Research Article

# Effect of Substitution of *Moringa oleifera* Leaf in Feed on the Production and Reproductive Performance of Native Chickens

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**Abstract** | The present study was carried out to evaluate the effect of substituting *Moringa oleifera* (moringa) leaf meal in feed on the production and reproductive performance of native chickens. The study was conducted in two stages: In the first stage, the effects of moringa administration on male reproductive traits were assessed, including semen volume, sperm concentration, and sperm motility. In the second stage, the effects of moringa on the performance of female chickens were investigated. In the first experiment, a total of 20 male native chickens were randomly divided into five groups (n = 4 per group). The first group received a diet containing 0% moringa leaf meal (control), while the 2nd, 3rd, 4th, and 5th groups were fed diets supplemented with 2%, 4%, 6%, and 8% moringa, respectively. In the second experiment, 60 chickens (50 females and 10 males) were divided into two groups, each consisting of 25 females and 5 males. One group served as the control (0% moringa), while the treatment group received 6% moringa in the feed. The results of the first study showed that semen volume, sperm concentration, and sperm motility were significantly improved ($p < 0.05$) in the moringa-supplemented groups compared to the control, with the highest values observed in the 6% moringa group. Additionally, sperm viability and morphology also showed statistically significant improvements ($p < 0.05$) in the moringa-treated groups compared to control, although the differences were less pronounced. In the second study, no significant differences ($p > 0.05$) were observed between control and treatment groups in terms of fertility, hatchability, or day-old chick (DOC) weight. However, egg weight and hen-day egg production were significantly higher ($p < 0.05$) in the moringa-fed group compared to the control. The study concluded that the inclusion of 6% *Moringa oleifera* leaf meal in the diet of native chickens had positive effects on semen quality in males, and significantly improved egg weight, and hen-day egg production in females.

**Keywords** | *Moringa oleifera*, Native chicken, Sperm quality, Hen day production, Egg weight, FCR

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## INTRODUCTION

Almost all countries in the world, including Indonesia, have native chickens with different breeds. There are several advantages of native chickens compared to commercial chickens, including adaptability to the environment, resistance to disease, a promising market, and lower cholesterol and fat content (Alam *et al*., 2020). The main actors in free-range chicken farming are typically local breeders who operate on a non-industrial,

household scale, using semi-intensive rearing systems. These breeders often maintain control over both market access and pricing (Wilson *et al*., 2022). Reproductive performance in male and female chickens begins at sexual maturity (Lei at al., 2021). While, decreased fertility is a major problem in chicken farming due to old age, oxidative stress, and disruption of steroid formation (Malik *et al*., 2013). Furthermore, Lagares *et al*. (2017) stated that native chicken sperm quality would decrease after 50 weeks of age. Therefore, the breeder livestock industry or local farmers always anticipate so that the roosters can be maintained optimally.

Feed quality is one of the important factors in determining the success of native chicken productivity. The moringa tree is known to have high nutritional values and medical benefits due to hypocholesterolemic properties (Bibia *et al*., 2023). Moringa leaves are rich in minerals such as calcium, zinc, potassium, magnesium, iron, copper, vitamins such as beta-carotene from vitamin A, B vitamins such as folic acid, vitamins C, D, E, pyridoxine, and nicotinic acid (Shivangi *et al*., 2023). Moringa contains essential amino acids, minerals, vitamins, fatty acids, fiber, and different compounds of antioxidants, including poly-phony, pro-anthocyanidins, flavonoids, and flavonols (Khalafalla *et al*., 2010; Dhalaria *et al*., 2020). *Moringa oleifera* is very useful as a feed supplement for animals, as its leaves are highly nutritious, and the plant is widely grown in tropical and subtropical regions, including Indonesia (Leone *et al*., 2015). Sharmin *et al*. (2020), and Gobezie (2021) reported that the use of moringa leaves in broiler chickens can reduce the feed conversion ratio (FCR) while improving growth performance and meat quality.

