



# Evaluation of Semen Quality of Free-Range Chickens After Addition of Putri Malu Leaf Herbal Extract (*Mimosa pudica* Linn)

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**Abstract:** This research was conducted to determine the effect of female shy daughter (*Mimosa pudica* Linn) leaf extract supplementation on the semen quality of native chickens. In this study, 18 male native chickens aged approximately 1 year with an average weight of 2.0 kg were used, divided into 6 treatments, each with 3 replications, each consisting of 1 chicken. Basal feed in this study used BR-1 chicken feed produced by PT. Japfa Comfeed Indonesia Tbk. As an adjustment before treatment, the chickens were reared for 1 week with basic feed. As a control before being given treatment, sperm was taken from chickens and used as a control. Furthermore, the chickens began to be given treatment by giving shame daughter leaf extract supplementation. The study was conducted experimentally using a completely randomized design by allocating 18 free-range male chickens which were divided into 6 treatments, each treatment consisting of three replications, one chicken each. The conclusion of this study is that the supplementation of Putri Mal leaf extract as much as 0.50 cc to 2 cc per day per chicken can increase sperm count per ml of free-range chicken semen, viability, motility, thickness, and color density, while the volume and pH of semen do not experience change.

**Keywords:** cement production; embarrassed daughter leaf extract; free-range chicken

## Introduction

Free-range chicken is a potential germplasm and genetically has a high adaptability to the environment. This indicates that free-range chicken has a significant role in the development of livestock in Indonesia, as well as being the economic basis for rural farmers for the needs of meat and eggs (free-range chicken) for the community (Wong et al., 2017). Maintenance of free-range chickens is mostly diumbar. This makes malnutrition a major problem. For hens this will affect egg production, while for male hens it will affect sperm quality. Therefore, so that free-range chickens can produce well, one of them must be given adequate feed, both nutrition and quantity. Increasing the amount of nutrients will accelerate puberty and body growth. In adult livestock, lack of food can cause physiological function disorders, both in the testes and accessory glands, and can reduce libido so that semen production decreases. Therefore, it is necessary to maintain the completeness of nutrition because this will help manage

livestock reproduction which in turn will improve the function of the reproductive organs as well as improve semen quality.

Putri Malu plant (*Mimosa pudica* Linn) is a herbaceous plant that is often used in traditional medicine (Arfiandi et al., 2022). This plant contains alkaloids, saponins, flavonoids, tannins, and phenolics. The embarrassed daughter plant can be used in all parts because it contains medicinal compounds (Twaij & Hasan, 2022). The phytochemical screening of the ethyl acetate fraction in the shy daughter showed the presence of compounds belonging to the class of flavonoids, tannins, polyphenols, monoterpenoids, and steroids. In the shy daughter plant there are 3 kinds of steroids namely ergost-5-en-3 $\beta$ -ol, stigmast-5,22-dien-3 $\beta$ -ol and stigmast-5-en-3 $\beta$ -ol. Steroids in the leaves of Putri Malduda have an aphrodisiac effect, so this plant is often used as a strong medicine to increase virility.

The Zn mineral contained in the leaves of the embarrassed daughter is needed in the process of spermatogenesis. The Zn mineral content in the leaves of

Putri Maluku is 14.0 mg/kg. Zn minerals stimulate Leydig cells in the testes to produce testosterone (Santos & Teixeira, 2020), in the process of spermatogenesis Zn minerals play a role in ribonuclease activity, maturation of spermatozoa and increasing sperm motility and maintaining the germinative epithelium and seminiferous tubules. Zn is important for the normal development of the testes, maintenance of the germinal epithelium, and sperm motility. Several studies also reported a significant increase in normal sperm count in subfertile males after being given folic acid in combination with zinc sulfate (Zhao et al., 2016).

Another benefit of *Mimosa pudica* is as an aphrodisiac or libido enhancer. This has been studied in male rats compared with the standard drug (sildenafil). The result is that the administration of 500 mg/kg bw of Putri Maluku extracts once a day for 1 week significantly increases the libido of the rats and increases their testosterone level. The leaves of the embarrassed daughter (*Mimosa pudica*) function as a trigger for increased testosterone production, which is very necessary for sperm production and virility in chickens. These herbs have been studied for their efficacy in experimental animals (male rats). However, the effect on the production and quality of spermatozoa is not known with certainty. Therefore, in this study, we wanted to know the effect of giving putri embarrassment leaf extract on the sperm quality of male native chickens. This is related to the abundant availability of the embarrassed daughter plant so that if it is proven to be able to increase the production of chicken sperm, it will produce cheap and efficacious aphrodisiac herbal medicines.

