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Full Length Research Paper

Spermatozoa quality of half blooded Ettawah goat fed with three day old green bean sprout

Djoko Winarso[1]*, Budi Purwo[2] and Y. Rina Kusuma[2]

[1]Faculty of Veterinary Medicine of Brawijaya University, Indonesia.
[2]Extension Agriculture College, Magelang, Indonesia.

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The research aims to study the sperm quality of local Ettawah goat fed with three day old green bean. Sixteen males of local Ettawah goats that are one and half year old were used in this study, with one female as the libido inducer. Randomized studies were designed using four kinds of treatment groups arranged in four trial blocks. However, there were four dietary treatments: K diet without sprout was used as the control diet; PI diet contains 1 g sprout/kg body weight; PII diet contains 2 g sprout/kg; and PIII contains 3 sprout/kg body weight. Four goats were allocated to each treatment diet and all goats underwent four times sperm collection during a seven day period. Variables recorded were sperm motility, concentration, and sperm liveability percentages, while scrotum size and body weight gain were measured as the supporting data. The data obtained were analyzed statistically following analysis of variance (ANOVA). There were significant differences, and the Duncan's multiple range test was used to observe the level of their influence on the treatments. The results indicated that the use of green pea in the diets significantly increased ($P < 0.01$) motility and sperm liveability. Decreasing sperm concentration was observed on goats receiving 1 g sprout/kg body weight, but followed by inconsiderable increase on goats receiving 2 and 3 g sprout/kg body weight. It could be concluded that the use of three day old green pea sprout in the diets increased goat sperm quality, especially in the case of motility and sperm liveability, but did not increase sperm concentration, scrotum size, and body weight gain.

Key words: Local Ettawah goat, spermatozoa quality, scrotum size, body weight gain, green bean sprout.

INTRODUCTION

Artificial insemination (AI) is an entry or delivery of semen into the female genital tract by using man-made tools or AI gun (Martin et al., 2011). This technique has been introduced in Indonesia since the beginning of the fifties. Benefits expected using the AI technique include avoiding disease transmission through direct contact sex, male animal maintenance cost savings, as well as the improvement of genetic quality with the use of superior males (Djanuar, 2005). For success in marriage with the AI technique, the production of semen in the right amount or quantity/quality, if not to say high, is required. Quantity, especially the declining semen quality also reduces conception rates. There are several factors that have been commonly discussed in relation to the quantity and quality of semen, one of which is the feed factor (Batista et al., 2009).

Food is needed quantitatively and qualitatively in male animals. However, food for reproduction need not exceed the needed amount for animal growth either in young or adult animals to sustain life in a healthy condition. In addition to forage, goats feed also requires reinforcement to meet nutritional needs. Food that is perfect is a fairlywell-balanced diet of carbohydrates, proteins, fats, minerals, water and vitamins that are essential for reproduction (Shi et al., 2010).

Green bean, as a food ingredient amplifier, besides having high enough protein is also rich in vitamin E. Many say vitamin E serves to increase fertility, as well as prevention of aging, whereas Purwanto et al. (2004) said that vitamin E is needed for reproduction in rats.

The content of alpha tocopherol in 3 days old mung bean sprouts also helps to prevent sterility and muscular

*Corresponding author. E-mail: djwinarso@yahoo.com.

dystrophy of the reproductive organs and improve the quality of spermatozoa. Active alpha-tokopherol can be regenerated by interaction with vitamin C, which inhibits free radical oxidation peroxysalts in the reproductive tract. The oxidation of free radicals is inhibited in the reproductive tract peroxysalts so that the motility and percentage can increase the spermatozoa's life. Thus, one of the two peroxysalts free radicals found in the reproductive organs becomes glucoronat conjugated when there is excretion in the kidney (Agawal, 2010). Vitamin E also strengthens the walls of blood capillaries and prevent damage to the red blood cells due to toxins in the intestine tract so that blood circulation in the manufacture of spermatozoa can run well (Rink, 2010).

