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The Effect of Betel Nut Extract (*Areca catethu L*) on Spermatozoa Quality (Macroscopic and Microscopic) in Male Goats

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ABSTRACT

The purpose of this study is to determine the effect of betel nut extract on the macroscopically and microscopically quality of spermatozoa. Materials and methods were used for 16 male Ettawa goats at the age of 2 years. The preparation of betel nut extract and macroscopic observations of spermatozoa via the use of odors, colors, and volumes, as well as microscopic observations of spermatozoa through the use of mass motion, mass motility, and individual motility. The betel nut extract was administered orally for 15 days. The treatments in this study were T0 (without the provision of betel nut extract), T1 (provision of betel nut extract (90 mg / goat / day)), T2 (provision of betel nut extract (180 mg / goat / day)), T3 (provision of betel nut extract (270 mg / goat / day)). The results showed that the addition of betel nut extract was significantly different than without extracts ($P < 0.05$). The goats without betel nut extract had the lowest quality of spermatozoa, whereas the goats treated with a betel nut extract (270 mg / goat / day) had the highest quality of spermatozoa of all the treatments. There was an increase in spermatozoa in the 10th data collection, including smell, colour, volume, mass motility, and individual motility. Furthermore, male goats were given betel nut extract at a dose of 270 mg/goat/day, which improved spermatozoa quality both macroscopically and microscopically. Subsequently, we assumed that betel nut extract could improve reproductive quality in male goats.

Keywords: Betel nut, Individual motility, Male goats, Mass motility, Spermatozoa

Introduction

A decline in livestock productivity may lead to a decrease in animal reproduction (Wijayanti *et al.*, 2018). The reproductive quality of females and males was also influenced by environmental factors, feed, and body condition of the livestock (Wijayanti *et al.,* 2017). Male livestock productivity decreased because it was influenced by age, free radicals, and drugs, which means the quality of male fertility decreased. Mating success in a livestock population, both natural and artificial insemination, was closely related to spermatozoa quality. Spermatozoa are produced by male reproductive organs, namely the tests and complementary glands (Liu *et al.*, 2019). Decreasing the quality and quantity of spermatozoa will reduce the number of conceptions achieved. Therefore, it is necessary to maintain spermatozoa quality so that fertility does not decrease. The use of herbal plants has been widely used to increase reproduction in animals.

Betel nut was included in the category of herbal plants. Betel nut contains alkaloids such as arecoline, arekolidine, arecaine, guvakoline, guvasine and isoguvasine, condensed tannins, and hydrolysed tannins flavans, phenolic compounds, gallic acid, latex, lignin, volatile oil that does not evaporate, as well as salt (Shih *et al.,* 2020). Furthermore, the betel nut contained proanthocyanins, condensed tannins belonging to the class of flavonoids (Salehi *et al.*, 2020). It is known that proanthocyanins have antibacterial, antiviral, anticarcinogenic, anti-inflammatory, anti-allergic, vasodilating dan for reproduction (Berger *et al.*, 2016). Previous studies using herbal plants have also been carried out, namely less than 80% cell viability at concentrations of 500 mg/L and 1,000 mg/L of *Rubus coreanus Miquel* and *Cuscuta chinensis Lam* (Kim *et al.*, 2013). The use of betel nuts in mammals to increase reproduction in male goats has never been studied. For this purpose, it is necessary to study the quality of spermatozoa from the goats in the field. If it turns out that spermatozoa quality was not high, then efforts need to be made to improve

it, but if it turns out that spermatozoa quality was high, then it must be maintained. The population can also be maintained or even increased by increasing the reproducibility of livestock. With good livestock productivity, the increasing number of livestock will be faster. One thing that is closely related to increasing livestock productivity is spermatozoa quality. The success of fertilization depends on the quality and concentration of spermatozoa (Partodihardjo, 1992). The concentration of spermatozoa has a positive correlation with the viscosity of semen. This indicates that the higher the level of the semen thickness, the higher the concentration level of spermatozoa (Cindy *et al.*, 2015).

