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Research article

Effect of minerals and concentrate supplementation on the fertility of Pote goat during the dry season

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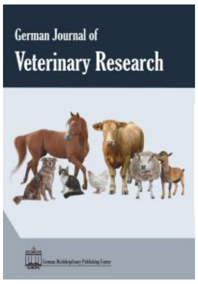

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Abstract

This study aimed to determine the effect of minerals and concentrate supplementations on the fertility of Pote goats in the long dry season. For this purpose, sixty healthy and not pregnant goats were divided randomly into three groups, designated T0, T1, and T2. All animals were fed forage 3 kg/animal/day; additionally, animals kept in T1 were supplemented with a mineral mixture at a dose of 0.5 g/kg BW/day, while animals kept in T2 were supplemented with concentrate at a dose of 0.5 g/kg BW/day for 45 days. The efficacy was assessed based on the body weight, blood urea nitrogen, total protein and albumin, two estrus synchronization, pregnancy rate, and litter size. The result showed that the body weight, blood urea nitrogen, total protein, and albumin were not significantly different among groups. In the first estrus synchronization, the T1 and T2 groups exhibited a higher estrus rate compared to group T0. For goats that were supplemented with concentrate (T2), the onset of estrus was faster ($p<0.05$) both in the first and second estrus synchronization. All goats were inseminated artificially. Pregnancy examination based on progesterone levels (21-day post-insemination) and Ultrasonography (USG at 45-day post-insemination) showed a variation in pregnancy rate. All goats diagnosed as pregnant based on USG examination had kidding with 1-2 kids per doe. In the T0 and T2 groups, serum progesterone levels on the 21st day in singlets were lower ($p<0.05$) than in twin kidding. It could be concluded that mineral or concentrate supplementations enhanced the performance parameters of goats during the long dry season.

Keywords: Estrus rate, Kidding rate, Pregnancy rate, Regional climate, Sustainable food consumption

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Introduction

Global warming can threaten the availability of animal protein for global consumption through disruption to livestock production (Godde et al., 2021). Climate changes reduce the quantity and quality of forage for livestock, which can disrupt the livestock reproductive system (Khurshid et al., 2023). As a model, Patengteng Village, Modung District, Bangkaalan Regency, Madura Island, Indonesia, is an area that experiences the problem of drought every year, which has not yet been resolved. Every dry season, land and rice fields dry out, and residents always have difficulty getting clean water because springs dwindle and residents' wells dry up. Sources of animal feed, including those from agricultural production (rice and corn), are becoming scarce. Feed plays an important role in around 70% of total livestock production in livestock businesses.

Lack of feed nutrition is the main obstacle to increasing livestock productivity. Lack of feed affects health and production, as well as fertility and animal immunity. Consumption of less energy and protein than required leads to loss of body weight, decreased body condition score, and negative energy balance (McGrath et al., 2017). In goats, carbohydrates, fats, proteins, vitamins, water, and minerals are required (Wang et al., 2020). Animals fed on a protein-deficient diet result in a low concentration of ammonia in the rumen (around 50 mg/L) and can disturb the intestinal microbiota, which subsequently hamper digestion and metabolism (Putri et al., 2021).

One of the nutritional elements of feed that needs to be considered in supporting goat production is minerals. Minerals are divided into two, namely essential and non-essential minerals. Essential minerals are divided into two types, namely macro-minerals (Ca, Mg, Na, K, and P) and microminerals (Zn, Mn, Co, Cr, Ni, Fe, and I) (Byrne and Murphy, 2022). Some of these micro-minerals that function in enzymatic processes are Fe and Mn. The Fe mineral used in the enzymatic metabolism process of hemoglobin is 15% and is stored in the form of ferritin around 70-80%. The Mn mineral functions as a carbohydrate synthesizer, mucopolysaccharide, and enzyme systems, for example, pyruvate carboxylase and arginine synthetase (Pugh, 2023). Apart from enzymatic reactions, Mn is important for livestock growth and reproduction.