Mung bean sprout as good source of vitamin E (alpha-tocopherol), has sufficient potential. Vitamin E is an antioxidant that protects cells from free radical attack. By eating the sprouts, there is the possibility that vitamin E will protect the cells of spermatozoa from damage caused by free radical attack, so that there will more motility and higher power of life (Made, 2003).

The research aims to determine the effect of 3 days old green bean sprouts on the quality of goat spermatozoa. Given that the green bean sprouts are 3 days old, they can improve the quality of spermatozoa in Ettawah goats.

MATERIALS AND METHODS

The tails of 16 PE goats (aged one and half year) with initial body weight and an average of 31.5 kg were maintained for 120 days in the stable stage, with the plasma of each tail placed separately in a box (inside a cage). The livestock feed had 10% of body weight, and it consisted of king grass and polard feed (waste grain) with a ratio of 75 versus 25%. In addition to polard feed and bean sprouts, feed supplement was given first before king grass. Green bean sprouts are manufactured by soaking the green beans in water for one night, and then placed in a container positioned in the hangar at the dark. The container is washed and drained every 3 hours for juice, after which it is ready for use. Bean sprout green was added to the polard feed/*waste grain* in accordance with treatment doses ranging from 0 g / kg bw to 3 g / kg bw for the benefit of sperm quality tests conducted for four treatments with the addition of bean sprouts (3 days old) as follows: Control (K) = 0 grams / kg bw, Treatment I (PI) = 1 gram / kg bw, Treatment II (PII) = 2 g / kg bw, Treatment III (PIII) = 3 g / kg bw. It was discovered by chemical analysis in the Chemical Analysis Laboratory of the Faculty of Pharmacy, Gadjah Mada University that every gram of the bean sprout green (3 days old) has 0.0155 mg vitamin E.

For each treatment, 4 goats were used and semen was collected from each goat in the first, second, third and fourth month of the maintenance period by using artificial vagina for microscopic examination, and the sperm motility, sperm concentration and percentage of live spermatozoa were evaluated. However, the data supporting the process of sperm production was measured by scrotal circumference and weight gain.

The motility of spermatozoa was evaluated by looking at the movement and motion of individual spermatozoa, with the end result classified into six groups of scores: special (6), very good (5), good (4), less good (3), sufficient (2) and not good (1), where the stipulation was synchronized with the percentage of the number of progressively moving spermatozoa. The score is "very good" if the mass movement of spermatozoa is + + + + and the motion of the individual is above 90%; it is "good", if the mass movement of spermatozoa is + + + and the motion of the individual is 80 to 90%; it is "less good", if mass movement of spermatozoa is + + and the motion of the individual is 70 to 80%; it is "sufficient", if the mass movement and motion of the individual spermatozoa is + 60 to 70%; and it is "not good", if there is no mass movement and gestures of individuals less than 60%. Semen, prior to review, was previously 0.1 ml diluted with 0.9 ml Na citrate. Dilution was done to facilitate the calculation of sperm motility. A drop of semen was placed on a glass object, and was made thin. The glass was covered with glass cover, and the movement of the semen was viewed under a microscope with 45 x 10 magnification.

The concentration of spermatozoa was assessed by direct counting using a hemocytometer and was recorded in a million / cc. Erythrocyte pipette filled with semen (without dilution first) until a 0.5. 3% NaCl solution was sucked up to mark 101 on the pipette. The mixture was shaken gently to form the number 8 for 2 to 3 min.

A few drops were again discarded, and then one drop was placed under a glass cover at room count Neubeur. By using a microscope with a magnification of 40 x., the number of spermatozoa was counted in the top right, top left, middle, bottom right and bottom left of the room count in a single view. Sepermatozoa concentration was obtained from the sum total count of spermatozoa in the room Neuber multiplied by 10^6.

