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Research Article

Improving Sperm Quality of Jawarandu Goats by Supplementation of Vitamin E and L-Carnitine

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Abstract | The fertility of goats can be seen from various parameters of sperm quality. Good sperm quality is very influential on reproductive efficiency. Vitamin E plays a role in male fertility because it can increase testicular weight, sperm count or concentration, sperm motility, and vitality. L-carnitine is an amino acid compound and is able to provide energy so that it can help improve sperm quality. The objectives of this research were to find out the sperm quality of Jawarandu goats after oral administration of vitamin E and L-carnitine. The research used 16 Jawarandu male goats, with a completely randomized design (CRD) using 4 treatments, control (P0), administration of vitamin E 50 IU (P1), administration of L-carnitine 50 mg (P2), and administration of a combination of vitamins E 50 IU and L-carnitine 50 mg (P3). The data from the research on sperm consistency and mass motility were analyzed descriptively. In contrast, the volume, individual motility, concentration, and percentage of live sperm were analyzed using analysis of variance ANOVA. The results showed that the administration of vitamin E and L-carnitine exerted a very significant effect on motility ($P<0.01$), the concentration, and the percentage of live sperm ($P<0.05$) but did not affect the sperm volume and abnormalities ($P>0.05$). In conclusion, the combination of vitamin E and L-carnitine gave the best results on the sperm quality of the Jawarandu Goat.

Keywords | Jawarandu Goat, sperm quality, vitamin E, L-carnitine

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INTRODUCTION

Goat is one of the livestock that plays a major role in meeting the meat needs of the community which is currently always increasing along with the increase in population and increasing public awareness about the importance of protein, including animal protein for growth and brain development so that livestock populations including goats need to be increased. In addition, goats are also suppliers of meat and milk to support the national food security program. Therefore, it is very important to make efforts to accelerate the increase in the goat population.

The natural increase in population is strongly influenced by reproductive performance. Reproductive performance is reflected in several parameters, for male cattle is sperm quality, while for female cattle such as age of puberty, age at first mating, postpartum estrus, post partum mating, litter size, and survival of offspring until weaning (Sodiq and Abidin, 2012). Increasing the population and productivity of goats is the main goal of goat maintenance and this can only be done if the reproductive performance goes well or the level of livestock reproduction efficiency is high, but there are always problems in livestock development, one of which is the low reproductive performance which results in the increase in livestock population being low.

Sperm is one of the factors that play an important and very decisive role in the success of mating in goats. Sperm quality inhibit fertilization, causing pregnancy failure. The results of Susilawati (2011) showed that sperm quality with 30-40% motility resulted in a higher pregnancy rate than 5-20% motility. In addition, the results of Fatah et al. (2018) showed that sperm quality showed a very significant relationship to the success of pregnancy.

Vitamin E is an important vitamin in ruminant nutrition which is not synthesized by rumen microbes. This vitamin is also called the anti-sterility vitamin or vitamin for fertility and functions as antioxidant and as a protective cell membrane, food substances such as vitamin A and unsaturated fatty acids so as to avoid damage due to the oxidation process. Vitamin E deficiency causes gonadal atrophy and inhibits the formation of sperm cells, and causes infertility in male animals. Vitamin E has various functions, biochemically the function of vitamin E is as a coenzyme and biologically it functions as an antioxidant that protects sperm cell membranes so that it can help penetrate egg cells (Tillman et al., 1991).

Vitamin E is a line of defense against the peroxidation of polyunsaturated fatty acids present in cellular and subcellular membranes. Vitamin E acts as an antioxidant by breaking free radical chain reactions as a result of its ability to transfer phenolic hydrogen to peroxyl free radicals and peroxidized polyunsaturated fatty acids (Mayes, 1995). According to Winarno (1997), antioxidants are materials used to prevent fat oxidation. Antioxidants are effective in preventing peroxidation by providing hydrogen to free radicals. In sperm cells, the effect of peroxidation causes loss of sperm motility, inhibits fructolysis and respiration, releases trans-cellular enzymes and damages the plasma membrane, especially for the acrosome (Combs, 1992).