Animals that experience mineral deficiencies exhibit a decrease in body weight and production, as well as display reproductive failure (Khalil et al., 2019). The essential micro and macro minerals needed by ruminants are found in forage plants, so if livestock are not given additional mineral feed, more attention needs to be paid to the quality and quantity of forage (Arthington and Ranches, 2021). The soil where the forage grows is a source of nutrients for plants. If the soil is poor in mineral elements, then livestock that consume the forage show symptoms of mineral deficiency (Capstaff and Miller, 2018). Essential mineral elements, both macro and micro, are needed for the physiological processes of livestock, especially ruminants that depend on forage. Forage that grows on soil that is poor in mineral elements has reduced mineral content, especially grass types (Darch et al., 2020).

Low feed intake in female goats, both in quantity and quality, can be followed by a deficit in nutritional needs, namely the need for basal metabolic is greater than the feed intake, causing a decrease in body weight (Sow et al., 2020). Nutritional deficiencies in female goats also impact other physiology, including the reproductive system, and reduce fertility (Khalil et al., 2019). This study aims to determine the effect of minerals and concentrate supplementations on the fertility of Pote goats in the dry season.

Material and methods

Ethical approval

This research has been approved by the Universitas Airlangga Research Ethical Clearance Commission, certificate number: 1250/HRECC.FODM/XI/2023.

Experimental animals

This research was conducted on Pote goat farming in Patengteng Village, Modung District, Bangkalan, Madura Island, Indonesia. It is a coastal area with a height of between 2-10 m above sea level, located at the coordinates 7°43'53"S and 111°27'54"E. The study was conducted in the dry season, from July to October 2023. The air temperature was 27–34 °C, with relative humidity levels of 68%–83%. The tropical climate is wet and dry with two seasons, namely the rainy season and the dry season. The dry season takes place in the May–October period, with the driest month being August. In the dry season, river flow decreases and even dries up. The rainy season takes place in the period November–April, with the wettest month being January. Annual rainfall is between 1,200 and 1,800 mm per year, with a total of 80–120 rainy days per year. Analysis of the composition of animal feed was carried out in the Feed Technology Laboratory, Department of Animal Nutrition and Feed Science, Faculty of Animal Husbandry, Gadjah Mada University, Yogyakarta, Indonesia.

This study used sixty Pote goats (20 goats per treatment group) that were healthy, non-pregnant, and had a normal estrous cycle aged 1-2 years, weighing 20-35 kg, parity more than once. The goats were given albendazole (PT Kimia Farma, Cikarang, Indonesia) at a dose of 1 gr/20 kg body weight (BW) a week before treatment. Standard feed consists of 2-3.5 kg of forage, and drinking water is always available. Animals were divided randomly into three groups. In the control group (T0), goats were fed only a standard feed forage of 3 kg/day/animal. In addition to the standard feed forage, the T1 group was supplemented with a mineral mixture (PT Agromix Lestari, Yogyakarta, Indonesia) of 0.5 g/kg BW/day, while the T2 group was supplemented with a concentrate (PT Agromix Lestari, Yogyakarta, Indonesia) of 0.5 g/kg BW/day. The composition of the minerals mixture is shown in Table 1, and the composition (%) of concentrate and forages is shown in Table 2. The feeding treatment was carried out for 45 days. The mineral mix or concentrate was dissolved in 100 mL of drinking water and then given orally. Goats are given standard feed in the form of forages consisting of elephant grass, field grass, mango leaves, and mangrove leaves. Drinking water was always available. Goat body weight was measured using a hanging spring scale with a scale of 0-100 kg (Nops, China) before treatment (day 0), day 21, during estrus, and day 45.

Table 1: Composition of 100 g mix minerals.

Mineral's content	Amount
Calcium (Ca)	50 g
Cobalt (Co)	3 mg
Copper (Cu)	4 mg
Iodine (I)	0.3 mg
Iron (Fe)	11 g
Potassium (K)	2.4 g
Magnesium (Mg)	6 g
Manganese (Mn)	5.4 mg
Phosphor (P)	17 g
Selenium (Se)	2 mg
Sodium (Na)	3.6 g
Sulfur (S)	0.7 g
Zinc (Zn)	19 mg

Table 2: Composition (%) of forages and concentrates.