On the other hand, the use of *Moringa oleifera* leaf extract as a sperm diluent has been shown to enhance motility, viability, and antioxidant capacity of rabbit sperm (El-Desoky *et al*., 2017; El-Seadawy *et al*., 2017; Ajuogu *et al*., 2019). Furthermore, the addition of methanolic extract from moringa seeds to sheep semen has been reported to improve post-thaw sperm motility (Carrera-Chavez *et al*., 2020). Venkatesh *et al*. (2019) also found that administering moringa leaf extract to stressed mice significantly improved sperm quality, testosterone levels, and sexual behavior. Similarly, Iqbal *et al*. (2022) reported that supplementation with 15% moringa leaves offered substantial protection during freezing and significantly increased post-thaw sperm motility in water buffalo. Given these promising effects on male reproductive parameters across species, it is important to investigate the potential benefits of moringa supplementation in native chickens to improve their reproductive performance. Therefore, the present study evaluated the effects of substituting *Moringa oleifera* leaf in feed on the production and reproductive performance of native chickens in Indonesia.

## MATERIALS AND METHODS

### Feed, birds, and management

The present study was conducted in the Animal Production Laboratory, Faculty of Agriculture, University Islamic of Kalimantan (UNISKA), and the BIB lab, Banjarbaru, Province of South Kalimantan, Indonesia, with coordinates (3°15' to 3°22' S,114°32' E). During the study, the mean ambient temperature ranged from 30 to 34°C, and relative humidity ranged from 65 to 70% (Maksindo Ltd. Jakarta. Indonesia). The present study was divided into two stages. The first stage aimed to evaluate the effects of adding moringa to the feed at different doses on the performance of male native chickens. A total of 20 male native chickens aged 40 weeks with an average body weight of 1.3 kg were selected from local farms and randomly assigned to five groups. Each group consisted of four chickens. The first group was given a 100% basal ration (T1) without moringa, the second group was given a 98% basal ration + 2% moringa leaf flour (T2), the third group was given a 96% basal ration + 4% moringa leaf flour (T3), the fourth group was given a 94% basal ration + 6% moringa leaf flour (T4), and the fifth group was given a 92% basal ration + 8% moringa leaf flour (T5). Feeding pellet form was done twice a day with 120 g/head/day. The basic ingredients and nutritional content of male chicken feed in the first study was listed in Table 1. The proximate analysis of feed ingredients to determine crude protein (CP), Fat, Fiber, Ca, and P content was conducted based on the Association of Official Analytical Chemists method (AOAC, 2003). while, metabolizable energy (ME) content in feed ingredients was calculated based on the chemical composition (NRC, 1994). Water was freely available for drinking. While, the adaptation period to changes in feed treatment was two weeks in both the first and second stages of the present study. Semen collection was performed during the third week, with two collections each week for four weeks. The parameters were evaluated, including volume of semen, sperm viability, concentration, sperm morphology, and motility.

The second stage of this study was continued to applying the best findings from the first study, with namely effects of adding moringa at 6% on the production performance of female native chickens. A total of 60 native chickens aged 50 weeks, consisting of 50 females and 10 male chickens, were selected from local farms. All chickens were randomly divided into two groups, each group containing 25 females and 5 male chickens.

The first group was given basal feed without moringa leaf substitution, while the second group was given 6% moringa leaf substitution. The composition of the basal ration used in the second study was presented in Table 2. Meanwhile, data collection on female chickens began in the third week. The parameters were observed, including

production performance, hen day production (HDP), and feed conversion ratio (FCR). While, reproductive parameters include egg weight, fertility rate, hatching rate, and hatching weight were also investigated.

**Table 1:** Basic ingredients and nutritional content of male chicken feed in the first study.

| Feed ingredients | % substitution of moringa in feed | | | | |
|---|---|---|---|---|---|
| | T0 (0 %) | T2 (2 %) | T4 (4%) | T6 (6%) | T8 (8%) |
| Concentrate [1] | 400 | 392.0 | 384.0 | 376.0 | 368.0 |
| Rice bran | 290 | 284.2 | 278.4 | 272.6 | 266.8 |
| Corn | 280 | 274.4 | 268.8 | 263.2 | 257.6 |
| Vitamin premix[2] | 20 | 19.6 | 19.2 | 18.8 | 18.4 |
| Mineral premix[3] | 10 | 9.8 | 9.6 | 9.4 | 9.2 |
| Moringa | 0 | 20.0 | 40 | 60 | 80 |
| Total | 1,000 | 1,000 | 1,000 | 1,000 | 1,000 |
| **Chemical composition** | | | | | |
| Metabolizable energy (kcal/kg) | 2,766 | 2,752 | 2,737 | 2.723 | 2,709 |
| Crude protein (%) | 18.96 | 19.14 | 19.31 | 19.49 | 19.66 |
| Ca (%) | 1.35 | 1.38 | 1.40 | 1.43 | 1.45 |
| P (%) | 0.73 | 0.73 | 0.72 | 0.72 | 0.72 |