Materials and Methods

This research was conducted in Malaganti Village, Sukaharja Village, Sariwangi District, Tasikmalaya Regency. This study used 16 male Ettawa goats at the age of 2 years. The percentage of feed consisted of 10% fresh forage and 3% dry forage by body weight. The grass to legume ratio is 3:4. The grass given consisted of king grass, odot grass, Setaria grass, and legumes consisting of Indigofera, Lamtoro, and Centrosema. Feeding is given twice a day in the morning and evening. Artificial spermatozoa and a spermatozoa reservoir tube create a vaginal reservoir. Tools and materials used for spermatozoa examination were microscopes, object glasses, and covers glass. The treatments in this study were: T0: Without the provision of betel nut extract; T1: Provision of betel nut extract (90 mg / goat / day); T2: Provision of betel nut extract (180 mg / goat / day); T3: Provision of betel nut extract (270 mg / goat / day). Giving extracts was carried out orally for 15 days in the morning. Data collection was carried out on day 1, day 5, day 10 and day 15. Observed variables include smell, colour, volume, mass motility, and individual motility.

Extraction of betel nut

Betel nuts were washed, cut into pieces, and dried in an oven at 70°C. The dried betel nuts were macerated with 96% ethanol. The liquid ethanol extract obtained was thickened with a *rotary evaporator* and dried over a water bath (Meiyanto *et al.*, 2008; Wijayanti *et al.,* 2018).

Macroscopic observation

The semen volume was measured from the boundary line. The semen liquid was equivalent to the scale on the glass measure. Usually, the new semen was accommodated where there was still foam—calculating the volume added as much as half of the thickness of the foam (Husin *et al.*, 2007). The colour of the semen can be seen directly from the spermatozoa tube. The semen can be milky white, creamy, or yellowish (Husin *et al.,* 2007). Normal semen generally has a distinctive fishy odor, accompanied by odors from these animals (Husin *et al.*, 2007).

Microscopic observation

Microscopic observation utilizing fresh spermatozoa was poured slightly into the object-glass, covered with a glass cover, and then seen by the mass and individual motility. Mass motility observed by dripping semen on a warm object glass was then observed under a microscope with a magnification of 40 x 10 (Husin *et al.*, 2007). Individuals' motility was observed by dripping semen on a glass of warm objects and then observed under a microscope with a magnification of 40 x 10 (Husin *et al.,* 2007).

Data analysis

The data will be analyzed using ANOVA based on a randomized block design with four treatments and four replications. If significantly different, it will be tested further with Duncan. All values were expressed as the mean ± standard error (SE). The results were presented as mean and standard error, whereas $P < 0.05$ was considered statistically significant.

Results and Discussion

Effect of betel nut extract on the macroscopic quality of goat spermatozoa

In Table 1, macroscopic results of spermatozoa change after the addition of betel nut extract. Retrieval of the first data until the 5th data retrieval has increased. In the fifth data collection, there was an increase in odor, volume, and colour with the treatment of betel nut extract. The result showed that the provision of betel nut extract with doses of 90, 180 and 270 (mg / goat / day) increased. However, goats with treated betel nut extract 270 (mg / goat / day) had significantly increased macroscopic quality ($P < 0.05$) than goats without betel nut extract.

Colours. Goats that had a good semen colour were goats with 270 mg of extract (mg / goat / day) because they changed from cream to yellow while in the treatment. Goats with 90 (mg / goat / day) and 180 (mg / goat / day) had a normal colour. Kartasudjana (2001) stated that the colour of goat semen was creamy white, and when reddish was found, it indicated that it had semen concentrate, which had fresh blood. The colour of the cream in the semen was normal. The cream colour of semen was caused by the presence of riboflavin from the secretion of the vascular veins. The quality of semen was stated to be good if it had a yellowish colour.

Smells. The above study results indicated that all treatments produce a normal semen odor, generally a typical fishy smell and an odor from the animal. Normal semen generally has a distinctive fishy odor, accompanied by the smell of the animal. Foul odor occurs when semen contains pus caused by an infection of male reflux organs (Lopes, 2002).

