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Short communication

Ovarian activity, metabolic and physiological parameters of canindé goats submitted to short-term supplementation with licuri oil

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ABSTRACT

This study aimed to evaluate the estrous behavior, ovarian activity, feed intake, metabolic and physiological parameters of Canindé goats supplemented for nine days with Licuri oil (*Syagrus coronata*). Thirty-six adult female goats were distributed randomly into two groups: control group (n = 18) without Licuri oil and the oil group (n = 18) receiving a supplement containing 3% Licuri oil. The short-term food supplementation was associated with an estrus synchronization protocol. The number of ovulations and follicle diameter were evaluated by ultrasonography. Dry matter intake (DMI) and nutrient intake, water ingestion, and physiological parameters such as rectal temperature (RT), respiratory rate (RR), heart rate (HR), and serum concentrations of cholesterol and non-esterified fatty acids (NEFA) were also determined. As concern statistical analysis, ANOVA followed by Fisher's LSD was used and the differences were considered significant when $P < 0.05$. The control group showed higher HR and RR in the afternoon shift compared to the Oil group. The Oil group showed lower DMI but higher ether extract intake, and serum cholesterol and NEFA concentration than control group. Treatments did not affect estrus response and ovulation rate, but the Licuri oil increased the number of small follicles. The supplementation of Licuri oil improved thermal comfort during the dry season and a marginal increase in the ovarian activity was observed.

1. Introduction

Nutrition plays a crucial role in regulating reproductive performance. It has been observed that the sudden intake of increasing levels of energy can increase ovarian activity and induce the manifestation of estrus (Nogueira et al., 2016). The energy value of a food supplement is the most important factor in the relationship between nutrition and reproduction in goats. Energy sources such as vegetable oils both increase energy intake and reproductive performance in ruminants (Thomas and Williams, 1996). According to Nogueira et al. (2016), when evaluating the reproductive response of goats in seasonal anoestrus supplemented with corn and/or hormonally synchronized, they observed that corn supplementation for only 9 days promoted an increase in the number of ovulations of goats by 43 %.

The use of vegetable oils in animal nutrition can improve reproductive performance, since these substances participate in the synthesis of steroid hormones. For example, palmitic acid and lauric acid, present in Licuri oil, affect serum cholesterol formation and steroidogenesis (Grundy, 1994; Vilanova et al. 2012). However, no data were found in the literature regarding supplementation with Licuri oil for nine days associated with an estrus synchronization protocol in goats.

We hypothesize that the addition of Licuri oil in the diet of Canindé goats for nine days during estrus synchronization could affect follicular development and the number of ovulations, as it happens in goats supplemented with other energy sources (corn, other vegetable oils), without losses in dry matter and nutrient intake. Therefore, this study aimed to evaluate the estrous behavior, ovarian activity, feed intake, serum concentrations of beta-hydroxybutyrate, cholesterol, and non-esterified fatty acids (NEFA), and physiological parameters of

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Canindé goats that received Licuri oil for nine days during estrous synchronization.

2. Material and methods

2.1. Location and animals

This study was approved by the Ethics Committee on Animal Experimentation (CEUA) of Embrapa Semiárido under protocol No. 01/2021. The experiment was conducted at the Animal Metabolism sector of Embrapa (09°13' S and 40°29' W). The climate is classified as *BSh* according to Köppen and Geiger (1928), with an annual rainfall of 443 mm. The meteorological data were obtained from the weather experimental station at Embrapa Semiárido in Petrolina-PE, Brazil.

At the beginning of the experiment, the animals were 5.66 ± 0.26 years old (68 months old); they weighted 33.81 ± 0.85 Kg and had a body condition score of 3.21 ± 0.08. The animals were placed in a ventilated, steel-covered shed, each in an individual pen (1.6 m × 1.0 m) containing individual feeders and drinkers, during 33 days, 21 for adaptation and 12 for data collection.

In total, 36 adult female goats distributed in two groups: Control group (n = 18) that received a concentrated supplement without Licuri oil, and an Oil group (n = 18) that received a concentrated supplement with Licuri oil. Before the experiment, goats were dewormed with levamisole hydrochloride (Ripercol® L – Zoetis) and the reproductive system was examined by ultrasonography. The animals were weighed at the beginning and at the end of the experiment to obtain the initial and the final body weight.

