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Original Article

Moringa oleifera Flour Fermentation as Feed Additive on Egg Quality in Mojosari Ducks with Different Storage Period

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ABSTRACT

Eggs are widely consumed by Indonesian people. High market demand will cause breeders to increase egg productivity and quality. Moringa leaf flour is high in crude fiber, so it needs to be fermented. Fermentation aims to reduce crude fiber and increase crude protein. This study aims to determine the effect of using fermented Moringa leaf flour on the egg yolk index of the Haugh Unit and the color of the Mojosari's duck egg yolk with different shelf life. This study used 60 ducks that divided into four treatment groups, namely P0 without the addition of fermented Moringa leaf flour, P1 with the addition of 0.5% Moringa leaf flour fermentation, P2 with the addition of 1% Moringa leaf flour fermentation, and P3 with the addition of 1.5%. Moringa leaf flour fermentation. The results showed that the addition of 1.5% fermented Moringa leaf flour (P3) gave the highest average value for the egg yolk index, Haugh Unit, and egg yolk color with different storage ages. Providing fermented *Moringa oleifera* leaf flour at 1.5% as a feed additive had the highest influence on increasing the egg yolk index, haugh unit value, and egg yolk color of Mojosari ducks with different storage period.

Keywords: *Moringa oleifera*, Mojosari ducks, yolk index, haugh unit, color of yolk

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Introduction

Eggs are widely consumed by Indonesian people. Eggs are used in various types of cooking. Apart from being easy to process, eggs also have many health benefits. Eggs have complete nutritional content ranging from protein, fat, vitamins and minerals. According to Suryana *et al.* (2021), egg consumption in Indonesia continues to increase, reaching 1.5 million tonnes from 1.4 million tonnes in 2016, and is expected to continue to rise in 2021 to reach 1.7 million tonnes. The population of laying ducks in East Java reached 5,696,190 in 2018. This population has continued to increase in the last five years. High market demand will cause breeders to increase egg productivity and quality.

To increase egg production, you can try adding *Moringa oleifera* leaves to the feed. Moringa leaves are easy to find in tropical countries like Indonesia. Islam et al. (2021) reported that Moringa leaves are a food source that is rich in β-carotene, protein, vitamin C, calcium, and potassium, and is a good source of antioxidants because of the various types of antioxidant compounds contained in Moringa leaves.

Moringa leaf flour is high in crude fiber, so it needs to be fermented. Fermentation has the aim of reducing crude fiber and increasing crude protein (Ali *et al*., 2020). Previous research by González-Burgos *et al*. (2021) showed that the amount of protein contained in Moringa leaves reached 27%. The high protein content of Moringa leaves contributes to egg weight gain. According to Batubara *et al*. (2021), cellulotic bacteria in the fermentation process can produce cellulase enzymes which function to hydrolyze cellulose, so that it can be easily digested by poultry.

Proteolytic bacteria in the fermentation process are able to increase crude protein, because they produce protease enzymes (Diether and Willing, 2019). This enzyme can break down proteins into polypeptides and then into amino acids which are used by microbes to reproduce themselves (Wang *et al*., 2020). An increase in microbes can increase crude protein because these microbes are single-celled. According to Montenegro *et al*. (2019), overall egg quality is determined by the quality of the contents and egg shell. Therefore, egg quality determination is carried out on both external and internal egg parts. External quality includes egg weight and egg shell thickness, internal quality includes egg yolk index, egg white index, egg yolk color and haugh unit value.

According to Nabila *et al*. (2023), the higher the protein content in the feed given, the higher the egg yolk index value. From various studies, it is reported that Moringa leaves contain a high composition of vitamins A, B, C, and calcium, iron and protein (Sultana, 2020). Based on research conducted by Khan *et al*. (2021), giving Moringa leaf flour to feed with a concentration of 0.50% increased the egg yolk index to 35.66 and giving Moringa leaf flour with a concentration of 1.5% increased the Haugh unit value to 87. 31. Garcia *et al*. (2021) reported that the use of 5% and 10% Moringa leaf flour had a significant effect on increasing the yolk color score of quail eggs.

The length of time an egg is stored determines the quality of the egg. The longer the egg is stored, the quality and freshness of the egg decreases (Adriaensen *et al*., 2022). Therefore, it is necessary to use various methods to improve the quality of eggs with a long shelf life. Egg quality can be improved by improving feed quality and preservation. The addition of fermented Moringa leaf flour to commercial feed is expected to increase the efficiency of feed consumption, thereby reducing production costs for laying ducks and breeders still making a profit even though there are additional costs. This research aims to determine the effect of using fermented *Moringa oleifera* leaf flour as a feed additive on the egg yolk index, haugh unit and egg yolk color with different shelf lives.

