

Research Article



The Influence of Differential Administration of Natural Vitamin E (Green Bean Sprouts) and Synthetic Vitamin E on the Macroscopic and Microscopic Quality of Semen in Sheep

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Abstract | The objective of this research was to investigate the impact of administering natural vitamin E (from green bean sprouts) and synthetic vitamin E on the macroscopic (volume, viscosity, color, odor, and pH) and microscopic (motility, live spermatozoa, abnormalities, and concentration) quality of sheep semen. The research involved an experimental study using a Randomized Complete Block Design (RCBD) consisting of 3 treatments, with each treatment being replicated 5 times. The treatments applied were as follows: P0; Complete Feed 60% + silage 40% (without the addition of natural and synthetic vitamin E), P1; Complete Feed 60% + Silage 40% + Natural Vitamin E 50 IU (equivalent to 223 grams of green bean sprouts), P2; Complete Feed 60% + silage 40% + Synthetic Vitamin E 50 IU. The acquired data was subsequently analyzed using descriptive methods for pH, consistency, color, and odor, while volume, motility, live spermatozoa, abnormality, and concentration were analyzed using ANOVA and followed by Bonferroni post hoc test at a significance level of 5%. The findings of this study indicate that there is no significant ($P>0.05$) difference in pH, consistency, color, and odor between natural vitamin E and synthetic vitamin E supplemented groups, and they do not significantly impact the motility, live spermatozoa, abnormalities, and semen concentration. However, there is a significant ($P<0.05$) difference in semen volume. The study concludes that inclusion of synthetic or natural vitamin E in sheep ration does not have an impact on the macroscopic and microscopic quality of the semen.

Keywords | Semen, Macroscopic quality, Microscopic quality, Vitamin E, Sheep

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INTRODUCTION

Sheep is one of the small ruminants that has a rapid development in Indonesia. Sheep are widely kept by farmers both on a small and large scale because these animals can adapt to various environments, besides that sheep are used by the people of Indonesia as a fulfillment of animal protein needs. Rusdiana and Praharani (2015) stated that sheep can adapt well to all agroecosystems in Indonesia. Sheep also have rapid breeding in which one sheep can

give birth to more than one tail, for that sheep are widely used as a livestock business or as a side income for farmers. There are many types of sheep raised by farmers, namely thin-tailed sheep, arrowroot sheep, fat-tailed sheep, priangan sheep, and dorking sheep. Therefore, sheep breeding or cultivation needs to be improved to achieve these things.

Efforts toward successful breeding or raising of sheep should prioritize maintenance management, particularly in feeding. Providing high-quality feed to sheep can sig-

nificantly contribute to their success in breeding or cultivation. The quality of feed directly impacts the reproductive response of livestock, particularly in male animals' ability to produce high-quality sperm. Sheep with superior sperm quality have a higher likelihood of achieving pregnancy rates. However, it's common for smallholder farmers to feed their sheep with field grass, which typically contains 11.55% protein, 40.97% crude fiber, and 22.61% dry matter (Nawang Sari and Hendarti, 2021).

Sheep semen is a suspension in which there are spermatozoa and various components produced by the reproductive organs of the male sheep, namely the testes and appendage glands. A successful mating in a sheep population both natural mating and artificial insemination is closely related to sperm quality. Good sperm quality can support the improvement of genetic quality in sheep. According to Hafez (1993) which states that, the reduction of conception rates in female livestock is closely related to the quality and quantity of semen that decreases in male livestock. For this reason, the improvement of nutritional elements in the ration given to sheep is very important. Nutritional elements that can be used to increase the volume and quality of semen in sheep are vitamin E. Vitamin E given can be in the form of natural ingredients derived from green bean sprouts and synthetic vitamin E, namely from one of the processed vitamin E products.

Vitamin E is very important for livestock reproduction, especially male livestock. Vitamin E if given to livestock regularly can maintain the reproductive system of livestock. Yuliyantika et al. (2019) Vitamin E functions as an intracellular balance and as an antioxidant. Vitamin E has an important role in the reproductive process, including preventing degeneration of the germinal epithelium in the testes, so that spermatozoa production and fertility can be maintained (Kamal et al., 2022). Green bean sprouts are plants which consider as rich source of vitamin E (α -tocopherol) in addition to antioxidant compounds, namely phytosterols, phenols, and several minerals (selenium, manganese, copper, zinc, and iron). Green bean sprouts are plants recognized as a rich source of vitamin E (α -tocopherol), along with an array of antioxidant compounds such as phytosterols, phenols, and various minerals including selenium, manganese, copper, zinc, and iron. Antioxidants work by donating one electron to oxidant compounds so that the activity of these oxidant compounds can be inhibited (Yuliyantika et al., 2019). The current study hypothesized that supplementation of natural Vitamin E in the form of green bean sprouts may have different consequences on the semen quality of sheep. Hence, this study incorporated natural Vitamin E (in the form of green bean sprouts) and synthetic vitamin E into the diets of sheep to investigate their impact on the macroscopic and microscopic quality of semen.

RESEARCH DESIGN

This research was conducted over a period of 60 days at the Department of Animal Husbandry, Faculty of Agriculture, University of Lampung, in compliance with ethical standards for animal research. The study was carried out experimentally using a Randomized Complete Block Design (RCBD) comprising 3 treatments with 5 replications, with each experimental unit consisting of 1 sheep. The research design is outlined as follows:

- P0: Complete Feed 60% + silage 40%
- P1: Complete Feed 60% + Silage 40% + Natural Vitamin E 50 IU (223 grams of green bean sprouts)
- P2: Complete Feed 60% + silage 40% + synthetic Vitamin E 50 IU

The treatment feed consisted of a complete feed comprising 60% and silage 40%. The composition of the feed treatment can be observed in Table 1.

Table 1: Composition of the treatment ration.