## Method

The study was conducted experimentally using a completely randomized design by allocating 18 free-range male chickens which were divided into 6 treatments, each treatment consisting of three replications, one chicken each. The treatments given were: Control group (T0) chickens were given basal feed (BR-1), (without giving Putri Mal extract); group T1: basal feed and supplementation of embarrassed daughter leaves 0.250 cc/head/day; group T2: basal feed and supplementation of the leaves of Putri Maluku leaf extract 0.500 cc/head/day; group T3: basal feed and supplementation of female embarrassment leaf extract 0.750 cc/head/day; T4: basal feed and supplementation of Putri Maluku leaf extract 1 cc/head/day; T5 basal feed and supplementation of embarrassed daughter's leaf extract 2 cc/head/day. Supplementation is done by force-feeding with a syringe.

## Result and Discussion

Cement appearance of free-range chickens in this study on a grass basis in both the control and treatment groups did not show any significant physical difference. Physically, the difference is seen in the thickness and density of the color, wherein the treatment group the color appears to be stronger towards beige, while for the thickness, the treatment group's chickens appear thicker.

### *Cement Volume*

The increase in the semen volume of free-range chickens after supplementing the embarrassed daughter's leaf extract can be seen in Table 1. From Table 1, it can be seen that the average volume of native chicken semen before treatment was 0.22 ml/ejaculate and after treatment it became 0.25 ml/ejaculate. There was an average increase of 0.03 ml/ejaculate, but after testing with SPSS it turned out that both the volume and the increase were not significantly different ( $P > 0.05$ ). The semen volume of the cock each time he ejaculated ranged from 0.16 – 0.72 ml. These results are also in accordance with the findings of our study on previous native chickens, namely between  $0.29 \pm 0.07$  ml. -  $0.40 \pm 0.03$  ml. According (Fernandez-Novo et al., 2021), semen quality is affected by temperature, age, sexual activity and genotype. (Abah et al., 2023) added that sperm quality and quantity are influenced by genetics, age, feed, temperature, ejaculation frequency, libido, physical factors, transport, testicular size, disease health and livestock breeds.

Provision of embarrassed daughter leaf extract did not increase the volume of native chicken semen (Table 2). This shows that semen volume was not affected by the supplementation of the shy daughter extract, which, when calculated, added nutrition was very small, as well as corroborating the opinion of above (Amrein et al., 2020). Furthermore (Chen et al., 2023), explains that nutrition has an effect on livestock reproduction is vitamin A, vitamin E and minerals. In this study, there was no additional intake of vitamin A and vitamin E from the supplementation of Putri malu leaf extract. (Mousa et al., 2019) added that the micro and macro nutrients needed to achieve reproductive success in addition to vitamin A, are vitamin B12, vitamin B9 (folic acid), vitamin D, selenium, nickel, manganese, chromium, copper, fatty acids, protein, arginine and carnitine. In this study, the addition of these elements to the shy daughter's leaf extract was only very small, so it did not affect semen volume.

**Table 1.** The appearance of free-range chicken semen after being supplemented with embarrassed daughter leaf extract.

Parameter	Treatment					
	T0	T1	T2	T3	T4	T5
Semen volume (ml)	0.20	0.20	0.20	0.20	0.20	0.20
Sperm count (Cell x 106)	2.63 <sup>a</sup>	3.20 <sup>ab</sup>	4.09 <sup>b</sup>	3.55 <sup>ab</sup>	4.17 <sup>b</sup>	4.32 <sup>b</sup>
Consistency	Medium - Thick	Thick	Thick	Thick	Thick	Thick
Color	Milky white	Milky white	Milky white	Milky white	Milky white	Milky white
Mass movement	+++	+++	+++	+++	+++	+++
Motility (%)	87.89±2.88 <sup>a</sup>	90.35±3.80 <sup>b</sup>	88.66±2.64 <sup>a</sup>	88.81±2.82 <sup>ab</sup>	91.34±3.53 <sup>b</sup>	91.22±4.27 <sup>b</sup>
Viability (%)	86.51±3.47 <sup>a</sup>	89.70±2.72 <sup>b</sup>	88.46±2.43 <sup>ab</sup>	89.91±2.57 <sup>b</sup>	90.37±2.64 <sup>b</sup>	90.28±2.44 <sup>b</sup>
pH	7.10	7.10	7.30	7.10	7.30	7.20

Note: The average value followed by different superscripts in the same line shows a significant difference (p <0.05).

