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Libido and Sperm Quality of the Etawah Cross-Breed Fed Urea Moringa Molasses Multinutrient Block Supplement

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Abstract: Moringa leaves contain high and complete nutrients, so they can be used as a constituentfor multi-nutrient blocks to increase libido and sperm quality. This study aimed to evaluate the effect of Moringa leaf flour in a multi-nutrient block on libido and sperm quality of the Etawah Cross-Breed (PE) goats. This study used a two-sample t-test on 8 PE goats aged 18.50±1.00 months, body weight 32±1.49 kg, kept in individual pens for 8 weeks, given the swamp forage ad libitum. A total of 4 animals were supplemented with *urea molasses multi-nutrient block* (UMMB) as a control, and 4 animals were supplemented with *urea moringa molasses multi-nutrient block* (UMMMB) as a treatment. Libido and semen quality were measured from week 4 to week 8. Measurement of scrotal circumference and blood sampling were performed at weeks 0, 5, and 8. The concentration of testosterone in plasma was analyzed using the ELISA technique. The measured variables were compared using the independent sample t-test. UMMMB supplementation did not significantly ($p>0.05$) increase scrotal circumference and testosterone levels. UMMMB supplementation did not significantly ($p>0.05$) decrease reaction time, but significantly ($p<0.05$) decrease mount (24.20±4.30 vs 12.93±1.58 min) and ejaculation (25.60±4.11 vs 13.97±2.23 min). UMMMB supplementation did not significantly ($p>0.05$) increase semen volume and sperm concentration, but significantly ($p<0.05$) increased total sperm motility (69.67±0.76 vs 74.67±1.46%) and sperm viability (80.37±0.68 vs 86.48±1.74%). It can be concluded that UMMMB supplementation markedly increased libido, total motility, and sperm viability of PE goats.

Keywords: libido, sperm quality, moringa, multi-nutrient block, Etawah cross-breed.

伊塔瓦杂交种饲喂尿素辣木糖蜜多营养素块补充剂的性欲和精子质量

摘要：辣木叶含有丰富而完整的营养成分，因此可用作多营养块的成分，以提高性欲和精子质量。本研究旨在评估多营养块中辣木叶粉对伊塔瓦杂交（藻红蛋白）山羊性欲和精子质量的影响。本研究对 8 只 18.50±1.00 月龄、体重 32±1.49 公斤、在单独的围栏中饲养 8 周的藻红蛋白山羊进行了两样本 t 检验，并随意给予沼泽草料。共有 4 只动物补充了尿素糖蜜多营养块作为对照，4 只动物补充了尿素辣木糖蜜多营养块作为治疗。从第 4 周到第 8 周测量性欲和精液质量。在第 0、5 和 8 周进行阴囊周长测量和采血。使用酶联免疫吸附测定技术分析血浆中睾酮的浓度。使用独立样本 t 检验比较测量变量。辣木糖蜜多营养块补充没有显着（$p>0.05$）增加阴囊周长和睾酮水平。尿素辣木糖蜜多营养块补充没有显着 ($p>0.05$) 减少反应时间，但显着 ($p<0.05$) 减少坐骑 (24.20±4.30 对比 12.93±1.58 分钟) 和射精 (25.60±4.11 对比 13.97±2.23 分钟) 。尿素辣木糖蜜多营养块补充没有显着（$p>0.05$）增加精液量和精子浓度，但显着（$p<0.05$）增加总精子活力（69.67±0.76 对比 74.67±1.46%）和精子活力（80.37± 0.68 对 86.48±1.74%）。可以得出结论，尿素辣木糖蜜多营养块补充剂显着提高了藻红蛋白山羊的性欲、总活力和精子活力。

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关键词：性欲、精子质量、辣木、多营养块、伊塔瓦杂交

1. Introduction

The feed technology multi-nutrient blocks have been developed for a long time and have been shown to increase the productivity and reproducibility of ruminants, especially goats. Multinutrient blocks have been shown to improve the production and reproductive performance of goats [1]. The multi-nutritional blocks can also be used to feed lactating goats to partially replace the concentrate [2]. Urea Molasses Multinutrient Block (UMMB) has been shown to improve milk composition and milk quality of Saanen crossbreed goats[3].

