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Display of Free-range Chicken Semen After Supplementation of Phylanthus Niruri Herbal Extract and Kaffir Lime Leaf Extract (Citrus Hystrix D. C)

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Abstract: This study was conducted to examine the supplementation of green meniran herb extract (Phylanthus niruri L) and kaffir lime leaf extract (Cytrus hystrix D. C.) on the appearance of free-range chicken semen. The study was conducted using a completely random design of unidirectional patterns, using 24 male free-range chickens aged about one year with an average weight of 1.8 kg with eight treatments, three replicates each. Each replicate consisted of 1 chicken. Basal feed uses BR-1 chicken feed produced by PT. Japfa Comfeed Indonesia Tbk. After 1 week of basal feeding, chicken sperm was taken and tested for control (H-0), then began to be treated with testosterone supplementation, as a positive control, meniran extract, kaffir lime leaf extract, or a mixture thereof. The following semen collection is carried out after 1 week of treatment. Semen collection is carried out every 3 days with five intakes. This study concludes that the supplementation of green meniran herb extract and kaffir lime leaf extract or a combination thereof, macroscopic, does not change the appearance of free-range chicken semen, both volume, color, consistent odor, and pH of semen, but microscopic significantly increase sperm concentration, motility, and Viability. There was no synergy between green meniran herb extract and kaffir lime leaf extract on the appearance of chicken semen.

Keywords: Free-range chicken; Kaffir lime leaf extract; Phylanthus niruri herbal extract; Semen display

Introduction

In the field of livestock, especially free-range chickens, the government has issued regulations to protect local chickens through Government Regulation (Perpres) No. 44 (2016) concerning the List of Closed Business Sectors and Open Business Fields with Requirements in the Investment Sector, which explains that free-range chicken farming business actors are limited to only a few micro, small, medium, and cooperative business systems (MSMEs). This regulation opens and encourages opportunities for free-range chicken farming for smallholder farmers.

This shows how vital free-range chickens are in livestock development in Indonesia, as well as the economic basis for rural farmers' meat and egg needs (free-range chickens) for the community (Nangoy & Karisoh, 2018). The traditional pattern of raising free-range chickens can make chickens malnourished (Desta, 2021).

This can affect their productivity, namely eggs for hens, while for roosters, it will affect the quality of their sperm (Assersohn et al., 2021). Improving and maintaining nutritional completeness will help manage livestock reproduction, which will enhance the reproductive organs' function while improving the

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quality of semen. At the same time, food shortages can result in impaired physiological functions, both in the testes and in the accessorial glands, and can reduce libido so that semen production decreases (Yendraliza, 2013).

The provision of stimulants in feed additives is often carried out to increase productivity (Dawood et al., 2018; Komarraju et al., 2024). However, commercial feed additives on the market are expensive, contain synthetic chemical compounds, and are often avoided by consumers. As a result, many consumers choose farm products labeled "organic" (Prache et al., 2022; Taddesse et al., 2016).

Natural additive feeds that have the potential to replace commercial additive feeds include medicinal plants (Komarraju et al., 2024; Pliego et al., 2022). People who like to use natural ingredients also support this because the side effects are lower than synthetic drugs (Cohen & Weinstein, 2018). Medicinal plants that meet the above criteria are meniran (Phyllanthus niruri Linn) and kaffir lime (Citrus hystrix D. C.).

Meniran (Phyllanthus niruri Linn), according to the results of the study by Rivai et al. (2013), contains steroids, namely the triterpenoid group that includes the cyclopentane perhydrophenantrene nucleus, which consists of three cyclohexane rings and one cyclopentane ring. Steroids are essential in maintaining salt balance, controlling metabolism, and improving the function of the sexual organs (Nasrudin, 2017). Meniran plants also contain flavonoids (quecertin, quercitrin, isoquercitrin, astragalin, rutin, kaempferol-4, rhamnopynoside), lignans (phylline, hypophylantin, nirantin, lintetratin), alkaloids, triterpenoids, fatty acids (ricinoleic acid, linoleic acid, linolenic acid), vitamin C, potassium, resin, tannins and geranin (Ahsan, 2020).

