# The inclusion of fermented Moringa leaves in feed on hen-day production, eggshell thickness, cholesterol and egg yolk color of laying hens

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## Abstract

*The combination of herbal plant compounds and probiotic microbes is expected to have a positive synergy in improving egg quality and production. It is interesting to study the impact of the inclusion of moringa leaves fermented with Saccharomyces spp (FML) in the diet on eggshell thickness, cholesterol, and yolk color of laying hens. Lohmann Brown of laying hens (n=240, 60 weeks old) divided into 4 treatments, namely: A, diets without FML; B, diets with 2% FML; C, diets with 4% FML; and D, diets with 6% FML. Laying hens supplementation with 4% FLM could significantly (P≤0.05) increase feed efficiency and hen-day production (HD), namely: 12.0 and 17.39% higher than control. Egg shell thickness, yolk color, and BC in yolk increased significantly (P≤0.05),namely: 10.39; 29.37; and 11.18% higher due to 4% FML supplementation compared to controls. It was concluded that supplementation of laying hens aged 60 to 70 weeks with 4% FLM could be beneficial in terms of increasing feed efficiency, HD, shell thickness, egg yolk color, and BC, due to FML supplementation.*

**Keywords:** Beta-carotene, Moringa, Fermentation, Probiotik, Yolk color.

## Introduction

The use of probiotics and phytochemical compounds from herbal plants in poultry feed has increased over the past two decades. This is due to the use of antibiotics in the feed already banned. Excessive use of antibiotic growth promoters (AGPs) to stimulate poultry growth, however, causes of both antibiotic-resistant bacteria and dangerous antibiotic residues in poultry products, thereby increasing consumer concerns in consuming poultry products [1,2]. The combination of the properties of herbal plant compounds and probiotic microbes is expected to have a positive synergy to improve egg quality that is preferred by consumers.

Bioactive compounds in plants play a key role as a substitute for the use of AGPs, due to the attributes of their active feed compounds. Inclusion of herbal leaf can improve digestibility, feed efficiency, hen-day production, but reducing cholesterol in yolks [3]. *Moringa oleifera* plants are widespread in the tropics, especially in Indonesia. Based on the nutritional potential, the Moringa tree is a versatile plant and has been widely used as a medicinal plant and consumed by humans. According to[4], *Moringa* leaves contain high protein (29.10%) and β-carotene: 2.556 mg/100g. The inclusion of herbal leaves in laying hens feed significantly increases feed efficiency and yolk color, while reducing cholesterol levels in yolks.

Herbal products that have been fermented by probiotic microbes have multiple benefits compared to those without fermentation [5,6]. It is interesting to study the impact of the inclusion of FML in the diet on eggshell thickness, cholesterol, and egg yolk color of laying hens.

## Materials and Methods

### Ethical Approval and Experimental Design

The animals and research procedures were approved by the head of the Animal Ethics Committee of the Faculty of Veterinary Medicine, Udayana University, Denpasar.

Lohmann Brown (LB) laying hens (n=240, 60 weeks old) divided into 4 treatments. The treatment studies involved four different experimental diets, namely: four levels (0, 20, 40, and 60 g/kg diet) of fermented *Moringa* leaves (FML) were added to an basal diet *viz.,* diet A (FML 0%); B (FML 2%); C (FML 4%); and D (FML 6%).

The experimental diet (Table 1) was compiled based on the calculations according to [7]. All birds were housed in individual cages (35 cm long, 40 cm high, and front side width 20 cm) tiered and made of aluminum wire. Each pen is equipped with a feeder and waterer made from PVC pipe. All hens are given free access to the waterer and the finely ground feeder.

