Effect of *Moringa oleifera* leaf powder in diets on laying hens performance, β-carotene, cholesterol, and minerals contents in egg yolk

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Abstract. The present study was designed to investigate the influence of *Moringa oleifera* on Laying Hens Performance, β-carotene, cholesterol, and minerals contents in egg yolk. Two hundred and forty 30 weeks of healthy laying hens with homogeneous body weight in a complete randomized design with four treatments and 6 replications. Laying hens were randomly divided into four groups: M0: diets without administration of *Moringa oleifera* leaves, M1: diets with 2% *Moringa oleifera* leaves; M2: diets with *Moringa oleifera* leaves 4%; and M3: diets with 6% *Moringa oleifera* leaves, respectively. Each treatment consisted of six replication cages with 10 birds randomly assigned to each cage. This study showed that administration of the *Moringa* leaves powder were increased significantly different on egg productions, egg mass, feed efficiencies, yolk color, shell thickness, Mg and Ca contents in eggshell, but not the efficiency of feed consumption. The administration of 2-6% *Moringa* leaves powder in diets results in significantly lower yolk cholesterol contents. It was concluded that supplementation of 4-6% *Moringa* leaves powder in diets, increased egg production, egg mass, feed efficiencies, yolk color, shell thickness, β-carotene, Mg and Ca contents in the yolk, but decreased yolk cholesterol contents in laying hens.

1. Introduction

High consumer demand for food safety, such as eggs with the dangers of antibiotic residues, has made researchers look for other ways to replace antibiotics by creating animal feed that not only provides for the nutritional needs of poultry (energy, amino acids, vitamins, and minerals) but also safe for consumers. The use of antibiotics or antimicrobials as additives in poultry feed has lasted more than 40 years. The antibiotic is used as a growth promoter in a relatively small amount but can improve feed efficiency and productivity of poultry so that with the use of these additives, farmers can benefit more.

In Indonesia, the use of Antibiotic Growth Promoter (AGP) has been banned since 2018, even in some developed countries since the past has banned the use of antibiotics as additives in animal feed. This is due to two main factors, namely (1) the possibility of residues from antibiotics that will be toxic to consumers, and (2) antibiotics can create micro-organisms that are resistant in the human
body. With the negative impact of the use of AGP, experts began to look for replacements focused on natural ingredients, such as the efficacy of herbs. Herbs that have received much attention lately are *Moringa oleifera* leaves. *Moringa* leaves contain 7 times vitamin C orange, 4 times Calcium milk, 4 times carrot vitamins, 3 times Potassium banana, and 2 times Yogurt protein [18]. The leaves contain quality proteins so they can compete with soybeans because *Moringa* leaves contain all essential amino acids in the right proportions. Carbohydrates, vitamins, minerals, and fatty acids (essential and non-essential), are very high.

The use of *Moringa* leaves in feed laying hens is expected to be able to improve egg quality and feed efficiency. Eggs are a product of laying hens that can experience a rapid loss of quality during the period of collection and storage before consumption. Thus, increasing and extending the shelf life of eggs is very important for farmers. Various efforts have been made to increase the thickness of eggshells and reduce cholesterol levels in eggs. Because of the high cholesterol content in egg yolks is an inhibiting factor for consumers to consume eggs. Reducing cholesterol in egg yolk and increasing the yolk color will benefit the poultry industry and public health [38,40].

The use of *Moringa oleifera* leaves as a mineral source for eggshell thickness, and sources of provitamin A and natural beta-carotene to increase the color of egg yolks can be used as an alternative natural fortification feed to overcome vitamin A deficiency, and natural provitamin A and beta-carotene compounds safer to consume than synthetic vitamin A [1,9,7,16]. The phytochemical compounds contained in *Moringa* include flavonoids, saponins, tannins, and several other phenolic compounds that have antimicrobial activity [1,15]. Phytochemical compounds in herbal plants have antimicrobial and antioxidant properties, can be used in the treatment of diseases in poultry as a substitute for antibiotics [5,50,53,39]. The antimicrobial activity of phytochemical compounds in *Moringa* leaves has been investigated and is likely to use it to develop new antimicrobial drugs [19,21,26]. Several previous studies confirmed that extracts or compounds isolated from *M. oleifera* had antioxidant, anti-carcinogenic, anti-diabetic, anti-inflammatory, and anti-hypertensive properties, as well as the ability to protect against liver damage [10,18,19,21,27].