Measurement of the percentage of live spermatozoa was based on observations made with the microscope for the unit value of percent (%). Inspection was carried out by dripping a drop of eosin - nigrosin on the object glass, after which one drop of semen was then added and smear preparations were made. The object glass was immediately aerated to dry. Sperm was counted under a microscope with a magnification of 10 x 10. Scrotal circumference measurement was done by using a tape measurement with cm scale in the middle of the scrotum (the bulk). Body weight was measured using scales with measurements every week for four times to obtain a weight gain (kg). The data obtained from the four kinds of treatment analyzed descriptively and statistically with Random Group Draft were arranged in four trial blocks on sperm motility, sperm concentration and percentage of live spermatozoa, as the treatments were given doses of bean sprouts at 3 days with a size of K = 0 g / kg bw, PI = 1 gram / kg bw, PH = 2 g / kg body weight, and P III = 3 g / kg bw. Each treatment uses 4 goats, and the examination of each tail is repeated with semen shelter as much as 4 times the time interval of one month. The data obtained were the resource persons being statistically analyzed following analysis of variance (ANOVA). If there are significant differences, the Duncan's Multiple Range Test is used to determine the influence among the treatments.

RESULTS AND DISCUSSION

Motility spermatozoa

Based on the results of microscopic examination of semen collection during the study, the data obtained are given in Table 1 and Figure 1. Figure 1 shows the mean percentage motility of spermatozoa with 3 days old green bean sprouts at different doses, while Table 1 shows a change in sperm motility setup dose for bean sprouts at 3 different days. Here, we can see that the higher dose of bean sprouts when they are 3 days old also increased sperm motility.

Results of the microscopic examination of motility in PI, PII and PIII show that the movement of spermatozoa is fast, strong and progressive, with a mean percentage of

Table 1. Average of spermatozoa motility percentage Goat PE during 4 times semen collection at different dose giving 3 days old sprout green peanut.

Animal	Percentage of spermatozoa motility (%)			
	K	PI	PII	PIII
A1	69	75.25	79	80.5
A2	71	76	78.75	81
A3	69.25	74	78	81
A4	69.5	75.25	80.25	81
Average	69.5625[a]	75.125[b]	79[c]	80.875[c]
Total	1113	1202	1264	1294

[a,b,c] Different superscripts at the same row indicate significant differences (P < 0.05).

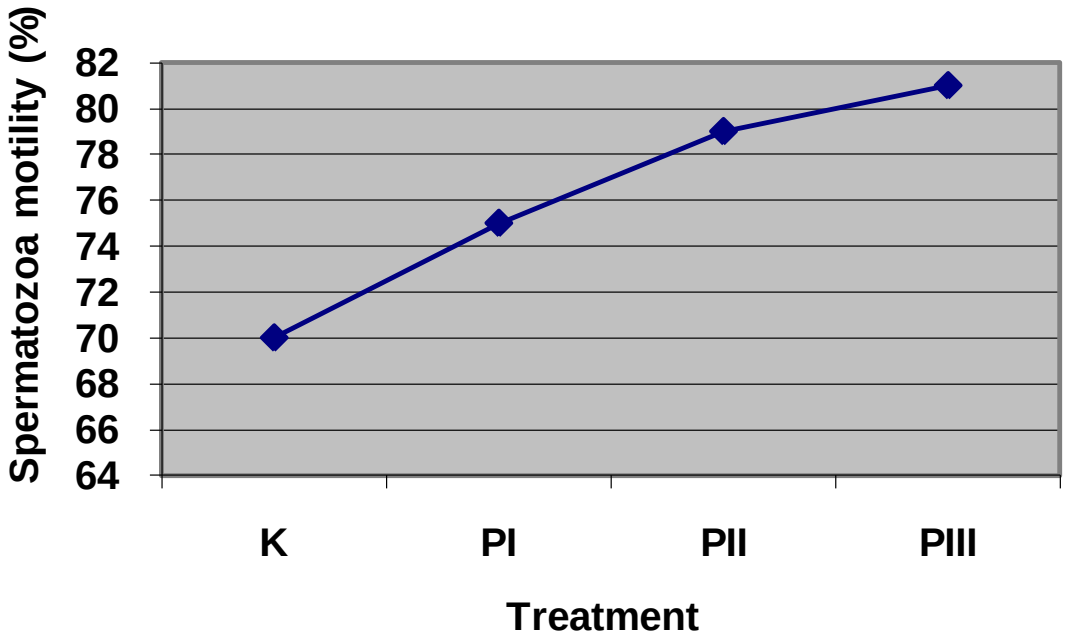

Figure 1. Sperm motility of goats given 3 days old sprout green peanut at K = 0 gram/kg bw, PI = 1 gram/kg bw, PII = 2 gram/kg bw, and PIII = 3 gram/kg bw.