Moringa (*Moringa oleifera* Lam) is a tree species, has great potential to be used as feed, due to its high protein contentwhich ranges between 226.0 to 268.0 g kg^{-1}dry matter (DM) [4] and its biomass production can reach 2871.8 $kgha^{-1}cut^{-1}$ DM [5]. Moringa leaves have been used as feed to sperm fertility of rabbits [6] increasing libido and sperm quality in Bali bulls [7] and semen characteristics of buffalo [8]. The libido and semen quality are influenced among others by nutritional factors. Nutrition controls hormone testosterone secretion, sperm production, and may promote testicular development [9].

Moringa leaves contain high and complete nutrition. Moringa leaves contain amino acids, fatty acids, macrominerals, microminerals [10], and phytochemicals [11]. Moringa leaves contain the amino acid arginine essentials for the process of spermatogenesis, putrescine precursors, spermidine, and spermine synthesis which is important for sperm motility and the capacitation of spermto fertilize ova [12].

Moringa leaves contain minerals Zn and Se, which are important for the process of spermatogenesis. Zn minerals can function to stimulate Leydig cells in the testes to produce the hormone testosterone. Mineral Se functions as a strong antioxidant combined with amino acids to form seleno-proteins and enzymes to form selenoenzymes affecting sperm quality by preventing oxidative damage.

Moringa leaves contain lipid (DHA) as a major component in sperm tail, important in sperm motility, and fertility capacity. Moringa leaves contain vitamins B12, B9, A, E, and C which play an important role in the process of spermatogenesis[13].

Therefore, this study will use Moringa leaf flour as a constituent of multi-nutrient block supplement feed in the form of *Urea Moringa Molasses Multinutrient Block* (UMMMB) to increase the libidos and sperm qualities of PE goats.

2. Materials and Methods

2.1. Animal

This study used eight healthy PE goats aged 18.50±1.00 month, bodyweight 32±1.49 kg obtained from Breeding Center Pelaihari. The goats were kept in individual pens measuring 1.25 m × 1.00 m in the form of a stage with a height of 1 m from the ground.

2.2. Experimental Design

The PE goats were divided into two groups, the first group of four goats was supplemented with UMMB as a control and the second group of four goats was supplemented with UMMMB as a treatment. The goats were given the main feed of swamp forage a mixture of *Polygonum barbatum L* and *Ischaemum polystachyum*. This study used *the independent sample t-test* to compare each observed variable.

Scrotal circumference measurements were carried out at 0, 5, and 8 weeks to know the growth of the scrotal circumference. Measurement of scrotal circumference using a measuring tape with a scale of 1 mm according to the procedures of [14]. Blood sampling in the jugular vein using a vacutainer tube at 0, 5, and 8 weeks to determine the increase in testosterone hormone levels. The plasma sample was stored at -20C until the hormonal assay. Testosterone was analyzeby using the ELISA technique in accordance procedure of [15]. Measurements of libido and semen quality were carried out once a week for five weeks from the fourth to the eighth week. Measurement of libido and semen collection was carried out at 07.00 - 10.00 WITA before feeding. Libido observation was begun from the first time to sniff the teaser (*reaction time*), to mount (*mount*), and finally to ejaculate in an artificial vagina (*ejaculation*).Semen collection and semen quality assessment macroscopically and microscopically according to [16].

2.3. Diet and Feeding

The PE goats kept for eight weeks were given rations according to the nutritional requirements for goats [17]. Four goats were given the main feed of swamp forage plus one block of UMMB weighing 200g, and four goats were given the main feed of swamp forage plus one block of UMMMB weighing 200g. Supplementary feed was given in the morning before forage feeding. Forage feeding is carried out after the supplement feed runs out. Provision of forage and drinking water on an *ad-lib* basis. The composition of feed supplements for UMMB and UMMMB are

shown in Table 1. The nutritional content of swamp forage,UMMB, UMMMB, used is shown in Table 2.