Nutrient content	Forages	Concentrates
Ash	8.09±1.61	10.80
Crude fat	2.64±0.14	3.37
Crude fiber	29.23±4.97	20.92
Crude protein	10.72±0.58	18.03
Dry material	-	61.94

Estrus synchronization

Estrus synchronization was carried out by intramuscular injection of 200 µg of Prostaglandin (PG) F2α (Sigma-Aldrich Chemicals Private Limited, Jakarta, Indonesia). Signs of estrus were assessed to determine the onset of estrus and the duration of estrus. Estrus detection was monitored every 12 hours using a teaser buck for three consecutive days after PGF2α injection. Does were determined in estrus when exhibiting standing heat as the gold standard of estrus sign. The estrus rate was calculated in percent (%). The onset of estrus was estimated as the interval (h) from PGF2α injection to the first time the doe expresses standing heat. The duration of estrus was estimated from the start of standing heat to the end of sexual receptivity.

Ultrasonography

Ultrasonography was performed to check the presence and describe the functional structure of the ovaries. It is performed before the first PGF2α injection was performed at the time of the first heat, before the second PGF2α injection, and then at the time of the second heat. Ultrasonography (PT Agro Primalab Indonesia, Jakarta, Indonesia) was performed on day 45 after the female goat was inseminated. The ultrasound instrument used is a Draminsky ultrasound scanner with a 5 MHz transducer probe and B-mode image mode, which is displayed on a 6.4-inch LCD.

Blood samples

5 mL blood samples were taken from the jugular vein before treatment, on day 21 (just before the first PGF2α injection), at the time of the first estrus, on day 32 (before the second PGF2α injection), at the time of the second estrus, and on day 22 after the female goat was mated. Blood chemistry analyses, including blood urea nitrogen (BUN), total protein, and albumin, were performed on day 0 and day 45. Progesterone levels were measured on samples before PGF2α injection and on day 22 after the female goat was inseminated, while estrogen levels were measured on blood samples taken during estrus. Progesterone and estrogen levels were measured using the enzyme-linked immunosorbent assay (ELISA, Thermo

Fisher Scientific, PT.GENECRAFT LABS, Jakarta, Indonesia).

Diagnosis of pregnancy

The non-return rate (NRR) was observed on days 21-24 after the female goat's insemination. Serum progesterone levels of more than 1.5 ng/mL on day 21 were used as a guideline for pregnancy detection (Susilowati et al., 2020). Pregnancy diagnosis was based on the presence of fetal images using ultrasound examination.

Statistical analysis

The data were analyzed using a one-way ANOVA followed by Tukey's Honest Significant Difference test at a 95% confidence level (SPSS Version 23, IBM, New York, United States).

Results

Body weight, blood urea nitrogen, total protein, and albumin

Supplementations with minerals (T1) or concentrate (T2) for 45 days were not able to increase the body weight (p>0.05) of goats (Table 3). Also, blood urea nitrogen, total protein, and albumin were not significantly changed compared to the control group (T0) (Table 4).

Table 3: Effect of minerals and concentrates supplementations on body weight (kg) of Pote.

Group	D0	D21	Estrus	D45
T0	22.54±2.94	21.89±1.86	22.99±1.70	23.60±1.79
T1	24.41±4.82	29.37±6.87	30.25±7.20	30.22±7.39
T2	27.5±5.07	29.83±6.07	30.59±6.04	30.72±5.65

No significant difference (p>0.05) among groups. D0: before treatment, D21: 21 days after treatment, estrus: day at estrus, D45: 45 days after treatment. T0: control group as animals were standard feed forage of 3 kg/day, T1 and T2: animals were fed forage 3 kg/day and supplemented with minerals (0.5 g/kg BW/day) or concentrate (0.5 g/kg BW/day), respectively.

Table 4: Effect of minerals and concentrates supplementations on the level of blood urea nitrogen (BUN), total protein (TP), and albumin (Alb) of Pote.

Group	BUN (mg/dl)		TP (g/dl)		Alb (g/dl)	
	Before	After	Before	After	Before	After
T0	12.65±3.38	14.19±3.94	6.63±1.20	8.57±1.31	3.55±0.80	4.89±0.79
T1	13.83±5.01	15.08±1.64	7.82±0.83	8.87±0.47	3.51±0.36	5.47±1.73
T2	11.31±3.95	16.15±5.07	7.61±1.16	9.24±2.93	5.08±1.74	4.78±0.97

No significant difference (p>0.05) among groups BUN: blood urea nitrogen, TP: total protein, Alb: albumin. Before: before treatment, After: 45 days after treatment. T0: control group as animals were standard feed forage of 3 kg/day, T1 and T2: animals were fed forage 3 kg/day and supplemented with minerals (0.5 g/kg BW/day) or concentrate (0.5 g/kg BW/day), respectively.