motility for each treatment; although, the movement is higher than that observed by Toelihere (1998) which states that the ram has 60 to 70% motile spermatozoa.

From the results obtained by statistical tests, it was observed that different doses of 3 days old green bean sprouts (Figure 1) had an effect on the motility of spermatozoa with increased number of real or significant (P < 0.01) differences observed in the control (without giving 3 days old green beans sprouts). However, significant difference was observed between treatments II and III, but was not for treatment I.

As stated by Bearden and Naing et al. (2010), the part which provides energy for life and movement of spermatozoa is the tail of spermatozoa through the metabolic processes that take place in the mitochondrial helix. Supported by Men (2001), a reaction that produces energy in the semen only takes place in spermatozoa. The energy itself to sperm motility by Toelihere (1998) originated from within the renovation of the sheath mitochondria ATP through its disposal reactions to ADP and AMP. He also added that most of the vital physiological activism is given by the energy source of carbohydrate or fat.

The use of 3 days old bean sprouts in the implementation of the study, other than as an energy source for the fat content of 5.997 mg / gram also has vitamin E that amounted to 1.55 mg/100 g in accordance with the results of the chemical analysis performed at the Chemistry Laboratory, Faculty of Pharmacy UGM Analyst. According to Fuquay (2002), vitamin E contains alpha-tocopherol that affects sperm trajectory where the deficiency of vitamin E will shorten the path down which affects sperm motility. Besides, it is said also by Made (2003) that deficiency of vitamin E results to loss of

movement of sperm and embryos growth disruption in mice, in addition to sterility in mice and muscle damage in animals, such as: dogs, guinea pigs and rabbits, including the muscles of the reproductive tissues. The content of alpha tocopherol in 3 days old mung bean sprouts also helps to prevent muscle dystrophy sterility in reproductive organs, and it inhibits free radical oxidation peroxysalts in the reproductive tract so that the motility and percentage could increase spermatozoa life.

In addition, one of the two peroxysalts free radicals found in the reproductive organs becomes glucoronat when there is excretion in the kidney (Agawal, 2010). Vitamin E deficiency can cause reduction in the power of the body, decreased sexual activity, and abnormal fat deposits in the muscles (Epoch, 2008) so as to reduce the motility of spermatozoa. Also, Sediaoetama (2001) said that green bean sprouts contain lecithin that serves to improve the function of the hypophysis gland in the brain to stimulate hormonal secretion included in the reproductive hormones.

The concentration of spermatozoa

The data obtained from the collection of semen are shown in Table 2. Figure 2 shows an increase in the concentration of spermatozoa in the delivery of 3 days old green bean seedling for 2 g / kg body weight and 3 g / kg body weight when compared with controls, but the figure decreased in the provision of green bean sprouts at 3 days with a weight of 1 g / kg bb.

Statistical analysis showed that sperm concentration decreased the 3 days old bean sprouts at increments of 1 g / kg bw, and also showed that the numbers are neither real nor significant ($P < 0.01$). An increase was observed for the 3 days old green bean sprouts in control, followed by an increase in the concentration of spermatozoa with significant changes ($P < 0.01$). Decrease in sperm concentration occurred in the delivery of 3 days old green beans in increments of 1 g / kg bw as compared to the control, probably due to the time of the semen-making erratic weather with occasional hot climate and a sudden rain, thus affecting the concentration of spermatozoa. Toelihere (1998) observed that hot weather and climate with low humidity tends to decrease the concentration of spermatozoa. Also, he added that the concentration of spermatozoa is influenced by climate and weather, as well as by the volume of semen, age, weight, individual health, food, frequency of relocation and genetic factors.

