J I T A A
Journal of the Indonesian Tropical Animal Agriculture
Accredited by Ditjen Riset, Teknologi dan Pengabdian kepada Masyarakat No. 164/E/KPT/2021

J. Indonesian Trop. Anim. Agric.
pISSN 2087-8273 eISSN 2460-6278
http://ejournal.undip.ac.id/index.php/jitaa
50(2): 122-130, June 2025
DOI: 10.14710/jitaa.50.2. 122-130

Physicochemical, microbiological, and organoleptic characteristics of quail eggs given moringa leaf flour (*Moringa oleifera* L.) in Feed

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Received March 3, 2025 ; Accepted May 3, 2025

ABSTRACT

Moringa leaf (*Moringa oleifera* L.) has excellent potential as an alternative source of protein for animal feed because of its abundant nutritional content. It is high in protein biological value, and has good properties. The research aims to evaluate the effects of adding moringa leaf flour to feed on quail eggs' physical, chemical, microbiological, and organoleptic properties. A total of 120 female quail aged 36 days were used in the study, which lasted for 4 weeks. The experiment was conducted in a fully randomised design. The treatment included four levels of moringa leaf flour addition: P0 (without moringa leaf flour), P1 (2.5% moringa leaf flour), P2 (5% moringa leaf flour), and P3 (7.5% moringa leaf flour). Variance analysis and descriptive analysis were used to examine the data. The findings revealed that moringa flour could reduce egg yolk fat, increased antioxidant activity, and reduced bacteria in eggs. The 7.5% moringa leaf treatment produced the best quail egg quality.
Keywords: Antioxidants,Bioactive compounds, Laying quails, Moringa leaf

INTRODUCTION

The fulfilment of animal protein requirements is crucial in improving community nutrition. Livestock products like meat, milk, and eggs are the primary sources of animal protein, with increasing demand at the global and regional levels.

Quail (*Coturnix coturnix japonica*) is a poultry species with great potential as a source of animal protein, especially quail eggs. Quail starts laying eggs at 42 days of age and can produce 250–300 eggs/head/year (Gül *et al*., 2022). Based on PKH Statistics data (2022), the contribution of quail eggs in Indonesia reached 0.41% in 2021, showing that quail has promising prospects to be developed as a source of animal protein.

Genetic and environmental factors influence productivity in quail. Environmental factors include feed quality, rearing management and microclimate conditions. Microclimate conditions, including airflow, humidity and ambient temperature, influence quail productivity (Santos *et al*., 2019). The ambient temperature in Indonesia ranges from 26-33 °C (BMKG, 2023). These temperatures far exceed the comfort zone of poultry, especially quail, which is 18-26 °C (Silva *et al*., 2020). This high temperature can trigger oxidative stress in quail.

The oxidative stress arises when free radicals generation, such as reactive oxygen species

(ROS), exceeds the body's antioxidant capabilities (Radomska-Leśniewska *et al*., 2016). Although ROS plays a role in biological processes, excess ROS can damage proteins, lipids, and DNA and cause cell death (Zhao *et al*., 2023). This imbalance can trigger various diseases (Sahin *et al*., 2023). The addition of antioxidant compounds helps reduce the impact of oxidative stress through a free radical neutralising mechanism. Antioxidants donate electrons to stop free radical reactions (Raza *et al*., 2020). Antioxidants can be obtained from plants, including the Moringa plant.

Moringa contains active compounds such as flavonoids, alkaloids, tannins, terpenoids, and steroids (Pareek *et al*., 2023). These compounds function as antioxidants, antibacterials, and anti-inflammatories, strengthening the immune system by counteracting free radicals from oxidative stress (Akbari *et al*., 2022).

Lu *et al*. (2016) examined moringa flour levels in laying hen feed, with 5%, 10%, and 15%. As a result, adding moringa flour starting from 5% significantly improved egg yolk colour. Shen *et al*. (2021) examined the moringa flour in laying hen feed with 2.5%, 5%, 7.5%, and 10%. As a result, adding moringa flour starting from 2.5% in feed can strengthen eggshell thickness and improve feed conversion value in laying hens.