[1]Ingredients of concentrate were soybean meal, fish meal, meat and bone meal, corn protein meal, limestone meal, oil, sodium bicarbonate, vitamins, trace minerals, and amino acids purchased from a commercial company (PT. New Hope, Makasar, Indonesia). Nutrient composition was moisture content: max 13.0%, protein: min 35.0%, fat: min 2.0%, crude fiber: max 9.0%, ash: max 35.0%, calcium: 9.0-12.0%, total phosphorus: min 0.80%, lysine: min 1.70%, methionine: min 0.80%, methionine + cystine: min 1.30%, tryptophan: min 0.34%, and threonine: min 1.10%. [2]Per 10 g contained: Vitamin A, 12,500 IU; Vitamin D3, 2,500 IU; Vitamin E, 10 mg; Vitamin K3, 2 mg; Vitamin B1, 2 mg; Vitamin B2, 4 mg; Vitamin B6, 1 mg; Vitamin B12, 12mg; Vitamin C, 40 mg; Niacin, 4 mg; Ca-d-pantothenate, 0.2 mg; biotin, 0.2 mg; L – arginine, 10 mg; L – threonine, 15 mg; DL-methionine, 50 mg; L-lysine, 125 mg; choline, 20 mg; folic acid, 0.5 mg; zinc, 70 mg; Ferrum, 30 mg; Manganese, 60 mg; Copper, 5 mg; Iodine, 0.2 mg; Selenium, 0.2 mg; Cobalt, 0.2 mg; antioxidant carrier, 0.01 mg. [3]Each kg contains: Ca, 32.5 %; P, 1.0%; Fe, 6 g; Mn, 4 g; I, 0.075 g; Cu, 0.3 g; Zn, 3.75 g; Vitamin B12, 0.5 mg; Vitamin D3, 50,000 IU; Excipients, up to 1 kg.

**Table 2:** The ingredients and chemical composition of basal diet used in the second study.

| Composition of feed | % substitution of moringa in basal diet | |
|---|---|---|
| | T0 (0%) | T 6(6%) |
| Concentrate[1] | 400 | 376.0 |
| Rice bran | 290 | 272.6 |
| Corn | 280 | 263.2 |
| Vitamin premix[2] | 20 | 18.8 |
| Mineral premix[3] | 10 | 9.4 |
| Moringa | 0 | 60 |
| Total | 1,000 | 1,000 |

1, 2, and 3 see Table 1 footnote.

## Semen collection

Semen collection was obtained using the abdominal massage technique described by Malik *et al.* (2013). Then, semen was placed into 2 mL centrifuge tubes. The samples were immediately transported to the laboratory, where the tubes were immersed in a water bath at 37°C and immediately subjected to laboratory examination including pH, consistency, and volume.

## Semen volume and sperm concentration

Volume of semen was measured using a graduated tube (with an accuracy of 0.1 mL). The technique of measuring semen volume was used in a graduated tube (with an accuracy of 0.1 mL). Meanwhile, sperm concentration was determined using a Neubauer hemocytometer (Ghadimi *et al.*, 2024).

## Sperm viability

Sperm viability was evaluated using the eosin-nigrosin staining method adopted by Mehdipour *et al.* (2021). A total of 10 mL of semen and an equal amount of eosin-nigrosin were mixed, fixed, and allowed to air dry on a slide. Sperm evaluation was performed using a phase contrast microscope at 400x magnification (Olympus, Tokyo, Japan). From each sample, 200 sperm were counted. Sperm that did not absorb the stain were classified as alive, while spermatozoa that absorbed the stain were grouped as dead.

## Sperm morphology

A total of 20 mL of semen was put into a tube containing 1 mL of Hancock's solution, including 500 mL of double-distilled water, 150 mL of saline solution, 150 mL of buffer solution, and 62.5 mL of 37.5% formalin. Then, 5-10 mL of processed sperm was placed onto a slide, and 200 sperm were counted using a phase-contrast microscope at 400x magnification (Olympus, Tokyo, Japan). The sperm morphology showed damage, including defects in the mid-piece and tail, detached heads, and other acrosome abnormalities (Mehdipour *et al.*, 2021).