Materials and methods

Research design

Experimental animal rearing was carried out in Kebonsari Village, Candi District, Sidoarjo. Moringa leaf flour fermentation carried out at the Animal Feed Laboratory, Faculty of Veterinary Medicine, Airlangga University, Surabaya. Examination of egg quality research materials carried out at the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Airlangga University, Surabaya. This research was conducted for 3 months from April – June 2020. This experiment used 60 ducks who were randomized into 4 (four) treatment groups (P0, P1, P2, P3), each consisting of 5 (five) repetitions, Each replication consists of 3 (three) tails. P0: 100% commercial feed, without fermented Moringa leaf flour; P1: 100% commercial feed + 0.5% fermented Moringa leaf flour; P2: 100% commercial feed + 1% fermented Moringa leaf flour; P3: 100% commercial feed + 1.5% fermented Moringa leaf flour.

Preparation for making feed

Moringa leaf flour is obtained from fresh, stemless Moringa leaves dried indoors. The Moringa leaves are grinded using a shredder into flour. The flour then analyzed proximately to determine its nutritional content.

Making fermented Moringa leaf flour by using fine Moringa leaf flour and Bio MC4 solution. Preliminary research began with fermented Moringa leaf flour with Bio MC4 solution by random sampling with doses of 0%, 2.5% and 5% Bio MC4 plus 3% molasses and 22% water in 100% Moringa leaf flour. Then the best 5% dose was obtained because of the results of proximate analysis. Appendix 1 with a dose of 5% increases protein and reduces crude fiber. The probiotics used contain the following microbial composition: *Enterobacter* sp., *Bacillus* sp., *Cellulomonas* sp., and *Actinomyces spp*. *Enterobacter* sp. bacteria. used in this research is capable of producing three types of cellulase enzymes (endoglucanase, exoglucanase, and β-gucosidase) (Lokapirnasari *et al*., 2018).

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Moringa leaf flour that has been given 5% Bio MC4 + 3% drops + 22% water is then stirred and leveled until the Moringa leaf flour becomes moist, and then packed into a plastic bag, closed tightly without air and placed in a dark place. protected from sunlight. The fermentation process is carried out for seven days. Next, dry it by airing it without direct sunlight for approximately 2 days. Next, fermented Moringa leaf flour was divided according to treatment groups.

Preparation of experimental animals or acclimatization

The experimental animals were 60 female ducks. The ducks were given sugar water to drink with a ratio of 20 grams of sugar water/L. Then the ducks were adapted to the conditions of the research location and food for 2 weeks. The experimental animals drawn into 4 treatments (P0, P1, P2, and P3), each consisting of 5 (five) replications, each replication consisting of 3 ducks.

One week before the cage is used, the cage is cleaned and sprayed evenly with disinfectant. Equipment for feeding and drinking water that will be used must be cleaned first (sterilized), then prepared in the cage. Lamp with 40 watt incandescent installed in the center of each cage plot.

Sample data collection

Fresh eggs are broken on day 1 (day of egg collection), day 5 and day 10 of egg storage. Eggs were broken one by one to collect data on yolk height, yolk index, yolk color and Haugh Unit value. The data was collected using a spherometer, caliper and egg yolk color fan.

Data analysis

The research design used was a completely randomized design. Data were analyzed using the Factorial RAL test and continued with the Duncan test. This analysis uses the SPSS software application.

Result

Egg yolk index

The percentage of egg yolk index is obtained from the results of measuring eggs after being stored for 1, 5 and 10 days. The results of statistical tests using a Completely Randomized Factorial Design on the egg yolk index showed significantly different results for the doses used, with a significance value of 0.000 (≤0.05). The influence of the day factor also showed significantly different results with a significance value of 0.000 (≤0.05), and there was an interaction between the dose factor and the day factor with a significance value of 0.000 (≤0.05).

Based on the average egg yolk index data in **table 1**, P3 day 1 showed the highest average egg yolk index, namely 0.417 ± 0.014 and was statistically significantly different from the other treatments. The lowest average egg yolk index was shown by P0 on day 10, namely 0.272 ± 0.002. These results show that the administration of 1.5% fermented Moringa leaf flour (P3), showed the highest influence on the average value of the egg yolk index.