The use of *Moringa* can be an alternative to reduce fat and cholesterol levels in eggs and meat of poultry [32,33,42,20] and replace antibiotics, because of the rich and diverse phytochemical compounds, and efficacious as an antibacterial agent and can increase immunity [27,19,70]. It has been thought to control many diseases, as an endothelial vasodilator and inhibits the activity of HMG Co-A (3-hydroxy-3-methyl-glutaryl Co-A), thereby inhibiting lipase lipoprotein which is responsible for plasma lipid hydrolysis and has health benefits in connection with their ability to change the fat profile and carcass yield of broilers [3,20,45].

Previous research found that the total carotene in *Moringa* leaves was 24735 μg/100 g. The high content of beta-carotene is very good for increasing the color and content of β-carotene in egg yolks. Beta-carotene supplementation is effective in increasing serum beta-carotene concentrations with little effect on serum retinol concentrations, reducing serum oxidative stress, and increasing biological antioxidant capacity [51]. β-carotene is provitamin A, the highest carotenoid in food and human tissue. This provides a number of beneficial functions for livestock, including humans, because of their ability to produce vitamin A [61]. When added to feed, bioactive compounds along with other phytochemicals can improve egg quality and have a positive effect on chicken health and performance. *Moringa* leaves can be used as an effective feed supplement for poultry to encourage results in relation to total weight gain and feed efficiency, reduce abdominal fat and cholesterol in race chicken and laying hens [8,20,30]. Herbal extracts in drinking water increase egg production and can reduce cholesterol in serum and egg yolks in laying hens [14,33,38,69].

Based on this, the study was conducted to determine the effect of *Moringa* leaves in the diet on egg production, content of β-carotene, cholesterol, mineral in egg yolks of Lohmann Brown laying hens up to 30-weeks old.
2. Material and Methods

**Animals, treatments, and experimental design:** This study was a feeding trial using two hundred and forty 30 weeks of healthy laying hens with homogeneous body weight 1685 ± 18.37 grams obtained from commercial poultry farms in a complete randomized design with four treatments and 6 replications. Laying hens were randomly divided into four groups: M0: diets without administration of *Moringa oleifera* leaves, M1: diets with 2% *Moringa oleifera* leaves; M2: diets with 4% *Moringa oleifera* leaves; and M3: diets with 6% *Moringa oleifera* leaves, respectively. Each treatment consisted of six replication cages with 10 birds randomly assigned to each cage at 150×70×45 cm (length×width×height). Each experimental diet was in the form of mash and birds have free access to feed and water during the experiment.

All diets were isonitrogenous and isoenergetic. Diets are formulated to meet nutritional requirements for poultry [43] for 12 weeks of trials. The ingredients and chemical composition of the feed are shown in Table 1.

**Table 1.** The ingredient and calculated nutrient content of the feed of laying hens up to 30 weeks old

| Basal Diets | M0     | M1     | M2     | M3     |
|------------|--------|--------|--------|--------|
| *Ingredients (%):* |       |        |        |        |
| Yellow corn | 61.00  | 60.00  | 59.60  | 58.10  |
| Rice bran  | 15.40  | 14.40  | 13.50  | 13.50  |
| Soybean    | 7.00   | 8.00   | 7.70   | 8.00   |
| Fish meal  | 10.80  | 9.80   | 9.40   | 8.70   |
| Coconut oil| 0.32   | 0.20   | 0.10   | 0.14   |
| *Moringa oleifera* | 0.00   | 2.00   | 4.00   | 6.00   |
| Mineral-B12 | 5.48   | 5.60   | 5.70   | 5.56   |
| **Total**  | 100    | 100    | 100    | 100    |

**Chemical composition (**):**

| Metabolizable energy (kcal/kg) | 2901.7 | 2900.9 | 2901.4 | 2901.5 |
| Crude protein (%)              | 16.02  | 16.07  | 16.04  | 16.08  |
| Crude Fiber (%)                | 3.53   | 3.65   | 3.74   | 3.94   |
| Ether Extract (%)              | 6.93   | 6.83   | 6.61   | 6.68   |
| Calcium (%)                    | 3.56   | 3.65   | 3.76   | 3.74   |
| Phosphor (%)                   | 1.30   | 1.28   | 1.28   | 1.24   |
| Arginine (%)                   | 1.15   | 1.17   | 1.19   | 1.22   |
| Histidine (%)                  | 0.41   | 0.42   | 0.42   | 0.43   |
| Isoleucine (%)                 | 0.85   | 0.86   | 0.87   | 0.87   |
| Leucine (%)                    | 1.58   | 1.59   | 1.61   | 1.62   |
| Lysine (%)                     | 1.11   | 1.11   | 1.11   | 1.12   |
| Methionine (%)                 | 0.38   | 0.38   | 0.38   | 0.37   |
| Phenylalanine (%)              | 0.82   | 0.83   | 0.85   | 0.86   |
| Threonine (%)                  | 0.73   | 0.73   | 0.74   | 0.75   |
| Tryptophan (%)                 | 0.18   | 0.19   | 0.19   | 0.20   |
| Valine (%)                     | 0.87   | 0.87   | 0.88   | 0.89   |