Table 1.Compositions of the multi-nutrient block

No	Feed ingredients (%)	UMMB	UMMMB
1.	Moringa Leaf Flour	-	30
2.	Oil Palm meal	25	-
3.	Concentrate Laying Ducks (CP144®)	5	-
4.	Fine rice bran	33	33
5.	Molasses	20	20
6.	Lime	5	5
7.	Salt	5	5
8.	Urea	5	5
9.	Mineral Mix	2	2
	Total	100	100

Table 2. Nutrient content of swamp forage, UMMB, and UMMMB (as % DM)

No	Nutrient composition (%)	Swamp Forage	UMMB	UMMMB
1.	Dry matter	28.86	69.32	66.68
2.	Ash	16.39	15.24	14.14
3.	Crude Protein	13.23	19.24	21.27
4.	Crude Fiber	22.41	6.45	7.72
5.	Ether Extract	1.22	1.95	2.45
6.	Ca	0.28	3.68	4.21
7.	P	0.34	2.15	2.72
Continuation of Table 2				
8.	Nitrogen Free Extract	46.75	57.12	54.42
9.	TDN	55.33	67.78	69.82

Note: Analyzed by Laboratory of Nutrition and Animal Feedstuff, Faculty of Agriculture, Animal Science Department, Lambung Mangkurat University.

2.4. Statistical Analysis

Data (increased scrotal circumference, level testosterone, libido, and semen quality) were reported as the mean and standard error of mean analyzed using *theindependentsample t-test*with SPSS® Version 22 Software.

3. Results

3.1. Dry Matter Consumption, Nutrients Consumption, and Average Daily Gain

Table 3 shows that the consumption of dry matter, crude protein, TDN, and the average daily gain of the PE goats, supplemented with UMMB and UMMMB, were no significant differences ($p>0.05$). However, the Ca and P minerals consumption was significantly higher ($p<0.05$) in the PE goats supplemented with UMMMB compared to supplemented with the UMMB.

Table 3 The consumption of dry matter and nutrients, average daily gain, of the PE goats supplemented with UMMB and UMMMB (g/day)

No	Parameters	UMMB	UMMMB	Requirement [17]
1	Dry matter	1.338 ± 0.01	1.328 ± 0.03	810
2	Crude protein	186.42 ± 1.86	188.32 ± 3.48	63
3	TDN	654.25 ± 7.83	648.50 ± 14.55	448
4	Ca	0.009 ± 0,00[a]	0.010 ± 0,00[b]	0.002
5	P	0.007 ± 0,00[a]	0.008 ± 0,00[b]	0.001
6	Average daily gain	0,066 ± 0,06	0,074 ± 0,08	-

Note: Means in the same row with different superscripts differ significantly ($p<0.05$).

3.2. The Scrotal Circumference, Testosterone Level, and Libido

Table 4 shows thatincreased scrotal circumference, testosterone levels, and reactions time of the PE goats were supplemented with UMMB and UMMMB were no significant differences($p>0.05$). However, the time to mount and to ejaculation was significantly faster ($p<0.05$) for PE goats supplemented with UMMMB compared to PE goats supplemented with UMMB.

Table 4 The increased scrotal circumference, level testosterone, and libido of the PE goatssupplemented with UMMB and MMMB

No	Parameters	UMMB	UMMMB	*p*-Value
		Means (± SEM)		
1.	Increased scrotal circumference (cm)	0.25 ± 0.22	0.48 ± 0.14	0.51
2.	Testosterone (ng/ml):			
	a. 0 weeks	0.74 ± 0.05	1.00 ± 0.02	0.10
	b. 5 weeks	0.76 ± 0.13	2.51 ± 0.97	0.15
	c. 8 weeks	6.58 ± 2.53	6.71 ± 3.93	0.98
3.	Libido:			
	a. Reaction time (min)	12.45 ± 3.59	6.38 ± 1.21	0.15
	b.Mount (min)	24.20 ± 4.30[a]	12.93 ±1.58[b]	0.04
	c.Ejaculation (min)	25.60 ± 4.11[a]	13.97±2.23[b]	0.04

Note: Means in the same row with different superscripts differ significantly ($p<0.05$).

Fig. 1 shows the growth of scrotal circumference during rearing. The average scrotal circumference at week 0 was 23.05 vs 23.13 cm and after being reared for eight weeks the average scrotal circumference was 23.30 vs 23.60 cm, so obtained the addition of 0.25 vs 0.48 cm.

The testosterone level in PE goats also increased along with the time of rearing and feeding with multi-nutrient block supplements. Testosterone levels of PE goats fed UMMMB supplementation were higher than UMMB. The increase in testosterone levels is shown in Fig. 2.