In addition to functioning as an antioxidant, vitamin E also plays a role in the reproductive process, namely preventing testicular degeneration. Vitamin E deficiency in animals can cause muscle weakness, stunted growth, embryonic degeneration, low egg hatchability, degeneration and release of testicular germinative epithelial cells, infertility, decreased hormone from the testes, decreased cell permeability, spur death and nerve (Lehninger, 1993).

L-carnitine is a form of amino acid that is produced naturally in the body or can also be obtained from certain foods and supplements. L-carnitine has various benefits for the body. However, you need to know the safe dosage for consumption so as not to cause adverse side effects**.** L-carnitine has an important role in producing energy, namely by carrying fatty acids into the mitochondria in body's cells. Mitochondria then act as fat-burning machines to create energy that the body can use for activities. In addition, L-carnitine is also useful in many processes in the body, such as heart and brain function, and muscle movement. Several studies have shown that taking L-carnitine supplements regularly can improve sperm quality, increase sperm count, and maximize sperm motility (Elokil et al., 2019).

The addition of L-carnitine to feed has a positive potential to increase growth and fat catabolism (Chatzifotis and Takeuchi, 1997). L-carnitine plays a role in the metabolic intermediates required to oxidize long-chain fatty acids in the mitochondria which produce metabolic energy (Owen et al., 2001). L-carnitine also plays a role in regulating the CoA/CoA-SH ratio which is important in carbohydrate and fat catabolism (Chatzifotis et al., 1996; Vaz et al., 2002).

Until now, a lot of research has explored the production performance, while research on reproductive performance, especially the quality of goat sperm, has not been done much. High and low reproductive performance for males can be determined by assessing sperm quality. Information on livestock reproductive performance is needed as a basis for further reproductive management. Livestock productivity is closely related to performance. Reproductive performance is an expression of reproductive characteristics, therefore it is necessary to explore information about reproductive performance so that efforts can be made to improve reproductive efficiency in livestock. The purpose of this study was to determine the sperm quality of Jawarandu goats after administration of vitamin E and L-carnitine.

MATERIALS AND METHODS

Materials

This research took place during June and July 2021 in Rejo Asri Village, Seputih Raman District, Central Lampung Regency, Lampung Province. The materials used were 16 heads of Jawarandu male goats, aged approximately 2 years that weigh about 25 kg. The basal feed was consisted of corn, cassava leaf silage, pollard, coffee husk, cassava flour, copra meal, oil palm cake, molasses, salt, vitamin E (alpha tocopherol), and L-carnitine. The goats were equally divided into four groups. The research design used was a completely randomized design (CRD) with four treatments: (i) P0 = control, (ii) P1 = vitamin E 50 IU/head/day (iii) P2 = L-carnitine 50 mg/head/day, and (iv) P3 = vitamin E 50 IU/head/day and L-carnitine 50 mg/head/day.

Research in cages was carried out for 45 days (10 days of adaptation and 35 days of treatment). Goats are housed in individual cages and given rations as needed that goat were equally divided into four groups. Feed analysis data are in Table 1. Drinking water is given in free quantities (*ad libi*

Table 1: Nutrient content of feed used

Composition	Dry Matter	Crude Protein	Extract Ether	Ash	Crude Fiber
			% (DM)		
Content	81.39	18.36	11.69	13.62	12.98

Note: Feed analysis was carried out according to method of (Nutrition and Animal Feed Laboratory, 2021).

Table 2: Semen consistency of Jawarandu Goats after administration of Vitamin E and L-carnitine

	Semen Consistency			
	P0	P1	P2	P3
1	Thick	Thick	Thin	Thick
2	Watery	Thick	Thick	Thick
3	Watery	Thick	Thick	Thick
4	Watery	Thick	Thick	Thickness

P0 = control, P1 = vitamin E 50 IU/head/day, P2 = L-carnitine 50 mg/head/day, P3 = vitamin E 50 IU/head/day and L-carnitine 50 mg/head/day.

tum). Vitamins E and L-carnitine

were given orally in the morning. A mixture of vitamins E and L-carnitine is given in capsule form according to the treatment dose.