**Table 1. Nutritional content in feed for laying hens aged 60-70 weeks.**

| *Ingredients* (%) | FML content (%) in diet. | | | |
|---|---|---|---|---|
| | 0 | 2 | 4 | 6 |
| Yellow corn | 55.5 | 55.7 | 55.1 | 54.7 |
| Rice bran | 15 | 14.5 | 15 | 15.5 |
| Coconut meal | 6 | 5.8 | 4.9 | 3.6 |
| Soybean | 4.5 | 3 | 3 | 2.1 |
| Fish meal | 13 | 13 | 12 | 12 |
| Fermented Moringa Leaves | ***0*** | ***2*** | ***4*** | ***6*** |
| Mineral | 6.0 | 6.0 | 6.0 | 6.1 |
| Total | 100 | 100 | 100 | 100 |
| ***Nutrients **** | | | | |
| Metabolizable energy (Kcal/kg) | 2751 | 2750 | 2751 | 2751 |
| Crude protein (%) | 17.08 | 17.1 | 17.01 | 17.1 |
| Ether extract (%) | 6.2 | 6.19 | 6.44 | 6.63 |
| Crude fibre (%) | 4.17 | 4.18 | 4.27 | 4.26 |
| Calcium (%) | 3.80 | 3.85 | 3.83 | 3.94 |
| Phosphorus (%) | 1.41 | 1.54 | 1.64 | 1.79 |
| Isoleucine (%) | 0.89 | 0.91 | 0.93 | 0.97 |
| Lysine (%) | 1.24 | 1.29 | 1.33 | 1.38 |
| Methionine + cysteine (%) | 0.74 | 0.75 | 0.78 | 0.78 |
| Trypthophan (%) | 0.19 | 0.20 | 0.21 | 0.23 |

***** Calculations according to table Scott et al. (1982)

**Fermented Moringa leaves.**

Fresh, dark green Moringa leaves were taken from Moringa plantations, then dried in an oven at $75^0$C. Furthermore, *Moringa* leaves were ground to a size of 1-2 mm. The process described involves increasing the water content in moringa leaf flour to 30% by spraying it with a 5% molasses solution, than fermenting with *Saccharomyces spp* 5%, and incubated for 3 days. The fermented *Moringa* leaves powder was then used for the experimental treatments.

**Egg quality**

Egg production and feed intake (FI) in each cage were tabulated daily. Production performance (FI, egg numbers, and egg weight) was monitored weekly. Measurement of egg quality using an egg multitester machine (model EMT-7300, Japan) which includes (albumen, yolk, shell, yolk color, haugh unit, and egg shape) and the shell thickness was measured using a vernier caliper (mm). Cholesterol in egg yolk was analyzed according to the Lieberman-Burchard method [8]. The concentration of cholesterol in the yolk, expressed in mg/100 g yolk.

**Beta-carotene content in yolk**

The working step to calculate the total carotene in the yolk is to follow the steps of [3]. Total carotene was read using absorbent spectrometry (abs) at = 450 m. Total carotene (μg/100 g) = (total volume x abs x100)/(0.2 x sample weight).

One-way ANOVA was conducted to see whether there was a significant difference ($P<0.05$) between all treatment means, and if there was, Duncan's Multiple Range Test (DMRT) was used to precisely identify treatment pairs that had a significant difference at the significance level of $P<0.05$ [9].

## Results and Discussion

Table 2 presents the results that indicating that the inclusion of FML did not statistically impact on LWG, FI, and average egg weight between treatment groups. This result is in accordance with [6] observed that supplementation of FML had no impact on FI. Egg mass and egg production in the groups that were supplemented with 2-6% of FML more heigher ($P\leq0.05$) compared with the unsupplemented FML or control group (Tabel 2). Increased egg mass and egg production in chicken groups that received FML, caused by active compounds contained in Moringa leaves, such as alkaloids, flavonoids, saponins, phenolics and tannins [3,10]. Similar findings have been reported by [11], egg weight increased with the addition of moringa flour to the diet. Fermented feed products can affect microbial ecology in the intestine and suppress the number of pathogens [6]. Adding fermented Ginkgo leaf flour to laying hen feed can improve egg quality, thereby increasing eggshell strength and yolk color [12].

**Table 2. Impact of feeding feed containing FML to laying hens aged 60-70 weeks on egg production.**

| Variables | FML levels in diet (%) | | | | SE |
|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | |
| Weight gain (g) | 149.42 | 148.58 | 153.01 | 151.92 | 2.719 |
| Feed intake, g/bird | 9455.25 | 9478.16 | 9389.81 | 9480.20 | 30.904 |
| Egg mass, g/bird | 3306.41a | 3631.48b | 3639.46b | 3646.23b | 82.971 |
| Egg weight, g/head | 60.27a | 62.97b | 63.01b | 63.16b | 0.509 |
| Egg production, egg/bird | 54.86a | 57.67b | 57.76b | 57.73b | 0.703 |
| Hen-day production, % | 78.37a | 82.38b | 82.51b | 82.47b | 1.025 |
| FCR (FI/egg mass; g/g) | 2.86a | 2.61b | 2.58b | 2.60b | 0.052 |

a,b mean values with the same superscript in the same row do not differ at probability P = 0.05.