2.2. Experimental diets and feeding management

The diets consisted of spineless cactus (*Opuntia stricta* Haw.), elephant grass (*Pennisetum purpureum*), soybean meal, and ground corn (Table 1). Mineral salt was provided ad libitum, and the diet was formulated according to the control group (60 % roughage, 40 % concentrate; 0% Licuri oil) and the Oil group (60 % roughage; 37 % concentrate; 3 % Licuri oil). The diets were provided as a mixture twice a day: at 9:00 am and at 3:00 pm (Table 1). The goats were fed according to the dry matter intake of the previous day by daily weighing the leftovers in the feeders and adjusting the amount to be provided in order to ensure a 10 % leftover fraction.

Table 1 –
Proportion of ingredients and chemical composition of the feed supplementation.

Ingredient (%)	Control	Licuri oil
Cactus	10	10
Elephant grass	50	50
Soybean meal	10	9
Ground corn	30	28
Licuri oil	0	3
Nutrient (%)		
DM %*	60.95	59.00
OM	92.88	90.02
CP	11.07	10.50
$_{NDFap}$	42.50	42.00
ADF	23.50	23.34
EE	4.38	7.14
CE (cal/g)	4.00	4.15
TC	77.43	75.37
NFC	32.06	30.65
CF	45.37	44.72
LIG	8.62	8.55
TDN	76.3	76.7

* Percentage of DM; DM – dry matter; OM – organic matter; CP – crude protein; FDNcp – neutral detergent fiber, corrected for ash and protein; ADF – acid detergent fiber; EE – ether extract; CE – crude energy (calorie/gram); TC – total carbohydrates; NFC – non-fiber carbohydrates; LIG – lignin. TDN – Total Digestible Nutrients.

Supplementation with Licuri oil represented 3% of the dry matter in the diet provided to the Oil group, corresponding, on average, to 30 mL per day, split into two aliquots of 15 mL. A dosing syringe was used for oral administration of the oil few minutes after the diet. On the other hand, the control group received water with the dosing syringe. The fatty acids were determined according to ISO 5509, as previously described by Lima et al., (2013). The saturated fatty acids present in Licuri oil were lauric acid (52 %) and myristic acid (16 %), followed by palmitic acid (7 %), capric acid (5 %), caprylic acid (3 %), and traces of tridecanoic acid, stearic acid, eicosanoic acid, and lignoceric acid, also showing 10 % oleic acid and traces of linoleic acid (unsaturated fatty acids).

2.3. Chemical composition and determination of dry matter, nutrient and water intake

Forage, concentrate and leftover samples were analyzed as described by the Association of Official Analytical Chemists (AOAC, 2016) for dry matter (DM), organic matter (OM), crude protein (CP) and ethereal extract (EE). The content of neutral detergent fiber (NDF), Neutral detergent fiber with corrections for protein and ash (NDF_{ap}), acid detergent fiber (ADF) and lignin were determined according to Van Soest et al. (1991).

Non-fiber carbohydrates (NFC) were calculated as described by Hall (2000). Total carbohydrates (TC) were calculated as described by Sniffen et al. (1992) and fibrous carbohydrates (FC) were obtained through the difference between TC and NFC. Total digestible nutrients (TDN) were calculated as described by Chandler (1990). The gross energy (GE) was estimated by calorimetric bomb, as described by Silva and Queiroz (2009).

The dry matter intake (DMI) was determined by the difference between the diet provided and the leftovers on the following day. The intake of nutrients (DM, OM, CP, EE, NDF_{ap}, ADF, CFN, TC) was obtained by the difference between the total of each nutrient/DM provided in the diet and the total contained in the leftovers, expressed as kilograms per day (Kg/day). The total water intake was determined by the sum of the free water intake with the water intake via the food. The total water intake was determined by the sum of the free water intake with the water intake via the food, expressed as milliliters per day (mL/day).

2.4. Estrus synchronization, ovarian follicular dynamics and estrous behavior

Estrus synchronization was performed with an injection containing 100 μg of cloprostenol, vaginal sponge with medroxyprogesterone acetate and 100 IU of equine chorionic gonadotropin, following the synchronization and nine days supplementation protocol described by Nogueira et al. (2017) (Fig. 1). During the three days that preceded ovulation, the ovarian follicular dynamics was monitored by ultrasonography following the methodology adapted from Nogueira et al. (2016). The ovarian ultrasound images were obtained with a B-mode ultrasound scanner (SAEVO, model FP 102, Alliage S/A, São Paulo, 2019). The follicles were classified into three categories: small (≥ 2 and ≤ 3 mm), medium (> 3 and < 4 mm), and large (≥ 4 mm). The number of corpora lutea (ovulations) was determined by ultrasonography seven to 11 days after the sponges were removed. Estrus detection was performed by 2 bucks, every four hours, from 12 h to 64 h after sponge removal. The females were considered in estrus when they accepted mounting by males.

