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Effect of cassava leaf silage (*Manihot esculenta*) on ram reproductive performance: Utilization of waste-based feed

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Abstract. Cassava leaves, which are high in protein but contain anti-nutritional compounds such as cyanide and tannins, were ensiled to enhance nutrient availability. This study examined the impact of cassava leaf (*Manihot esculenta*) silage on ram reproductive performance, emphasizing semen quality and scrotal circumference. Twenty rams aged 7–8 months (18–20 kg) were reared for 12 weeks under a Completely Randomized Design (CRD) with four treatments: T0 (unfermented cassava leaves) and T1–T3 (fermented using different concentration of Effective Microorganisms 4 (EM4), NASA Liquid Organic Fertilizer (POC NASA), and Indigenous Microorganisms (IMOs)), each with five replications. Observed parameters included semen characteristics and scrotal circumference. T1 significantly affected ($P < 0.05$) semen volume (0.83 ± 0.21 mL), sperm concentration (3136.00 ± 218.02 million/mL), and scrotal circumference (25.00 ± 3.46 cm). No significant effects ($P > 0.05$) were observed on semen color, odor, consistency and pH, also sperm motility, viability, and abnormalities. Positive correlations were detected between scrotal circumference-semen volume ($r = 0.699$; $P < 0.05$), semen consistency-sperm concentration ($r = 0.880$; $P < 0.01$), mass motility-progressive motility ($r = 0.802$; $P < 0.01$), mass motility-viability ($r = 0.590$; $P < 0.05$), while sperm viability showed a negative correlation with abnormalities ($r = -0.579$; $P < 0.05$). The use of cassava leaf silage (T1) can enhance livestock sustainability through the utilization of agricultural waste-based feed, while also supporting national food security.

1. Introduction

Sheep are one of the animal protein sources that are in great demand by the community, especially North Sumatra. The sheep population in North Sumatra has increased in line with the increasing demand for sheep. The sheep population in 2022 in North Sumatra reached 778,816 heads, an increase of around 10,555 heads compared to the previous year [1]. The productivity of small ruminant livestock such as sheep is greatly influenced by the quality and availability of feed. One of the main challenges in sheep rearing system is the high cost of feed and the limitation of feed materials quality, especially during the dry season. Dependence on conventional feed causes farmers to look for cheaper and sustainable alternatives. Therefore, the use of agricultural waste



as an alternative feed material is a potential solution to reduce production costs while supporting livestock production capacity and national food security.

Cassava leaves (*Manihot esculenta*) are abundant agricultural waste and have high nutritional content, especially crude protein which is very important for the growth and reproduction of livestock. However, raw cassava leaves contain antinutrient compounds such as cyanide and tannins that can harm livestock. Fermentation of cassava waste can reduce antinutrient levels, while increasing the shelf life and palatability of these feed ingredients [2–4]. Thus, cassava leaf silage has the potential to be an alternative, safe, and nutritious feed solution for sheep.

The reproductive performance of sheep is highly dependent on the adequacy of nutrition, especially in terms of protein and energy. Providing good feed is expected to be able to improve body and hormonal conditions that support optimal ram reproduction. The use of cassava leaves as animal feed has been applied to various commodities and is reported to be able to increase livestock productivity such as body weight, carcass and non-carcass weight, and feed efficiency [5–7]. However, so far, the effectiveness of the use of cassava leaf silage on ram reproductive performance still needs to be studied more deeply.

Seeing the significant potential of the increasing sheep population and the need of feed, this study is necessary. The aim of this study was to evaluate the impact of providing cassava leaf silage on reproductive performance of rams. The results of this study are expected to provide an efficient and environmentally friendly alternative feed that can enhance livestock reproductive productivity, thereby increasing livestock population to meet market demand. Furthermore, this study is also consistent with the principle of livestock sustainability and contributes to Indonesia's national food security program.

2. Materials and Methods

2.1 Animals

This study was conducted using 20 clinically healthy rams with a uniform age range of 7–8 months and an average body weight of 18–20 kg. All experimental procedures involving animals were reviewed and approved by the Animal Ethics Committee of the Faculty of Mathematics and Natural Sciences, as documented under ethical clearance No. 0810/KEPH-FMIPA/2025.