Table 1. Average percentage and standard deviation of egg yolk index

Treatment	Day 1	Day 5	Day 10
P0	0.343 ±0.002^{d}	0.326 ±0.008^{e}	0.272 ±0.002^{h}
P1	0.350 ±0.002^{cd}	0.329 ±0.007^{e}	0.274 ±0.004^{gh}
P2	0.379 ±0.015^{b}	0.341 ±0.001^{d}	0.283 ±0.002^{g}
P3	0.417 ±0.014^{a}	0.359 ±0.014^{c}	0.294 ±0.003^{f}

Note: Different superscripts in the same column indicate a significant difference (p < 0.05); Std dev: Standard deviation.

Haugh unit value

Table 2. Mean and standard deviation of haugh unit values

Treatment	Day 1	Day 5	Day 10
P0	73.02± 0.19^{d}	70.96± 0.72^{ef}	69.91± 0.71^{f}
P1	73.57± 0.72^{cd}	71.45± 1.35^{e}	70.05± 0.57^{f}
P2	74.97± 0.29^{ab}	73.01± 0.83^{d}	71.53± 0.34^{e}
P3	75.87± 0.54^{a}	74.53± 0.75^{bc}	73.05± 0.81^{d}

Note: Different superscripts in the same column indicate a significant difference (p < 0.05); Std dev: Standard deviation.

The percentage of haugh unit values showed on **table 2**. The values obtained from the results of measuring eggs after being stored for 1, 5 and 10 days. The results of statistical tests using a Completely Randomized Factorial Design on the haugh unit value showed significantly different results for the doses used, with a significance value of 0.000 (≤0.05). The influence of the day factor also showed significantly different results with a significance value of 0.000 (≤0.05), however there was no interaction between the

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dose factor and the day factor with a significance value of 0.902 (≥0.05).

Table 2 showed that P3 on day 1 shows the highest Haugh Unit value, namely 75.87 ± 0.54. The lowest average haugh unit value was shown by P0 on day 10, namely 69.91 ± 0.71. These results show that the administration of 1.5% fermented Moringa leaf flour (P3), showed the highest influence on the haugh unit value.

Egg yolk color

Egg yolk color data was obtained from egg measurements after being stored for 1, 5 and 10 days. The results of statistical tests using a Completely Randomized Factorial Design on egg yolk color showed significantly different results for the doses used, with a significance value of 0.000 (≤0.05). The influence of the day factor also showed significantly different results with a significance value of 0.000 (≤0.05), however there was no interaction between the dose factor and the day factor with a significance value of 1.000 (≥0.05).

Table 3. Average percentage and standard deviation of egg yolk color

Treatment	Day 1	Day 5	Day 10
P0	13.50± 0.57^{b}	12.50± 0.57^{c}	11.50± 0.57^{d}
P1	13.50± 0.57^{b}	12.50± 0.57^{c}	11.50± 0.57^{d}
P2	14.50± 0.57^{a}	13.50± 0.57^{b}	12.50± 0.57^{c}
P3	14.50± 0.57^{a}	13.50± 0.57^{b}	12.50± 0.57^{c}

Note: Different superscripts in the same column indicate a significant difference ($p < 0.05$); Std dev: Standard deviation.

Based on the average egg yolk color data in **table 3**, P3 day 1 shows the highest average egg yolk color value, namely 12.50 ± 0.57. The lowest average value of egg yolk color was shown by P0 on day 10, namely 11.50 ± 0.57. These results show that the administration of 1.5% fermented Moringa leaf flour (P3), showed the highest influence on the average value of egg yolk color.

Discussion

Egg yolk index

The egg yolk index is related to the ideal egg shape that consumers prefer. Narushin *et al.* (2021) states that the egg yolk index is an index of freshness quality which is measured by the height and diameter of the egg yolk. The fresh egg yolk index ranges from 0.33-0.52 (Nabila *et al.*, 2023). The lowest average egg yolk index was shown by P0 on day 10, namely 0.272 ± 0.002. This shows that if duck eggs are stored for more than 10 days, the egg yolk index value is <0.3, which is an egg yolk index value below the average for fresh eggs, this shows that the egg quality has decreased.

Agustono *et al.* (2023) stated that the egg yolk index is influenced by the protein, fat and essential amino acids contained in the ration. Marzec *et al.* (2016) said that the egg yolk index indicates a progressive decline in the function of the vitelline membrane in the egg. The smaller the egg yolk index, the lower the egg quality. Egg yolk is composed of fat and protein, forming lipoproteins which are synthesized by the liver under the influence of estrogen.

