Synergistic Effect of Moringa Leaf Extract (*Moringa Oleifera Lam*) and Purwoceng (*Pimpinella alpine* Molk) Supplementation on the Quality of Rural Chicken Semen

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Abstract

This study was conducted to examine the synergistic effects of *Moringa oleifera* and purwoceng (*Pimpinella alpine molk*) supplementation on the quality of chicken semen. In this study used roosters aged about 1 year weighing an average of 2.0 kg as much as 18 roosters divided into 6 treatments, each 3 repeats, each repeat consists of 1 rooster. The first group of chickens were used as controls, not given treatment, only given basal feed (T0). The second group of chickens were given moringa leaf extract as much as 1 g/L of drinking water (T1), chicken group 3 was given purwoceng extract as much as 1 g/L of drinking water (T2). Furthermore, chickens in group 4 (T3) were given moringa leaf extract supplements of 250 mg/L of drinking water and 750 mg/L per head per day and group 5 (T4) were given moringa extract supplements as much as 500 mg/L of drinking water and purwoceng 500 mg/L of drinking water ml every day (T5). The last group (T6) supplemented moringa leaf extract of 750 mg/L of drinking water and purwoceng of 250 mg/L of drinking water. Basal feed in this study used BR-1 chicken feed produced by PT. Charoen Pokphand. As a pre-treatment adjustment the chicken is maintained 1 week with basal feed. Furthermore, chickens began to be treated by supplementation of moringa leaves, purwoceng and the mixture. On the 7th day after treatment, semen begins to be taken and tested. Semen collection is done every 3 days with the amount of 3 times the taking. The observed variables were semen volume per ejaculation, ejaculatory spermatozoa count (cells/ml), percentage of living sperm, motility, mass movement, color, viscosity and pH of semen. Results from semen volume and sperm count, motility and percentage of living sperm were analyzed with variance analysis using the spss series 25 program, while viscosity, color and pH were reported in a descriptive manner. From the results of observations it can be concluded that the synergistic effect between moringa leaf extract and purwoceng extract only occurs in the increase in the concentration of spermatozoa, but does not occur in other fractions. Supplementation of moringa leaf extract and its combination with purwoceng extract can increase the production and quality of chicken semen, but the influence of moringa leaf extract is more dominant than purwoceng extract.

Keywords: Roosters, *Moringa oleifera*, purwoceng (*Pimpinella alpine Molk*), semen quality

Introduction

Free-range chicken - another term for native chicken, is a type of poultry that has become popular in the community and is spread throughout the archipelago (Sarwono, 1995). Free-range chicken is liked by people because of its chewy and "stuffy" meat,

not soggy and not fatty like purebred chicken. Village chickens are kept by the community, especially as a source of animal protein in the form of eggs and meat (Sarwono, 1995).

Malnutrition is a major problem in umbaran chickens. For hens this will affect egg production, while for roosters it will affect the quality of their sperm. Therefore, in order for native chickens to produce well, one of them must be given sufficient feed (Rasyaf, 1992).

Roosters to be able to produce sperm properly require adequate nutritional intake. Protein is a nutrient that has an important role in spermatogenesis. Protein deficiency has an impact not only on the normal function of the gonads and spermatogenesis, but also decreases the production of gonadotropins, either impacting on testosterone or LH levels, also has a negative effect on testicular development and low sperm count (Zambrano et al., 2005).

Moringa leaves are annual plants that contain balanced essential nutrients, so that Moringa leaves have hope in the future as an alternative source of animal feed supplements. Moringa leaves (*Moringa oleifera*) have a fairly high crude protein content (25.1 – 30.29%) (Krisnadi, 2015). The amino acid elements contained in *Moringa oleifera*, such as scordinine, methionine, lysine and cystine, can stimulate chicken growth, increase body weight, and increase energy (Kusmardika, 2020; Desy et al., 2021). Furthermore, it was explained that Moringa also contains vitamins A, B1, B2 and Vitamin C which are quite high.

Various studies have been carried out using Moringa leaves as feed ingredients with the aim of increasing libido and semen quality. Lalas and Tsaknis (2002) reported that, Moringa leaves have been traditionally used to increase libido. Abu et al., (2013) succeeded in improving the sperm quality of the male rabbit epididymis. The results of the study by Syarifuddin et.al., (2016) showed that, Moringa leaf supplementation significantly increased libido and sperm motility of Bali cattle.