Estrus synchronization

In the two synchronizations, high progesterone levels were obtained before PGF2α injection and high estrogen levels during estrus (Table 5), confirmed by the USG examination. Before PGF2α injection, corpus luteum was seen, and during estrus, follicle growth was seen, both in the first and second estrus synchronizations. In the first estrus synchronization, supplementations with minerals (T1) or concentrate boosters (T2) resulted in a higher estrus rate compared to control (T0). However, in the second estrus synchronization, all groups produced a 100% estrus rate (Table 6). In goats that were supplemented with concentrate, the onset of estrus was faster (p<0.05) both in the first and second estrus synchronization. In all groups, the duration of estrus from the second estrus synchronization was longer (p<0.05) compared to the first estrus synchronization (Table 6). The functional structures of the ovaries identified based on ultrasonography examination in this study are as follows: Before the first PGF2α injection, the composition of the functional structures in the ovaries varied; all goats had Graafian follicles and corpus luteum; only goats in the T2 group also had dominant follicles.

In the first estrus (after the first PGF2α injection), all goats had Graafian follicles and dominant follicles; only goats in the T0 group still had corpus luteum. Before the second PGF2α injection, all groups of goats had corpus luteum and dominant follicles; only goats in the T0 group still had dominant follicles. In the second estrus (after the second PGF2α injection), the corpus luteum and dominant follicles were no longer visible. At this time, all goats had 1-2 growing follicles (Table 7).

Pregnancy rate and litter size

In goats diagnosed as pregnant on the 21st day after insemination, progesterone levels were higher ($p<0.05$) compared to goats that were not pregnant (Table 8).

Pregnancy estimation based on the absence of estrus showed that all did not return to estrus on the 21st day after mating. However, the results of pregnancy examination based on progesterone hormone levels on the 21st day after insemination showed that only group T2 was pregnant, while 25% of groups T0 and T1 were diagnosed as not pregnant.

Based on ultrasound examination on day 45 after insemination, only group T1 maintained pregnancy the same as the diagnosis results using progesterone levels on day 21. In groups T0 and T2, the pregnancy diagnosis results on day 45 were lower than the pregnancy diagnosis on day 21. All goats diagnosed as pregnant based on ultrasound examination had given birth with 1-2 kids per doe (Table 10). There was no significant difference ($p>0.05$) in progesterone hormone levels on day 21 between goats that gave birth to a single kid and those that gave birth to twins.

Table 5: Effect of minerals and concentrates supplementations on progesterone (ng/mL) serum levels in Pote.

Group	First synchronization		Second synchronization	
	progesterone	**Estrogen**	**progesterone**	**Estrogen**
T0	4.74±0.89	150.05±29.91	6.13±1.26	168.01±30.72
T1	5.36±1.21	154.66±36.39	4.02±0.34	186.17±20.95
T2	4.43±1.03	132.03±10.74	6.10±1.02	165.52±39.47

T0: animals were fed forage 3 kg/day, T1 and T2: animals were fed forage 3 kg/day and supplemented with minerals (0.5 g/kg BW/day) or concentrate (0.5 g/kg BW/day), respectively.

Table 6: Effect of minerals and concentrates supplementations on the rate onset and duration of estrus after synchronization using prostaglandin F2α of Pote.

Group	First synchronization			Second synchronization		
	Estrous rate	Onset of estrus	Duration of estrus	Estrous rate	Onset of estrus	Duration of estrus
T0	50% (10/20)	48.15±2.06^{a}	11.87±0.52^{A}	100% (20/20)	48.60±0.91^{a}	24.10±0.45^{B}
T1	70% (14/20)	46.80±2.80^{a}	12.40±1.26^{A}	100% (20/20)	43.60±1.55^{a}	24.30±0.98^{B}
T2	75% (15/20)	36.90±1.60^{b}	12.13±0.52^{A}	100% (20/20)	36.13±1.12^{b}	24.20±0.89^{B}

Different superscripts a and b in the same column and A and B in the same row were significantly different ($p<0.05$). T0: animals were fed forage 3 kg/day, T1 and T2: animals were fed forage 3 kg/day and supplemented with minerals (0.5 g/kg BW/day) or concentrate (0.5 g/kg BW/day), respectively.