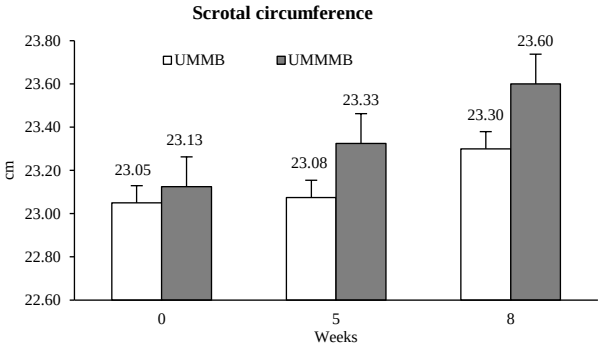

Fig. 1 Growth scrotal circumference of the PE goats

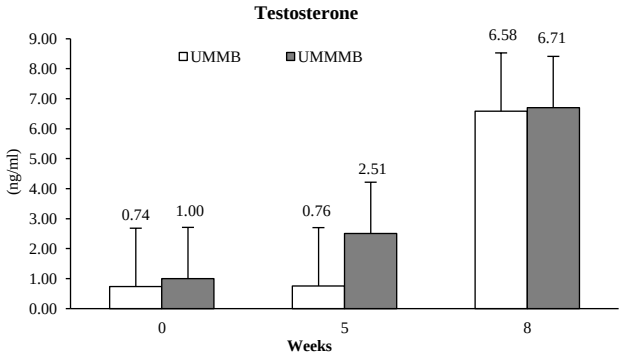

Fig. 2 Increased testosterone levels of PE goats

Comparison of the time required by the PE goats fed UMMB and UMMMB supplements to sniff a teaser, to mount, and ejaculate in an artificial vagina at 4 to 8 weeks are shown in Fig. 3.

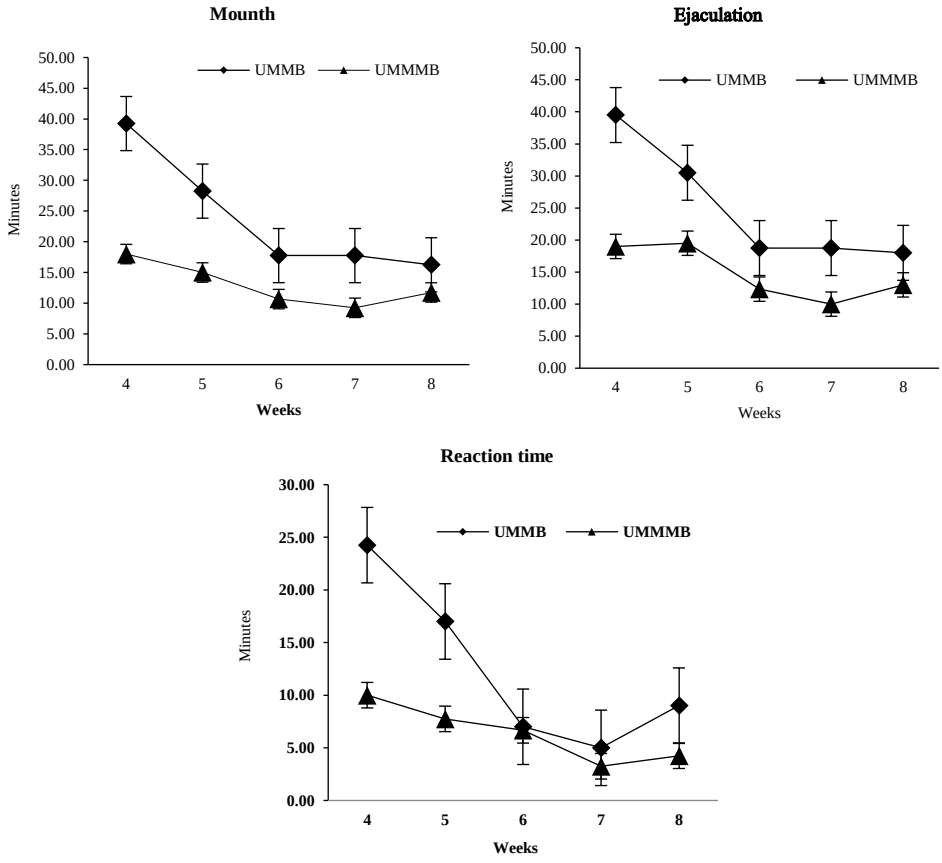

Fig. 3. Measurement of reaction time, mount, and ejaculation of PE goats

3.3. Semen Quality

Table 5 shows that semen volume, concentration, and the sperm abnormalities of PE goats supplemented with UMMB and UMMMB were no significant differences ($p>0.05$). However, the total motility and viability of sperm were higher ($p<0.05$) in PE goats supplemented with UMMMB compared to the control goat supplemented with UMMB.