*) The mineral-B12 composition per 10 kg contains: Calcium: 49%; Phosphor 14%; Iron: 40000 mg; Manganese: 27500 mg; Mg: 27.500 mg; Zinccum: 25 mg; Vit-B12: 4.50 mg and Vit D3: 500000 IU. (PT. Eka Farma. Deptan RI No. D 8109127 FTS)

**) Based on calculation according to Scott *et al.* (1982)

**Process of making flour *Moringa leaves***: *Moringa oleifera* leaves were dark green, thinly sliced, and dried at room temperature for 1-2 days, then dried in an oven at 50°C for 24 hours. Then the *Moringa* leaves were pounded into fine powder.

**Live performance.** Continuous lighting and access to feeding and water were provided during the experiment. The hens were weighed at the start (age 30 weeks) and the end (age 42 weeks) of the
experiment. Eggs were collected every day and egg production was expressed on a day-to-day basis (% of chicken days). Individual egg weights were recorded and then used to calculate the average egg weight for all trial periods. The total egg mass was calculated by multiplying the weight of the egg with egg production. Feed intake was measured based on cages (hens) every week. Daily feed intake per bird was calculated based on the total cage intake for the entire trial period and the number of days in all periods. Feed conversion ratio (gram feed/gram egg mass) for all periods was calculated based on the cage of egg production, egg weight, and feed consumption. Egg quality parameters were measured using a multi-egg tester.

Quality of eggs and yolk minerals. Eggs are collected and labeled every day at 08.00 and 14.00 hours during the trial period. The percentage of egg production was calculated. Examination of egg and eggshell quality (shell weight, eggshell thickness, egg yolk weight, and albumin, egg yolk color, albumen, and egg yolk height) were carried out at the end of the experiment. For this purpose, two eggs placed between 08.00 and 12.00 hours were taken randomly from each group on the 42nd day of the week (a total of 12 eggs per group during the experiment). Eggs were weighed individually and the specific gravity of the egg, as the index of the thickness of the shell, was measured. After the egg was broken on the EQM measurement stand, the albumen and yolk height was measured. The intensity of the yolk color was evaluated and recorded according to Roche's egg yolk fan method. Albumen's weight was calculated by reducing the weight of the yolk and shells of the overall egg weight. To measure the weight of the shell, the eggshell was cleaned from the albumen which attaches to the membrane was removed; Eggshells were then dried at room temperature and expressed as a percentage of all eggs. Evaluation of egg quality was carried out on individual eggs, similar to the weight of the eggs measured. The mineral concentration of egg yolk (Ca and Mg) was measured by the AAS method. Egg yolk samples were analyzed for dry matter content (DM) by drying the sample at 105°C for 24 hours in a forced-air oven. Crude proteins were determined by the Kjeldahl method[11] and crude fat was determined by the Soxhlet method [11].

The cholesterol content of egg yolk is evaluated from a sample of egg yolk. These samples are mixed until smooth and homogeneous then stored for cholesterol analysis. Samples of egg yolks frozen in sample bottles were used to determine yolk total cholesterol levels with commercial kits [23]. Cholesterol levels were analyzed according to the Lieberman-Burchard method [34]. The cholesterol of egg yolk was calculated and expressed in milligram/gram of egg yolk. Measurement of Beta-carotene content: namely by entering as much as 0.10-0.50 into the centrifuge tube then adding 5 ml of acetone and 5 ml of pure petroleum ether (PE), then stirring evenly and centrifuging for 5 minutes at a speed of 3000 rpm. The supernatant was taken and stored in a test tube, while the sediment was added 5 ml of acetone and then centrifuged again until the supernatant was colorless (the supernatant becomes clear). The collected supernatant was then inserted into a separator tube and rinsed with 15 ml of distilled water and repeated three times. The rinsing water was then removed and the top of the tube (clear) was inserted into the test tube, then 1 g of NaSO, was added, then vortex. Then the clear part was taken and the PE solution was added until the volume becomes 10 ml and then read on the spectrometry absorbent (abs) at λ = 450 nm. Total carotene (ug/100 g) = (total volumexabsx100)/(0.2 x sample weight).