Kaffir lime leaves are used as the main ingredient in traditional medicine. Kaffir lime leaves (Citrus hystrix D. C.) contain tannins, steroids, triterpenoids, and essential oils 1 – 1.5 % (Hendarwati, 2014). Kaffir lime leaves also contain alkaloids, polyphenols, essential oils, tannins, flavonoids that have pharmacological effects as antiseptics and antioxidants and antibacterial.

In addition to what has been explained above, kaffir lime leaves have traditionally been believed to be an aphrodisiac that functions to increase libido. According to Harmusyanto (2013), flavonoids, terpenes, and polyphenols are active compounds that stimulate steroid hormones, including progesterone and testosterone, essential in regulating male libido. In addition to steroids, the content of tannins and alkaloids can also increase and facilitate blood flow to the male genital organs (Enema et al., 2018; Toyin et al., 2014). Kaffir lime leaves are known to have a steroid content of 1-1.5% (Kurniawati et al., 2015), and on the peel of the fruit, 2-2.5% (Herbie, 2015).

Supplementation of meniran leaf extract and kaffir lime leaf is expected to have a synergistic effect so that it can improve the performance of both, which in turn can improve the quality of sperm with a smaller dose and do not cause side effects for free-range chickens.

Method

This study, 24 free-range chickens were used with a body weight range of 1.6 kg – 2 kg with an average body weight of 1.8 kg. Green meniran herb extract and kaffir lime leaf extract were obtained by water extraction. The feed provided is commercial feed from PT. Charoen Pokphand BR-1 series adlibitum.

The equipment used in this study includes individual cages measuring 80 cm X 80 cm X 80 cm equipped with feed and drinking containers, test tubes, label paper, microscopes, haemocytometers, suction pipettes, scissors, tube shelves and refrigerators, disposable syringes, glasses, mini tubes, cotton, physiological NaCl, eosin-negrosin and alcohol 70%.

The study was conducted experimentally using a Complete Random Design design by allocating 24 male free-range chickens divided into eight treatments, and each treatment consisted of three replicates, each replicating one chicken. The treatment given is as follows:

T0 : Control (without additional materials)
T1 : Testosterone Supplementation 1 mg, 2 doses
T2 : Meniran extract supplementation 1 ml /day
T3 : Supplementation of kaffir lime leaf extract 1 ml/day
T4 : Meniran extract supplementation 2 ml
T5: Lime leaf extract supplementation 2 ml
T6 : Meniran extract 0.5 ml and kaffir lime 0.5 ml
T7 : Supplementation of meniran extract 1 ml and kaffir lime leaf extract 1 ml

Research Implementation

Preparation of green meniran herb extract and kaffir lime leaf

The extraction of meniran herbs and kaffir lime is carried out by water extraction, with a ratio of 1 part meniran herb powder or kaffir lime leaf powder plus five parts water, then boiled and kept heated at a temperature of about 90-100 0 C for 30 minutes, then filtered. The filtration results are heated again at a temperature of 90 O until a viscous extract is obtained.

Adaptation and treatment

The adaptation stage is carried out in chickens for a week. On the 8th day, the treatment begins by giving supplements to the chicken according to the design, by

being cut once a day in the morning. Testosterone is administered by injection on days 8 and 15.

Sampling and analysis of semen quality

Semen collection is carried out in the morning. Before collecting, cut the fur around the cloaca and clean the cloaca with 70% alcohol using cotton. Then, prepare tools and materials used for semen storage in glass cups. Two people carried out the semen shelter. Semen was collected 5 times on day 8 (as control), 15, 18, 21, and 24 (3-day intervals, as replication). Furthermore, the semen obtained was evaluated, including macroscopic display (volume, consistency, pH, color) and microscopic display (concentration, mass movement, individual motility, viability).