Table 5 The quality of the fresh semen of the PE goats supplemented with UMMB and UMMMB

No	Parameters	UMMB	UMMMB	*p*-Value	References [18]
		Means (± SEM)			
1.	Volume (ml)	0.83 ± 0.06	0.86 ± 0.07	0.75	0.5 – 1.2
2.	Concentration (million/ml)	2.708 ± 293.73	3.258 ± 152.31	0.14	2,500 – 5,000
3.	Total motility (%)	69.67 ± 0.76[a]	74.67 ± 1.46[b]	0.02	70 – 90[1]
4.	Viability (%)	80.37 ± 0.68[a]	86.48 ± 1.74[b]	0.00	60 - 80[2]

Note: Means in the same row with different superscripts differ significantly ($p<0.05$)

A comparison of the semen quality of PE goats fed UMMB and UMMMB supplements during rearing at 5 to 8 weeks is shown in Fig. 4.

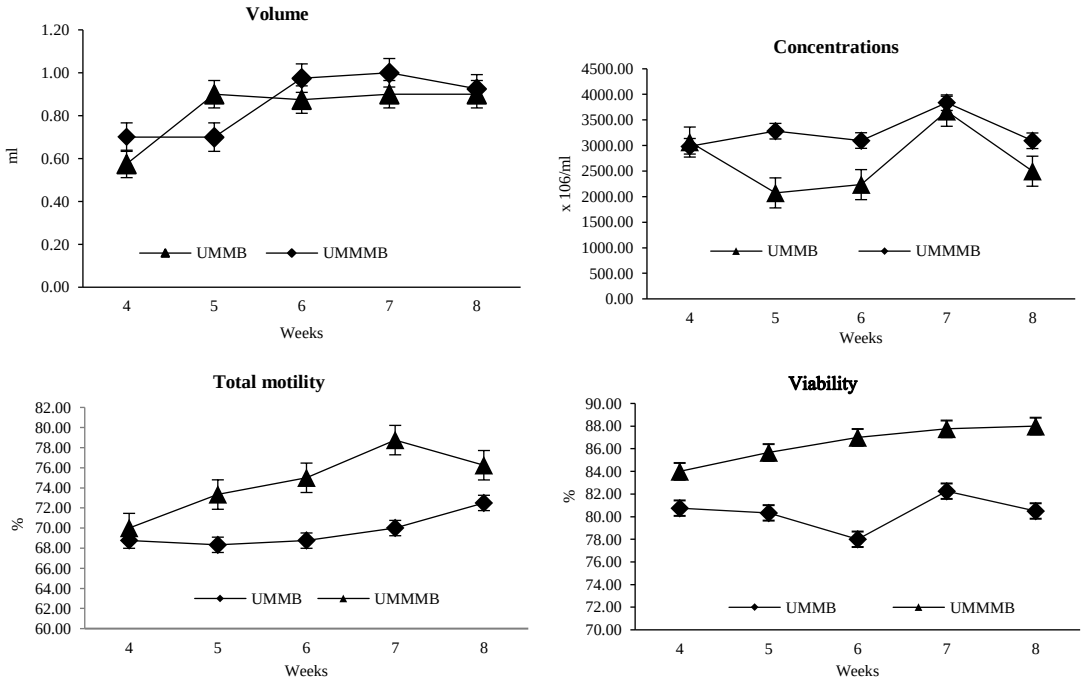

Fig. 4 Semen quality measurement results

4. Discussion

4.1. The Scrotal Circumference, Testosterone Level, and Libido

The results of this study showed that the supplementation of UMMMB significantly increased the libido of PE goats. The time required the goats to mount the teaser and ejaculate in the artificial vagina was significantly shorter than the control. However, all other reproductive variables observed such as scrotal circumference,testosterone concentration, also increased and reaction time decreased but did not show a significant difference.