Sperm collection was carried out twice, namely before and after treatment. Semen collection was performed using an artificial vaginal method (Toelihere, 1993). Semen that is collected is immediately checked for quality which includes volume, motility (movement), concentration, percentage of live sperm and percentage of abnormalities.

Parameters

Volume: Volume of semen (ml) that is accommodated in each ejaculation or each collection and can be read directly on the collection tube.

Sperm Motility: Examination of sperm motility or motility is used as a basis for determining the ability to fertilize an egg. Sperm motility examination includes mass movement and individual movement. To determine the mass movement is done by dripping a drop of sperm on the object and examined using a microscope with a magnification of 10 times. For individual movements, sperm was dripped onto glass an object and covered with a cover and then examined using a microscope with a magnification of 40 times. Motility assessment was based on Toelihere (1993).

Semen Concentration: Concentration was calculated using a haemocytometer by sucking semen with a pipette to the 0.5 mark then sucking the physiological NaCl solution up to the 101 mark, then shaking until homogeneous and discarding a few drops. The calculation is done by dripping the mixture into the counting chamber which has been given a cover glass. According to Toelihere (1993), semen concentration is calculated by the formula:

$$Y = Z \times 400/80 \times 200/0{,}1$$

description:
Y: semen concentration (million cells/ml)
Z: number of semen cells counted
400: number of small squares in counting room
80: the number of small squares from the five counted boxes
200: the number of dilutions

Percentage of live sperm: Sperm examination was carried out by differential staining using eosin and smear preparations were made and then observed using a microscope with a magnification of 40 times. Live sperm are transparent and dead sperm are dark red. According to Toelihere (1993), the calculation of the percentage of live sperm is carried out using the formula:

$$\text{Percentage of live sperm} = \frac{Y - X}{X} \times 100\%$$

Description:
Y: the number of sperm counts
X: the number of dead sperm

Abnormal sperm percentage: Observation of abnormal sperm (abnormal morphological shape) was carried out on the smear preparations that had been made. Observations were made using a microscope with a magnification of 40 times. According to Toelihere (1993) to calculate the percentage of abnormal sperm:

$$\text{Percentage of abnormality:} \frac{D}{C} \times 100\%$$

Description:
C: counted sperm count
D: abnormal sperm count Sperm
Quality data for consistency and mass motility were ana-

lyzed descriptively, while for volume, concentration, individual motility and percentage of live spermatozoa were analyzed using analysis of variance and continued with BNT test at 5% and or 1% level for significantly different variables (Steel and Torrie, 1991).

RESULTS AND DISCUSSION

Semen volume

The results showed that the average volume of Jawarandu Goats for each treatment was 0.75±0.28 ml (P0); 0.78±0.32 ml (P1); 1.05±0.90 ml (P2); and 1.1±0.68 ml (P3) with an average of 0.93 ml. The volume of sperm can be seen from the volume contained in the sperm tube. Data on the average volume of semen of Jawarandu Goats can be seen in Figure 1.

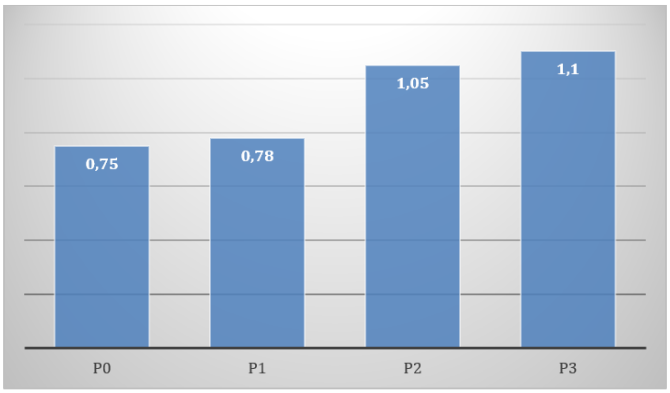

Figure 1: Semen volume (ml) of Jawarandu goats given vitamin E and L-carnitine.
P0 = control, P1 = vitamin E 50 IU/head/day, P2 = L-carnitine 50 mg/head/day, P3 = vitamin E 50 IU/head/day and L-carnitine 50 mg/head/day.