Research related to the moringa flour addition effect in feed on quail egg characteristics is still limited. Thus, the study aims to assess the addition of moringa flour to the physical, chemical, microbiological and organoleptic characteristics of quail eggs.

MATERIALS AND METHODS

Time and Place

The study was conducted from December 2023 to January 2024. Phytochemical testing of moringa leaf flour was conducted at the Laboratory of the Research Centre for Spice and Medicinal Plants, Bogor City. Quail rearing was conducted at Arkan Quail Farm, Ciampea District, Bogor Regency. Furthermore, physical analyses of eggs, including pH and water activity (Aw) measurements, were conducted at IPB's Poultry Nutrition Science Laboratory. Antioxidant and Malondialdehyde (MDA) activity analyses were conducted at the Integrated Laboratory Unit, Baranangsiang Campus, IPB. Egg microbiological analysis was conducted at the Culture Collection Laboratory, Department of Biology, IPB.

Phytochemical Content Analysis

Quantitative phytochemical analysis of moringa leaf powder, including phenols, flavonoids, tannins and saponins, was done following the procedure of Senguttuvan *et al*. (2014), and the results were measured using UV-Vis Spectrophotometry.

Quail Maintenance

This study used moringa leaf flour obtained from CV. Pusaka Madura mixed until homogeneous with New Hope feed in the form of mash according to the treatment (0%, 2.5%, 5%, 7.5%). Information regarding the nutritional composition of feed with moringa leaf flour substitution can be seen in Table 1.

One hundred twenty female quails aged 36 days were kept for 4 weeks in a cage measuring 45×35×60 cm with 12 compartments, each containing 10 quails, equipped with feed and drink containers. Temperature was recorded daily in the morning (06.00-07.00 am), afternoon at 12.00-13.00 pm and 17.00-18.00 pm throughout the study.

Quail Egg Sample

A total of 360 eggs were tested for pH, Aw

Table 1. Nutritional Composition of Feed with Moringa Leaf Flour Substitution

Component	New Hope*	Moringa Leaf Flour**	Substitution of Moringa leaf Flour***		
			2.5%	5%	7.5%
Protein	20.00	29.34	20.23	20.47	20.70
Fat	7.00	7.44	7.01	7.02	7.03
Fiber	7.00	8.59	7.04	7.07	7.11
Ash	14.00	9.52	13.88	13.76	13.64

* PT. New Hope Indonesia; ** Biochemistry Laboratory IPB, 2023; *** By calculation

test (24 eggs), antioxidant activity and MDA (36 eggs), microbiological (180 eggs) and organoleptic (120 eggs) tests. Samples were taken weekly during the study.

Physical Characteristics of Eggs

The physical testing of eggs measured included the egg weight, which was determined by weighing the egg (g) using a digital scale (Osuka -HWH, Japan). The thickness of the shell was measured with a micrometre in three sections (blunt end, centre, sharp end) and then averaged. Yolk colour was measured with a *yolk colour fan*. Haugh Units were applied using the equation of Eisen *et al*. (1962).

HU = 100 logs (H+7.57-1.7 W0.37)

Description:
HU = *Haugh Unit*;
H = Egg white height (mm);
W = Egg Weight (g)

Chemical Characteristics of Eggs

Egg chemical testing included pH measurements using a Schott Lab 850 Ph meter calibrated at pH 4 and 7. Water activity (Aw) was measured using a Novasina ms1 Aw meter by inserting egg samples into the device and waiting about five minutes until the Aw value was visible.