**Table 2.** Increase in volume after supplementation of embarrassed daughter leaves

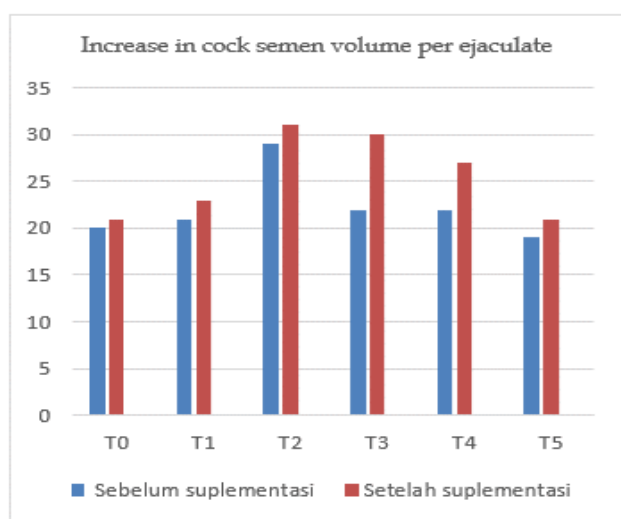
Treatment	Volume before treatment (ml/ejaculation)	Volume after treatment (ml/ejaculation)	Increase (ml/ejaculation)
T0	0.20	0.21	0.01
T1	0.21	0.23	0.02
T2	0.29	0.31	0.02
T3	0.22	0.30	0.08
T4	0.22	0.27	0.05
T5	0.19	0.21	0.02
Average	0.22	0.25	0.03

Description: Not significantly different (P > 0.05).

*Sperm Count*

Unlike the volume of semen, the sperm count of free-range chickens with the difference in the administration of the shy daughter's leaf extract showed an increasing trend along with the higher dose of the embarrassed daughter's leaf (Table 3). The average sperm count of free-range chickens ejaculating before treatment was 2,354 million cells/ml, a very significant increase (P <0.01) after being supplemented with Putri malu leaf extract (3,661 million/ml semen) or an increase of 56% from the initial amount, but this is still within normal limits according to the report of (Zong et al., 2023), reported that the sperm count of chickens is around 0.03 - 11 billion cells/ml. It can be seen here that the addition of the extract at a dose of 0.250 cc per day has not been able to increase the sperm count of chickens. However, the addition of 0.5 cc of extract has been able to significantly increase sperm count, as good as adding extract up to 2 cc per day. This gives an illustration that there is a steroid content in the leaves of Putri Maluku which is suspected to have

Aphrodisiac effect (Tvrdá et al., 2021), plays a role in increasing sperm production. This is probably because one of the steroid hormones, namely testosterone, has a function in stimulating sperm production (spermatogenesis) and is anabolic (Desai et al., 2022). These results are in accordance with our previous findings (Tutubalang et al., 2022) which reported that supplementation with Moringa leaf powder and extract was proven to significantly increase the sperm count of free-range chickens. These findings provide an illustration that the leaves of Putri Maluku



**Figure 1.** Graph Of Increase in Chicken Semen Volume Per Ejaculate

are as good as the ability of Moringa leaves in terms of increasing the sperm count of free-range chickens.

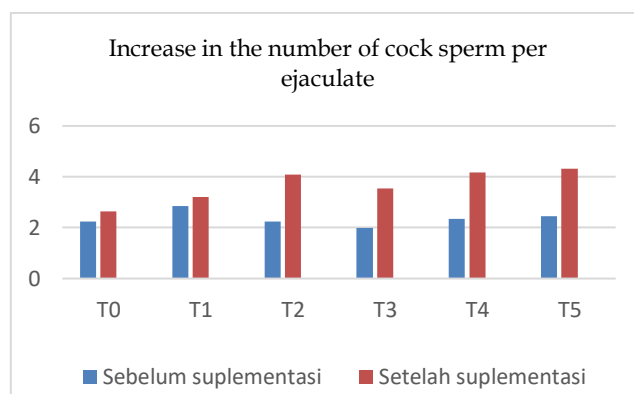
**Table 3.** Increase in the number of chicken spermatozoa per ejaculate

Treatment	Before supplementation (Million cells/ml)	After Supplementation (million cells/ml)	Increase (million/ml)
T0	2.25	2.63	380 <sup>a</sup>
T1	2.84	3.20	351 <sup>a</sup>
T2	2.24	4.09	1.85 <sup>b</sup>
T3	1.99	3.55	1.55 <sup>b</sup>
T4	2.34	4.17	1.82 <sup>b</sup>
T5	2.44	4.32	1.87 <sup>b</sup>
Average	2.35	3.66	1.30

Description: Different superscripts in the same column show very significantly different ( $P < 0.01$ ).