Table 7: Ultrasonographic features of ovarian morphology of Pote goats before and after estrus synchronization.

Stages	**Group**	**Corpus luteum**	**subordinate follicle**	**Graafian follicle**
Pre-Synch 1	T0	2.80±0.45	–	4.40±1.51
	T1	1.07±0.26	–	3.95±0.22
	T2	1.10±0.32	1.73±0.46	2.10±1.20
1st estrus synchronizazion	T0	1.20±0.45	1.10 ±0.32	3.93±2.15
	T1	–	1.20±0.45	1.95±1.19
	T2	–	1.40±0.51	3.80±0.45
Pre-Synch 2	T0	1.75±0.50	1.27±0.47	1.57±0.76
	T1	1.07±0.26	–	3.10±0.85
	T2	1.10±0.31	–	4.10±1.45
2nd estrus synchronizazion	T0	–	–	1.05±0.22
	T1	–	–	1.10±0.31
	T2	–	–	1.10±0.31

Pre-Synch: before treatment

Table 8: Effect of minerals and concentrates supplementations on the serum progesterone levels in pregnant and non-pregnant Pote goats without or with the addition of minerals or concentrate boosters.

Group	Pregnant	Not pregnant
T0	8.77±2.01^a (n=15)	0.20±0.09^b (n=10)
T1	5.86±0.47^a (n=15)	0.17±0.03^b (n=10)
T2	8.89±1.4 2^a (n=20)	n.a.

T0: animals were fed forage 3 kg/day, T1 and T2: animals were fed forage 3 kg/day and supplemented with minerals (0.5 g/kg BW/day) or concentrate (0.5 g/kg BW/day), respectively.

Table 9: Effect of minerals and concentrates supplementations on the pregnancy of Pote goat based on progesterone levels at 21 days and ultrasonography at 45 days after artificial insemination

Group	Non-return rate	Pregnancy diagnosis		Early embryonic death	Litter size
		P4	USG		
T0	100% (20/20)	75% (15/20)	50% (10/20)	25% (5/20)	1.50 (15/10)
T1	100% (20/20)	75% (15/20)	75% (15/20)	0% (0/20)	1.00 (15/15
T2	100% (20/20)	100% (20/20)	75% (15/20)	25% (5/20)	1.67 (25/15)

P4: progesterone, USG: ultrasonography. T0: animals were fed forage 3 kg/day, T1 and T2: animals were fed forage 3 kg/day and supplemented with minerals (0.5 g/kg BW/day) or concentrate (0.5 g/kg BW/day), respectively.

Table 10: Effect of minerals and concentrates supplementations on serum progesterone levels at 21 days from the time of artificial insemination

Group	Single	Twin
T0	7.89±1.92 (n=5)	9.87±0.89 (n=5)
T1	5.86±0.47 (=15)	n.a.
T2	8.56±2.17 (n=5)	9.86±0.32 (n=10)

T0: animals were fed forage 3 kg/day, T1 and T2: animals were fed forage 3 kg/day and supplemented with minerals (0.5 g/kg BW/day) or concentrate (0.5 g/kg BW/day), respectively.

Discussion

Feed treatment in this study was carried out for 45 days. Research to determine the effect of nutrition on the reproductive physiology of livestock was carried out during a period of two estrus cycles, or around 36-42 days (Hyder et al., 2013) or for 35 days (Sitaresmi et al., 2023). In this study, even though it was the dry season, farmers always fed their goats with green fodder in the fields so that the goats did not lack feed in terms of quantity. In the dry season, where the quality and quantity of feed decreased, the goat's weight did not decrease if the physiological basal metabolic needs were still met (Underwood et al., 2015). There is no change in body weight even though there is the addition of minerals or booster concentrates. During the study, the goats were at body condition scoring (BCS) 3-4 on a scale of five. BCS is a simple indicator of the fat reserves available under the skin, around the base of the tail, backbone, and hips, which describes the nutritional condition of the goat. The ideal BCS range in pregnant females is 3.0-3.5 (Ghosh et al., 2019). Goats should not be under conditions (<2.5) during the dry season so as not to interfere with their reproductive system (Sahoo et al., 2022).