2.2 Location and time of research

This research was carried out at the Department of Animal Science, Faculty of Agriculture, Universitas Sumatera Utara, specifically in the sheep pens in Greenhouse and Field Laboratory, and Livestock Production Laboratory. This study was conducted for 4 months with a rearing period of 12 weeks.

2.3 Treatment

This study used an experimental design arranged in a Completely Randomized Design (CRD) with 4 treatments and 5 replicates. The treatments investigated were as follows:

T0: Cassava stems and leaves without fermentation.

T1: Cassava stems and leaves fermented with Effective Microorganisms 4 (EM4) 20 mL, NASA Liquid Organic Fertilizer (POC NASA) 10 mL, and indigenous microorganisms (IMOs) 20 mL.

T2: Cassava stems and leaves fermented with EM4 10 mL, NASA 10 mL, and IMOs 20 mL.

T3: Cassava stems and leaves fermented with EM4 20 mL, NASA 20 mL, and IMOs 10 mL.

2.4 Preparation of cassava leaves silages

Cassava leaf silage is made with reference to [8] with modifications, where the process is shown in Figure 1.

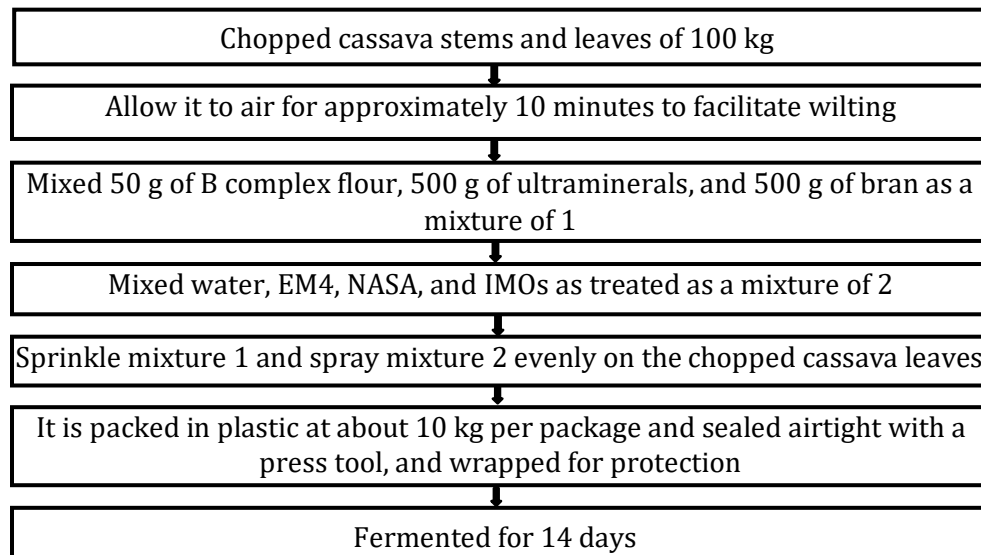


Figure 1. Flow chart of cassava leaf silage making.

2.5 Animal rearing

The rams were managed under an intensive rearing system in individual pens, each measuring 50 x 100 cm. The rams underwent a two-week adaptation period. During the initial week, they were provided with forage and concentrate feed. Subsequently, concentrates, forage, and treated feed (cassava leaf silage) were gradually introduced, with careful consideration of the animals' health and physiological condition.

The feed was provided twice daily and consist of cassava leaf silage and concentrate. Concentrate is given every morning as much as 750 g, and cassava leaf silage is given every afternoon as much as 5% of the body weight of the rams. Drinking water was supplied ad libitum, and the health status of the animals was monitored regularly. Additionally, the temperature and humidity within the pens were controlled to ensure optimal environmental conditions. The rams were maintained under these conditions for a period of 12 weeks. According to [9], the process of spermatozoa formation takes 60 to mature.

2.6 Fresh semen collection

The semen collection is carried out using artificial vagina, referring to [10], where this method has the best quality ejaculation compared to other methods. The semen is taken in the morning, then the quality of fresh semen is checked macroscopically and microscopically.

2.7 Scrotum measurement

Measurements of scrotal circumference refer to [11]. The scrotum is measured periodically once a week. This measurement is performed to assess the development of testicular size in a non-invasive manner.