Increasing the concentration of spermatozoa with numbers that are not significant ($P > 0.01$) in 3 days old bean sprouts with a dose of 2 and 3 g / kg bw as compared to the controls is likely due to differences in the dose level, which is not too flashy but demands that the feed meets its required ration in order to increase the concentration of spermatozoa, because, as expressed by Gutrie (2000), animals should be fed properly for the production of spermatozoa. Supported by Djanuar (2005), feeding needs to spur the gonadothropine hormone secretion from the pituitary gland, so that spermatogenesis can be run perfectly.

The percentage of live spermatozoa

Examination of the percentage of live or dead spermatozoa is important, because it not only shows live die (Partodihardjo, 2000). According to Toelihere (1998), the preparations made for shaking time affect the percentage of live spermatozoa. This is reinforced by the opinions of Priyono et al. (1994) which state that the increasing time and shock will reduce the motility and percentage of live spermatozoa. Results of the data obtained by semen collection percentage for live spermatozoa are shown in Table 3 and Figure 3.

The picture shown in Figure 3, and the statistical tests, showed that any change in the dosage of 3 days old bean sprouts affected the increase in the percentage of live spermatozoa from the numbers significantly ($P < 0.01$). As stated by Sediaoetama (2001), the 3 days old green bean sprouts, rich in alpha-tocopherol content, can prevent infertility. It is reinforced by Purwanto et al. (2004) that vitamin E may help the regeneration of cells, including reproductive cells, especially the test organ cells and sperm cells, so that they will maintain the quality of life spermatozoa including the percentage of spermatozoa. Also, the 3 days old green bean sprouts contain lecithin that works to improve the tissue function regulated by the pituitary gland associated with expenditure mechanisms of the reproductive hormones (Sediaoetama, 2001). The content of alpha tocopherol in 3 days old mung bean sprouts also helps to prevent muscle dystrophy sterility in reproductive organs, and it inhibits the free radical oxidation peroxysalts in the reproductive tract, so that the percentage of live spermatozoa motility may increase (Agarwal, 2010).

An increasing percentage of life on the provision of bean sprouts is possible. This is supported by the opinion of Burns (2005) which states that the increased activity on the hormone testosterone metabolisms have an influence on the spermatogenesis process of improvement by increasing the trajectory of spermatozoa in the male genital tract so that the accessory gland secretion will increase and provide energy substrate for spermatozoa. However, this has more impact on increasing the vitality of spermatozoa.

Scrotal circumference

The results of scrotum circumference are shown in Table 4. It was observed that giving 3 days old green bean sprouts to Ettawah goat did not affect the size of the circumference of the scrotum. Scrotal circumference measurements were taken to support these observations.

This is because the increase in scrotal circumference

Table 2. Average of spermatozoa concentration Goat PE during 4 times semen collection at different dose giving of sprout green peanut old age 3 days.

Animal	Spermatozoa concentration (million/cc)			
	K	PI	PII	PIII
A1	225	202.50	216.25	221
A2	206	265.5	230.5	248.50
A3	264.50	211.75	305.25	250
A4	249.25	224	262.5	266.5
Average	236.1875[a]	225.9375[a]	253.5625[a]	246.625[a]
Total	3779	3615	4057	3946