Statistical analysis. All data were analyzed by ANOVA to determine the difference between treatments. If differences were found, further analysis is carried out with Duncan's multiple range test.

3. Results
The results are presented that the final body weight, feed consumption, egg production, and feed efficiency in groups fed the experimental diets are shown in Table 2. The treated laying hens exhibited higher significantly different (p<.05) on egg production, egg mass, egg numbers, and feed efficiencies (feed consumption/egg mass) than the control bird. No significant differences (p>.05) in the final body weight and feed consumption were observed among the dietary treated groups. Laying hens in M2 and M3 groups corresponded with higher egg production, egg mass, egg numbers (p<.05) and better feed efficiencies (p<.05) than those Group M0 and M1.
Table 2. Effect of *Moringa oleifera* leaves powder in diets on egg production and feed efficiencies in laying hens up to 30 weeks of ages

| Variables                          | Groups¹ | SEM²  |
|-----------------------------------|---------|-------|
| Initial body weight (g/hens)      | M0      | M1    | M2    | M3    |       |
|                                   | 1682.35a| 1690.71a | 1685.04a | 1687.42a | 18.631 |
| Final body weight (g/hens)        | 1713.81a| 1742.63a | 1736.28a | 1745.35a | 20.062 |
| Feed consumption (g/hen/d)        | 128.46a | 127.35a | 117.47a | 115.60a | 4.072  |
| Egg production (%)                | 88.37a³ | 88.75a  | 90.92b  | 91.17b  | 0.306  |
| Egg mass (g/hens)                 | 55.61b  | 56.10b  | 57.30a  | 57.51a  | 0.285  |
| Feed efficiency (feed consumption/egg mass) | 2.31a  | 2.27a  | 2.05b  | 2.01b  | 0.017  |

¹M0: The diet without *Moringa* powder (control); M1: The diet with 2% *Moringa* powder; M2: The diet with 4% *Moringa* powder; and M3: The diet with 6% *Moringa* powder, respectively.

The effects of dietary supplementation with *Moringa* leaves powder on external egg quality characteristics of laying hens are presented in Table 3. The treated laying hens exhibited higher significantly different (p<.05) on shell thickness, Mg and Ca in eggshell, yolk color and yolk cholesterol than the control of laying hens. No significant differences (p>.05) in the albumen, haugh unit, egg shape, and specific gravity, Mg and Ca in egg yolk were observed among the dietary treated groups. Laying hens in M2 and M3 groups correspond with higher shell thickness, Mg and Ca in eggshell and yolk color, but lowering in yolk cholesterol (p<.05) than those Group M0 and M1.

In addition, the results show that an additional *Moringa* leaves powder (group M2 and M3) in diets resulted in a significant (P<0.05) increase in shell thickness of birds were: 22.28% and 26.08%, respectively higher than control (Table 3). The mineral content of Ca in the eggshell of the M2 and M3 groups were: 18.27% and 18.23% significantly different (P<0.05) higher than control. The mineral content of Ca in yolks group M2 was 21.15%, followed by group M3 was 22.89%, respectively significantly different (P<0.05) higher than group M0 (control).

Table 3. Effect of *Moringa oleifera* leaves powder in diets on external egg quality, yolk color and yolk cholesterol in laying hens

| Variables                      | Groups¹ | SEM²  |
|--------------------------------|---------|-------|
| Shell thickness (mm)           | M0      | M1    | M2    | M3    |       |
|                                | 0.395b  | 0.408b | 0.483a | 0.498a | 0.024  |
| Magnesium in eggshell (ppm)    | 0.3925a | 0.3901a | 0.3953a | 0.3948a | 0.117  |
| Magnesium in egg yolk (ppm)    | 0.1472a | 0.1469a | 0.1502a | 0.1527  | 0.103  |
| Calcium in eggshell (ppm)      | 27.821b | 28.037b | 32.905a | 32.892a | 1.073  |
| Calcium in egg yolk (ppm)      | 12.138b | 12.207b | 14.705a | 14.916b | 0.509  |
| Yolk colour (1-15)             | 6.35b   | 8.21b  | 8.35a  | 8.51a  | 0.513  |
| β-carotene (µg/100 g)          | 129.35b | 168.19a | 179.37a | 185.39a | 10.502 |
| Egg yolk cholesterol (mg/g yolk) | 8.92a | 8.65a  | 7.14b  | 7.29b  | 0.116  |

¹M0: The diet without *Moringa* powder (control); M1: the diet with 2% *Moringa* powder; M2: the diet with 4% *Moringa* powder; and M3: the diet with 6% *Moringa* powder, respectively.