MDA testing used the TBARs method modified by Singh *et al*., (2002). The preparation of PBS-KCl solution used 0.0243 g KH_2PO_4, 0.0203 g KCl, 0.144 g Na_2HPO_4, and 8 g NaCl. The reagent solution was prepared using 0.25N HCl containing 10 g TCA, 0.38 g TBA, 0.5 g BHT, and 2.23 ml of concentrated HCl. Egg yolk samples (5 g) were put in a glass cup, added with 2 ml of cold PBS-KCL, stirred until smooth with a stirrer, and centrifuged for 20 minutes at 10,000 rpm. The supernatant formed was put in an Eppendorf tube for MDA assay. 0.5 ml of supernatant was taken, added with 2 ml of mixed solution (2.23 ml concentrated HCl, 10 g TCA, 0.38 g TBA, 0.5 g BHT in 100 ml distilled water) and homogenised. Then, the solution was heated using an oven. After 1 hour, it was removed and cooled to room temperature. The centrifuge was carried out for 5 minutes at 3500 rpm. The supernatant was poured into another tube to read the absorbance on a UV-Vis spectrophotometer with a wavelength of 540 nm. The absorbance values of the samples were entered into the TEP (tetraethoxypropane) standard curve equation: Y = aX + b. MDA concentration was calculated based on the formula:

$$MDA = \frac{(Absorbance - b) / a}{Sample\ volume} \ x\ Fp$$

Description:
Fp = Dilution factor;
a,b = coefficients in the regression equation of the TEP curve (Y= aX + b)

Antioxidant activity testing used a DPPH solution. A total of 2.5 mg of sample was weighed, then dissolved in absolute methanol, homogenised, and the volume was adjusted to reach 25 mL. Dilutions were made to produce five concentration series (100, 150, 200, 250 and 300 ppm). In measuring antioxidant activity, 2 mL of sample solution from each concentration was taken using a micropipette and put into a test tube. 2 mL of DPPH solution was then added at a concentration of 50 ppm. The mixture was homogenised and allowed to stand for 30 minutes in a dark place. Absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm. Antioxidant activity was determined based on the level of DPPH radical inhibition, which was calculated through the percentage (%) of DPPH absorption inhibition using the formula of Molyneux (2004):

$$\%\ Inhibition = \frac{abs\ blanko - abs\ Sample}{abs\ blanko} \ x\ 100\%$$

The calculation of IC_{50} values for each sample concentration used a linear regression equation. On the graph, the sample concentration was plotted on the x-axis, while the percentage inhibition was on the y-axis of the equation: Y = a + bX. The IC_{50} value was obtained through a calculation using the following formula:

$$IC_{50} = \frac{(50 - a)}{b}$$

Description:
Y = % Inhibition (50);
a = Intercept (the intersection of the line on the Y-axis);
b = Slope;
X = Concentration

Microbiological Characteristics of Eggs

Microbiological analysis of eggs included testing for Total Plate Count (TPC), *Escherichia coli* and *Salmonella* sp. referring to the Indonesian National Standard method (SNI 2897-2008).

Organoleptic Characteristics of Eggs

Organoleptic testing of eggs was conducted through the hedonic and hedonic quality tests, including smell, taste, white and yolk colours, and texture. The hedonic test aims to assess the level of panellists' liking for a food product using a 7-level rating scale: very dislike, dislike, somewhat dislike, neutral, somewhat like, like, very like. The hedonic quality test aims to assess the acceptability and quality of food products using the rating test method with a 5-level scale. The variables assessed included smell (very fishy, slightly fishy, fishy, less fishy, very fishy); taste (very unpleasant, unpleasant, tasty, more tasty, very tasty; yolk colour (very not yellow, slightly yellow, yellow, more yellow, very yellow); egg white colour (very not white, slightly white, white, more white, very white); the texture of yolk and white eggs (very not chewy, less chewy, chewy, more chewy, very chewy). The assessment was conducted by 30 semi-skilled panellists on 120 hard-boiled egg samples with treatment codes (Wahyuni and Sudrajat, 2021).