Another study conducted by Satria (2017), proved that the use of 2% Moringa leaf extract can inhibit the growth of Escherichia coli bacteria, while 2% Moringa leaf powder in feed has the effect of increasing feed consumption and reducing feed conversion and improving exterior egg quality. (Egg mass) and egg interior quality (lowering egg yolk cholesterol).

Purwoceng is a commercial medicinal plant that can be used as an aphrodisiac, diuretic and tonic. This plant is a native Indonesian plant that grows endemic to the Dieng plateau, Central Java, Mount Pangrango, West Java and mountainous areas in East Java. (Darwati and Ika, 2006). Furthermore, they also reported that the most efficacious parts of purwoceng are sterol derivatives, saponins and alkaloids. Purwoceng roots also contain coumarin derivatives, namely bergapten, isobergapten and saponins which are used in the modern medicine industry as analgesic, antipyretic, sedative, anthelmintic, anti-fungal, anti-bacterial and anti-cancer compounds. namely alkaloid compounds, coumarins and saponins. (Darwati and Ika, 2006). Juniarto (2004) reported that purwoceng root extract given to Spraque Dawley rats could increase the degree of spermatogenesis in the testes, the number and motility of spermatozoa compared to controls, but tended not to differ from the pasak bumi treatment. Based on pharmacological studies, it has been tested preclinically and clinically and has been patented that purwoceng extract functions as an aphrodisiac.

Kosin (1992) reported that purwoceng root extract had an androgenic effect on male chicks which was characterized by an increase in comb size supported by an increase in testicular weight. It was also reported that purwoceng triggers an increase in levels of the hormone LH (Luteinizing Hormone) and Testosterone (Taufiqqurrachman, 1999). Taufiqqurrachman also reported that purwoceng has a synergistic effect on the effect of increasing testosterone levels which is higher than other treatments when given together with pasak bumi at the same dose (Taufiqqurrachman, 1999).

Materials and Methods

Research Tools and Materials

This research was conducted at the Animal Practice Unit of the Karanganyar Animal Husbandry Academy. The equipment used in this study consisted of: individual cages measuring 70 cm x 70 cm x 70 cm, equipped with feed and drinking places. For semen examination, test tubes, microscopes, glass and deck glass objects were used, haemocytometer, suction pipettes and disposable syringes, glass, cotton, physiological NaCl, eosin and 70% alcohol.

This study used native chickens aged about 1 year with a body weight range of 1.84 kg - 2.16 kg with an average body weight of 2.0 kg as many as 18 tails. The feed given is commercial feed from PT. Comfeed BR-1 series ad libitum.

Research methods

The study was conducted experimentally using a completely randomized design by allocating 18 free-range chickens which were divided into 6 treatments, each treatment consisted of three replications, each with 1 chicken.

The treatments were: The first group of chickens was used as a control, not given any treatment, only given basal feed (T0). The second group of chickens were given basal feed and Moringa leaf extract as much as 1 g/L of drinking water (T1), group 3 chickens were given basal feed and purwoceng extract of 1 gram/liter of drinking water (T2). Furthermore, the chickens in group 4 (T3) were given basal feed and supplements of Moringa leaf extract as much as 250 mg/L and 750 mg/L of drinking water per head per day, while group 5 (T4) was given basal feed and supplements of Moringa extract 500 mg/L. liter and purwoceng 500 mg/L of drinking water every day (T5). The last group (T6) received basal feed and supplementation with 750 mg/L Moringa leaf extract and 250 mg/L purwoceng drinking water.

Research implementation :

Moringa and purwoceng leaf extraction

This is done by extracting water, with a ratio of 1 part of Moringa leaves or purwoceng powder plus 5 parts of water, then brought to a boil and kept heated at a temperature of around 90-100ºC for 30 minutes, then filtered. The filtering results are heated again at a temperature of about 90-100^{o} C until a thick extract is obtained.

Adaptation and treatment

The adaptation stage was carried out on chickens for a week. The activities carried out were preparing the cage, weighing the chickens, putting them into individual cages and giving them new feed (BR-1) and drinking, within 1 week. On day 8, the treatment was started by giving supplements to the chickens according to the design, mixed with drinking water.

Sampling and analysis of cement quality

Before carrying out the shelter, the chickens were first cut feathers around the cloaca and cleaned the cloaca with 70% alcohol using a cotton swab, then prepared the tools and materials used for the semen collection, in the form of sterile glass cups. Semen collection is carried out by two people, once every 3 days. Semen was accommodated 3 times, namely on the 7th, 10th and 13th days after giving the extract. Furthermore, the semen obtained was assessed for semen quality which included macroscopic (volume, consistency, pH, color) and microscopic (concentration of mass movement, individual motility, viability) quality.