²SEM: standard error of treatment means

The content of β-carotene in egg yolk increased significantly (P<0.05) in the presence of *Moringa* leaf flour in feed at Group M1, M2, and M3 (Table 3). The β-carotene content in hens in Group M1, M2, and M3 was increased significantly different (P<0.05) than Group M0. Dietary *Moringa* leaf
powder increased yellowness in yolk color (P<0.05) and decreased yolk cholesterol in egg-laying hens. The egg yolk color in M2 and M3 hen groups were increased by 31.50% and 34.02% than group M0. Yolk cholesterol content was tended to decrease by about 19.96% and 18.27% (P<0.05) in M2 and M3 groups rather than group M0, respectively.

4. Discussion

There was no significant difference in feed consumption between treatments. The same was reported by [57] and [20] which shows that *M.oleifera* does not have a toxic effect or contains factors that limit intake which is opposite to the absorption of nutrients. However, Ayssiwede *et al.* [12] reported that the use of 24% *Moringa* leaves in the ration was a decrease in feed intake.

The use of *Moringa* in real feed increases feed efficiency in groups M2 and M3 rather than M0 and M1 groups. *Moringa* leaf extract can be useful to be used as an effective feed supplement in poultry to improve feed efficiency in poultry [8,57]. The main way of action of this active ingredient is the inhibition of microbial pathogens and endotoxins in the intestine and increased pancreatic activity, resulting in better metabolism and utilization of nutrients [68,28]. *Moringa oleifera* extract was found to be more effective in controlling gram-negative bacteria tested than gram-positive bacteria [19].

According to [26], the antimicrobial activity of plants is mainly caused by the presence of secondary metabolites. Plants are rich in various secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties. These active compounds in the digestive tract of poultry will be able to help absorb nutrients. As reported by [2], that herbal active compounds (Garlic) can increase villous height and crypto depth, and reduce the epithelial thickness and the number of villous cells in the duodenum, jejunum, and poultry ileum. Increased height of villi, as well as the thickness of epithelium in the duodenum, jejunum and ileum, will increase nutrient uptake. Results from [8] found that the administration of *Sauropus* leaf extract in drinking water can significantly improve feed efficiency in laying hens.

Increased egg production and egg weight in chickens given *Moringa* leaf is caused by the presence of phytochemical compounds on *Moringa* leaves, as reported by [53] that *Moringa* leaves are also a source of vitamin A, riboflavin, nicotinic acid, folic acid, pyridoxine, ascorbic acid, β-carotene, calcium, iron, and α-tocopherol. The same thing was reported by [8] that *Moringa oleifera* plants are also efficacious to strengthen the heart, eyes, brain, bile, and immune system. Supplementation of *Moringa* flour affects egg productions and egg mass [24] and egg yolk bioactive compounds [4]. Antioxidants, flavonoids, carotenoids, amino acids, proteins, and energy levels that result in a decrease in egg water content can be the reason for increasing nutrient density in egg yolk [45,44].

According to [37], *Moringa oleifera* seed flour inclusions can improve the quality of external eggs, and increase fatty acid profiles. Some of the results of research on the effect of herbal extracts in poultry were carried out by [8,30] that administration of *Moringa* leaf can significantly increase body weight and feed efficiency in broiler chickens. Siti *et al.* [59,60,41] reported that *Moringa* leaves can increase egg production and egg quality, but decreasing yolk cholesterol in laying hens. Bidura *et al.* [14] reported that administration of 5 cc/100 cc herbal extracts (*Sauropus* and garlic leaves) in drinking water increased egg production and total egg weight. The results of [69] reported that the addition of garlic powder at level 5 or 10 g/kg in feed, showed an increase in chicken egg production. The same was reported by [33] that laying hens given garlic (2-8%) showed higher egg productions.