Semen Volume and pH checks are carried out by measuring directly after the semen is stored in a microtube. The semen volume was calculated from each rooster by looking at the scale on the microtube. The acidity (pH) degree is measured by dripping semen on the pH indicator paper. Then, the color change on the indicator paper is compared to the standard color.

Mass movement was evaluated by dripping semen as much as one drop on a clean glass object, covered with a glass deck, and observed under a microscope with a magnification of 100 times. Observations were made based on the wave thickness of the spermatozoa mass and the speed at which the waves moved from place to place. The criteria for assessing mass movements are divided based on three criteria, namely 3 (+++), 2 (++), and 1 (+).

The individual movements (motility) of spermatozoa were evaluated by adding one small drop of semen and physiological solution (NaCl) with a ratio of 1:1. Then, the two solutions were mixed evenly and covered with deck glass. Observations were made using an objective lens with a magnification of 400 times. The assessment was carried out from several fields of view by looking at the percentage of speed and direction of spermatozoa movement that were fast and progressive.

Evaluation of Spermatozoa Viability was carried out by eosin-negrosin differential staining at 4%. Samples of semen and dye (about 1:3) are mixed in the glass of the object, and a thin clove preparation is made on the glass of the other object. The preparation is then fixed (dried) using a bunsen heater. Observations were made under a 10x40 magnifying light microscope. Living spermatozoa are characterized by a light-colored head, while dead ones with a pink head.

Data analysis

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

Result and Discussion

Macroscopic View

The macroscopic display of chicken semen includes volume, consistency, color, odor, and pH. Semen consists of two main parts: male gamete cells or spermatozoa and semen plasma (Garner & Hafez, 2000). Spermatozoa, with a length of 100 µm generated inside the testicles through spermatogenesis, consists of the head, acrosomes, the middle, and the tail. Hoesni (2016) said that semen plasma has a function as a means of transporting spermatozoa during ejaculation and as a buffer for spermatozoa to stay alive after being deposited into the female reproductive tract. The compounds contained in semen plasma are organic and inorganic substances.

Table 1. Macroscopic view of free-range chicken semen after supplementation of green meniran herb extract (Phylanthus niruri Linn) and kaffir lime leaf extract (Citrus hystrix D. C)

Treatment	Parameter				
	Volume (ml)	Consistency	Color	Smell	pH
T0	0.28 ± 0.04	Medium-thick	Milk white	Typical semen	7.2
T1	0.29 ± 0.03	Thick	Milk white	Typical semen	7.3
T2	0.34 ± 0.04	Thick	Milk white	Typical semen	7.2
T3	0.27 ± 0.02	Thick	Milk white	Typical semen	7.0
T4	0.29 ± 0.06	Thick	Milk white	Typical semen	7.2
T5	0.27 ± 0.01	Thick	Milk white	Typical semen	7.3
T6	0.32 ± 0.04	Thick	Milk white	Typical semen	7.3
T7	0.35 ± 0.03	Thick	Milk white	Typical semen	7.2
Average	0.31 ± 0.03	Thick	Milk white	Typical semen	7.2

Information: No real difference (P > 0.05)

Semen Volume

The volume of semen obtained in this study ranged from 0.27 + 0.01 ml to 0.35 + 0.03 ml, with an average of 0.31 + 0.03 ml per ejaculate (see Table 1). These results align with the findings of Suripta et al. (2023). The volume of chicken semen each time ejaculated ranged

from 0.16 – 0.72 ml. This result is similar to the findings of our previous study on free-range chickens, which are between 0.29±0.07 ml. - 0.40± 0.03 ml, but lower than the volume of semen of the Merawang type of free-range chicken produced by the massage method of 0.35±0.07 reported Suripta & Astuti (2021), as well as the reported volume of 38-week-old male free-range chicken semen Azizah et al. (2023) which is 0.38±0.15 ml.

The fertility of roosters begins to decline from 40 weeks of age (Leeson & Steven, 2009). Ansari et al. (2018) said that the decrease in fertilization ability of old roosters is due to several factors, namely weight gain, decreased semen concentration, lower testosterone circulation level, Sertoli cell dysfunction, and a decrease in the total antioxidant capacity of semen.