Apart from steroids, the leaves of the embarrassed daughter also contain other substances that can increase sperm concentration, namely the mineral Zn. This mineral functions to stimulate the Leydig cells in the testes to produce testosterone, because this mineral is a protein component involved in the synthesis and secretion of testosterone (Zamir et al., 2021). Zinc is an important component for more than 200 enzyme systems whose metabolic activities include carbohydrate metabolism and protein metabolism, protein synthesis, nucleic acid metabolism, cell repair and division, transport and utilization of vitamins A and E. In males, Zn deficiency causes a decrease in the average ejaculate volume, sperm concentration and percentage of spermatozoa motility (Osadchuk et al., 2021).

In addition to helping maintain the function of sexual organs, sperm production and agility of sperm in humans, Zn also functions in livestock as an enhancer of sperm protection against damage caused by free radicals and increases spermatogenesis by actively participating in the process of sperm maturation and preservation of germinative epithelial tissue and is involved in the development anatomy and normal function of the male reproductive organs (Bronson, 2011).



**Figure 2.** Graph of increasing the number of chicken sperm

*Color and Consistency*

To find out the color of the cement, you can see it by comparing the color of the cement with the color of sweetened condensed milk for cream-colored cement and fresh milk for white cement. The results of examining the color and consistency of free-range chicken semen after the addition of Putri Maluku leaf extract in this study were milky white in color with a thick consistency. In general, the color of the fresh semen of free-range chickens obtained in this study was milky white, and some of the others were rather pale. The color of the cement has a positive correlation with the consistency or thickness of the cement. (Wiyanti et al., 2013) said that free-range chicken semen has a milky white or slightly creamy color. The creamy color of semen is formed due to the dense concentration of spermatozoa. In this study, even though the concentration of each treated semen was different, on a grass basis there was no difference in the color of the semen, although the turbidity appeared to be more turbid in the semen containing more sperm.

In this study, the average cement consistency is thick. This opinion is in accordance with the report of (Promket et al., 2016) who found that the fresh semen of native chickens has a thick consistency. It was also explained that good cement, the degree of thickness is almost the same or slightly thicker than milk, while bad cement, both in color and thickness, is the same as coconut water. Semen consistency is related to the concentration or number of spermatozoa, with the assessment of dilute (<1,000 million spermatozoa per ml of semen), moderate (1,000 - 1,500 million spermatozoa per ml of semen) and concentrated (> 1,500 million spermatozoa per ml of semen). In this study, all chickens, including the control, produced sperm above 2,000 million per ml of semen, so they were included in the thick category.

*Mass Movement*

The mass movement of spermatozoa is an illustration of the motility or movement of individual

spermatozoa. The more active and the more spermatozoa that move forward, the better the mass movement and the faster the movement. There were differences in the average mass movement of free-range chicken spermatozoa after the addition of Putri malu leaf extract between the treatment groups. In the control group (addition of 0.25 cc of extract) and T1 (addition of 0.5 cc of extract) mass movement was at the level (+++), while in group T2 (addition of 0.75 cc of extract), T4 (addition of 1 cc extract) and T5 (addition of 2 cc of extract) is at the level (++++). This finding is similar to the results of research by (Boni, 2019), who reported mass movement in free-range chicken sperm ranging from good (+++) to very good (++++) marked by progressive spermatozoa movement and forming thick, fast-moving mass waves. Sperm with these criteria include good to very good criteria (Bold & Swinburne, 2022). This seems to have something to do with the high - low concentration of spermatozoa in each treatment group and the more active and more moving spermatozoa, resulting in thicker mass movements and faster movements. (Martin & Touaibia, 2020) said that the mineral Zn, which is contained in the leaves of the embarrassed daughter, can stimulate Leydig cells in the testes to produce testosterone, while in the process of spermatogenesis the mineral Zn plays a role in ribonuclease activity, maturation of spermatozoa and increases sperm motility and maintains sperm motility. germinative epithelium and seminiferous tubules. The administration of putri malu leaf extract is thought to be able to increase the nutritional value of the rations so as to enable the metabolism of spermatozoa to take place more optimally which in turn increases the motility of spermatozoa, which is indicated by the mass movement of sperm.