Increased egg production and feed efficiency due to the role of *Moringa* in the digestive tract of chickens can increase feed digestibility. As reported by [31,45], that plant extract supplements can increase the digestibility of nutrients in the digestive tract of poultry. Herbal extracts (Garlic) can increase the activity of pancreatic enzymes and microenvironmental conditions for better utilization of nutrients in mice [54]. *Moringa oleifera* leaves are known to be very poor in anti-nutritional content and have been used in ruminant rations [62] and other poultry or monogastric. This result is contrary to that reported by [64] that the high use of *Moringa* leaves in feed can cause increased levels of saponin as an antinutrient which can reduce digestion and absorption of nutrients, especially lipids.
The yolk colors in Groups M1, M2, and M3 are significantly higher compared to the control. The increase in egg yolk color in this study showed that *Moringa* leaves are rich in vitamin A or carotenoid pigments which are efficiently absorbed and utilized by chickens. The yellow color increase can be attributed to the carotenoid content of *Moringa* leaf powder. In this study, the carotene of *Moringa* was: 24735 µg/100 g. Besides that, *Moringa* pods are enriched with carotenoids and flavonoids, which are powerful natural antioxidants that can modify the levels of β-carotene and quercetin egg yolk [24,35,29]. According to [6,55,56], β-carotene in *Moringa* pods ranges from 2.7 to 3.10 mg/100 g dried pods. When added to feed, this bioactive, along with phytochemicals, increases egg production and has a positive effect on chicken health. Carotenoids play an important role in the development of different color scores in egg yolk. Especially, lutein is an active yellow dye. According to [17] that increasing the amount of olive leaf powder in food results in a linear increase in the color of the yolk. This increase in the yolk color can be attributed to the carotenoid content of olive leaf powder [44].

Some researchers report that herbal extract supplements show the potential for an increase in the yolk color. *Moringa oleifera* seed inclusion increases egg yolk color [37], also mulberry leaves, ginkgo [36,72], *Allium sativum* and *Sauropus androgynus* [14], olive leaf powder [71], and carrots in feed [29]. This observation is supported by the findings of [48,49] that the use of *Moringa oleifera* leaves 10-20% in broiler feed or laying can significantly increase the yellow color of the skin and egg yolk.

The results showed that *Moringa* leaf supplementation at a level of 2-6% in feed could reduce cholesterol levels of egg yolk. According to [52] that reduction of cholesterol and triglycerides by alkaloids is partly due to a reduction in lipogenic enzyme activity and an increase in excretion of bile acids in feces. The ability of β-carotene to reduce cholesterol is related to the hydroxymethyl glutaril-CoA enzyme. This enzyme plays a role in mevalonic formation in cholesterol biosynthesis. The synthesis of cholesterol and β-carotene is together through the mevalonic pathway derived from acetyl CoA. If the consumption of β-carotene is greater than saturated fatty acids, the biosynthesis process by the HMG-CoA enzyme will be directed to the synthesis of β-carotene, so that saturated fatty acids are not converted to cholesterol [3,27,54]. Mevalonic is needed in the process of cholesterol synthesis by inhibiting enzymes, thus inhibiting cholesterol formation [63].

Several studies have shown that supplementation of *Moringa oleifera* leaves affects egg mass, serum biochemistry, and egg yolk bioactive compounds positively [4,39,20,67], as well as reducing cholesterol and triglycerides [22,32]. Some researchers report the use of herbal compounds can reduce cholesterol, as released by [18] that *Olea europaea* leaf powder can be used to reduce cholesterol levels of egg yolks, *Sauropus* leaves [63], Garlic [66], *Tanacetum balsamita* [46].

Egg cholesterol levels are also influenced by antioxidants (flavonoids and carotenoids) in the feed. Antioxidants potentiate the production of bile salts, which produce fat emulsification and decrease fat absorption, thereby reducing cholesterol levels [58,46]. Sterols and antioxidants in plants, can reduce the absorption process and accelerate the disposal of cholesterol through feces [13]. In addition, a decrease in egg cholesterol levels can be caused due to fiber contents of *M. oleifera*, which plays an important role in binding and removing cholesterol [25,47,48]. On the other hand, Zangeneh and Torki [71] found no effect of herbs (olive leaves) on blood cholesterol levels in laying hens.

5. Conclusion
We conclude that supplementation in diets of 4-6% *Moringa oleifera* leave powders was increased egg production, egg mass, feed efficiencies, yolk color, shell thickness, Ca and β-carotene contents in the yolk, but decreasing yolk cholesterol contents in laying hens up to 30 weeks of ages.

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