In this study, the levels of blood urea nitrogen, total protein, and albumin were still within the normal range of healthy goats. In healthy goats, the values of BUN, total protein, and albumin were 20.8±1.5 mg/dl, 8.32±0.25 g/dl, and 3.64±0.17 g/dl (Rashmi et al., 2022). More specifically, in female goats, Akyüz et al. (2020) blood urea nitrogen levels were reported at 20.03-32.09 mg/dL, total protein at 6.32-7.96 g/dL, and albumin at 1.94-3.01 g/dL. BUN levels are a reflection of crude protein intake in goats and other ruminants (Torres-Cavazos et al., 2023). In pathological conditions, BUN values are higher, while total protein and albumin values are lower (Rashmi et al., 2022), for example, due to gastro-intestinal parasite infection disorders (Chovanová et al., 2021). The goats in this study had been dewormed before treatment. Inadequate feed intake and nutrient supply lead to low blood protein levels and adversely affect the reproductive cycle of Kacang goats (Khalil et al., 2019).

Synchronization of estrus in female goats can be done by injecting PGF2α intramuscularly on the condition that the female goat is not pregnant and has a corpus luteum in its ovary. In this study, these conditions were confirmed by an ultrasound examination that the goat was not pregnant, had a corpus luteum, and had high progesterone levels. Synchronization of estrus can be done by injecting PGF2α intramuscularly twice with an interval of eleven days. Although supplementations with minerals and concentrate did not affect weight gain and blood chemistry, a higher estrus rate was recorded in treated groups compared to control (T0). Nutrition, as one of the environmental factors, induces epigenetic modifications in oocytes, embryos, and their development (Cai et al., 2021). Genes show specific expressions that are very sensitive to epigenetic changes. These changes are mediated by DNA methylation patterns and histone protein modifications that work together with chromatin structure to determine their transcriptome in the antral follicle phase. Methylation is completed at the metaphase II stage of oocyte nuclear maturation (Sendžikaitė and Kelsey, 2019). The metabolic state of the mother during the follicle growth period before ovulation can have an important influence on the methylation process. More specifically, the amino acid tyrosine functions as a nutritional signal that affects the nerve center, controlling the release of GnRH, which controls gonadotrophin secretion (Radovick et al., 2012). Disruption of FSH secretion affects the growth of gonadotrophin-dependent follicles. Insufficient follicle growth produces insufficient 17-B estradiol to cause signs of estrus and feedback to the anterior pituitary for LH surge for ovulation (Lee et al., 2021).

In this study, the maturity of the corpus luteum before the first estrus synchronization still varied, so its sensitivity to PGF2α stimulation also varied. After the first PGF2α injection, goats with mature corpus luteum responded to estrus, while those with immature corpus luteum experienced estrus later. At the time of the second PGF2α injection, which occurred 11 days after the first injection, the corpus luteum was 8 to 9 days old. As a result, estrus occurred only a few hours apart. A corpus luteum of this age is particularly susceptible to regression caused by PGF2α, as it was observed that the goat corpus luteum could be detected at just five days old (Dogan et al., 2020), and without PGF2α injection, the corpus luteum regressed on days 17-19 after estrus (Chao et al., 2008). Thus, in the results of the second estrus synchronization with PGF2α injection, all goats were in estrus.

In two estrus synchronizations, the onset of estrus in goats, given additional minerals and concentrate, was faster. The duration of estrus in all groups of the second estrus synchronization results was longer than the results of the first estrus synchronization. The average duration of estrus in goats was 36 hours (between 24 and 48 hours) (Arrebola et al., 2022). The onset and duration of estrus in goats are influenced by nutrition. Adequate nutrition in quantity and quality can meet the physiological needs of the body's metabolism, including smooth folliculogenesis. Microalgae supplementation to improve nutrition in Anglo-Nubian crossbred goats showed an increase in the intraovarian blood perfusion area, an increase in the ovarian artery pulsatility index, and an increase in follicle development (Silva et al., 2023). Follicular development in goats can consist of four waves, with ovulation occurring in the fourth wave. The emergence of follicular waves is closely related to increased FSH secretion (Medan et al., 2003). Short-term supplementation increases follicle growth rates (Alves et al., 2019). It is suspected that the addition of concentrate booster nutrients increases the rate of folliculogenesis and shortens the follicle growth wave to two or three waves so that the onset of estrus is shorter.