Chemical composition*	Complete Feed	Silage
Dry matter (%)	88.50	93.66
Crude protein (%)	11.84	7.34
Crude fat (%)	1.78	3.88
Crude fiber (%)	19.13	33.94
Ash (%)	8.32	10.43

* Analysis results at the Nutrition and Animal Feed Laboratory, Department of Animal Husbandry, Faculty of Agriculture, University of Lampung (2022).

RESEARCH PROCEDURE

The pre-trial period was carried out for 2 weeks prior to the sheep entering the observation phase in order for the sheep to adapt to the treatment given. The maintenance stage of sheep carried out by providing rations according to the treatment design in the form of basal feed supplemented with green bean sprouts and additional vitamin E. Feeding was done three times a day, and treatment was given every morning before feeding the animals during the maintenance period with the predetermined dosage. The ad libitum availability of drinking water was ensured.

The collection of ram semen was carried out in weeks 7 and 8 during the maintenance period. Every week, all the sheep used to collected semen and then subjected to macroscopic examination. The examination of semen was done macroscopically to ascertain its quality. Herdiawan (2004) states that the macroscopic quality of semen includes volume, pH, consistency, color, and odor.

OBSERVED VARIABLES

The variables observed in this study are the macroscopic

ic quality of semen in sheep, tested both macroscopically (volume, viscosity, color, odor, and pH) and microscopically (motility, live spermatozoa, abnormalities, and semen concentration).

DATA ANALYSIS

The data obtained were then analyzed using descriptive methods for pH, consistency, color and odor, while volume, motility, live spermatozoa, abnormality, and concentration were analyzed using ANOVA and followed by Bonferroni post hoc test at a significance level of 5%.

RESULTS AND DISCUSSION

The fresh semen observed was obtained from sheep kept in the Animal Husbandry Department Stables, Faculty of Agriculture, University of Lampung for one month. Examination of fresh semen produced from sheep can be known based on macroscopic examination. According to Nahriyanti et al. (2017), one of the efforts to determine the quality of fresh semen produced by male animals is by macroscopically evaluating fresh semen. Macroscopic evaluation of semen is the observation of semen quality with the naked eye. Macroscopic tests of semen include volume, pH, consistency (viscosity), color, and odor. Microscopically tests of semen include motility, live spermatozoa, abnormalities, and semen concentration.

VOLUME

The volume of fresh semen obtained from collection is one of the standards in determining the quality of semen in sheep. Semen volume is one of the standards in determining semen quality in livestock (Nubatonis et al., 2022). The volume of semen containing gel and not can be seen through its color and concentration. Semen volume is not important in fertilization but the total ejaculated spermatozoa determine the success of fertilization (Susilawati, 2011). Average semen volume of sheep in this study can be seen in Table 2.

Table 2: Semen volume of male sheep.

Group	Treatment*		
	P0	P1	P2
	------(ml)-----		
1	0.5	1	0.8
2	0.5	1.9	2
3	0.3	1.8	1.3
4	0.7	0.8	0.9
5	0.7	2	0.9
Average:	0.54±0.17 ^b	1.50±0.56 ^a	1.18±0.50 ^a

* P0: Complete Feed 60% + silage 40%; P1: Complete Feed 60% + Silage 40% + Natural Vitamin E 50 IU (223 grams of green bean sprouts); P2: Complete Feed 60% + silage 40% + Synthetic

Vitamin E 50 IU. Different superscript letters within the average line indicate significant differences (P<0.05).

The volume of semen ejaculated by sheep treated with 223 grams of green bean sprouts is an average of 1.50 ml and those treated with vitamin E 50 IU have an average of 1.18 ml while untreated sheep have an average of 0.54 ml. Semen ejaculated by sheep shows that the volume of sheep semen in this study is in the normal range. This is in accordance with the statement of Susilawati (2011) that the semen volume of adult sheep ranges from 0.5—2.0 ml, while the young ones range from 0.5-0.7 ml. Meanwhile, according to Khairi et al. (2021) that sheep semen has a volume range of 0.37-0.80 ml with an average of 0.62 ml. The results of analysis of variance in semen volume of sheep showed that the provision of green bean sprouts and synthetic vitamin E had a significant effect (P < 0.05). The results further showed that the provision of green bean sprouts and synthetic vitamin E was different from the control. Semen volume produced by male cattle is influenced by the accessory glands. Some of the accessory glands consist of seminal vesicle glands (vesicularis), prostate and bulbourethral (cowper's gland). The vesicular glands are the glands that produce the largest portion of semen plasma compared to the secretions of other genital accessory glands. The volume of ejaculate in cattle can vary according to the degree of maturity of the male animal and more specifically with the length of lambing stimulation and frequency of collection (Hunter, 1995).

Vitamin E has a role in male fertility because it can increase testicular weight, increase sperm count and motility (Anggorodi, 1994). Vitamin E given to livestock can work against oxidative stress in livestock. Vitamin E is also important in maintaining the health of male reproductive organs and spermatid survival. The role of vitamin E in the reproductive system is to enhance the development of reproductive organs by increasing the weight of the epididymis, epididymal ductules and seminiferous tubule diameter, spermatogenic cells and interstitial cell density which is very important in the smooth progress of spermatogenesis (Wang et al., 2007). Vitamin E is a vitamin that has the first line of defense against the peroxidation process of polyunsaturated fatty acids contained in phospholipids of cellular and subcellular membranes. Phospholipids in mitochondria, endoplasmic reticulum and plasma membranes have an affinity for vitamin E, and vitamin E acts as an antioxidant that can break various free radical chain reactions due to its ability to transfer phenol hydrogen to peroxide free radicals from polyunsaturated fatty acids that have undergone peroxidation (Mayes, 1995).

pH

pH (degree of acidity) is a parameter that can affect spermatozoa survival. Dethan et al. (2010) stated that pH greatly affects sperm survival and pH has a correlation with concentration, if the concentration is high then the resulting pH will be slightly acidic. The pH of semen produced from male animals has a pH value that varies depending on the species of livestock itself. The average pH of sheep semen in this study can be seen in Table 3.

Table 3: pH of semen of male sheep.