The results of the analysis of variance in semen volume of the Jawarandu Goat showed that the administration of vitamin E and L-carnitine had no significant effect (P>0.05). The volume of semen produced is influenced by the accessory glands. Some of these accessory glands consist of the seminal vesicles (vesicularis), prostate and bulbourethral (*cowper's gland*) glands. The vesicular glands produce the largest portion of semen plasma compared to the secretions of other sex accessory glands. The volume of ejaculate in livestock can vary according to the degree of maturity of the male animal and more specifically the duration of oestrus stimulation and the frequency of holding (Hunter, 1995).

The results of this study are good and in accordance with Toelihere (1993), which states that normal goat semen has a volume between 0.7--3.0 ml; while Hardjopranjoto (1995), stated that the volume of goat semen ranged from 0.25-1.25 ml. The results of this study are not much different from the results of research by Suharyati and Hartono (2013) on Boer goats which produced a volume of 0.77-1.13 ml but higher than the results of Sutriana et al. (2020) which shows the volume of semen in Boerka goats from 0.7 to 0.9 ml.

Figure 1 shows that the average volume of semen produced in the combination treatment of vitamin E and L-carnitine (P3) resulted in a higher volume than P0, P1, and P2, which is related to the function of vitamins E and L-carnitine. Vitamin E plays a role in male fertility because it can increase testicular weight, and increase sperm count and motility. Vitamin E plays a role in male fertility because it can increase testicular weight, and increase sperm count and motility (Anggorodi, 1994). L-carnitine is a compound that plays a role in converting fat into an energy source so that it can improve sperm quality. According to Owen (2001), L-carnitine plays a role in the transfer of long-chain fatty acids across the inner mitochondrial membrane. L-carnitine functions as an intermediary in metabolic processes, having a major role in the formation of acyL-carnitine esters from long-chain fatty acids. The results showed a positive relationship between early sperm motility and increased LC in the epididymis and L-acetyl in sperm (Ahmadi et al., 2016).

Semen Consistency

The consistency of Jawarandu Goat semen given vitamin E, L-carnitine and a combination of vitamins E and L-carnitine was relatively thick, however in the untreated group it had a runny consistency (Table 2).

Based on Table 2, it can be seen that the administration of Vitamin E and L-carnitine (P1; P2: P3) showed a thick consistency compared to the control. The thick consistency of the Jawarandu Goat semen is because the Jawarandu Goat semen obtained has a high concentration level. The concentration *of* spermatozoa produced by Jawarandu Goats given vitamin E and L-carnitine shows a higher concentration. According to Lubis et al. (2013), consistency is the degree of viscosity that is closely related to the concentration of spermatozoa. Consistency assessment can provide an overview of the concentration of spermatozoa contained in the semen. Kartasudjana (2001) also stated that consistency or viscosity is one of the characteristics of sperm that has a relationship with the concentration of spermatozoa in it. The thicker the sperm, the higher the concentration. 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 if the semen is found to be too dilute, it can be assumed that the semen has a low concentration of spermatozoa and vice versa.

Administration of vitamin E and L-carnitine increased the

production of spermatozoa cells, this is because vitamin E functions as an antioxidant and plays a role in male fertility because it can increase testicular weight, increase count and motility (Anggorodi, 1994). L-carnitine is a compound that plays a role in converting fat into an energy source, thereby increasing the availability of energy for the formation of spermatozoa cells. According to Owen (2001), L-carnitine plays a role in the transfer of long-chain fatty acids across the inner mitochondrial membrane.