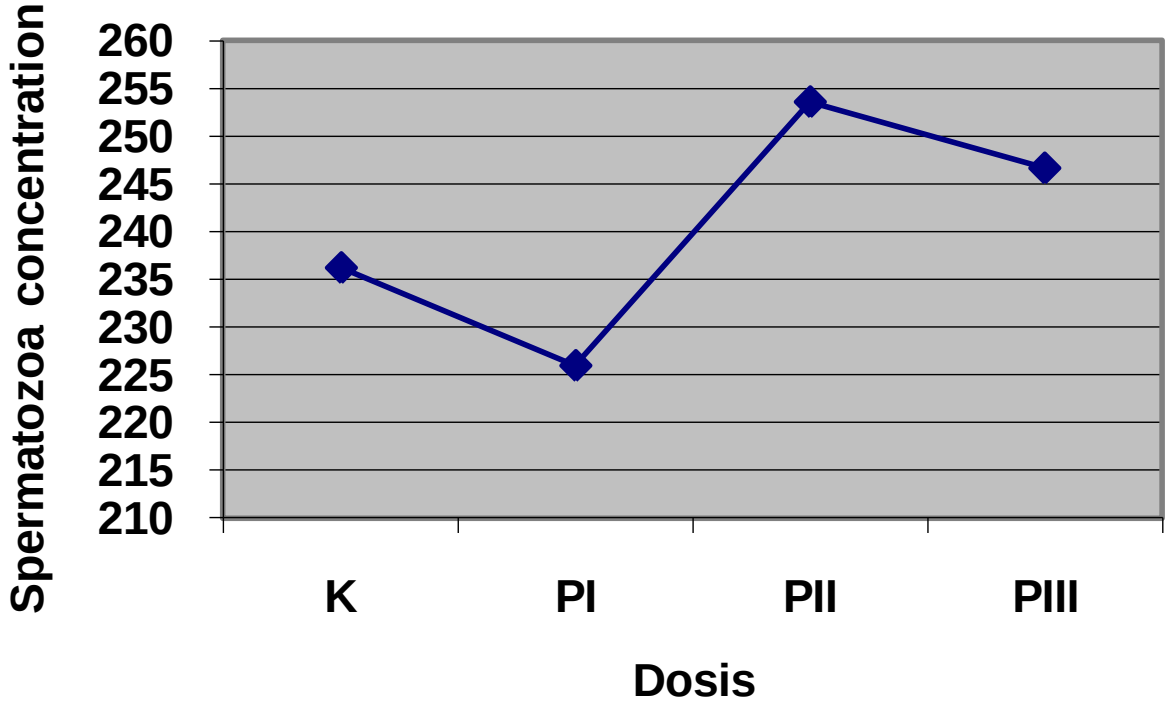

Figure 2. Spermatozoa concentration of goats given 3 days old sprout green peanut at K = 0 gram/kg bw, PI = 1 gram/kg bw, PII = 2 gram/kg bw, and PIII = 3 gram/kg bw.

size has a fair correlation with the quality and quantity of spermatozoa in young males (Gipson et al., 2001; Diwyanto et al., 1992); although, scrotal circumference size is influenced by many factors such as the nation (Coulter and Bailey, 1988), age (Vogt ct tit., 1984; Diwyanto, 1992), body weight (Diwyanto, 1992), as well as season and management (Coulter and Bailey, l988). In North America, the size of scrotal circumference is seen as one parameter used in the study of talon, such that if there is an increase in scrotal circumference sizedue to the 3 days old green bean sprouts, it can be recommended to a Shaman who can help improve thequality of male candidates as an additional information about the relationship between scrotal circumference and sperm motility, but moving spermatozoa that are about to quality spermatozoa.

Weight

The results of the analysis of weight gain of Ettawah goat (PE-) are shown in Table 5. The mean weight gain between treatments, both for control (without addition of bean sprouts at 3 days) and for other treatments (with addition of green bean sprouts), showed a slight increase in body weight on the provision of green bean sprouts (1 g / kg body weight), when compared with the control (3.92 kg: 3.82 kg). Overall, the statistical test results showed that the administration of green bean sprouts had no effect on weight gain, because the green bean sprouts provide the main influence on the reproductive tract by enhancing trajectory of the spermatozoa hormone (Bearden and Fuquay, 2002), so that the quality of spermatozoa increases. Weight gain was used in almost

Table 3. Average of spermatozoa live percentages goat PE during 4 times semen collection at different dose giving of sprout green peanut old age 3 days.

Animal	Percentage of spermatozoa live (%)			
	K	PI	PII	PIII
A1	81	83.50	87	90
A2	80.5	84.25	87.25	90.25
A3	80.25	84	85.25	90
A4	80.25	84.5	87.25	90.75
Average	80.5625[a]	84.0625[b]	86.6875[c]	90.25[d]
Total	1289	1345	1387	1444

Different superscripts at the same row indicate significant differences (P < 0.05).