In this study, the duration of estrus in the second estrus was longer than the first estrus. This is different from the report of Wondim et al. (2022), in which the duration of estrus from PGF2α single injection was 41.6±1.39 hours, not significantly different from double injection 42.1±1.56 hours. The difference in the duration of estrus between the first and second cycles observed in this study is not likely due to nutritional factors, as there were no significant variations in estrus duration among the groups during the same observation period. Cadena-Villegas et al. (2023) reported that metabolic reconstituent during estrus synchronization did not affect the duration of estrus. The duration of estrus in the second estrus was longer than the first estrus, suspected to be due to endocrine factors related to the uniformity of functional structures in the ovaries. At the time of the first estrus synchronization, the ovaries contained corpus luteum with varying ages. At the time of the second estrus synchronization, the ovaries contained corpus luteum with relatively the same age as a result of the first PGF2α injection.

A wave of follicle growth followed the second PGF2α injection. The number of days to ovulation is influenced by the presence of a dominant follicle in producing E2 (Kaneko et al., 2020). It is suspected that the level of estrogen in the circulatory system in the second estrus is sufficient to cause signs of estrus, but it takes longer to reach the threshold value that can trigger the LH surge; thus, the ovulation and the duration of estrus became longer.

The duration of estrus of 48 hours is more advantageous for farmers than 36 hours because there is more time to detect estrus. In the first estrus, all goats had Graafian and dominant follicles. However, the control group goats still had a corpus luteum. Before the second PGF2α injection, all groups of goats also had a corpus luteum. In the second estrus, there was no more corpus luteum; all goats had 1-2 growing follicles.

The non-return rate is an estimate of pregnancy following the mating of goats, which is a common practice among breeders. In this study, none of the goats returned to heat on the 21st day after mating. However, using non-return to heat as a method for estimating pregnancy is the least reliable approach. Early pregnancy diagnosis can be performed on the 21st day after insemination, indicated by higher progesterone levels compared to non-pregnant goats. A progesterone concentration of 1.5-2.5 ng/mL in goats distinguishes pregnant from non-pregnant animals within one estrous cycle after insemination (Boscos et al., 2003). In goats with serum progesterone levels of less than 1.5 ng/mL within 18-22 days after insemination, they were diagnosed as non-pregnant with 100% accuracy. Pregnancy diagnosis by measuring serum progesterone levels on day 22 after insemination has an accuracy of 83.33-94.73% (Susilowati et al., 2020), and using progesterone test strips with blood samples 18-22 days after insemination with 100% accuracy (Melia et al., 2023) when confirmed by ultrasound and birth. The difference in pregnancy diagnosis results based on progesterone levels compared to ultrasound examination is suspected to be due to early embryonic death (Susilowati et al., 2020).

At the peak of estrogen levels in the blood, a positive feedback mechanism stimulates the anterior pituitary gland to release luteinizing hormone, which triggers ovulation. Additionally, the granulosa cells and theca cells in the remaining ovarian tissue undergo luteinization to form the corpus luteum, which produces the hormone progesterone (Holesh et al., 2023). If the goat is not inseminated or is inseminated but not pregnant, then on days 17-19 after estrus, the hormone PGF2α is secreted from the endometrium. Corpus luteum regression is followed by the return of estrus in non-pregnant female goats. In non-pregnant goats, on day 21, progesterone hormone levels reach basal levels. If the female goat is inseminated, fertilization follows to produce a zygote, which then develops into an embryo. Embryos aged 14-17 days produce interferon tau, which prevents the release of the PGF2α hormone from the endometrium so that the corpus luteum is maintained and does not regress (Susilowati et al., 2020). In this study, the pregnant goats on the 21st day of pregnancy showed higher levels of progesterone hormone.