Semen Concentration

The spermatozoa concentration of Jawarandu Goat after being treated with Vitamin E and L-carnitine is presented in Figure 2. The results showed that semen concentration ranged from 1,910 to 3,290 million/ml with an average of 2,611.88 million /ml. The average concentration of semen in this study was higher than the results of the study by Husin et al. (2007) which showed that the concentration of spermatozoa in Nubian goats was 2,546.67 ± 130.51 million/ml and the results of Sutriana et al. (2020) which resulted in a concentration in Boerka goat of 2,405.56 million/ml.

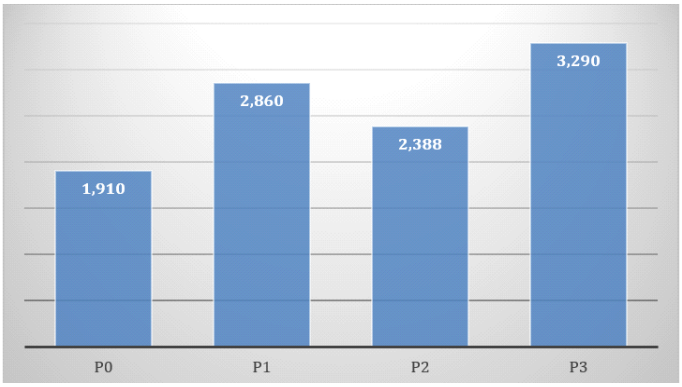

Figure 2: The semen concentration (10^9/ml) of Jawarandu goats after the administration of vitamin E and L-carnitine. P0 = control, P1 = vitamin E 50 IU/head/day, P2 = L-carnitine 50 mg/head/day, P3 = vitamin E 50 IU/head/day and L-carnitine 50 mg/head/day.

The results of the analysis of variance showed that the administration of vitamin E and L-carnitine showed a significantly different effect (P<0.05) on the semen concentration of the Jawarandu Goat. The results of LSD further test showed that the administration of a combination of vitamin E and L-carnitine was different from the administration of L-carnitine and control but not different from the administration of vitamin E and L-carnitine.

The combination of vitamin E and L-carnitine produces the highest concentration. Administration vitamin E and L-carnitine increased the production of spermatozoa cells. According to Church (1983), vitamin E can prevent the regeneration of cells in the testes. Anggorodi (1994) stated that vitamin E functions as an antioxidant. Antioxidants are effective in preventing peroxidation by providing hydrogen to free radicals. Further, Ogbuewu et al. (2010) stated that vitamin E was able to prevent damage to spermatozoa in male cattle and maintain zygote development in female goat. In the process of spermatogenesis, vitamin E functions as an antioxidant capable of neutralizing free radicals resulting from aerobic metabolism.

Sperm Mass Motility

Motility or motility of spermatozoa can be assessed immediately after storage and is generally used as a measure of the ability to fertilize a semen sample. Mass motility on administration of vitamin E, L-carnitine, or the combination of vitamin E and L-carnitine showed better motility than the control, this was due to the vitamin E content given to increase sperm motility (Anggorodi, 1994). Giving L-carnitine cause an increase in the energy produced, this is because L-carnitine is a compound that plays a role in converting fat into an energy source so that it can provide more energy. According to Owen (2001), L-carnitine plays a role in the transfer of long chain fatty acids across the inner mitochondrial membrane.

Individual Motility of Sperm

The results of the study of administering vitamin E and L-carnitine on individual motility are presented in Figure 3. The average individual motility ranged from 72.50 to 83.75% with an average of 78.13%. The results of this study are higher than the results of Kostaman and Sutama (2006) which showed the results of sperm motility of Boer Goats were 71.67% and 73.33%, respectively.