According to Cheah & Yang (2011), semen quality is affected by temperature, age, sexual activity, and genotype. Ismaya (2014) added that the quality and quantity of sperm are influenced by genetics, age, feed, temperature/season, frequency of ejaculation, libido, physical factors, transport, testicular size, disease health, and livestock.

The administration of green meniran herb extract and kaffir lime leaf extract did not increase the volume of free-range chicken semen. This shows that the volume of semen is not affected by the supplementation of green meniran herb extract or kaffir lime leaf extract, which, when calculated, the additional nutrients are indeed minimal, as well as strengthening the opinion of Ismaya (2014).

Romero-Sanchez et al. (2008) reported that the nutritional content of feed, especially energy-protein content, affects the production and quality of rooster semen. The nutritional content of feed is related to the reproductive function of roosters because the nutritional content of feed is closely related to the weight achievement of roosters. Djermanovic et al. (2013) said a negative relationship existed between rooster weight and mating activity and between rooster weight and fertility rate.

Huda et al. (2018) 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 green meniran herb extract and kaffir lime leaf extract is minimal, so it does not affect the volume of semen.

One way to test the consistency of semen is to tilt the tube and re-enact it a few moments later. If the semen attached to the tube drops slowly after it is re-erected, it indicates that the semen has a thick consistency.

Consistency and Color of Semen

Semen is said to be good if the consistency is thick. Semen consistency is related to the color and number of spermatozoa. Semen with a viscous consistency has a high concentration, while semen with a diluted consistency has a low concentration of semen (Lubis, 2011). In this study, the consistency of semen is almost all dense, except for the control, which is somewhat thick semen. This is by Sopiyana et al. (2006), who state that the consistency of the semen is slightly thick to thick.

Several factors affect the viscosity of semen, including the age factor, where the older the age, the quality of semen, including consistency and fertility level, also becomes lower, 3-year-old chickens are chickens that still have good semen quality (Rivai et al., 2013). Feed is another factor that affects the quality of semen. The better the quality of the feed provided, the better the ability of chickens to produce quality semen. Next is the frequency of ejaculation, which will also affect the consistency of semen. The frequency of ejaculation also affects the consistency of semen, where too close to the frequency of ejaculation, the semen produced is not good because the body needs time to produce semen again. In free-range chickens, it takes approximately three days to make good semen.

Bebas & Laksmi (2013) reported that the frequency of ejaculation had a very significant effect on semen volume, spermatozoa concentration, and spermatozoa motility of green partridges, where the frequency of ejaculation every 3 days resulted in better semen quality than once every 1 and 2 days. According to Yuwanta (2016), The journey of spermatozoa from Tubuli seminiferous to the Vas deferens takes 1-4 days. The maturation process occurs in the proximal part of the Vas deferens for several hours and then stored in the distal part of the Vas deferens.

Fresh semen of free-range chickens has a white/beige color. There is a correlation between the color of the semen and the concentration of sperm, where the beige indicates a dense concentration of sperm su. In this study, the average sperm is white (Suripta et al., 2023). The semen in this study can be expected because there is no mixture of redness and brown color, indicating that the semen is contaminated with blood. The standard color of fresh semen for free-range chickens is thick white. This corresponds to Kartasudjana (2001), which states that if the semen is reddish, it is a sign that the semen is contaminated by fresh blood, while if the color is close to brown, it can be a sign that the blood that contaminates the semen has been decomposed. The greenish color is a sign of decaying bacteria in the semen. Variations in semen color can occur between males and in the same male from different ejaculated semen.

The acidity (pH) of semen is related to the motility or Viability of sperm. According to Susilawati, (2013), The pH of chicken semen ranges from 7-7.6. If the pH of the semen is too acidic <7, the semen will die quickly, as well as if the pH of the semen is too alkaline >8, the semen will also die soon.