#### *Spermatozoa Viability and Motility*

The results showed that there was a significant increase in the percentage of live spermatozoa (viability) of free-range chickens after administration of putri malu leaf extract. The increase in the percentage of live spermatozoa in the T1, T4 and T5 treatment groups was significantly higher ( $P < 0.05$ ) compared to T0, (control). While the increase in the percentage of live spermatozoa at T2 and T3 has not indicated any significant difference. This illustrates that the administration of putri malu leaf extract is able to optimize the process of spermatogenesis, so as to create optimal conditions from the seminiferous tubules to the epididymis which in turn are able to maintain the life of spermatozoa optimally. Increased production of testosterone by Leydig cells in the testes due to steroid and Zn stimulation from the leaves of Putri Maluku, functions among other things to maintain the complementary sex organs which will

produce seminal plasma which is a medium for the ongoing metabolism of spermatozoa and as a source of nutrition for spermatozoa to live (Qiu et al., 2016).

Semen quality assessment can be done by testing the motility or motility of spermatozoa and the percentage of viable spermatozoa. In this study, the average motility rate in the treatment group (89.6%) was higher than the control group (87.89+2.88%). These results indicate that the percentage of chicken spermatozoa motility after the addition of putri malu leaf extract experienced a significant increase. The Zn mineral and steroids contained in the leaves of Putri Maluku can stimulate Leydig cells in the testes to produce testosterone, while in the process of spermatogenesis Zn minerals play a role in ribonuclease activity, maturation of spermatozoa and Increases sperm motility and maintains the germinative epithelium and seminiferous tubules. The administration of putri embarrassment leaf extract is thought to be able to increase the nutritional value of the ration so as to allow for more optimal spermatozoa metabolism which in turn increases spermatozoa motility. The results of this study are in accordance with the results of (Qazi et al., 2019) who provided an additional 0.02% vitamin E and up to 20% additional protein was able to significantly increase the percentage of live sperm.

#### *Degree of Acidity (pH) of Cement*

The pH of the semen of free-range chickens in this study was in the range of 7 and 8. This shows that the supplementation of Putri Maluku leaf extract had no effect on the acidity of the semen. The average pH of cement at T0 was 7.1; T1 of 7.1; T2 is 7.3 and T3 is 7.1, T4 is 7.3 and T5 is 7.2. Native chicken semen was around 6.82 – 7.88, (Mussa et al., 2023) who reported that the pH of fresh native chicken semen was around 6.83–7.87. The average pH of native chickens in this study was still at the normal standard (7.2). The provision of embarrassed daughter leaf extract did not cause abnormal semen pH of free-range chickens. Semen of chickens that were given embarrassed daughter leaf extract every day had a pH that tended to be alkaline. According to (Chakraborty & Saha, 2022), semen pH affects sperm motility. Changes in semen pH are caused by the metabolism of spermatozoa in the form of lactic acid. (Nechipurenko et al., 2021) said that high levels of lactic acid can cause a decrease in pH. This is closely related to the ability of spermatozoa to survive after leaving the body. A decrease in semen pH due to an increase in the amount of lactic acid can affect the osmotic pressure in the semen plasma, causing a decrease in the permeability of the spermatozoa cell membrane and increased damage to the spermatozoa cell membrane

and can affect the ability of spermatozoa cells to move. Semen pH has an important role in the survival of spermatozoa. Semen conditions that are too acidic cause high spermatozoa mortality (Pimprasert et al., 2023).

## Conclusion

The conclusion of this study is that 0.5 cc to 2 cc per day per chicken can improve the semen quality of free-range chickens in terms of sperm count, thickness and color density as well as viability and motility, while the pH of the semen does not change.

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## Author Contributions

Conceptualization, H.S.; methodology, P.A.; soft, I.N.; validation, H.S., P.A. and I.N.; formal analysis, H.S.; investigation, H.S.; resources, P.A.; data curation, H.S.; writing – original draft preparation, I.N.; writing – review and editing, H.S.; visualization, P.A.; supervision, I.N.; project administration, H.S.; funding acquisition, I.N.

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## Conflicts of Interest

The authors declare no conflict of interest.

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