Table 1. Macroscopic assessment result of semen with treatment of different dosage

Treatment (mg/goat/day)	Volume (ml)			
	Day 1	Day 5	Day 10	Day 15
T0	1.000 ± 0.001	0.726 ± 0.001	1.000 ± 0.001	0.500 ± 0.001[a]
T1	1.000 ± 0.001	0.751 ± 0.050	1.000 ± 0.001	0.825 ± 0.236[b]
T2	0.800 ± 0.355	0.526 ± 0.391	0.800 ± 0.355	0.975 ± 0.125[b]
T3	0.850 ± 0.173	0.576 ± 0.310	0.950 ± 0.173	1.025 ± 0.206[b]

[a,b] Different superscripts in the same line showed significant differences (P <0.05), T0 = Without the provision of betel nut extract, T1 = Provision of betel nut extract 90 mg / goat / day, T2 = Provision of betel nut extract 180 mg / goat / day , T3 = Provision of betel nut extract 270 mg / goat / day.

Table 2. Semen volume is given betel nut extract

Treatment (mg / goat / day)	Day 1		Day 5		Day 10	
	Colour	Smell	Colour	Smell	Colour	Smell
0	C	Normal	C	Normal	C	Normal
90	W	Normal	W	Normal	C	Normal
180	W	Normal	C	Normal	C	Normal
270	C	Normal	Y	Normal	Y	Normal

Cream (C), White (W), and Yellow (Y).

Volume. Based on research conducted on goats with betel nut extract, the volume was obtained at 0.9-1.2 ml. The volume was significantly increased in goats with a betel nut extract (270 mg / goat / day) (P < 0.05) on 15 days treatment showed that the more valuable 15 days was more excellent produced compared with other treatments that were inconsistent (Table 2). The semen volume in this study was 1 ml, and this was still normal following Sekosi *et al.* (2016), which stated that the volume of goat semen every time ejaculation ranges from 0.5 to 1.5 ml. According to Hafez and Hafez (2000), the volume of goat semen ranges from 0.5 to 1.2 ml / ejaculate. The volume of sperm varies depending on the country, age, body size, frequency of shelter, environment, livestock condition, shelter, and feed time. Gangyi *et al.* (2001) stated that the volume of semen in Boer goats ranged from 0.45 to 1.15 ml. The number of spermatozoa per ejaculate varies according to several factors, including nation and body size (Toelihere, 1981; Ax *et al.*, 2000).

Effect of betel nut extract on the microscopic quality of Ettawah goat spermatozoa

The microscopic quality was determined in Table 3, Table 4, Table 5, and Table 6. The microscopic results of spermatozoa have increased from the first day of data collection to day 1 (Table 3). In the retrieval of day 5th data, there was a significantly increased between mass motility and individual motility with the treatment of 270 betel nut extract (mg / goat / day) compared to without betel nut extract by increasing the individual motility height from +1 to +3 (P < 0.05).

Mass motility. On the first day, the goat with a betel nut extract (270 mg / goat / day) had the highest average of the others treated on the first day, 5th day, and 10th day (Table 3, Table 4 and Table 5). In addition, on the 15th day of the goat with a betel nut extract (270 mg / goat / day) significantly increased (P <0.05) the mass motility than with another treatment, namely experiencing instability every day. The lowest results were shown in the goat without betel nut extract (Table 6). *Spermatozoa* motility was an indicator of the degree to which *spermatozoa* move (as an indicator of the level or percentage of live and active spermatozoa) in semen. According to Soeprapto (2006), *spermatozoa* motion was enhanced if a large, dark, thick, and active presence, such as black clouds, fast-moving rain, and moving places. The motility of spermatozoa was demonstrated by mass motility. Tendency of *spermatozoa.* Move together in one direction to form waves that move thick or thin. The fast or slow motility of *spermatozoa* depends on the concentration of *spermatozoa* that live in them. Assessment of mass motility between 0, 1 +, 2 +, and 3+. This was under the statement of Yusuf *et al.* (2006), which stated that the best mass motion with a value of 3+ if the *spermatozoa* move rapidly around 70% -80%, the mass motility is good with a value of 2+ if the *spermatozoa* move fast 50% -70%, and the motion was bad with a value of 1+ if the *spermatozoa* move weakly, up to 20%, while the motion was slight (0) if the *spermatozoa* do not move at all.