The results showed that the pH of the chicken semen studied had an average of 7.2. This is by the statement of Getachew (2016), which states that the quality of good rooster semen has a volume ranging from 0.2-0.5ml, a pH value of 7.2-7.6, and a motility of 60-80%. Some factors that affect the pH of semen include storage time and phenolic compounds. Green meniran herb extract contains alkaloid compounds, flavonoids, saponins, steroids, tannins, and phenolics (Rivai et al., 2013).

According to Awad et al. (2019), A decrease in pH can occur due to phenol compounds with acidic properties that can lower the pH of the semen. Phenolic compounds are included in compounds that are toxic to plants and can have an effect when used at high levels. In this study, the pH did not change even though the chicken was given 2 grams of green meniran extract and 2 grams of kaffir lime leaf extract, and the combination did not affect the quality of the semen.

Microscopic Display

The microscopic appearance of semen includes concentration, mass movement, individual motility, and Viability.

Sperm Concentration

A significant increase in the number of sperm in free-range chickens ($P < 0.05$) occurred in chickens supplemented with testosterone propionate, and all chickens were given meniran leaf extract and kaffir lime leaf extract of 1 ml or more and a combination thereof. However, the increase in sperm count did not occur in the administration of a combination of 0.5 ml of meniran extract and 0.5 ml of kaffir lime leaf extract (table 2). This shows that there is no synergy between the two. But a mixture of the two with a dose of green meniran herb alone or kaffir lime leaves alone can have an equally good effect on increasing sperm count. The administration of 2 ml of green meniran extract gave the highest increase in amount, and this confirmed that the administration of 2 ml of green meniran extract had a better effect than kaffir lime leaf extract at the same dose.

The average free-range chicken sperm in the study was 4,431 million cells/ml, which is higher than the report by Nataamijaya & Setioko (2002), which reported an Arabic chicken sperm count of 1.86+/ml. Also higher than the report by Kusumawati et al. (2018), which found the number of spermatozoa in free-range chickens was 1.43 + 0.15 million cells/ml and slightly higher than the statement by Junaedi et al. (2016) which states that the concentration of spermatozoa in native chickens is 3,126 million cells/ml.

Differences in sperm concentration can be influenced by age, livestock nation, body weight, and frequency of shelter. In this study, it is suspected that meniran has the same effect as our previous research (Suripta & Astuti, 2021). Meanwhile, the combination with kaffir lime does not further strengthen the impact on both, so it cannot significantly increase the number of perm in a small amount. In this study, it was seen that chickens that received enough supplementation of green meniran leaves and kaffir lime showed a significant increase in sperm count. This is suspected to be due to the influence of the green meniran supply, which is given equally well compared to kaffir lime leaf extract.

Green meniran leaves have substances that can increase sperm concentration. One of them is the mineral Zn, which stimulates Leydig cells in the testes to produce testosterone because this mineral is a component of proteins involved in the synthesis and secretion of testosterone. In males, Zn deficiency increases the average volume of ejaculation, sperm concentration, and percentage of motility (Bindari et al., 2013). Folate also plays a vital role in the development of germ cells. The Zn content and folic acid contained in moringa leaves can improve the quality of semen, where the Zn content increases sperm concentration (Bindari et al., 2013). Folic acid increases sperm production and fertility in males (Rivai et al. (2013) state that the green meniran herb contains metabolite compounds, including steroids.

The presence of steroid content in green meniran leaves is suspected of having an aphrodisiac effect, and this is stated by Arini (2021) that some secondary metabolite compounds can affect the action of testosterone hormones, namely flavonoid compounds, saponins, and steroids. According to Ferlinahayati (2001), In the green meniran plant, there are three types of steroids, namely Ergost-5-en-3β-ol, Stigmast-5,22-Dien-3β-ol and Stigmast-5-En-3β-ol. Animal steroids are generally found in hormones that affect growth and reproduction. Steroids in the medical world are used as medicinal and contraceptive ingredients. For example, androgens are steroid hormones that can stimulate male sexual organs, and estrogen can stimulate female sexual organs.