Group	Treatment*		
	P0	P1	P2
1	7	7	7
2	7	6	7
3	7	7	7
4	7	7	7
5	7	7	7
Average:	7.0±0.0	6.8±0.45	7.0±0.0

* P0: Complete Feed 60% + silage 40%; P1: Complete Feed 60% + Silage 40% + Natural Vitamin E 50 IU (223 grams of green bean sprouts); P2: Complete Feed 60% + silage 40% + Synthetic Vitamin E 50 IU.

Sheep treated with 223 grams of green bean sprouts have an average pH of 6.8 while sheep treated with vitamin E 50 IU and untreated have an average pH of 7. Semen ejaculated by Sheep shows that the pH of sheep semen in this study is in the normal range. This is in accordance with the statement of Dethan et al. (2010) states that the normal pH of semen is 6.2 - 7.0 According to Alvionita et al. (2015), the average pH in sheep that have different ages ranges from 6.4 - 6.9. This is thought to be because the rations given to experimental animals both using natural vitamin E and synthetic vitamin E or not, can support the metabolic process of spermatozoa normally. The difference in pH results is influenced by several factors. neutral pH in semen then the feed given contains food substances that can support the metabolic process of spermatozoa normally (Hersade, 2012). The accumulation of lactic acid from carbohydrate metabolism will cause a decrease and increase in pH, while an increase in pH in semen can be caused by bacterial contamination or the death of many spermatozoa which will form ammonia (Handarini, 2005). Normal or not the pH of the semen is determined by the balance of cations and anions in the accessory glands (Elya et al., 2010).

pH in the normal range is a requirement for spermatozoa to be able to live longer. pH greatly affects sperm survival and pH has a correlation with concentration, if the concentration is high then the resulting pH will be slightly acidic (Dethan et al., 2010). The antioxidant content con-

tained in green bean sprouts can increase antioxidants in the body of the doma. Antioxidant compounds contained in green bean sprouts are phytosterols, vitamin E (*α-tocopherol*), phenols, and several minerals (selenium, manganese, copper, zinc, and iron) (Yuliyantika et al., 2019). Vitamin E as a fat-soluble antioxidant that has a major protective role against free radical oxidation and prevents the production of lipid peroxides by counteracting free radicals (especially strong hydroxyl radical counteraction) which are toxic by-products of many metabolic processes in membrane biology (Gupta et al., 2004). Vitamin E acts as an antioxidant and can protect the action of free radical damage to biological membranes. Vitamin E protects unsaturated fatty acids in phospholipid membranes such as cell membranes in sperm, thus providing more energy for spermatozoa (Dutta-Roy et al., 1994).

CONSISTENCY

Consistency or degree of viscosity can be seen by gently shaking the collection tube containing fresh semen. Age in sheep does not affect the consistency of semen. Semen consistency in sheep that have different ages of each age is thick (Alvionita et al., 2015). The results of the observation of fresh semen consistency of Sheep can be seen in Table 4. The results of the evaluation of the consistency of fresh semen of Sheep in this study were obtained dilute to thick. The semen consistency of sheep treated with P1 has a thick consistency and P2 has a slightly dilute average semen consistency while P0 has a dilute average. The results of this observation show the consistency of semen is still in a normal state. Nahriyanti et al. (2017) stated that sheep semen which has a slightly thick to thick consistency indicates that sheep semen is still in a normal state and has a lot of spermatozoa. The color of the semen is getting faded, the consistency of the semen will be thinner and the concentration of spermatozoa in the semen will decrease (Pamungkas et al., 2008).

Table 4: Results of semen consistency in male sheep

Group	Treatment*		
	P0	P1	P2
1	Dilute	Viscous	Viscous
2	Viscous	Dilute	Viscous
3	Viscous	Slightly watery	Dilute
4	Dilute	Viscous	Dilute
5	Dilute	Slightly watery	Slightly watery

* P0: Complete Feed 60% + silage 40%; P1: Complete Feed 60% + Silage 40% + Natural Vitamin E 50 IU (223 grams of green bean sprouts); P2: Complete Feed 60% + silage 40% + Synthetic Vitamin E 50 IU.

Sheep treated with Complete Feed 60% + Silage 40% + Natural Vitamin E (green bean sprouts) 50 IU (equivalent to 223 grams of green bean sprouts) (P1) and Complete

Feed 60% + Silage 40% + Synthetic vitamin E 50 IU (P2) had a thick semen consistency and slightly diluted so that the semen concentration was high. According to Feradis (2007) which states that the viscosity of the semen is influenced by the concentration of spermatozoa, the higher the concentration of spermatozoa, the viscosity of the semen will be thicker. Dethan, et al. (2010) sperm consistency has a relationship with sperm concentration. Semen consistency is thick because the concentration of spermatozoa affects the degree of viscosity of sperm and sperm color. Sonjaya et al. (2005) stated that the degree of viscosity of semen has a positive correlation with the content of spermatozoa in the semen so that if the observation found semen that is too thin, it can be assumed that the semen has a low concentration of spermatozoa and vice versa.

Vitamin E is one of the antioxidants that can protect spermatozoa from various damages caused by free radical attacks, so that in the presence of vitamin E in bean sprouts, the number of spermatozoa produced will increase because there is a possibility that vitamin E will protect spermatozoa from various damages (Basir et al., 2013). Vitamin E functions as an anti-oxidant and plays a role in male fertility because it can increase testicular weight, increase sperm count and motility (Anggorodi, 1994). Vitamin E given to livestock can work against oxidative stress in livestock. Vitamin E is also important in maintaining the health of male reproductive organs and spermatid survival. The role of vitamin E in the reproductive system is to improve the development of reproductive organs by increasing the weight of the epididymis, epididymal ductules and seminiferous tubule diameter, spermatogenic cells and interstitial cell density which is very important in the smooth progress of spermatogenesis (Wang et al., 2007).