The success of fertilization is influenced by the quality of the ovum ovulated by the female goat. The effect of nutrition on oocyte quality begins when the ovarian follicles emerge from the primordial pool and begin to grow. Lack of nutrition during this period reduces the number of follicles that emerge and the number of ovulations. In the dry season, the nutrient content of forage is low and higher in crude fiber (Lee, 2018). High fiber intake interferes with steroid synthesis and reduces its levels in circulation. Fiber can bind cholesterol (as a steroidogenesis material) in the intestine or change the activity of bacterial enzymes and interfere with enterohepatic estrogen circulation (Durník et al., 2022). Low concentrations of estradiol in the circulatory system reduce the negative feedback of estradiol on the hypothalamic-pituitary axis to provide LH impulses, reduce ovarian gonadotrophs, and reduce oocyte maturity. Changes in the LH profile can change the composition of follicular fluid (Konstantinidou et al., 2023). Decreased oocyte quality caused by malnutrition is positively related to oocyst maturity and follicular fluid estradiol concentration, fertilization failure, and pregnancy failure.

Pregnancy failure after the insemination of goats can also be caused by early embryonic death, namely the death of embryos within one estrus cycle after the insemination of goats. Embryo death can be caused by malnutrition and vitamin deficiencies (Gernand et al., 2016). Retinoids are the primary metabolites of vitamin A, playing roles in cell proliferation, differentiation, expression of growth factors, gene transcription, and steroidogenesis, all of which are essential for maintaining embryonic survival (Gudas, 2022). Folic acid is essential for nucleic acid synthesis during embryonic development and growth. Vitamin C can enhance luteal function, supporting progesterone synthesis for early pregnancy maintenance (Coker et al., 2023). Nutrition affects the methylation process in the early stages of embryonic development. The quantity and quality of food consumed by the mother can have a major and lasting impact on the development of the embryo and fetus. Changes in food intake lead to fluctuations in the levels of metabolic hormones like glucose, insulin, leptin, and IGF-1, as well as reproductive hormones, which subsequently influence the composition of secretions in the reproductive tract (Athar et al., 2024). Early-phase embryos depend on histotrophic nutrition from the uterine lumen environment. There is a mechanical endocrine relationship between increased dietary fiber before mating and increased prenatal survival. Malnutrition reduces ovulation rate, oocyte maturity, blastocyst development, and function (Ashworth et al., 2009).

Correcting selenium deficiency reduces embryonic mortality during implantation. Selenium enhances the fertilization rate, aids uterine contractions, and facilitates sperm transport within the female reproductive tract (Dahlen et al., 2022). Malnutrition disrupts endometrial expression of progesterone receptor function genes.

The accuracy of pregnancy diagnosis based on progesterone levels one estrus cycle after mating is 75–86% in pregnant goats and 90–100% in non-pregnant goats (Singh et al., 2021). In addition, the failure to return to estrus cycles occurs at a very early stage after mating, and the progesterone concentration is 1.5 ng/mL at 25–30 days of pregnancy (Yamamoto et al., 2018).

Malnutrition can lead to abnormalities in the ratio of oestradiol to progesterone, interfering with embryonic development. Maternal mRNA expression is essential for regulating maternal development until the mid-blastocyst stage, which is influenced by progesterone levels (Winata and Korzh, 2018). Lack of food reduces the availability of progesterone and interferes with protein synthesis in the embryo. Embryo development is inhibited if there is a disruption in the progesterone-dependent gene (Wetendorf and DeMayo, 2014). Changes in the quantity of food consumed or the composition of food affect blastocyst survival.

All goats diagnosed as pregnant based on ultrasound examination have given birth with 1-2 kids per mother. The progesterone hormone levels on day 21 between goats that gave birth to single kids and those that gave birth to twins were relatively the same. The average luteal phase progesterone levels in non-pregnant goats were 8.6±1.02 ng/mL, in single pregnant goats was 9.74±1.6 ng/mL, and in twin pregnancies was 12.5±1.3 ng/mL (Fernández et al., 2021).

Environmental disturbances can impact the development of oocytes and embryos by altering gene expression. In this study, goats that were maintained during the dry season, either without additional support or with the inclusion of minerals and concentrates, did not show significant changes in body weight or various blood chemical levels. However, the treatment did improve fertility. Therefore, during the dry season or in times when animal feed becomes scarce due to climate change, it is crucial to provide additional concentrates and minerals to support fertility in goats.

Conclusions

Although supplementation did not significantly increase body weight or cause noticeable changes in blood chemical parameters, it did have a positive impact on fertility indicators, including estrus rates, ovarian activity, pregnancy rates, and kidding rates. Further research is necessary to confirm these findings in various animal species and environments, which could enhance reproductive and health management strategies.

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