Data analysis

The data collected were analyzed using analysis of variance (ANOVA) with SPSS version 25, to determine the effect of treatment on the observed variables. Qualitative data are reported descriptively.

Result and Discussion

The results of the study on the synergistic effect of Moringa (*Moringa oleifera*) and purwoceng (*Pimpinella alpine Molk*) leaf supplementation on the quality of native chicken semen can be seen in the table.

Semen Volume

The results of observations during the study showed that there was no synergistic effect between Moringa leaf extract and purwoceng extract on the roosters semen. The semen volume in control chickens (T0) averaged 0.29±0.06 ml per ejaculation, not significantly different from the semen volume given a combination of Moringa leaf extract and purwoceng (T3, T4 and T5). This semen volume was in accordance with the findings of Bah et al., (2001) who reported that the average volume of roosters semen was 0.28±0.14 mL. A significant difference actually occurred in chickens given only 1 gram of Moringa extract (T1) which increased to 0.41±0.02 ml/ejaculate ($P<0.05$). This result is in accordance with the results of previous research conducted by researchers by giving 2 g of Moringa leaves in powder form or 0.2 g of Moringa leaf extract (Suripta and Puji, 2021). The increase in nutrients from Moringa leaves is thought to have an effect on increasing the volume of semen. This is in accordance with the report by Kismiati (1997), which says that the quantity and quality of semen is influenced by the protein and energy content of the feed. Moringa leaves contain 25.1-30.29% protein

(Krisnadi, 2015), so the addition of Moringa leaf powder is thought to play a role in increasing the volume of cement.

Table average quality of free-range chicken semen after supplemented with Moringa leaf extract and purwoceng extract

Parameter	Treatment					
	T0	T1	T2	T3	T4	T5
Volume (ml)	0.29±0.06[a]	0.41±0.02[b]	0.33±0.01[a]	0.31± 0.02[a]	0.29± 0.04[a]	0.32± 0.06[a]
Consistency	medium - thick	thick	thick	thick	thick	thick
Mass movement	+++	++++	+++	++++	++++	++++
Color	milky white	milky white	milky white	milky white	milky white – cream	milky white - cream
pH	7.42±0,16	7.44±0.25	7.56±0.16	7.45±0.22	7.30±0.20	7.20±0.60
Sperm concentration (million sel/ml)*	2.704±1.30[a]	5.275±1.25[b]	3.186±2.27[a]	5.176±3.13[b]	6.915±7.35[c]	7.490±4.19[c]
Motilitas (%)	85.87±2,88[a]	88.33±3.80[b]	86.66±2.64[a]	86.83±2.82[ab]	89.34±3.53[b]	89.22±4.27[b]
Live spermatozoa (%)	84.50±3,45[a]	87.70±2.74[b]	85.46±2,43[ab]	87.51±2.55[b]	88.77±2.64[b]	87.78±2,46[b]

Note : * Different superscripts on the same line indicate significant differences (P<0.05)
* Different superscripts on the same line indicate a very significant difference (P<0.01)

The increase in semen volume may also be supported by the relatively high Zn content in Moringa leaves (31.03 mg/kg) (Moyo et al., 2011). According to Bindari et al. (2013) Zn supplementation causes an increase in the average volume of ejaculate, semen concentration and percentage of sperm motility. Besides this, it is suspected that the high folic acid found in Moringa leaves can increase fertility in males, especially with regard to its role in the period of cell division and growth and protect DNA from oxidative damage, as reported by Young et. al. (2008). In this study, it is suspected that the content of Zn and folic acid contained in Moringa leaves plays a role in increasing the volume of semen, in addition to the high protein content of Moringa.