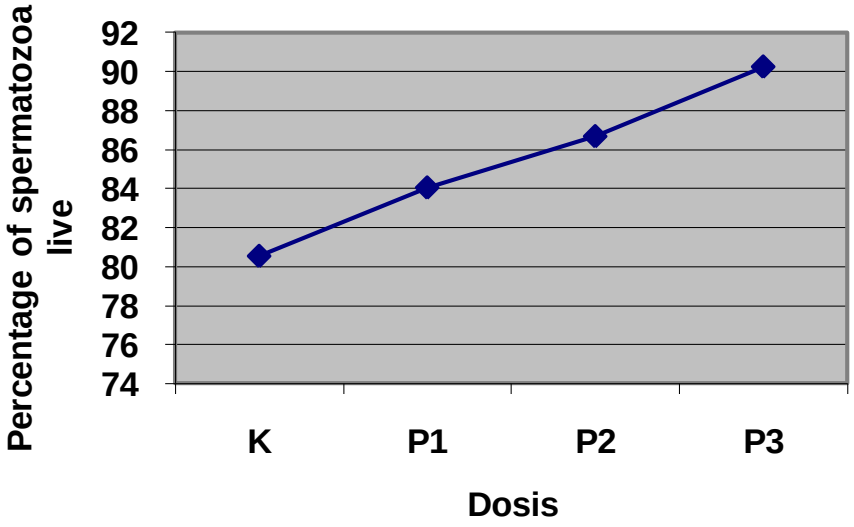

Figure 3. Percentages of spermatozoa live given 3 days old sprout green peanut at K = 0 gram/kg bw, PI = 1 gram/kg bw, PII = 2 gram/kg bw, and PIII = 3 gram/kg bw.

Table 4. Average of circular scrotum goat during 4 times semen collection at different dose giving of sprout green peanut old age 3 days.

Animal	Scrotum circular (cm)			
	K	PI	PII	PIII
A1	23.25	23.25	23.125	23.5
A2	21.25	21.5	21.75	21.875
A3	22.75	23	22.125	23.25
A4	21.25	21.375	21.375	21.625
Average	22.125[a]	22.281[a]	22.094[a]	22.562[a]
Total	254.0	356.5	353.5	361.0

all the treatments, because during the study, forage and concentrate feed that met the nutritional content of food (as seen in the results of the proximate analysis on the instrument) were used with a standard dose or dose maintenance (maintenance). According to Church and Pond (2003), the level of feed requirements for goats used in this research (in accordance with a maintenance dose) has been fulfilled by the protein concentrate, calcium, phosphorus, TDN, fat and raw fiber given to them, so the average weight gain during maintenance at the research activities is still within normal limits. Besides, green bean sprouts contain less energy, protein and fat than soy sprouts, and the germination process that occurs during the process of protein hydrolysis is lower

Table 5. Average of gain goat PE during 4 times semen collection at different dose giving of sprout green peanut old age 3 days.

Animal	Average body weight gain (kg)			
	K	PI	PII	PIII
A1	3.70	4.05	3.35	3.5
A2	3.725	3.7	3.775	3.6
A3	3.825	3.85	3.9	3.85
A4	4.075	4.075	4.025	3.975
Average	3.831[a]	3.9187[a]	3.762[a]	3.731[a]
Total	61.3	62.7	60.2	59.7

and is unable to support the weight growth (Made, 2003).

CONCLUSIONS AND SUGGESTION

From the results of this study, it is concluded that 3 days old green bean sprouts should be provided to Ettawah goat (PE) in order for it to:

1. Be able to improve sperm motility.
2. Not significantly affect the concentration of spermatozoa.
3. Be able to increase the percentage of live spermatozoa.

For study purposes, in order to improve the quality of spermatozoa, 3 days old green bean sprouts (2 g / kg bw) can be added to the feed of goat.

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