eggshell thickness between the eggs of hens fed fermented spent mushroom substrate added to feed and a control. In addition, Mužić et al. (2005) found that feed supplementation with *Lentinus edodes* did not affect egg quality. In a similar study with green tea, Uuganbayar et al. (2005) reported that shell thickness was reduced by the addition of different levels (0.5–2.0%) of green tea. The results of this experiment revealed that eggshell breaking strength was influenced by MOL added in the diet. In a previous study, Panda et al. (2008) found that eggshell quality improved when probiotics were given to laying hens. Albumen height is an important parameter for evaluating albumen quality and egg freshness. In our study, albumen height was increased in response to an increase in MOL added to feed. The results are in partial agreement with those of Yamane et al. (1999) and Biswas et al. (2000), who reported that the inclusion of Japanese green tea in layers’ diet improved the Haugh unit score and albumen height of eggs. In contrast, Uuganbayar et al. (2005) reported no changes in the albumen index and Haugh unit in alfalfa extract- and green tea-fed layer eggs. The yolk colour value increased linearly as the MOL addition increased due to a high concentration of xanthophylls, which is in agreement with the findings of Abou-Elezz et al. (2011). Pasaporte et al. (2014) reported that a strong yolk colour is a preferable trait for consumers. However, Lu et al. (2016) found that a higher level of MOL (15%) was needed to improve egg albumen quality and yolk colour. This variation might be due to differences in botanical origin, animal species and animal age.

The serum lipid profile of the hens was also favourably altered by MOL added to feed (Table 5). A highly significant ($p < .05$) reduction in serum cholesterol levels was noted upon feeding with MOL 1.5% added to the diet, which may be due to hypocholesterolemic effect of MOL. Among the additive groups, MOL 1 and MOL 1.5% were found to be most effective in reducing yolk cholesterol levels. The hypolipidemic effect is due to the high saponin content in MOL (Foidl et al. 2001). Teteh et al. (2013) found saponin to be the most active anti-nutritional substance in these leaves. Since plant saponin binds with cholesterol, as mentioned above, it can be expected that saponin reduces the level of cholesterol in the body. Eskandar et al. (2015) also reported that some saponins can prevent hypercholesterolaemia, a phenomenon that results from complex formation with cholesterol. Complex formation between cholesterol and starfish saponin has been investigated for the past years (Sharmin, et al. 2020). Our results showed that the addition of MOL at a level of 1.5% to feed reduced the cholesterol levels in egg yolk. However, Bidura et al. (2020) found 2–6% MOL was needed to reduce cholesterol levels. Our results showed that high-density lipoprotein content increased, and low-density lipoprotein and triglycerides were reduced with the addition of MOL to the layers’ diets. According to Patil et al. (2010), the reduction of cholesterol and triglycerides by alkaloids is partly due to a reduction in lipogenic enzyme activity and an increase in the excretion of bile acids in the faeces.

Several studies have been published since the last century on the effects of feeding flaxseed meal, flaxseed oil or oils rich in ω-3 PUFA on egg performance and the fatty-acid composition of eggs (Van Elswyk 1997). The concept of enriching eggs with ω-3 PUFA through dietary manipulation is still gaining the attention by the researchers. In the present study, the addition of MOL meal to the hens’ diet improved the fatty-acid profile of eggs, especially the ω–3 PUFA content. The fatty-acid composition of eggs is closely related to fatty acids in feed. According to Naber (1979), the main egg component (lipids) may be easily changed by dietary manipulation. Hall and Mckay (1993) found that egg lipid content is influenced by age in domestic fowl. The $\omega–3$ content was increased in the yolk as MOL increased in the diet, although slightly lower concentrations of DHA were found in diets containing 0.5% MOL. Surprisingly, EPA was found in only trace amounts or under the detection limit in this study. This may be because, as Herber and Van Elswyk (1996) suggested, DHA is more readily incorporated into membranes than EPA. However, the inclusion of MOL at levels of 0.5 and 1.5% in laying hens’ diets were significantly increased the PUFA content, and these PUFAs has the ability to control cholesterol. *Moringa oleifera* leaves are known to have very little anti-nutritional content and have been used in ruminant rations (Soliva et al. 2005) and other
poultry or monogastric animal feed. Thus, supplementing hen diets with this natural source of fatty acids is a promising solution for increasing the ω–3 PUFA content of eggs.

**Conclusion**

The results obtained in this study revealed that the addition of different levels of MOL in the hens’ diets had no effect on production performance in terms of egg mass, egg nutritional composition and external quality parameters of egg. Fatty-acid composition, especially ω–3 fatty acid, was improved, and egg cholesterol concentration was reduced at the higher levels, i.e. 1.5% of MOL added to diet. Therefore, it can be concluded that diets up to 1.5% dietary MOL could be used in layers’ diets. However, further follow-up research is required to know the mechanisms of various pathways of reducing cholesterol and enrichment of ω–3 fatty-acid content in the egg yolk.

**Ethical approval**

The experimental protocols, bird management and sample collection were reviewed and approved by the Animal Care Committee of Bangladesh Livestock Research Institute, Savar, Dhaka, Bangladesh (BLRI/SP RDP-ARDDL/2019).

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

**Funding**

This research was supported by the ‘Strengthening of Poultry Research and Development Project’, Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka under the Ministry of Fisheries and Livestock, Bangladesh.

**Data availability statement**

All data used in this manuscript are provided in table and as supplementary data. The data that support the findings of this study are available from the authors upon reasonable request.

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