Color, Viscosity and Mass Movement

There is a positive correlation between the color of semen and the thickness or consistency of semen, in other words, the color of semen is a reflection of the consistency of semen. Roosters semen has a milky white or slightly cream color. The cream color in semen is formed as a result of the dense concentration of spermatozoa (Wijayanti et.al., 2013). Sperm consistency has a relationship with the quality and

number of spermatozoa (Sopiyana et al. 2006). Semen with a thick consistency has a high concentration, while semen with a watery consistency has a low concentration of semen. The thicker the consistency of the semen, the darker the color of the semen will be. And vice versa, a slightly pale colored semen will get a lower semen consistency. In general, the color of fresh free-range chicken semen obtained in this study was milky white (T0, T1, T2 and T3), some were slightly creamy (T4 and T5) with thick consistency. Garner and Hafez, (2000). Says that good cement has a degree of viscosity that is almost the same or slightly thicker than milk, while bad cement, both in color and thickness is the same as coconut water. In this study all semen had a thick consistency, this is similar to that reported by Nataamijaya et. al., (2003) and Mustafa et. al., 2017

Spermatozoa mass movement is the movement of a swarm of spermatozoa on fresh semen, and forms a wave-like shape, which is a picture 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 (Toelihere, 1993). The mass movement of free-range chicken spermatozoa after supplementation with Moringa leaf extract and purwoceng extract showed differences between the treatment and control groups. At T0 (control chickens) mass movement was at level 3 (+++) the same as T3 (given purwoceng extract), while chickens receiving Moringa leaf extract supplementation or a combination of both showed more progressive mass movements (++++). These results are similar to the results of Sopiyana et al. (2006), who reported that the mass movement of free-range chicken sperm ranged from good (+++) to very good (++++) characterized by progressive spermatozoa movement and forming thick and fast-moving mass waves. Sperm with these criteria according to Toelihere (1985) including very good criteria. This seems to have something to do with the high concentration of spermatozoa in each treatment group and the more active and the more moving spermatozoa, resulting in thicker mass movement and faster movement.

Spermatozoa Motility

The simplest semen assessment for artificial insemination is the motility or motility of spermatozoa. In this study, the average number of motility in the control group was 85.87 + 2.88%, as good as the group of chickens given purwoceng extract (T2 = 86.66 + 2.64%) This finding is higher than that reported by Sopiyana et al. (2006) namely 81.63 + 3.54%. In the chickens in the treatment group with 1 g of Moringa leaf extract supplementation (T1), the spermatozoa motility was 88.33+3.80%, the same high as the treatment group with the combination of Moringa leaf extract and purwoceng, namely T4 (89.34+3.53%) and T5 (89.22+4.27%), significantly different from chickens in the control group and chickens with purwoceng supplementation. These results indicated that the percentage of motility of chicken spermatozoa after the addition of Moringa leaf extract or its combination with purwoceng extract increased, but did not show any synergism between the two. In this study, it was seen that Moringa leaf extract had a more dominant effect on increasing the percentage of motility of free-range chicken spermatozoa than purwoceng extract. This can be seen in T3 who received 250 mg of Moringa extract and 750 mg of purwoceng extract did not show a significant increase in motility. This finding strengthens the opinion of Widhyari et al., (2015), which said that the mineral Zn contained in Moringa leaves can stimulate

Leydig cells in the testes to produce testosterone, while in the process of spermatogenesis, Zn minerals play a role in ribonuclease activity, spermatozoa maturation and increase motility. sperm and maintains the germinative epithelium and seminiferous tubules. Giving Moringa leaf extract is thought to be able to increase the nutritional value of the ration so as to allow the metabolism of spermatozoa to take place more optimally which in turn increases the motility of spermatozoa.

Percentage of Live Spermatozoa

The results showed a significant increase in the percentage of live spermatozoa of roosters after administration of Moringa leaf extract and the combination of Moringa leaf extract with purwoceng extract. The increase in the percentage of live spermatozoa in the treatment groups T1, T3 and T4 and T5 was significantly higher ($P<0.05$) compared to T0, (control). Meanwhile, the increase in the percentage of live spermatozoa at T2 did not indicate a significant difference, and there was no synergism between Moringa leaf extract and purwoceng extract. This illustrates that the administration of Moringa leaf extract or combination with purwoceng extract in drinking water is able to optimize the process of spermatogenesis, so as to create optimal conditions from the seminiferous tubules to the epididymis which in turn is able to maintain the optimal life of spermatozoa. The increased production of testosterone by Leydig cells in the testes (Widhyari et al., 2015) due to Zn stimulation from Moringa leaves, serves, among other things, to maintain the complementary sex organs which will produce seminal plasma which is a medium for the metabolism of spermatozoa and as a source of nutrition for spermatozoa for life (Louis et al. (1994). The results of this study are in accordance with the results of research by Mustafa et al. (2017) which provides additional 0.02% vitamin E and additional protein up to 20% can increase the percentage of live sperm significantly.