## Natural mating and fertility

The mating system in the second stage of this study was carried out naturally with a ratio of 1:5 (one male: five females) for 14 days. Egg collection began on the second day after mating and continued for two weeks. A total of 150 eggs from the first group and 150 eggs in the second group were used for the study. After that, the eggs were incubated at 37.5°C and a relative humidity of 55%. The fertility rate was the percentage of fertilized eggs per incubated egg. Hatchability was the percentage of hatched eggs divided by the total number of fertile eggs. Hatching weight was obtained by weighing the newly hatched day-old chick after its feathers dried. Hen-day production was calculated as the number of eggs produced daily divided by

**Table 3:** Semen quality parameters of roosters supplemented with different doses of moringa leaf.

| Parameters | % substitution of moringa in feed | | | | |
|---|---|---|---|---|---|
| | T0 (0%) | T2(2%) | T4(4%) | T6(6%) | T8(8%) |
| Volume (mL/bird) | 0.61± 0.16[a] | 0.62± 0.03[b] | 0.63±0.04[c] | 0.74±0.02[d] | 0.64±0.03[c] |
| Concentration (x $10^9$cells/mL) | 1.79± 0.61[a] | 1.82± 0.28[b] | 1.86±1.01[c] | 2.02±0.85[d] | 1.91±0.05[d] |
| Viability (%) | 78.01± 0.65[a] | 78.39±0.50[b] | 79.49±0.68[b] | 79.53±0.45[b] | 79.55±0.49[b] |
| Motility (%) | 74.71± 0.52[a] | 75.02±1.26[b] | 77.16±0.89[c] | 79.72±0.52[d] | 79.70±0.52[d] |
| Morphology/Abnormality (%) | 10.90± 0.89[a] | 10.97±0.52[a] | 10.67±0.52[b] | 10.37±0.52[c] | 10.35±1.26[c] |

[e] mean ± SE values within the same rows followed by different superscript letters differ significantly ($p < 0.05$).

the daily population, multiplied by 100%. Meanwhile, the FCR was calculated from the total feed consumed divided by the total egg production.

### Statistical analysis

Statistical analysis was performed using the SAS Program (version 9.1). Data normality was checked using the Shapiro-Wilk test. If there was a significant difference, the Tukey test was used for further analysis. A general linear model was used to examine fertility rate, hatchability, and hatching weight (PROC GENMOD, chi-square test), with a significance level of $p < 0.05$.

## RESULTS

The average pH of rooster semen across all treatments ranged from 7.0 to 7.3, while semen consistency remained within expected norms for all groups. The effects of moringa leaf substitution in rooster feed are detailed in Table 3. Semen volume, sperm concentration, and motility increased significantly ($p < 0.05$) in the groups supplemented with moringa compared to the control, with the greatest improvements seen at the 6% supplementation level. Furthermore, sperm viability and morphology also demonstrated statistically significant ($p < 0.05$) enhancements in the moringa-treated groups, though these changes were less marked. Notably, sperm motility peaked in the 6% moringa treatment group (79.72%), whereas the 8% group showed a slight, non-significant decline to 79.70%.

**Table 4:** Reproductive performance of female native chickens supplemented with 6% moringa leaf in the diet.

| Parameters | % substitution of moringa in feed | |
|---|---|---|
| | T0 (0%) | T6 (6%) |
| Egg weight (g) | 43.35 ± 0.77[a] | 44.08 ±0.95[b] |
| Fertility (%) | 90.10 ± 0.23 | 90.21 ± 0.16 |
| Hatchability (%) | 83.76 ± 0.93 | 84.31± 0.25 |
| Hatch weight (DOC) (g) | 28.87 ± 0.49 | 28.95 ± 0.21 |
| Hen day production (HDP) (%) | 54.05± 0.81[a] | 59.10± 0.92[b] |
| FCR | 3.21 ± 0.61 | 3.15±0.87 |

[a-b] mean ± SE within the same row followed by different superscript letters differ significantly ($p < 0.05$).

The results of the second study, which assessed reproductive performance in female chickens, are presented in Table 4. Supplementation with 6% moringa leaves showed no significant differences ($p > 0.05$) in fertility, hatchability, or day-old chick (DOC) weight compared to the control. However, egg weight and hen-day production (HDP) were significantly improved ($p < 0.05$) in the treatment group. Feed conversion ratio (FCR) did not differ significantly ($p > 0.05$) between groups, although there was a tendency for a reduction in FCR with moringa supplementation (Table 4).