Statistical Analysis

The present study used a completely randomised design (CRD) with four treatments and three replicates, each replicate consisted of 10 quails. Treatments included P0 (commercial feed without moringa flour), P1 (feed + 2.5% moringa flour), P2 (feed + 5% moringa flour), and P3 (feed + 7.5% moringa flour). Physical, chemical, and TPC characteristic data were analysed with ANOVA. Duncan's Multiple Range Test was used if there were significant differences (Steel and Torrie, 1993). *Escherichia coli* and *Salmonella* sp. data were analysed descriptively. Organoleptic data was tested using non-parametric test of Kruskal-Wallis (Ostertagová *et al*., 2014).

RESULTS AND DISCUSSION

Research Environmental Conditions

The average temperature in the cages during the study varied: 21-24 °C in the morning, 33-39 °C in the afternoon and 28-31 °C in the afternoon. The afternoon temperatures were above the quail comfort zone of 18-26 °C (Silva *et al*., 2020). Quail faces challenges maintaining body heat balance in high temperatures, which can trigger oxidative stress. Oxidative stress can be overcome by adding antioxidants to the feed (Deng *et al*., 2023). The utilisation of antioxidant -rich moringa flour is a potential solution to reduce the effects of oxidative stress.

Phytochemical Content of Moringa Leaf Flour

The testing of the phytochemical content of moringa leaf flour analysed quantitatively is presented in Table 2. Phenols and flavonoids have antibacterial, anti-inflammatory, and antioxidant properties to inhibit free radicals, support cell healing, and inhibit bacterial growth (Chagas *et al*., 2022). Flavonoids act as antioxidants, preventing DNA oxidation and lipid peroxidation (Zhao *et al*., 2023). Tannins and saponins in plants are antibacterial compounds that damage the bacterial cell wall, reducing membrane permeability and inhibiting its activity to cause bacterial death (Hamzah *et al*., 2021). However, tannins can bind to protein, so excess consumption reduces quail productivity, with a safe limit in feed of 0.26% (Kumar *et al*., 2005).

Physical Characteristics of Quail Eggs

The results of the physical testing of quail eggs with the addition of moringa flour in the feed can be seen in Table 3. The statistical analysis indicated that treatment has no significant

Table 2. Phytochemical Content of Moringa Leaf Flour

Phytochemical Analysis	Result (%)
Phenol	2.32
Flavonoid	0.87
Tannin	8.52
Saponin	1.63

Test results at the laboratory of the Spice and Medicinal Crops Research Institute (2023)

Table 3. Physical Characteristics of Quail Eggs

Parameters	Treatments			
	P0	P1	P2	P3
Egg weight (g)	10.26±0.29	10.29±0.38	10.41±0.16	10.65±0.38
Shell thickness (mm)	0.181±0.00	0.18±0.00	0.18±0.00	0.186±0.00
Egg yolk color	7.17±1.15	7.33±0.29	7.67±0.29	7.83±0.29
Haugh unit (HU)	79.67±1.84	79.69±1.25	79.75±1.32	80.14±0.65

P0: moringa leaf flourless feed; P1: feed + moringa leaf flour 2.5%; P2: feed + moringa leaf flour 5%; P3: feed + moringa leaf flour 7.5%.

Table 4. Chemical Characteristics of Quail Eggs

Parameters	Treatments			
	P0	P1	P2	P3
pH	7.22±0,05	7.22±0,04	7.21±0,01	7.22±0.03
Water activity (Aw)	0.87±0.01[b]	0.86±0.00[b]	0.85±0.00[a]	0.85±0.00[a]
Antioxidant activity (%)	54.71±6,36[b]	48.63±2.20[b]	31.42±2.25[a]	27.25±1.24[a]
Malondialdehid (µmol/mg)	4.77±0.80[b]	4.56±0.52[b]	3.47±0.17[a]	3.04±0.56[a]

Different superscripts in the same row indicate significant differences (P<0.05); P0: moringa leaf flourless feed; P1: feed + moringa leaf flour 2.5%; P2: feed + moringa leaf flour 5%; P3: feed + moringa leaf flour 7.5%.