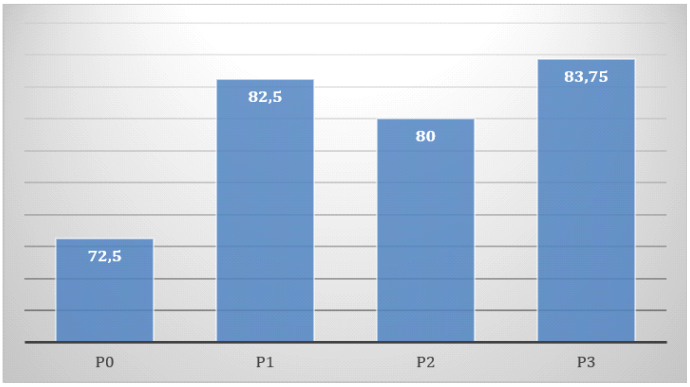

Figure 3: Individual motility (%) of the Jawarandu goats sperms after the administration of vitamin E and L-carnitine. P0 = control, P1 = vitamin E 50 IU/head/day, P2 = L-carnitine 50 mg/head/day, P3 = vitamin E 50 IU/head/day and L-carnitine 50 mg/head/day.

The results of the analysis of variance showed that there was a very significant effect (P<0.01). The results of the BNT further test showed that the administration of a combination of vitamin E and L-carnitine was different

from the administration of L-carnitine and control but not different from the administration of vitamin E.

Plays a role in male fertility because it can increase testicular weight, and increase sperm count and motility (Anggorodi, 1994). The addition of vitamin E can increase fertility (Iriyanti et al., 2007; Wahyuni et al., 2011). L-carnitine is a compound that plays a role in converting fat into an energy source, thereby increasing the availability of energy for the formation of spermatozoa cells. According to Owen (2001), L-carnitine plays a role in the transfer of long-chain fatty acids across the inner mitochondrial membrane. Motility is closely related to the level of fertility or fertility.

The percentage of live sperm

The results of the study of the administration of vitamin E and L-carnitine on the percentage of live sperm of the Jawarandu goat are presented in Figure 4. The average percentage of live spermatozoa ranged from 96.05 to 99.64 % with an average of 97.84%. This result is very good, according to Hardjopranjoto (1995) the number of viable spermatozoa is not less than 85%.

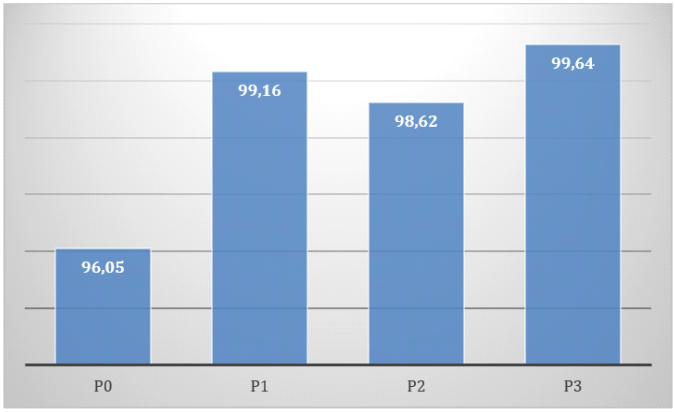

Figure 4: Percentage of Live sperm of Jawarandu goats given vitamin E and L-carnitine. P0 = control, P1 = vitamin E 50 IU/head/day, P2 = L-carnitine 50 mg/head/day, P3 = vitamin E 50 IU/head/day and L-carnitine 50 mg/head/day.

The results of the analysis of variance showed that there was a significant effect (P<0.05). The results of the BNT follow-up test showed that the combined administration of vitamin E and L-carnitine was different from that of the control group but did not differ from the administration of vitamin E and the administration of L-carnitine.