UMMMB supplementation did not significantly increase the scrotal circumference of the experimental goats, presumably, the goats were more than 18 months old, i.e. an average of 18.50±1.00 months. The study by [19] on Assam goats showed that the morphometrics of seminiferous tubules and Leydig cells experienced a significant increase at the age of 6-8 months. At the age of eight months and over, the scrotal circumference will be constant (between 17-18 cm) without further significant increase. This condition probably caused the use of Moringa leaves on UMMMB did not significantly affect the increase in the scrotal circumference of the experimental goats used.

The experimental goat scrotal circumference obtained was smaller than the standard Breeding Soundness Examination in the buck [14]. It was explained that the scrotal circumference was at least 25 cm for a breed that weighed 45 kg, and most dairy goat males had a scrotal circumference of 25-28 cm when the bodyweight reached 45 kg. The experimental goat used was only 32±1.49 kg, so the scrotum circumference obtained was also small (Fig. 1), because scrotal circumference was positively correlated with body weight [20]. However, the scrotal circumference obtained was still higher than the three indigenous breeds of goats in arid and semiarid agroecology of Ethiopia: Afar, Long-Eared Somali, and Woyto-Guji each; 20.5±2.10 cm; 21.4±1.67 cm; 20.6±1.93 cm; or an average of 20.8±1.94 cm [20].

Testosterone hormone in experimental goats increased along with an increase in scrotal circumference. This is because the hormone testosterone is formed in the Leydig cells of the testes. Scrotal circumference increases due to an increase in the number of Leydig cells. Leydig cells are still developing, so the production of the hormone testosterone continues to increase. The development of testosterone hormone production described by[21] it was starting from conception and the fetus is the highest peak producing testosterone is when differentiation is needed for male sex formation and masculinization in the brain. Testosterone production then declines to a nadir in the early postpartum period. After that, the production of the hormone testosterone then increases gradually through puberty to adulthood. Furthermore, testosterone production decreases due to aging and other conditions that result in decreased

testosterone levels (hypogonadism) that accompany changes in metabolism and quality of life.

Fig. 2 illustrates the increase in testosterone levels of experimental goats during maintenance and the highest at the eighth week after rearing. The testosterone level of experimental goats in the treatment group was higher than the control group but did not show a significant difference. The study by [22] showed that feed factors affect testosterone levels in male goats. The nutritional status of livestock has an impact on the growth performance and development of reproductive organs.Testicular mass, semen characteristics,spermatogenesis, and reproductive hormones can be influenced by feeding levels[23]. The testosterone level of experimental goats in the treatment group was higher than the control, possibly due to the effect of higher crude protein consumption, although it did not show a significant difference (Table 3) and the other possibility of Moringa leaves containing high Zn minerals in UMMMB. Moringa leaves contain the mineral Zn 25.5 - 31.03 mg/kg[10]. Zn mineralsstimulate Leydig cells to produce testosterone for the normal function of the hypothalamus-pituitary-testesaxis. Higher testosterone levels in the treatment group, similar to the results of our previous study in Bali cattle [7]. Diurnal changes in testosterone levels in Balinese cattle supplemented with Moringa leaves were significantly higher in the morning, afternoon, and evening. Therefore, the high protein content in UMMMB and the high Zn mineral content in Moringa leaves as a constituent of UMMMB are considered as possible triggers so that the testosterone levels of males supplemented with UMMMB are higher than the control, although it has not shown a significant difference.

Testosterone levels in experimental goats after being fed a multi-nutrient block supplement for 8 weeks were lower than those of [24] in Kacang goats, namely 18.51±19.4618 ng/ml, and 29.57±12.96 ng/ml. Factors affecting animal testosterone levels in the blood are a nation of origin, age, environment, disease, presence or absence of sexual stimulation, and the sensitivity of the methods [24].

Fig. 3 illustrates the better libido of PE goats during rearing fed UMMMB compared to controls. The time required to sniff a teaser, mount, and ejaculate in an artificial vagina was shorter than the control, which was 12.45 vs 6.38 min, 24.20 vs 12.93 min, and 25.60 vs 13.97 min, respectively. The time required to mount and ejaculate in an artificial vagina was significantly shorter, so the libido of PE goats supplemented with UMMMB was significantly better. Libido is influenced by testosterone levels [25] so the increase in libido in PE goats supplemented with UMMMB in this study was caused by an increase in testosterone levels. Thus, UMMMB supplementation can increase testosterone levels so that it can increase the libido of PE goats.