effect (P>0.05) on egg weight, shell thickness, yolk colour and haugh unit. The average egg weight in the study was between 10.26 to 10.65 g. Factors affecting egg weight were protein adequacy in the feed (Da Nóbrega *et al*., 2022). Chimezie *et al*., (2020) reported that quail egg weights were divided into three categories: large eggs (10.23 g), medium eggs (8.49 g), and small eggs (6.94 g). The thickness of the shell ranged from 0.181 - 0.186 mm, which was still within the normal range of 0.17 - 0.22 mm (Song *et al*., 2000; Sudrajat *et al*., 2014), where the adequacy of calcium impacted the thickness in the feed (Dawanto *et al*., 2024). The addition of moringa flour increases the yolk colour score, with the highest score of 7.83 in P3, due to the chlorophyll, carotenoids, and anthocyanins present in the plant (Rezaei *et al*., 2020). The *Haugh Unit* (HU) of eggs ranged from 79.67 to 80.14, which indicated AA grade according to USDA (2020). This result is because the eggs analysed were fresh, so the quality of the egg white was maintained. HU is influenced by albumin height, feed nutrition, protein intake, and egg weight (Bashir *et al*., 2015). The absence of significant differences between treatments was due to genetic factors, nutrition, age, and temperature that are not different (Pereira *et al*., 2022).

Chemical Characteristics of Quail Eggs

The chemical testing of quail eggs using moringa flour in the feed can be seen in Table 4. The statistical analysis of adding moringa flour to feed did not significantly affect the pH of quail eggs (P>0.05). Still, it significantly affected (P<0.05) the water activity (Aw), antioxidant activity, and MDA of quail eggs.

The average pH was 7.21-7.22. Based on the study of Kunaifi *et al*., (2019), fresh eggs with 0-14 days of storage have a pH of around 7 because no microbial activity breaks down protein or fat. According to Mutiar *et al*., (2023), the pH of good-quality eggs is 7.6-7.9. This study also showed a decrease in Aw value from P0 to P3, reflecting the reduction of free water that supports the growth of microorganisms. Aw values are influenced by chemical composition, humidity and temperature, with decreased Aw playing an essential role to inhibit microbial growth and extending the shelf life of eggs (Zhang *et al*., 2018).

The results also indicated that the antioxidant activity value of quail eggs was very strong, characterised by the smaller IC_{50} value, especially in the P2 (31,42) and P3 (27.25) treatments. This result indicated the role of bioactive compounds, such as flavonoids in moringa flour, as natural antioxidants that could neutralise free radicals in the body and accumulate in eggs. Higher antioxidant activity is associated with reduced levels of MDA P2 (3,47) and P3 (3,04), an indicator of lipid peroxidation often used to measure oxidative stress (Dromant *et al*., 2021).

Table 5. Microbiological Characteristics of Quail Eggs

Parameters	Treatments			
	P0	P1	P2	P3
TPC (log CFU/ml)	4.19 ± 0.32	3.85 ± 0.26	3.55 ± 1.04	3.12 ± 0.25
E. coli (MPN/ml)	3	3	<3.6	<3.6
Salmonella sp. (/25g)	Negative	Negative	Negative	Negative

TPC: Total place count; *E.coli*: *Escherichia coli*; P0: moringa leaf flourless feed; P1: feed + moringa leaf flour 2.5%; P2: feed + moringa leaf flour 5%; P3: feed + moringa leaf flour 7.5%.