2.8 Parameters

The quality of fresh semen was carried out macroscopically and microscopically, referring to [10]. The macroscopic examination, including volume, color, odor, consistency, and pH. While microscopic examinations include concentration, mass motility, individual motility, viability, and abnormalities.

The volume of the ejaculate collected in the tube was measured in milliliters (mL). The color of fresh semen was evaluated through visual observation of the ejaculate. The odor of fresh semen was assessed by carefully smelling the ejaculate. Semen consistency was determined by tilting the collection tube containing the ejaculate and then returning it to an upright position, followed by observing the flow rate of the ejaculate; a faster flow indicated a more dilute consistency. The pH was measured using a pH indicator.

Mass motility was assessed by placing a single drop of ejaculate on a glass slide and examining it under a microscope at 100× magnification. Progressive motility was evaluated by diluting the semen with a semen extender at a 1:1 ratio and observing it under a microscope at 400× magnification. Sperm viability and morphological abnormality were determined by preparing smear slides using the eosin-nigrosin staining technique, followed by microscopic examination at 400× magnification. The concentration of fresh semen was estimated using a haemocytometer.

2.9 Data analysis

The collected data were tabulated, and spermatozoa concentration data (expressed in million/mL) were first log-transformed before being analyzed using statistical tests. Then, data were analyzed using Analysis of Variance (ANOVA). When significant differences among treatments were identified, Duncan's Multiple Range Test (DMRT) at a 5% significance level was applied to determine differences. Correlation analyses between parameters were performed using Pearson's Correlation. All statistical analyses were conducted using SPSS software, version 29.0. The data is presented in the form of a mean $\pm$ standard error mean (SEM).

3. Results and Discussion

The quality of fresh semen is one of the important indicators in assessing male reproductive performance. Macroscopic parameters such as volume, color, odor, consistency, and pH as well as microscopic checks such as mass motility, progressive motility, viability, abnormality, and spermatozoa concentration reflect the quality of the semen produced. According to [12, 13], good quality of fresh semen can lead to a high conception rate, as this is closely related to male fertility. In various studies, physical appearance such as scrotal circumference has correlated with the quality of the semen produced [11, 12, 14].

3.1 Macroscopic quality of fresh semen

The results of the study demonstrated that providing fermented cassava leaf silage, combined with EM4, NASA, and IMOs, significantly influenced the semen quality of rams. The effects of providing cassava leaf silage on semen characteristics are presented in Table 1. The highest semen volume was recorded in treatment T1 (0.83 ± 0.21 mL), which differed significantly ($P < 0.05$) from the other treatments. In contrast, the lowest semen volume was observed in treatment T0 (0.47 ± 0.15 mL).

Table 1. Average macroscopic characteristics of fresh semen of rams.

Treatment	Volume (mL)	Color	Odor	Consistency	pH
T0	0.47±0.15 ^a	Milky white	Distinctive	1.33±0.58 ^{ns}	7.00±0.00 ^{ns}
T1	0.83±0.21 ^b	Milky white	Distinctive	3.00±0.00 ^{ns}	7.00±0.00 ^{ns}
T2	0.50±0.10 ^a	Milky white	Distinctive	1.67±1.00 ^{ns}	7.00±0.00 ^{ns}
T3	0.77±0.15 ^{ab}	Milky white	Distinctive	3.00±0.00 ^{ns}	7.00±0.00 ^{ns}

Note: Different letters in each column indicate significant differences at the 0.05 level; ns: non-significant differences. Consistency is assessed by scores (1.00: thin; 2.00: medium; and 3.00: thick).

The increase in volume and consistency of semen in fermentation treatment is due to the better availability of nutrients from the fermentation process. Fermentation with inoculant combinations can increase the availability of dissolved proteins, energy, and decrease crude fiber content, thereby supporting male metabolism and reproductive function [15, 16]. This is in line with [5], which reported that using cassava leaves as sheep feed should be limited because they contain antinutrients that can affect the animals' metabolism and physiological function, however, fermentation can reduce these antinutrients and improve the feed's nutritional value.

3.2 Microscopic quality of fresh semen

The quality of fresh semen that is microscopically examined can determine the success of fertilization. The average microscopic examination results of fresh semen of sheep fed with cassava leaf silage (*Manihot esculenta*) is shown in Table 2.

Table 2. Average microscopic characteristics of fresh semen of rams.