The addition of FML to the diet significantly improved feed efficiency in the experimental group, meaning the birds produced more egg mass for the amount of feed

consumed compared to without FML (Table 2). FCR in the control group was 2.86 and the FML group showed a significantly lower FCR, indicating a more efficient conversion of feed into eggs.

Inclusion of fermented feed in the diet can increase performance and feed efficiency [13]. Sugiharto et al. [14] reported that fermented feed can improve feed nutrition, and when given to poultry it can improve performance. Park et al. [15] reported that probiotic supplementation can increase hen-day-production (HDP) and eggshell thickness.

The administration of FML in the diet had no significant impact on HU, egg shape, and specific gravity (weight: volume) compared to non-FML or Group A (Table 3). However, the shell thickness in the FML group increased significantly compared to the control. Tesfaye *et al.*[16,17] reported that *Moringa* could increase feed efficiency, and had no effect on HU and eggshell thickness.

Herbal leaves containing flavonoids and carotenoids, can affect HU, shell thickness, and external quality of eggs [18,19]. Conversely [20] reported that the presence of anti-nutrient compounds in the *Moringa* plant, can cause disruption of the metabolieme Ca mineral which has an impact on decreasing the thickness of the chicken eggshell. According to [19], supplementing diets with Moringa whole seed flour can improve egg yolk color due to its high carotene content and improved external egg quality. Abdelqader et al. [21] reported that probiotic supplementation in the diet significantly increases production performance, eggshell quality, and mineral Ca retention in chicken eggs.

**Table 3. The effect of FML in laying hen diets on egg quality parameters**

| Variables | FML levels in diet (%) | | | | SE |
|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | |
| Haugh units (HU) | 75.01a | 76.27a | 76.12a | 76.45a | 0.613 |
| Egg shape | 76.26a | 76.49a | 75.02a | 76.05a | 0.904 |
| Specific gravity (weight : volume) | 1.035a | 1.043a | 1.045a | 1.040a | 0.032 |
| Shell thickness (mm) | 0.385b | 0.421a | 0.425a | 0.419a | 0.011 |
| Yolk color (1-15) | 9.31a | 9.75b | 11.27b | 11.92b | 0.375 |
| βeta-carotene in yolk (µg/g) | 1.293b | 1.305a | 1.522a | 1.549a | 0.031 |
| Yolk cholesterol levels (mg/100 g) | 1275a | 1286a | 1136b | 1124a | 35.171 |

a,b mean values with the same superscript in the same row do not differ at probability P = 0.05.

Provision of FML in diets can improve egg quality, because FML contains phytochemical compounds and also contains yeast probiotics. Probiotics significantly alter mucin movement and the gut microbial population in the small intestine of chickens, so that intestinal function and health, and nutrient uptake can be improved [22].

Table 3 shows that the addition of FML to laying hen feed at a concentration of 2% to 6% caused a significant increase in egg yolk color as well as its β-carotene content. This is due to *Moringa* leaves containing high protein and β-carotene [4]. In line with [19] that the use of herbal leaves in rations can improve yolk color. Herbal plants can increase the color of the yolk with β-carotene stored in the yolk [17,19,23].

Supplementation FML into the diet of laying hens, as indicated by a Table 3, can reduce the cholesterol content in egg yolks. According to [24], flavonoid and carotenoid compounds found in herbal leaves can suppress cholesterol synthesis in the yolk. Probiotics in feed can significantly reduce blood glucose and blood cholesterol levels [25], reduce egg yolk cholesterol and total saturated fatty [26]. In line [27] and other studies show that the supplementation of fermented feed, can reduce fat and cholesterol levels in broiler meat.

## Conclusion

Inclusion of 2-6% FLM in the diet can provide benefits in terms of increasing feed efficiency and egg production. Increased shell thickness, yolk color, and beta-carotene of eggs due to FML supplementation.

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