According to Herbie (2015) states that kaffir lime leaves also contain steroids as much as 1-1.5% and kaffir lime peel 2-2.5%. The steroid content in kaffir lime leaves can increase the hormones progesterone and estrogen, which work as a stimulator of rising levels of LH, FSH,

and testosterone. Steroids found in kaffir lime plants also act as a protector. In addition, kaffir lime leaves have traditionally been believed to be aphrodisiacs. Aphrodisiacs are stimulants that have the potential to increase sexual desire.

Table 2. Microscopic view of free-range chicken semen after supplementation of green meniran herb extract (Phylanthus niruri Linn) and kaffir lime leaf extract (Citrus hystrix D. C)

Treatment	Parameter			
	Sperm count (cells x 10^6)	Mass Movement (+)	Motility (%)	Viability (%)
T0	2.637 ± 238[a]	2.40 ± 0.08[a]	67.89 ± 2,88[a]	74.51 ± 3.47[a]
T1	4.446 ± 326[b]	2.60 ± 0.14[a]	79.35 ± 3.80[b]	82.70 ± 2.72[b]
T2	4.969 ± 224[b]	2.70 ± 0.22[a]	76.66 ± 2.64[b]	78.46 ± 2.43[ab]
T3	4.039 ± 424[b]	2.70 ± 0.16[a]	78.81 ± 2.82[b]	79.91 ± 2.57[ab]
T4	5.950 ± 186[c]	2.80 ± 0.07[a]	80.34 ± 3.53[b]	84.37 ± 2.64[b]
T5	4.447 ± 228[b]	2.60 ± 0.07[a]	74.22 ± 4.27[b]	80.28 ± 2.44[b]
T6	3.293 ± 544[a]	2.40 ± 0.14[a]	69.32 ± 6.27[a]	70.28 ± 2.44[a]
T7	4.669 ± 456[b]	2.60 ± 0.24[a]	73.28 ± 4.24[b]	78.28 ± 2.44[b]
Average	4.306 ± 337	2.60 ± 0.16	74.79 ± 3.80	78.47 ± 2.46

Information: The average value followed by different superscripts in the same column shows that there is a fundamental difference ($p<0.05$).

Aphrodisiac substances work by increasing blood flow to the reproductive organs so that they can increase sensitivity and response to sexual stimulation. Aphrodisiac in kaffir lime plants to increase libido. The increase in libido is influenced by the rise in the number of pituitary hormones and testosterone, thereby stimulating receptor domains and sexual behavior. Aphrodisiacs usually come from flavonoids, alkaloids, and steroids (Owaba et al., 2021).

Meanwhile, Harmusyanto (2013) states that flavonoids, terpenes, and polyphenols are active compounds in charge of stimulating steroid hormones, including progesterone and testosterone, essential in regulating male libido. In addition to steroids, the content of tannins and alkaloids can also increase and facilitate blood flow to the male genital organs (Enema et al., 2018).

Phyllanthus niruri itself has isoorientin compounds that can increase sperm production. In contrast, the sitosterol and stigmasterol compounds in the green meniran plant are androgenic, which can help improve the degree of spermatogenesis in the testes, increasing sperm count and motility, having an antioxidant effect and increasing levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone. Testosterone and LH hormones are the primary stimuli for testosterone secretion by the testes, and the FSH hormone mainly stimulates spermatogenesis (Aziman et al., 2015), thus increasing sperm concentration significantly.

Spermatozoa motility, Viability, and mass movement

Spermatozoa motility is an essential parameter in the assessment of semen quality for the success of IB (Putranto et al., 2020), so the motility of chicken spermatozoa that is less than 40% is not suitable for insemination (Isnaeni et al., 2019).