COLOR

The color of the semen produced by the ram is a reflection of the viscosity of the semen. Under normal circumstances, it is often said that the denser the color of the cement appears, the thicker the consistency of the cement. Similarly, the opposite is true for pale-colored semen, which will result in a more watery consistency (Toelihere, 1985). The determination of the color of the fresh thin-tailed sheep semen can be observed directly in the collection tube obtained after semen collection. The results of the observation of the color of thin-tailed sheep cement can be found in Table 5.

The results of the observation showed that the color of fresh semen of untreated and treated sheep had a milky white color. These results indicate that the color of fresh semen of sheep is said to be normal. This is in accordance with the opinion of Salmah (2014), which states that the color of normal semen in sheep is milky or whitish beige.

Table 5: Results of semen color of male sheep

Group	Treatment*		
	P0	P1	P2
1	Milky white	Milky white	Milky white
2	Milky white	Milky white	Milky white
3	Milky white	Milky white	Milky white
4	Milky white	Milky white	Milky white
5	Milky white	Milky white	Milky white

* P0: Complete Feed 60% + silage 40%; P1: Complete Feed 60% + Silage 40% + Natural Vitamin E 50 IU (223 grams of green bean sprouts); P2: Complete Feed 60% + silage 40% + Synthetic Vitamin E 50 IU.

This is supported by the opinion of Susilawati (2011) that sheep have a semen color that is milky white or light beige. The color of semen in livestock is influenced by several factors including the concentration of spermatozoa, the higher the concentration of spermatozoa, the more turbid the color of the semen will be because the semen is indicated to contain many spermatozoa (Feradis, 2007). The secretion of accessory glands is mainly influenced by the vesicular glands, and the color of semen in male livestock is influenced by the feed given (Arifiantini, 2012).

This is thought to be because the rations given to experimental animals both using natural vitamin E and synthetic vitamin E or not, can support the metabolic process of spermatozoa normally so as to produce normal sperm color. Feed that has a high protein content can increase the percentage of life and the integrity of the spermatozoa plasma membrane. The high protein can affect the plasma membrane of spermatozoa, considering the principle of viability testing based on plasma membrane permeability (Dethan et al., 2010). The fulfillment of PK needs in sheep can maintain the hormonal system including the process of spermatogenesis and the activity of the accessory glands in producing semen plasma (Nubatonis et al., 2022). Vitamin E which acts as an antioxidant and can protect the action of biological membrane damage due to free radicals. Vitamin E protects unsaturated fatty acids in phospholipid membranes such as cell membranes in sperm, so as to provide more energy for spermatozoa (Dutta-Roy et al., 1994).

ODOR

The determination of the odor of fresh sheep semen can be observed directly in the collection tube obtained after semen collection. Feradis (2010) stated that the quality of sheep semen collected can be evaluated through direct olfactory assessment. The results of the observation of the color of the thin-tailed sheep semen can be observed in Table 6.

Table 6: Observations of semen odor of sheep

Group	Treatment*		
	P0	P1	P2
1	Typical fishy sperm	Typical fishy sperm	Typical fishy sperm
2	Typical fishy sperm	Typical fishy sperm	Typical fishy sperm
3	Typical fishy sperm	Typical fishy sperm	Typical fishy sperm
4	Typical fishy sperm	Typical fishy sperm	Typical fishy sperm
5	Typical fishy sperm	Typical fishy sperm	Typical fishy sperm

The results showed that the smell of fresh sheep semen has a typical fishy smell of sheep. This is in accordance with the opinion of Apriyanti et al (2017) and Alvionta et al (2015) which states that fresh semen has a distinctive fishy smell like the smell of the animal. Junqueira and Carneiro (2007) state that the odor in semen (spermin) comes from alkaline produced by the prostate gland. The work of the prostate gland depends on testosterone levels as well as seminal vesicles. Vitamin E which acts as an antioxidant that can inhibit body cells from damage and can maintain fatty acids needed in the formation of prostaglandins which are mediators of gonadotropins, namely testosterone. Vitamin E can prevent damage to sperm DNA that can cause infertility (Dewantari, 2013).

MOTILITY

Motility is a general parameter that indicates the functional ability of spermatozoa cells which plays an important role in successful fertilization (Centola, 2018; Moradpour 2019). Motility is positively correlated with cell membrane morphology and strength (Moradpour, 2019). Measurement of spermatozoa motility is to estimate the viability of spermatozoa and the quality of the spermatozoa's ability to move to reach the ovum cells.

The motility value of fresh semen in ruminants which is declared good is 70-90%. Spermatozoa motility is influenced by various factors including livestock age, medical conditions, environmental temperature and pollution (Jaenudeen and Hafez, 2000; Centola, 2018). The results of the observational analysis of spermatozoa motility in sheep that have been carried out are shown in Table 7.

The results of analysis of variance (Table 7) show that natural and non-natural vitamin E was given at a dose of natural vitamin E (mung bean sprouts) 50 IU (equivalent to 223 grams of green bean sprouts)/head/day, and non-natural vitamin E 50 IU) /head/day had no significant effect (P>0.05) on spermatozoa motility. This shows that giving

natural vitamin E (mung bean sprouts) as much as 223 grams/head/day, and non-natural vitamin E 50IU/head/day has not been effective in increasing the motility of thin-tailed sheep spermatozoa.

Table 7: Results of sheep spermatozoa motility

Group	Treatment*		
	P0	P1	P2
	----- (%) -----		
1	70	70	70
2	75	70	60
3	70	70	55
4	75	70	60
5	65	60	60
To the track	70	68	61

* P0: Complete Feed 60% + silage 40%; P1: Complete Feed 60% + Silage 40% + Natural Vitamin E 50 IU (223 grams of green bean sprouts); P2: Complete Feed 60% + silage 40% + Synthetic Vitamin E 50 IU.