4.2. Semen Quality

The results of this study showed that UMMMB supplementation improved the quality of fresh semen of PE goats. Fig. 4 illustrates the semen volume, sperm concentration, total motility, and sperm viability of experimental goats supplemented with UMMMB during rearing better than controls. UMMMB supplementation significantly increased the total motility and viability of PE goats spermatozoa.

Semen volume, sperm concentration, total motility, viability, and abnormality of sperm obtained were all normal according to [18]. However, supplementation with UMMMB did not significantly increase the volume and concentration of semen. This condition is in line with the results of a study of our previous study, Moringa leaf supplementation did not significantly increase the volume and concentration of semen in Bali cattle [7].

The sperm concentration obtained in this study was higher than the results of research by [24] in Kacang goat, namely 2,763.0±395.0 $\times 10^6$. This condition accordance with the statement of [26] and[27] that various factors are known to influence the goat semen quality such as age, breed, season, method of semen collection, extender, and centrifugation, frequency of ejaculation, techniques of breeding and even by variation among individual goats within the same herd.

UMMMB supplementation significantly increased the total motility and viability of PE goats' sperm. This can strengthen the notion that Moringa leaves can significantly increase the total motility and viability of PE goat sperm. The results of our previous study also showedthat Moringa leaf supplementation significantly increased the total sperm motility of Bali bulls [7].Moringa leaves can increase total motility and viability possibly because Moringa leaves contain high Ca and P. Moringa leaves contain 2,003 mg/100 g Ca and 204 mg/100 g P [10]. As in the Bali cattle study, the results of this study showed that UMMMB supplementation significantly increased Ca and P consumption, so that the high Ca and P content in Moringa leaves could be suspected as the cause of significantly higher sperm motility of goats supplemented with UMMMB.As well [28] explained that Ca serves to increase sperm motility. Phosphorus plays an important role in fertility because it plays a role in energy (ATP) transfer processes and as a second messenger (cAMP). Phosphorus deficiency in males can cause testicular degeneration. cAMP is related to sperm motility and capacitation. In addition to the, Ca and P content, the high Zn content in Moringa leaves can also be expected to increase the total motility and sperm viability of PE goats in this study. The results of a review of[29] showed that high Zn concentrations were associated with high sperm motility and viability. Zinc in the testes is essential for spermatogenesis and spermatozoa physiology by maintaining the integrity of the inner genome and structure of spermatozoa. Zinc is

also effective in protecting sperm from bacteria and chromosomal damage. Due to its strong antioxidant properties, a sufficient amount of zinc in semen plasma shows a protective effect. Zinc is mainly derived from the prostate, and plays a key role in sperm motility, carrying out protective and antioxidant activities. It is also considered an antimicrobial factor against gram-positive and gram-negative bacteria. Deficiency of this element can lead to failure in spermatogenesis, atrophy of the seminiferous tubules, and thereforehypogonadism and a high incidence of changes in sperm morphology. Therefore, further research is needed to prove the effectiveness of the minerals Ca, P, and Zn in Moringa leaves to increase sperm motility and viabilityon PE goats.

5. Conclusion

This study has successfully proven the use of feed supplement UMMMB markedly increases libido, total motility, and sperm viability of PE goats. The PE goats were supplemented UMMMB required a shorter time to mount the teaser and ejaculate into the artificial vagina. The high Zn mineral content in Moringa leaves is strongly suspected to increase the libido of PE goats. Mineral Zn stimulates the formation of Leydig cells to produce the hormone testosterone, thereby increasing libido.Moringa leaf flour that constituent UMMMB contains the high minerals Ca, P, and Zn which are strongly suspected to increase total motility and sperm viability. Minerals Ca and P function to increase sperm motility and capacitation. The mineral Zn is essential for spermatogenesis, sperm motility, and protects sperm from bacteria and chromosomal damage.Thus, the spermatozoa will become more motile and can survive.

Therefore, Moringa leaves can be used as a constituent of UMMMB to increase the libido and sperm quality of PE goats. This research still needs to be continued to prove the minerals Ca, P, and Zn in Moringa leaves can increase libido, motility and sperm viability in PE goats using Ca, P, and Zn from extracted Moringa leaves.

5.1. Acknowledgment

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