The motility of spermatozoa in this study ranged from 67.89 + 2.88 - 80.34 + 3.53 (table 2). There was a significant increase ($P<0.05$) in positive control chickens (testosterone supplementation) and treated chickens supplemented with 1 ml or 2 ml of extract. However, there was no synergy in chickens with the addition of 0.5 ml and 1 ml of green meniran mixture with kaffir lime leaf extract. The results of sperm motility examination in this study were higher than the previous study in Gaok chickens by 57.22% (Komarudin et al., 2020) and Sentul chickens by as much as 71,95% (Hidayat & Sopiyana, 2010).

The age of livestock at the time of semen storage can affect the motility of spermatozoa (Adeoye et al., 2018). Livestock that are too young or too old will produce poor semen quality. In addition, external factors such as pH and temperature can also affect motility (Yang et al., 2019). Sperm flagella will move slowly at low pH, followed by decreased sperm viability (Sarkar, 2020).

The Viability of spermatozoa is a comparison between living and non-living spermatozoa. Viability is the vitality of spermatozoa that can be used to assess its quality. Viability can be seen as a measure of the ability of spermatozoa to fertilize eggs, so the percentage of Viability can be used as one of the indicators to determine the success of insemination. The Viability of spermatozoa is influenced by the use of oxygen in the process of metabolism and respiration to oxidize the primary substrates and restore phosphate bonds to re-establish ATP so that it is later converted into energy used by spermatozoa (Junaedi et al., 2016).

In this study, the average result was 78.47±2.46. Statistical analysis showed that the administration of

green meniran herb extract and kaffir lime leaf extract as much as 2 ml and a mixture of both as much as 1 ml each was able to increase sperm viability significantly (P< 0.05) with a value of (T4) 84.37±±2.64 (T5) 84.372.64% and (T7) 80.28±2.44%. This result is lower when compared to the findings of Mau et al. (2022), which obtained a yield of 93.7±1.75% in free-range chickens supplemented with L-lysine 0.6 g + L-Arginine 0.4 g. Still, according to Putranto et al. (2020), this concentration is enough to meet the needs of artificial insemination.

The intact plasma membrane determines the percentage of spermatozoa survival. The plasma membrane of spermatozoa functions to protect spermatozoan organelles and transport electrolytes in the process of spermatozoa metabolism. Damaged plasma membranes can affect the physiological function and metabolism of spermatozoa so that they cause spermatozoa to die, stating that the proteins in the feed consumed by poultry will be digested by pepsin inside the proventriculus and ventriculus which are subsequently processed by proteolytic enzymes (trypsin and chimotrypsin) into peptides and amino acids and then absorbed by the cells of the small intestine mucosa (Hidayat & Sopiyana, 2010).

High feed consumption is indicated in the fulfillment of poultry feed needs in quality and quantity. Djermanovic et al. (2013) reported that management that focuses on the body weight of roosters is critical to maintaining fertility, and management can be achieved by regulating the crude protein content (PK) followed by feed restriction techniques. Danang et al. (2012) stated that adding energy levels in feed can improve the quality of spermatozoa plasma membranes. This is by the statement of Haryuni et al. (2022), which states that the better the plasma membrane, the more spermatozoa live.

The results of observation of sperm mass movement in this study did not show a significant difference (P > 0.05). The movement of semen that forms thick and rapid mass waves contains many motile spermatozoa. It can be interpreted that although the motility and Viability have increased significantly, the visible semen wave population has not been able to improve. Hambu et al. (2016) reported that mass wave 3 (+++) is affected by the individual movement (motility) of chicken spermatozoa by more than 80%. Similar results have also been reported by Junaedi et al. (2016) on Sentul chicken semen. In this study, the height of mass movement was also influenced by spermatozoa motility, but there was no significant difference between treatments.

Conclusion

From the description above, it can be concluded that the supplementation of green meniran herb extract and kaffir lime leaf extract or a combination thereof, macroscopic does not change the appearance of free-range chicken semen, both volume, color, consistent odor, and pH of semen. Microscopic supplements of green meniran herb extract and kaffir lime leaf extract 1 ml and 2 ml increased sperm concentration, motility, and Viability, but the mass movement has not increased significantly. There was no synergy between the green meniran herb extract and kaffir lime leaf extract.

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