Individual motility. The study showed that on the first day, 5th day, 10th day, and 15th day of the goat with a betel nut extract (270 mg / goat / day) significantly increased (P <0.05) the individual motility than with other treatments (Table 3, Table 4, Table 5, and Table 6). In addition, the goat without betel nut extract had the

Table 3. Spermatozoa quality microscopically after 1 day of betel nut extract

Parameter	Treatment			
	T0	T1	T2	T3
Mass motility	1.500 ± 0.577	1.750 ± 0.500	1.500 ± 0.577	1.750±0.500
Individual motility	1.250 ± 0.500	1.250 ± 0.500	1.250 ± 0.500	1.250 ± 0.500

T0 = Without the provision of betel nut extract, T1 = Provision of betel nut extract 90 mg / goat / day, T2 = Provision of betel nut extract 180 mg / goat / day, T3 = Provision of betel nut extract 270 mg / goat / day.

Table 4. Spermatozoa quality microscopically after 5 days of betel nut extract

Parameter	Treatment			
	T0	T1	T2	T3
Mass motility	1.500 ± 0.577	1750 ± 0.500	1.500 ± 0.577	2.000 ±0.001
Individual motility	1.250± 0.500[a]	1.750 ± 0.500[ab]	1.750 ± 0.500[ab]	2.000 ± 0.001[b]

[a,b] Different superscripts in the same column showed significant differences (P <0.05), T0 = Without the provision of betel nut extract, T1 = Provision of betel nut extract 90 mg / goat / day, T2 = Provision of betel nut extract 180 mg / goat / day , T3 = Provision of betel nut extract 270 mg / goat / day.

Table 5. Spermatozoa quality microscopically after 10 days of betel nut extract

Parameter	Treatment			
	T0	T1	T2	T3
Mass motility	1.500 ± 0.577	2.000 ± 0.001	1.750 ± 0.001	2.000± 0.001
Individual motility	1.500 ± 0.577[a]	2.000 ± 0.001[ab]	2.000± 0.001[ab]	2.500 ± 0.577[b]

[a,b] Different superscripts in the same column showed significant differences (P <0.05), T0 = Without the provision of betel nut extract, T1 = Provision of betel nut extract 90 mg / goat / day, T2 = Provision of betel nut extract 180 mg / goat / day , T3 = Provision of betel nut extract 270 mg / goat / day.

Table 6. Spermatozoa quality microscopically after giving 15 days of betel nut extract

Parameter	Treatment			
	T0	T1	T2	T3
Mass motility	1.500 ± 0.577[a]	1.500 ± 0.001[b]	2.000 ± 0.001[b]	2.000 ± 0.001[b]
Individual motility	1.500 ± 0.577[a]	2.000 ± 0.001[a]	2.000 ± 0.001[a]	2.750 ± 0.500[b]

[a,b] Different superscripts in the same column showed significant differences (P <0.05), T0 = Without the provision of betel nut extract, T1 = Provision of betel nut extract 90 mg / goat / day, T2 = Provision of betel nut extract 180 mg / goat / day , T3 = Provision of betel nut extract 270 mg / goat / day.

lowest individual motility. Individual *spermatozoa* motility was used as a guideline in testing the quality of fresh sperm. The individual motility can be assessed by (0), meaning immortal or immotile *spermatozoa*, while immotile (1) means the *spermatozoa* move in a place or moves positively. 50% -80% and (3) positively and agilely moving motile *spermatozoa*, 90%-100%. According to Sonjaya *et al.* (2005), two factors influenced the motility of *spermatozoa*, namely endogenous and exogenous factors. Endogenous factors include age and energy sources, while exogenous factors include temperature and PH.

Conclusions

The male goats were treated with betel nut extract at a dose of 270 mg / goat / day had impacted to improve the quality of spermatozoa macroscopically and microscopically. Subsequently, we assumed that betel nut extract could improve reproductive quality in male goats.

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