Hafez (2000) stated that the microscopic quality of spermatozoa in normal sheep motility is 60-80%. Several other research results reported that the motility of sheep spermatozoa, namely Kusumawati et al. (2017) 70%; and (SNI 4869-1:2017) that the liquid cement used in the application of IB has a minimum motility of 70% (National Standardization Agency, 2017). The differences in the results of this study were influenced by livestock rearing patterns, the availability of nutrients provided, environmental temperature, and different types of experimental livestock for different livestock when the research was carried out. According to Dethan et al. (2010), differences in research results were caused by differences in experimental livestock breeds, length of research, environmental temperature during research and nutritional status of livestock. Spermatozoa motility or spermatozoa movement power is one of the determinants of the success of spermatozoa in reaching the ovum in the fallopian tubes and is the simplest way to assess sperm for artificial insemination (Hafez, 2000).

According to Centola (2018), the motility value of fresh semen in livestock that is declared good is 70-90%. Spermatozoa motility is influenced by various factors including animal age, medical conditions, environmental temperature, and pollution. Spermatozoa motility is influenced by various factors including livestock age, medical conditions, environmental temperature and pollution (Jaenudeen and Hafez, 2000; Centola, 2018). However, the spermatozoa motility standard that is suitable for AI is at least 40%. This is in accordance with the opinion of Hafez (1993), that the spermatozoa motility that is suitable for AI is at least 40%, while the percentage of survival below 40% is

no longer observed.

PERCENTAGE OF LIVING SPERM

Live spermatozoa are characterized by white heads (do not absorb dye) while dead spermatozoa have red or pink heads because they absorb eosin dye (Rizal and Herdis, 2008). Live spermatozoa can be identified by painting or staining using eosin. Eosin can be given by dissolving the eosin with distilled water, then the sperm to be observed is dropped with the eosin solution and spread evenly by covering the object glass with a cover glass. After that, observations were made under a microscope. Sperm that is recorded as having a red color means that the sperm is dead, while those that are not stained or have no defects means that the sperm is alive. The results of the percentage of live sheep spermatozoa in this study can be seen in Table 8.

Table 8: Results of live percentage of sheep spermatozoa

Group	Treatment*		
	P0	P1	P2
	(%).....		
1	70	75	80
2	70	70	60
3	70	75	70
4	75	70	65
5	70	70	70
To the track	71	72	67

* P0: Complete Feed 60% + silage 40%; P1: Complete Feed 60% + Silage 40% + Natural Vitamin E 50 IU (223 grams of green bean sprouts); P2: Complete Feed 60% + silage 40% + Synthetic Vitamin E 50 IU.

The results of the evaluation of the percentage of live spermatozoa in sheep in this study showed that the spermatozoa of sheep that had been given treatment P1 by giving fresh green bean sprouts 223 grams/gram/day had an average live percentage of 72% while P2 given non-natural vitamin E 50 IU/gram/day had an average percentage 67%, and those not given treatment had an average percentage of live spermatozoa of 71%.

Results of analysis of variance (Table 8) of giving natural and non-natural vitamin E with a dose of natural vitamin E (mung bean sprouts) 50 IU (equivalent to 223 grams of green bean sprouts)/head/day, and non-natural vitamin E 50 IU)/head /day. showed no significant effect ($P>0.05$) on live spermatozoa. This shows that giving natural vitamin E (mung bean sprouts) as much as 223 grams/head/day, and non-natural vitamin E 50 IU/head/day has not been effective in increasing live spermatozoa in thin-tailed sheep. This shows smaller results because according to Toelihere (1985), live spermatozoa in fresh sheep semen is 90% and the standard percentage of good sperm live cells is above

50%. The results of other research from the evaluation of live spermatozoa in pastured thin-tailed sheep were 74.5-89% Nubantonis et al. (2022). The percentage value of spermatozoa viability is slightly higher when compared to the percentage value of motility. This situation is caused by the presence of spermatozoa cells which are actually still alive but no longer motile, thereby increasing the percentage value of spermatozoa viability.

The results of the evaluation of spermatozoa viability values for male sheep grazing on dry land meet the criteria for retailing and freezing semen. The normal viability value for retailing and freezing semen is 60-75% live spermatozoa (Garner and Hafez, 2000). The percentage of viability of spermatozoa is determined by the integrity of the plasma membrane which functions to protect sperm cells. Wahy-uningsih et al. (2013), factors that influence the quality of live spermatozoa from fresh male semen are the age of the male, genetics, temperature, season, and lack of nutritional elements provided.

ABNORMALITY

Assessment of sperm abnormalities is important for semen analysis because it greatly affects semen quality. Centola (2018), spermatozoa abnormalities can be classified into three parts, namely abnormalities in the head, middle part and tail. Abnormalities in the head such as too big or small, pointed or blunt, two heads, acrosomal damage and amorphousness. Abnormalities in the middle such as a thick or thin neck, the tail not being in the middle of the neck, or a crooked neck. Meanwhile, abnormalities in the tail such as a bent tail, short tail, or curling from the tip of the tail. Normally fertile sheep semen should not contain more than 15% abnormal spermatozoa. Ariantie et al. (2014) confirmed that the percentage of sheep spermatozoa abnormalities was 6.40%. The results of observing spermatozoa abnormalities are in Table 9.

Table 9: Results of sheep spermatozoa abnormalities

Group	Treatment*		
	P0	P1	P2
	(%).....		
1	7	3	5
2	3	8	4
3	4	2	5
4	3	5	7
5	3	3	3
To the track	4	4	5

* P0: Complete Feed 60% + silage 40%; P1: Complete Feed 60% + Silage 40% + Natural Vitamin E 50 IU (223 grams of green bean sprouts); P2: Complete Feed 60% + silage 40% + Synthetic Vitamin E 50 IU.

The results of observations of spermatozoa abnormalities in sheep as a result of the research resulted in treatment P1 with green bean sprouts being given 4% abnormality, while in P2 with treatment giving non-natural Vitamin E a 5% abnormality was obtained, and P0 which was not given treatment found 4% abnormality. This shows that the semen evaluation results are in good condition, which is still within the range of research results by [Toelihere \(1985\)](#) which states that good sheep semen has an abnormality value range of between 5-15%.