Treatment	Mass motility	Progressive motility (%)	Viability (%)	Abnormality (%)	Sperm concentration (million cells/ mL)
T0	1.67±0.58 ^{ns}	75.00±5.00 ^{ns}	72.84±4.41 ^{ns}	3.39±1.72 ^{ns}	2232.00±416.44 ^{ab}
T1	2.33±0.58 ^{ns}	81.67±2.89 ^{ns}	80.69±8.99 ^{ns}	2.94±0.99 ^{ns}	3136.00±318.02 ^b
T2	1.33±0.58 ^{ns}	76.67±7.64 ^{ns}	75.44±2.78 ^{ns}	3.44±1.33 ^{ns}	2352.67±461.98 ^a
T3	1.67±0.58 ^{ns}	75.00±3.23 ^{ns}	80.47±5.11 ^{ns}	3.04±0.93 ^{ns}	3024.67±280.74 ^b

Note: Different letters in each column indicate significant differences at the 0.05 level; ns: non-significant differences. Mass motility was evaluated using a scoring system as follows: 0.00, no motile spermatozoa; 1.00 (+), individual sperm movement observed; 2.00 (++) , the presence of clouds of spermatozoa that were moderately dense with slow wave-like movement; and 3.00 (+++) , dense clouds of spermatozoa exhibiting rapid wave-like motion.

Based on Table 2, the providing cassava leaf silage had an effect on the rams semen quality observed through microscopic examination, where T1 (3136.00±318.02 million cells/mL) and T3 (3024.67±280.74 million cells/mL) showed the best results with a significant difference ($P < 0.05$) in sperm concentration. Although other parameters such as mass motility, progressive motility, viability, and spermatozoa abnormality did not show significant differences ($P > 0.05$), they consistently showed T1 to provide the best results.

The crude protein content in cassava leaf silage is degraded into amino acids, which are subsequently utilized for synthesis of new proteins within cells that are essential for spermatogenesis, thereby influencing sperm production. Dietary proteins enhance spermatozoa concentration by stimulating the secretion of gonadotropin-releasing hormone (GnRH), which in turn promotes the release of follicle-stimulating hormone (FSH) to regulate spermatogenesis, and luteinizing hormone (LH) to stimulate Leydig cell activity and testosterone production [17, 18].

Progressive motility in all treatments showed an average of 75-81%, which is still classified as good based on SNI 4869:2014 ($>70\%$). Progressive motility is one of the main indicators of

male fertility, as it indicates the ability of spermatozoa to move forward towards the egg. The increase in progressive motility at T1 ($81.67 \pm 2.89\%$) is thought to be associated with better nutritional quality of fermented feed, thus being able to provide sufficient energy for spermatozoa metabolism. Energy is essential for the spermatozoa movement, and in the process of capacitation and fertilization. Furthermore, proteins play an important role in maintaining motility, stabilizing the plasma membrane of spermatozoa, and improving spermatozoa's transport ability [17]. Therefore, providing good quality feed with sufficient nutrients, especially protein, is important in livestock reproduction management.

The percentage of viability shows a trend align with progressive motility, where T1 and T3 provide the highest scores ($>80\%$), while T0 has the lowest percentage (72%). The availability of sufficient nutrients plays a role in maintaining the integrity of the spermatozoa membrane, thereby increasing the survival of spermatozoa [19]. Spermatozoa abnormality in all treatments are relatively low, which indicates that the providing cassava leaf silage does not have a negative impact on the formation of spermatozoa morphology. The maximum percentage of abnormalities in semen is $<15\%$ [20]. Lack of feed nutrients, especially protein, can inhibit testicular development and spermatozoa production. This is in line with studies [17, 21], which reported that damage to the epididymis due to androgen hormone disruptions related to low protein content in feed can inhibit the activity of testosterone and LH, thereby interfering with the maturation process of spermatozoa which has an impact on imperfect morphology (abnormal).

3.3 Scrotal circumference

Scrotal circumference is one of the important indicators in assessing the reproductive potential of males, as it correlates with testicular size, semen volume, and spermatozoa production. The average scrotal circumference of rams fed cassava leaf silage with different treatment in week 1 and week 12 observations is presented in Table 3.

Table 3. Average scrotal circumference of rams in the first and last weeks of the study.