Sperm Concentration

The sperm concentration in this study was T0 : 2.704 + 130 million cells/ml semen; T1 : 5.275 + 125 million cells/ml cement; T2 : 3,186 + 227 million cells/ml cement; T3 : 5,176 + 313 million cells/ml cement; T4 : 6.915 + 735 million cells/ml cement; T5 : 7,490 + 419 million cells/ml semen. At T0 (without treatment) it can be seen that the sperm count is 2.704 (million cells/ml). This number is in accordance with Toelihere's (1993) statement, which states that the concentration of chicken spermatozoa ranges from 0.03 – 11 billion cells/ml of semen, but is smaller than the statement of Junaedi et al. (2016) which stated that the concentration of free-range chicken spermatozoa was 3.126 million cells/ml of semen. However, this result is still higher when compared to the results of previous studies, namely 1.410 cells/ml of cement (Suripta and Puji, 2021) and the findings of Isnaini (2000) which is 2.100 million/ml of cement.

A very significant increase in sperm count (Sig. $P<0.01$) occurred at T1, T3, T4 and T5. Here, there seems to be a synergy between Moringa leaves and purwoceng. But the new synergy occurs when the percentage of Moringa is at least 50 percent or more (see T4 and T5 in the table). In the combination of 250 mg Moringa with purwoceng 750 the effect was the same as that of chicken given Moringa alone (T1 and T3), while a

very significant synergy occurred at T4 (6.915±735 cells/ml semen) as well as T5 (7.490±419 cells/ml semen).), even the increase in sperm count in both of them reached more than 2 times the sperm count of control chickens. This result is higher than the report of Pratiwi et. al. (2019), who reported that the concentration of spermatozoa in local chickens of the Merawang breed was 4.240 ± 134 million cells / ml of semen.

Differences in sperm concentration can be influenced by age, breed of livestock, body weight and frequency of storage. In this study, it was seen that chickens receiving Moringa leaf supplementation showed a significant increase in sperm count (T1 and T3, T4 and T5). This is thought to be caused by the influence of Moringa supplementation which has substances that can increase sperm concentration. One of them is zinc mineral. 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 (Widhyari et al., 2015). In addition, Bindari et al. (2013) reported that Zn plays a role in the immune system and certain reproductive hormones. Furthermore, it is explained that Zn is known to be very important for the accuracy of sexual maturity. In males, Zn causes an increase in the average volume of ejaculate, sperm concentration, percentage of motility. Besides, it is supported by the role of folate which plays an important role in the development of germ cells. The content of Zn and folic acid contained in Moringa leaves can improve semen quality, where the content of Zn increases sperm concentration (Bindari et al., 2013) and folic acid which increases sperm production and increases fertility in males.

Acidity (pH)

The results showed that the pH of chicken semen was the lowest 7.20+0.60 (T5) and the highest 7.56+0.16 (T2) but the results of statistical analysis of the acidity (pH) of semen did not show a significant difference (P≥0.05). This result is slightly higher with the findings of Junaedi et al. (2016) who reported the acidity degree of fresh free-range chicken semen was 7.06 and the research result was slightly higher than the statement (Peters et al., 2008) that the average pH of semen chickens was 7.01±0.01, but similar to that of Bah et al. (2001) in native chickens in Nigeria. The degree of acidity (pH) of semen is influenced by the metabolic process of spermatozoa under anaerobic conditions. The end result of the spermatozoa metabolism process is lactic acid. The higher the lactic acid produced will cause the accumulation of lactic acid so that in the end it causes an increase in the degree of acidity or a decrease (pH) of the solution (Toelihere, 1993). In this study, the pH of the cement did not show a significant change so that it did not affect the quality of the cement. The degree of acidity (pH) of semen is related to the motility or vitality of sperm. According to Toelihere (1993) stated that the pH of chicken semen is between 7-7.6. If the semen pH is too acidic, less than 7, the spermatozoa will die quickly, and if the semen pH is too alkaline, higher than 8, the spermatozoa will also die quickly.

Conclusion

From the results of the research and discussion above, it can be concluded that the synergistic effect between Moringa leaf extract and purwoceng extract only occurred in increasing the concentration of spermatozoa, but did not occur in other fractions. Moringa leaf extract supplementation and its combination with purwoceng extract can increase the production and quality of native chicken semen, but the effect of moringa leaf extract is more dominant than that of purwoceng extract.

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