The results of this study show that there is no significant difference because the abnormalities that appear most are primary abnormalities, primary abnormalities occur during the spermatogenesis process, factors that influence the spermatogenesis process are nutrition and condition of the livestock. [Wahyuning \(2013\)](#), states that forms of spermatozoa abnormalities include abnormalities that occur in the spermatogenesis process, and factors that influence the quality of fresh semen are the age of the male, genetics, temperature, season, and the nutrition provided. The results of this study show that abnormalities are still in the good range. Good quality semen has an abnormal sperm count ranging from 5-15%. This was then confirmed by the opinion expressed by [Partodhihardjo \(2003\)](#), that spermatozoa abnormalities of more than 20% indicate poor results. From the results of [Susilawati's \(2003\)](#) examination, spermatozoa abnormalities were found to be heads without tails. Furthermore, spermatozoa morphology abnormalities were classified into five categories, namely: tail, abnormal head shape, abnormal tail shape, abnormal spermatozoa cell morphology.

CONCENTRATION

Spermatozoa concentration is the number of spermatozoa per unit volume or per milliliter of semen ([Ismaya, 2014; Centola, 2018](#)). Assessment of the concentration of spermatozoa per milliliter is very important, because this factor is used as a determining criterion for semen quality and determines the level of dilution in making frozen semen. Spermatozoa concentration is the number of spermatozoa cells contained in one milliliter of semen ([Apriyanti et al., 2017](#)). The normal concentration of sheep ranges from $3.5 \times 10^9 - 6 \times 10^9$ spermatozoa/ml ([Susilawati, 2011](#)). The concentration of sheep spermatozoa is grouped into 5 assessment categories, namely score 5 ($4.5-6.0 \times 10^9$), score 4 ($3.5-4.5 \times 10^9$), score 3 ($2.5-3.5 \times 10^9$), score 2 ($1-2.5 \times 10^9$), score 1 ($0.3-1 \times 10^9$) and score 0 (not calculated) ([Susilawati, 2011](#)). In the research of [Tambing et al. \(2000\)](#) it was reported that fresh semen of Etawah cross-breed goats has a concentration of 2.80×10^9 . The results of observing the concentration of sheep spermatozoa can be seen in [Table 10](#).

Results of analysis of variance ([Table 10](#)) of giving natural and non-natural vitamin E with a dose of natural vitamin E (mung bean sprouts) 50 IU (equivalent to 223 grams of green bean sprouts)/head/day, and non-natural vitamin E 50 IU/head/day. showed no significant effect ($P>0.05$) on spermatozoa concentration. This shows that giving natural vitamin E (mung bean sprouts) as much as 223 grams/head/day, and non-natural vitamin E 50IU/head/day has not been effective in increasing the concentration of spermatozoa in thin-tailed sheep. The concentration of spermatozoa in the study was influenced by the individual and physiological conditions of the experimental livestock as well as the nutrition of the feed consumed.

Table 10: Observation results of sheep spermatozoa concentration

Group	Treatment*		
	P0	P1	P2
	...(x10 ⁶ /ml).....		
1	1.290	2.210	3.010
2	2.320	2.170	2.650
3	2.160	3.120	2.300
4	2.230	2.370	1.090
5	2.120	2.610	2.230
To the track	2.024	2.496	2.256

* P0: Complete Feed 60% + silage 40%; P1: Complete Feed 60% + Silage 40% + Natural Vitamin E 50 IU (223 grams of green bean sprouts); P2: Complete Feed 60% + silage 40% + Synthetic Vitamin E 50 IU.

The concentration of sheep spermatozoa is grouped into 5 assessment categories, namely score 5 ($4.5-6.0 \times 10^9$), shoes 4 ($3.5-4.5 \times 10^9$), shoes 3 ($2.5-3.5 \times 10^9$), shoes 2 ($1-2.5 \times 10^9$), shoes 1 ($0.3-1 \times 10^9$), and a score of 0 (not counted) ([Susilawati, 2011](#)). The spermatozoa concentration value in this study was in the range of normal concentration values, namely $2-3 \times 10^9$ cells/mL ([Garner and Hafez, 2000](#)). [Toelihere \(1993\)](#) reported that the concentration of sheep spermatozoa ranged from $1500-3000 \times 10^6$ cells/ml. [Feradis \(2010\)](#) stated that differences in spermatozoa concentration were influenced by the individual and physiological conditions of the experimental livestock. [Salisbury and Vandemark \(1985\)](#), stated that the factors that influence spermatozoa concentration are the male's sexual maturity, semen storage distance, testicular size, age of the animal, season and the nutritional quality of the feed provided.

The spermatozoa concentration value in this study was in the range of normal concentration values, namely $2-3 \times 10^9$ cells/mL, this is in accordance with [Garner and Hafez \(2000\)](#), who stated that the normal range for spermatozoa concentration is $2-3 \times 10^9$ cells/mL added [Toelihere](#)

(1993), reported that the concentration of sheep spermatozoa ranged from 1500-3000x10⁶ cells/ml with a survival percentage of at least 50% can be continued in subsequent processes such as retailing and cement freezing. The results of this study showed that the concentration value was smaller than the normal range of spermatozoa concentration. Susilawati (2011), that the normal concentration of sheep is around 3.5 x 10⁹ - 6 x 10⁹ spermatozoa/ml (Susilawati, 2011). This difference in spermatozoa concentration is influenced by the individual and physiological conditions of the experimental animals. Salisbury and Vandemark (1985), stated that the factors that influence spermatozoa concentration are male sexual maturity, semen storage distance, testicular size, age of livestock, season and feed quality. Hormones in sheep are not optimal and these hormones also affect the spermatogenesis process in producing total spermatozoa concentration.

According to Heriyant et al. (2014), the concentration of spermatozoa in sheep is greatly influenced by the age of the male and tends to increase with increasing age up to 24 months. The total spermatozoa concentration in sheep aged 48-72 months will decrease. This happens because in old age, the activity of the spermatogenesis process decreases. This is strengthened by the opinion of Feradis (2007) that the difference in total spermatozoa concentration is caused by the individual influence of the condition of the livestock and the feed given.