The osmotic pressure of the egg yolk is greater than the egg white, so that water from the egg white moves towards the egg yolk continuously which will cause the viscosity of the egg yolk to decrease and make the egg yolk become flat until it breaks due to damaged vitelline membranes. The movement of water from egg white to egg yolk as much as 10 mg/day at a temperature of 10°C occurs due to too long storage (Xu *et al.*, 2017). Martínez *et al.* (2021) stated that the egg yolk index will decrease from 0.45 to 0.30 if stored for 25 days at a temperature of 25°C.

Giving 1.5% fermented Moringa leaf flour (P3) on day 1 showed the highest average egg yolk index, namely 0.417 ± 0.014. This is in line with what was reported by Yang *et al.* (2020), that there was an increase in the average value of the egg yolk index in the group of ducks that were given additional Moringa leaf flour in their experiments. This is thought to be related to the bioactive components of Moringa leaf flour which can stimulate the absorption and metabolism of nutrients, thereby accelerating blood flow and faster deposition of egg yolk forming material. The use of natural ingredients is also thought to be able to stimulate glucose uptake from the intestines and increase glucose uptake by liver cells from the circulatory system. Glucose is then processed into several forms, namely deoxidation as an energy source which is stored in the liver as glycogen or converted into fatty acids as raw material for the biosynthesis of egg yolk precursors.

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Haugh unit value

The Haugh unit is a unit used to determine the freshness of the contents of an egg, especially the egg white. The haugh unit value is one of the criteria for determining the quality of the inner egg by measuring the height of the egg albumen by the weight of the egg. The haugh unit of eggs produced is classified into quality groups, including quality I with a haugh unit value >72, quality II with a haugh unit value of 62-72, quality III with a haugh unit value of 50 – 60, and quality IV with a haugh unit value ≤ 50. The higher haugh unit value of an egg indicates that the quality of the egg is getting better (de Menezes *et al*., 2012).

The higher Moringa leaf flour usage, the higher the haugh unit value. A high haugh unit value indicates that the viscosity of the albumen is getting thicker. Albumen contains ovomucin which plays a role in binding water to form albumen gel, so that the albumen can be thick, if the ovomucin network is large and strong, and makes the albumen viscosity high. The higher the haugh unit value, the higher the ovomucin and the better the quality of the egg interior (Shan *et al*., 2020).

The longer the eggs stored, the haugh unit value will decrease due to the evaporation of CO2 gas which causes the thick egg white to become runny. Evaporation of CO2 through the pores of the egg shell causes the concentration of bicarbonate ions in the egg white to decrease and destroys the buffer. The egg white becomes alkaline (the pH of the egg rises) followed by damage to the ovomucin fibers, so that the viscosity of the egg white decreases. Ovomucin fibers which provide a thick texture (Wang *et al*., 2022).

Egg yolk color

The quality of the egg yolk color is determined visually, namely comparing the standards of the Roche Yolk Color Fan in the form of a standard color fan sheet with a score of 1-15 from pale to dark orange. The higher the egg yolk color score, the better the quality of the egg (Sözcü *et al*., 2021).

Moringa leaf flour in the ration improves the color intensity of the egg yolk. Egg yolk color determine the quality of eggs from consumer's perspective. The consumers prefer yellower or darker yellow egg yolk. Egg yolk color in this study has high value. Egg yolk with 7-8 value on the Roche scale is classified as good quality (Dvořák *et al*., 2009).

The egg yolk color influenced by the content of carotenoid compounds which are abundant in Moringa leaf flour, which are able to be absorbed and utilized efficiently by ducks (Lokapirnasari *et al*., 2022). Dansou *et al*. (2023) stated that feed containing carotenoid pigments, especially beta-carotene and xanthophyll, affects egg yolk color. This beta-carotene compound has a role as a precursor of vitamin A which functions as a pigment in egg yolk.

Moringa leaf flour also contains flavonoid compounds, vitamin C and other phenolic compounds which also act as natural antioxidants. The use of Moringa leaves in rations can significantly increase the color of egg yolks.

Conclusion

Providing fermented *Moringa oleifera* leaf flour at 1.5% as a feed additive had an effect on increasing the egg yolk index, haugh unit value, and egg yolk color of Mojosari ducks with different shelf lives.

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