The administration of vitamin E and L-carnitine showed a higher percentage of live sperm, this was because the administration of vitamin E and L-carnitine caused an increase in antioxidants in the goat's body. According to Dutta-Roy et al. (2004), vitamin E is an antioxidant that protects polyunsaturated fatty acids (PUFAs) and cell components and cell membranes from free radical oxidation. Vitamin E acts as an antioxidant and can protect against the action of damaging biological membranes due to free radicals. Vitamin E protects unsaturated fatty acids in phospholipid membranes such as the membranes of sperm cells, while being able to provide more energy for spermatozoa to live. According to Owen (2001), L-carnitine has an important role in producing energy, namely by bringing fatty acids into the mitochondria in the body's cells. Mitochondria then act as fat burning machines to create energy.

Figure 4 shows that the percentage of live spermatozoa from this study was very good because this result was higher than the results of Kostaman and Sutama (2006) which showed the results of live spermatozoa in goats. Boer goats range from 77-79%, the results of research by Suharyati and Hartono (2013) on Boer goats are 86.67%, and the results of research by Sutriana et al. (2020) on the Boerka goat by 79%.

Percentage of Sperm Abnormalities

The percentages of sperm abnormalities resulting from the study are presented in Figure 5. The results of the analysis of variance showed that the administration of vitamin E and L-carnitine had no significant effect (P>0.05), but these results were classified as very good because the percentage of abnormalities obtained is very low (average 0.82%). Hardjopranjoto (1995) stated that sperm is considered good if the number of abnormal shapes does not exceed 5%. The results of this study indicate a lower level of abnormality than the research of Kostaman and Sutama (2006) which showed sperm abnormalities in Boer Goats of 7.55% and the results of Suharyati and Hartono's research (2013) on Boer Goats showed sperm abnormalities of 1.61%.

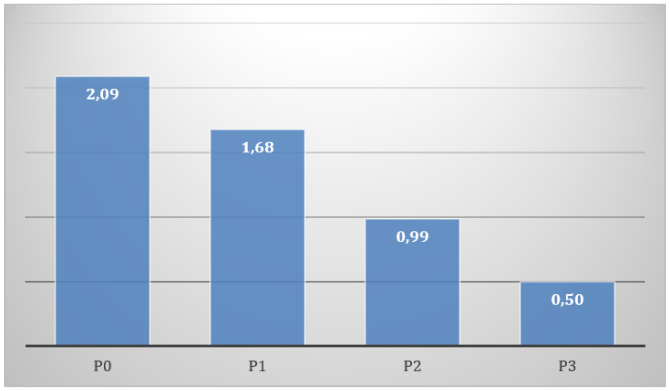

Figure 5: Percentage of abnormalities in the sperm of Jawarandu Goats given vitamin E and L-carnitine. P0 = control, P1 = vitamin E 50 IU/head/day, P2 = L-carnitine 50 mg/head/day, P3 = vitamin E 50 IU/head/day and L-carnitine 50 mg/head/day.

Figure 5 shows that the administration of vitamin E and L-carnitine resulted in the smallest abnormalities. This shows that the addition of vitamins E and L-carnitine plays an active role in the process of spermatogenesis so that the spermatozoa produced in the seminiferous tubules have a low level of abnormality. The lower the percentage of abnormal spermatozoa, the higher the quality of the semen produced. This is in accordance with the opinion of Toelihere (1993), which indicated that fertile sheep or goats should not contain more than 5-15% abnormal sperm.

CONCLUSION

The results showed that the administration of vitamin E and L-carnitine could increase motility, concentration and percentage of live sperm but had no effect on sperm volume and abnormalities. Vitamin E and L-carnitine each as much as 50 mg/head/day can be used for male goats to improve semen quality.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

NOVELTY STATEMENT

The main objective of this research work is to find out the impact suplementation vitamin E and L-carnitine on sperm quality. Even though many researchers have worked on improving sperm quality, but very few researchers reported with combination vitamin E and L-carnitine to improve sperm Jawarandu Goats in Indonesia.

AUTHORS CONTRIBUTION

All authors worked equally, SS designed research and drafted manuscript, Sis and MH collected, tabulated, and analysed data, and KA critically reviewed and revised final manuscript. All authors have read and agreed to the published version of the manuscript.

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