CONCLUSIONS

Based on the research results, it can be concluded that administration of both natural (in the form of green bean sprouts) and synthetic vitamin E have similar effect in semen parameters of sheep except semen volume.

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CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

NOVELTY STATEMENT

The research on supplementation of natural (in the form of green bean sprouts) and synthetic vitamin E in sheep diet to observe consequences on semen quality has not been conducted previously. It is hoped that this study will serve

as an initial step for further investigation of natural feed ingredients to enhance quality traits of semen.

AUTHORS CONTRIBUTION

All authors are equally sharing.

REFERENCES

- Alvionita C., S. D. Siti, N. Solihati. (2015). The quality of local sheep semen across different age groups. Students e-Journal., 4(3). <https://jurnal.unpad.ac.id/ejournal/article/view/6903>
- Ariantje O. S., T. L. Yusuf, D. Sajuthi dan R. I. Arifiantini. (2014). Kualitas semen cairkambing Peranakan Etawah dalam modifikasi pengencer tris denan trehalosadan rafinosa. J. Vet. 15 (1): 11 –22. <https://ojs.unud.ac.id/index.php/jvet/article/view/8780>
- Arifiantini R. I. (2012). Semen collection and evaluation techniques in animals. IPB Press. Bogor.
- Anggorodi R. (1994). General Animal Feeding Science. PT Gramedia. Jakarta.
- Apriyanti D., Samsudewa D., Ondho Y. S. (2017). Microscopic Examination of Fresh Ram Semen Quality in Flock Mating and Pen Mating. Indonesian J. Anim. Sci., 12(1): 64–70. <https://doi.org/10.31186/jspi.id.12.1.64-70>
- Basir A. A., M. S. Hassan, T. Buranda, E. W. Fariat. (2013). The Influence of Phaseolus radiatus L. Nutrient Administration on the Spermatozoa Density Level of Mus musculus L. Scient. J. Biol., 1(1): 70-73. DOI: <https://doi.org/10.24252/bio.v1i1.449>
- Centola G. M. (2018). Semen Analysis. In: Skinner, M. K (ed). Encyclopedia of Reproduction. Publisher Elsevier. Science Publishing Co Inc, USA
- Dethan A. A., Kustono, H. Hartadi. (2010). Sperm quality and quantity of male Bligon goats fed elephant grass with blood meal supplementation. Bullet. Peternakan, 34(3): 145-153. <https://doi.org/10.21059/buletinpeternakan.v34i3.83>
- Dewantari N.M. (2013). The role of nutrition in health production. J. Skala Husada, 10(2): 219-224. <https://www.poltekkes-denpasar.ac.id/files/JSH/V10N2/Ni%20Made%20Dewantari1%20JSH%20V10N2.pdf>
- Dutta-Roy A. K., M. J. Gorden, F. M. Campbell, G. G. Duthie, W. P. T. James. (1994). Vitamin E requirements, transport, and metabolism: role of a-tocopherol binding proteins. J. Nutrion. Biochem.. 5(12): 562-570. [https://doi.org/10.1016/0955-2863\(94\)90010-8](https://doi.org/10.1016/0955-2863(94)90010-8)
- Elya B., D. Kusmana, N. Krinalawaty. (2010). Quality of spermatozoa from *Polyscias guilfoylei* plant. Makara Sains. 14 (1): 51-56. <https://scholarhub.ui.ac.id/science/vol14/iss1/25>
- Feradis. (2010). Livestock Reproduction. Alfabeta. Bandung.
- Feradis. (2007). Characteristics of physical properties of semen of *St. Croix* sheep. *Croix sheep*. J. Anim. Husband. 4(1): 1-5. <https://ejournal.uin-suska.ac.id/index.php/peternakan/article/view/254/239>
- Garner D.L. E.S.E. Hafez. (2000). Spermatozoa and seminal plasma. In Reproduction in Farm Animals. Hafez, E.S.E. and B. Hafez (ed). 7th. Lippincott Williams and Wilkins, Philadelphia.
- Gupta R. S., E. S. Gupta, B. K. Dhakal, A. R. Thakur, J. Ahnn.