Treatment	T0	T1	T2	T3
1 st week average (cm)	19.00 ± 2.65^{ns}	18.33 ± 1.53^{ns}	19.33 ± 3.21^{ns}	15.67 ± 4.51^{ns}
12 th week average (cm)	20.67 ± 0.58^a	25.00 ± 3.46^b	24.00 ± 1.00^{ab}	21.00 ± 1.73^a

Note: Different letters in each column indicate significant differences at the 0.05 level; ns: non-significant differences.

Table 3 showed that the average value between treatments is relatively not much different. The average scrotal circumference between treatments did not differ significantly ($P > 0.05$) in the first week, but in the 12th week there was a significant difference ($P < 0.05$) with the highest average being T1 (25.00 ± 3.46 cm) and the lowest T0 (20.67 ± 0.58 cm) and T3 (21.00 ± 1.73 cm).

The results of the study showed that the provision of fermented cassava leaf silage had an effect on the growth of the ram's scrotal circumference during 12 weeks of rearing. An increase in scrotal circumference of about 6-7 cm in rams treated with T1-T3, indicates that cassava leaf silage may increase the size of the testicles. This is in line with [21] who reported that fermentation in silage is able to increase nutrient availability, which supports the growth of testicular tissue.

A large scrotal circumference is an important indicator in the selection of a male. The size of the scrotum circumference is one of the assessments in the Breeding Soundness Examination (BSE) in the selection of superior bulls [12]. [14] reported that scrotal circumference had correlation with semen production and quality.

3.4 Correlation of the quality of fresh semen and scrotal circumference

The correlation coefficient between the quality of fresh semen and the scrotal circumference of ram fed cassava leaf silage is presented in Table 4. The results of the analysis showed, there was a significant relationship between several parameters of semen quality and scrotal circumference. Positive and significant correlation between semen volume and scrotal circumference ($r=0.699$; $P<0.05$) supports the theory that testicular size represented by scrotal circumference is related to spermatogenesis capacity which impacts semen volume. The scrotal circumference is a reflection of the size of the testes, where 80% of the testes weight are seminiferous tubules, whose function is to produce spermatozoa [14].

Table 4. Correlation coefficient of fresh semen quality and scrotal circumference of rams.

Parameters	Correlation coefficient	P-value
Semen volume and scrotal circumference	0.699*	0.011
Semen consistency and sperm concentration	0.880**	<0.001
Mass motility and progressive motility	0.802**	0.002
Mass motility and viability	0.590*	0.043
Viability and abnormality	-0.579*	0.049

Note: *Correlation is significant at the 0.05 level and **Correlation is significant at the 0.01 level.

A very strong positive correlation was found between semen consistency and sperm concentration ($r=0.880$; $P<0.01$), indicates the viscous semen reflects a high concentration of spermatozoa. An increase in spermatozoa concentration will make the semen appear thicker [22]. A very strong association between mass motility and progressive motility ($r=0.802$; $P<0.01$), indicates that improved collective sperm motility is associated with a higher proportion of spermatozoa exhibiting progressive forward movement individually. A positive correlation between mass motility and viability ($r=0.590$; $P<0.05$) indicates that more living spermatozoa tend to have more active movements. In contrast, A significant negative correlation was observed between spermatozoa viability and abnormality ($r=-0.579$; $P<0.05$), indicate that an increase in the proportion of abnormal spermatozoa is associated with a decrease in the percentage of viable spermatozoa. This is supported by [23], morphological abnormalities affect the endurance and function of spermatozoa. Scrotal circumference is a heritable trait and an important measure of male fertility, as larger testes are linked to greater sperm output. Testicular growth and size are affected by nutritional factors, especially protein intake in the diet [12].

4. Conclusion

Providing cassava leaf silage has positive effect on the semen quality and scrotal circumference of rams. The combination of inoculants in T1 gave the best results which was reflected in the increase in semen volume, spermatozoa concentration, and scrotal circumference. Correlation analysis showed positive relationship between scrotal circumference with semen volume, semen consistency with sperm concentration, mass motility with progressive motility and spermatozoa viability, and negative relationship between spermatozoa viability and abnormality. The results of this study indicate that feed improvement through fermentation can improve the reproductive performance of rams. The use of cassava by-products as silage (T1) enhances livestock sustainability by transforming agricultural waste into valuable feed resources.

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