Table 6. Hedonic and Hedonic Quality of Quail Eggs

Parameters	Treatments			
	P0	P1	P2	P3
Hedonic				
Smell	3.97±0.56	4.03±0.41	4.10±0.61	4.00±0.46
Taste	4.33±0.48	4.30±0.47	4.40±0.50	4.37±0.56
Egg yolk color	4.80±1.10	4.73±0.91	4.87±1.07	4.93±1.06
Egg white colour	4.33±0.48	4.37±0.49	4.37±0.49	4.30±0.47
Egg yolk texture	4.43±0.68	4.40±0.72	4.50±0.68	4.50±0.57
Egg white texture	4.90±0,85	4.87±0,82	4.83±0,87	4.80±0.85
Hedonic Quality				
Smell	3.50±0,51	3.63±0.49	3.60±0.50	3.63±0.49
Taste	3.60±0.50	3.67±0.48	3.63±0.49	3.60±0.50
Egg yolk color	3.47±0.51	3.43±0.51	3.57±0.57	3.50±0.63
Egg white colour	3.70±0,47	3.73±0,45	3.70±0.47	3.70±0.47
Egg yolk texture	3.47±0.51	3.43±0.50	3.53±0.51	3.50±0.51
Egg white texture	3.63±0,49	3.63±0,49	3.67±0.48	3.63±0.62

P0: moringa leaf flourless feed; P1: feed + moringa leaf flour 2.5%; P2: feed + moringa leaf flour 5%; P3: feed + moringa leaf flour 7.5%.

Antioxidants stabilise free radicals and stop lipid peroxidation (Zhao *et al*., 2023). Nunes *et al*., (2019) also support the idea that antioxidant-rich ingredients such as *Acrocomia aculeata pulp* (0,5% flour) in feed can increase antioxidant activity (14,0%) and decrease MDA (9,64 mg/kg) in quail eggs. These results demonstrate the potential of Moringa leaf meal to reduce oxidative stress and produce functional eggs.

Microbiological Characteristics of Quail Eggs

The results of microbiological testing of quail eggs with moringa flour in the feed can be seen in Table 5. The results showed a decrease in TPC and E. coli values with negative results on Salmonella sp. from P0 to P3. The decrease in TPC and *E. coli* values indicated the effectiveness of bioactive compounds in moringa flour, such as flavonoids, polyphenols, saponins, and tannins, in inhibiting microbial growth in eggs. These compounds can reduce pathogenic bacteria in the digestive tract, lowering bacterial contamination of eggs (Batool *et al*., 2023). Besides, the antioxidant properties of Moringa leaves support the health of body tissues and the reproductive system of quail (Imtiaz *et al*., 2023). Hajati *et al*., (2020) reported that flavonoids, saponins, and tannins in spirulina also effectively reduced the *E. coli* population in the ileum of quail. The negative results of Salmonella sp. in all treatments showed that the eggs met the microbiological safety standards according to SNI 2897-2008. The absence of *Salmonella* sp. contamination is also supported by an environment that is less favourable to bacterial growth and the influence of bioactive compounds in moringa leaf flour.

Organoleptic Characteristics of Quail Eggs

The results of the hedonic quality test and quail egg hedonic test are presented in Table 6. The results of the Kruskal-Wallis analysis of panellists' responses to the hedonic test and hedonic quality showed no significant differences

(P>0.05). The hedonic test describes the panellist's liking test, while the hedonic quality test is more specific to egg quality (Wahyuni and Sudrajat, 2021). The average value of panellists' liking for the attributes of smell, taste, colour of egg yolk and white, and texture of egg white and yolk was in the range of 3.97-4.93, which was still a neutral trend. Egg quality test results showed similar panellist ratings on smell, taste, colour and texture. Panellists tended to rate the smell as fishy, the flavour as good, the yolk as yellow, the egg white as white, and the white and yolk as chewy. According to Randoni *et al*., (2020), the eggs the public prefers have yellow to orange yolks with good taste, chewy texture, and no fishy smell. The absence of differences in hedonic assessment and hedonic quality indicated that the eggs produced are acceptable to consumers.

CONCLUSION

Moringa leaf meal can be fed to quail to overcome oxidative stress. Adding up to 7.5 % of moringa flour positively reduced the number of bacteria in eggs, reduced fat oxidation, and increased antioxidant activity in quail eggs.

ACKNOWLEDGMENTS

The authors thank the Ministry of Finance of the Republic of Indonesia through the Education Fund Management Programme (LPDP) for funding this research.

CONFLICTS OF INTEREST

There is no conflict of interest in this research.

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