- (2004). Vitamin C and vitamin E protect the rat testes from cadmium-induced reactive oxygen species. *Mol. cells*, 17(1): 132-139. <https://pubmed.ncbi.nlm.nih.gov/15055539/>
- Handarini R. (2005). Dynamics of reproductive activity associated with growth stages of adult male timor deer (*Cervus timorensis*). Dissertation. Graduate School, Bogor Agricultural University, Bogor.
- Hafez E.S.E. (1993). Reproduction in Farm Animals. 6th ed. Lea and Fibiger, Philadelphia.
- Hafez E.S.E. (2000). Spermatozoa and Seminal Plasma. In: Reproduction in Farm Animals. E.S.E. Hafez (Eds.). 6th ed. Lea and Febiger, Philadelphia.
- Herdiawan I. (2004). The effect of freezing rate temperatures and kind of extenders on the quality of frozen sperm of Priangan Goat. *JITV*, 9(2): 98-107. <https://repository.pertanian.go.id/server/api/core/bitstreams/ad284d55-6f43-4a4a-b9fe-0236ead36d40/content>
- Heriyanta E., M. N. Ihsan, N. Isnaini. (2014). Pengaruh Umur Kambing Peranakan Etawah (Pe) Terhadap Kualitas Semen Segar. *Ternak Tropika J. Trop. Anim. Prod.* 14(2): 1-5. <https://ternaktropika.ub.ac.id/index.php/tropika/article/view/176>
- Hersade D. M. (2012). Overview of Spermatozoa Quality of Garut Sheep by Feeding Complete Rations Containing Green bean sprouts Waste and *Indigofera Sp.* Thesis. Faculty of Animal Husbandry. Bogor Agricultural University. Bogor.
- Hunter R.H.F. (1995). Physiology and Reproductive Technology of Domestic Female Animals. Translation by D.K. Harya Putra. Bandung Institute of Technology. Bandung
- Ismaya. (2014). Biotechnology in Artificial Insemination for Cows and Buffaloes. Yogyakarta's Gadjah Mada University Press.
- Jaenudeen M.R., E.S.E. Hafez. (2000). Cattle and Buffalo. in: Hafez E.S.E (ed). Reproduction in Farm Animals 7th Ed. Lippincott Williams & Wilkins, USA.
- Junqueira L. C., J. Carneiro. (2007). Basic Histology. EGC Publisher, Jakarta
- Kamal, M., Firdaus, Aldi. (2022). Addition of a combination of fermented feed from tofu dregs and soybean meal with vitamin E in the diet on sperm and testosterone of Arabian chickens. *J. Anim. Sci.* 6(1): 42-48. <http://jurnal.um-tapsel.ac.id/index.php/peternakan/article/view/6419>
- Khairi F., C. I. Dini, C. I. Novita, S. R. Ayuti. (2021). Effect of the addition of palm kernel and ammoniated lemongrass waste (*cymbopogon nardus*) on the quality of fresh semen of thin tailed sheep as a partial replacement of basal feed. *J. Med. Vet.*, 15(2): 103-112. DOI: <https://doi.org/10.21157/j.med.vet.v15i2.24563>
- Kusumawati E.D., K.N. Utomo A.T.N., Krisnaningsih, S. Rahadi. (2017). The quality of Kacang goat semen with different storage durations under room temperature using tris aminomethane and egg yolk. *J. Trop. Anim. Sci. Technol.* 4(3):42-51. <https://ojs.uho.ac.id/index.php/peternakan-tropis/article/view/3894>
- Mayes P. A. (1995). Structure and function of fat-soluble vitamins. In Harper's Biochemistry. Medical Book Publishers. EGC. Jakarta.
- Moradpour F. (2019). Review on animals semen characteristics: fertility, reproduction and development. *Asian J. Adv. Agric. Res.* 10 (2): 1-9. <https://doi.org/10.9734/ajaar/2019/v10i230024>
- Nahriyanti S., Y. S. Ondho, D. Samsudewa. (2017). Differences in macroscopic quality of fresh semen of Batur sheep in flock mating and pen mating. *J. Indonesian Anim. Sci.*, 12(2): 191-198. <https://doi.org/10.31186/jspi.id.12.2.191-198>
- Nawangsari D. N., E. N. Hendrarti. (2021). Proximate analysis of field grass as ruminant feed in Magelang District, Central Java. *J. Agric. Exten. Develop.*, 18(31): 25-31. <http://jurnal.polbangtanyoma.ac.id/index.php/jp3/index>
- Nubatonis A., T. I. Purwantiningsih, Y. Oki, B. Doarce. (2022). Assessment of Thin-Tailed Male Sheep Spermatozoa Grazed in Dry Land. *Indonesian J. Anim. Sci.*, 24(1): 55-65. <https://doi.org/10.25077/jpi.24.1.55-65.2022>
- Pamungkas F.A., F. Mahmilia, S. Elieser (2008). Comparison of Semen Characteristics of Boer Goats with Peanut. National Seminar on Animal Husbandry and Veterinary Technology. Research Institute for Slaughter Goats. <https://core.ac.uk/download/pdf/141497531.pdf>
- Partodihardjo S. (2003). Animal Reproduction Science. Mutiara Sumber Widya. Jakarta.
- Rizal M., Herdis. (2008). Artificial Insemination in Sheep. Rineka Citra. Jakarta.
- Rusdiana S., L. Praharani. (2015). Improvement of cattle sheep through crop diversification: economic income farmers. *Agriekonomika*, 4(1): 80-96. <https://doi.org/10.21107/agriekonomika.v4i1.676>
- Salisbury G.W, N.L. Van Demark (translated by R. Djanuar). (1985). Reproductive Physiology and Artificial Insemination in Cattle. W.H. Freeman and Company. San Fransisco and London.
- Salmah N. (2014). Motility, Live Percentage and Abnormality of Bali Cow Frozen Semen Spermatozoa in Andromed Diluent and Egg Yolk Tris. Thesis. Hasanuddin University.
- Susilawati T. (2011). Spermatology. UB Press. Malang.
- Susilawati T. (2013). Guidelines for Artificial Insemination in Livestock. Press of Universitas Brawijaya. Malang.
- Sonjaya H., Hasbi, Sutomo dan Hastuti. (2005). Pengaruh penambahan calcium ionophore terhadap kualitas spermatozoa kambing Boer hasil seksing. *J. Sains Teknol.*, 2: 90-101.
- Tambing S.N., M. R. Toelihere, T.L. Yusuf Dan I.K. Utama. (2000). Effects of glycerol in tris extender on frozen semen quality of crossbred Etawah bucks. *JITV*. 5(2): 1 – 8. <https://doi.org/10.14334/jitv.v5i2.203>
- Toelihere. (1985). Reproductive Physiology of Livestock. Sixth mould. Angkasa Bandung.
- Toelihere (1993). Reproductive Physiology in Livestock. Sixth edition. Angkasa Bandung.
- Wahyuningsih A., D.M. Saleh, Sugiyanto. (2013). The influence of male age and mating behavior the frequency of reception towards Volume and motility of fresh bovine semen Simmental cattle at the artificial Insemination Center Lembang. *Scient. J. Agric.* 1(3):947-953.
- Wang S., G. Wang, B. E. Barton, T. F. Murphy, H. F. S. Huang. (2007). Beneficial effects of vitamin E in sperm functions in the rat after spinal cord injury. *J. Androl.*, 28(2): 334-341. <https://doi.org/10.2164/jandrol.106.001164>
- Yuliyantika Y., I. R. S. Iswari, A. Marianti. (2019). Protective power of mung bean sprout extract on spermatozoa quality and superoxide dismutase enzyme levels of mice exposed to transfluthrin. *Life Sci.*, 8(2): 138-149. <https://doi.org/10.15294/lifesci.v8i2.37101>