## DISCUSSION

The results of the present study generally indicated that the substitution of moringa in male native chicken resulted an increase in sperm concentration, semen volume, and sperm motility. The current findings were strengthened by Dafaalla *et al.* (2016) who said that the effects of ethanol extract from moringa leaves could increase reproductive hormones and sperm quality. Furthermore, Najafi *et al.* (2018) and Supakorn *et al.* (2023) stated that chicken spermatozoa were susceptible to lipid per-oxidation due to a decrease in the antioxidant system, which was related to age and naturally increases poly unsaturated fatty acids (PUFA) levels in the plasma membrane. Adding moringa leaves at a level of 6% (T6) to male chicken feed indicated the best results in semen volume. This improvement can be attributed to the natural antioxidants and rich nutritional profile of moringa leaves. Satisfying the nutritional requirements of roosters positively influences reproductive performance, leading to increased semen volume compared to the control group. Supporting this, Khalil-Khalili *et al.* (2021) and Sabzian-Melei *et al.* (2022) reported that moringa leaves enhance productivity, efficiency, and reproductive outcomes, including an increase in chicken semen volume.

Furthermore, Ghadimi *et al.* (2024) reported that substituting feed ingredients with herbal antioxidants can enhance the quality of chicken sperm. However, the present study showed that supplementation with 8% moringa leaves (T8) resulted in a plateau and slight decline in sperm volume and concentration. The high antioxidant

content of moringa likely contributes to improved reproductive capacity and sperm quality, as evidenced by increases in sperm viability, concentration, and motility. Moringa leaves are rich in bioactive phenolic compounds, including flavonoids, phenolic diterpenes, essential oils, and phenolic acids (Verma *et al*., 2009; Leone *et al*., 2015). These phytochemicals exhibit strong antioxidant and free radical scavenging activities, which can protect reproductive tissues from oxidative stress-induced damage (Stohs and Hartman, 2015).

In addition, further investigation into the performance of female native chickens fed 6% moringa leaves (T6) revealed significant increases in egg weight and hen-day production (HDP) compared to the control group (Table 4). These findings align with those of Mahfuz and Piao (2019), who observed increased egg weight with 5% moringa leaf meal supplementation. However, increasing moringa to 20% resulted in decreased egg weight, potentially due to higher protein intake, feed conversion ratio (FCR), reduced egg production, and lower nutrient digestibility caused by anti-nutritional phytochemicals present in moringa leaves (Kakengi *et al*., 2007).

The results of the present study showed that supplementation with 6% moringa led to an increase in HDP compared to the control group. These findings are consistent with Elezz et al. (2011), who reported that adding moringa to the diet of laying hens enhances HDP. Similarly, Melesse *et al*. (2011) demonstrated that moringa supplementation improves egg mass, egg production, and yolk color, attributed to the high carotene content in moringa leaves. Additionally, moringa feeding increases feed and crude protein intake, which positively affects weight gain, FCR, and protein efficiency in laying hens, due to the rich protein, mineral, and amino acid content of moringa leaf meal.

## CONCLUSION

Based on the present study, it can be concluded that adding moringa at a level of 6% in male native chicken feed has a great impact on reproductive performance, especially spermatozoa concentration, motility, and semen volume in male chickens. Moreover, adding 6% moringa to female native chicken feed can increase hen day production, egg weight, and feed conversion ratio.

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## NOVELTY STATEMENT

Our research results was shown that substituting moringa leaves at a level of 6% can have a positive impact on hen day production and improve male native chicken reproductive performance.

## AUTHOR'S CONTRIBUTION

AP, P and AM: Conducted the experiments.
S: Observed sperm concentration, sperm viability, and sperm morphology.
AM and AG: Prepared and analyzed the data, and drafted the manuscript.

### Availability of data and materials

All data and materials are available on request from the corresponding author.

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### Ethical approval

The animal ethics committee's approval for the present study was obtained by the Animal Care and Use Committee of the Faculty of Agriculture, Islamic University of Kalimantan with number 23/U.CC/FP/IV/2024. The authors affirmed that all ethical issues in this manuscript have been addressed by all authors including consent to publish, and there is no duplicate publication.

### Generative AI and AI-assisted technology statement

The all author was declaration not generative AI to make in this manuscript.

### Conflict of interest

The authors have declared no conflict of interest.

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