2.5. Metabolic, physiological and environmental parameters

The serum concentrations of cholesterol, non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) in the females were determined in blood samples collected every morning on the days 0, 7 and 9 post-

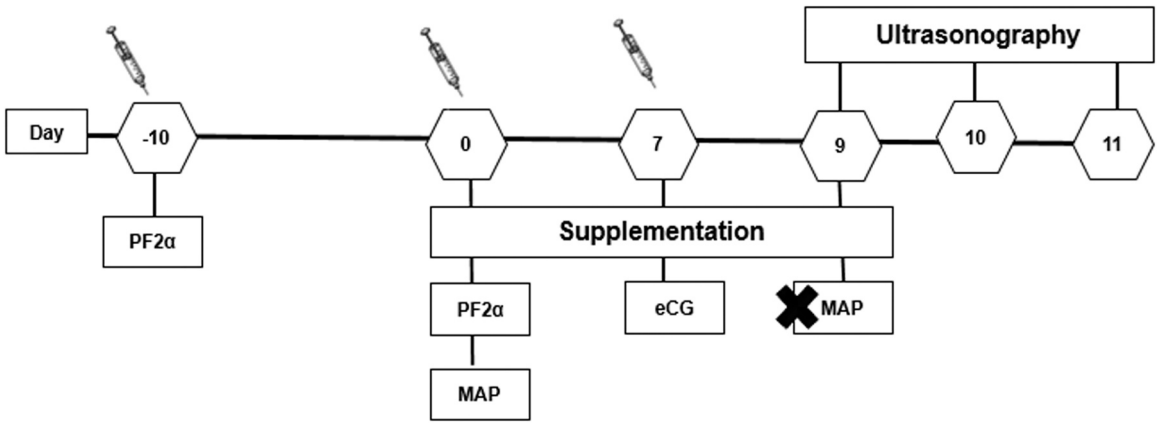

Fig. 1. Schematic design of the experiment, outlining the feed supplementation, estrus synchronization protocol, PGF2α (prostaglandin F2α), MAP (medroxyprogesterone acetate sponge) and ultrasonographic evaluations.
Source: Adapted from Nogueira et al. (2017).

insertion of the sponge. The physiological parameters (rectal temperature, respiratory and heart rates) were measured at 08:00 and 14:00 h on the days 2, 4 and 8 after sponge insertion. The environmental parameters of ambient temperature (AT), relative air humidity (RH), wind speed (WS), solar radiation (SR), and rainfall were obtained from the weather station at Embrapa Semiárido.

2.6. Experimental design and statistical analysis

Completely randomized design with two treatments (experimental groups) with 18 animals in each group was used. Analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) were used to compare the effects of the treatments on the following variables: the interval between the end of synchronization and the beginning of estrus, the duration of estrus, follicular diameter, number of follicles and ovulations. Repeated measures was used to compare the effects of treatments, time and interactions (Group x Time) for the plasma concentrations of Cholesterol, BHB, NEFA and number of follicles. The meteorological and physiological parameters were analyzed by 2 × 2 factorial arrangement (two diets and two times of the day). The online statistical software SAS® OnDemand for Academics (SAS 3.8 Enterprise Edition, EUA, 2020) was used in the statistical analyses, and the differences were considered significant when $P < 0.05$. The proportion of animals in estrus were compared by the chi-square test using the software Epi Info (Epi Info 7.2.5, Atlanta, GA, USA, 2021).

3. Results

The environmental parameters of AT, RH, WS, and SR differed between the morning and afternoon shifts, with the highest means during the afternoon for AT (26.19 °C vs 32.06 °C) and SR (340.73 w/m^2 vs 589.88 w/m^2) and the highest means during the morning for RH (72.75 % vs 48.16 %) and WS (3.02 m/s vs 1.45 m/s) ($P < 0.05$).

The control group showed higher HR and RR values ($P < 0.05$) during the afternoon compared to the morning. For the Oil group, the RR and RT were higher ($P < 0.05$) in the afternoon than in the morning (Table 2). For the comparisons between experimental groups, the control group showed higher HR and RR values ($P < 0.05$) in the afternoon compared to the oil group (Table 2). There was no significant difference ($P > 0.05$) for the final body weight (34.13 vs 33.36) and the total water intake (1883.6 vs 1592.7 mL), respectively, for control and Oil groups.

Compared to the Oil group, the control group showed a higher values ($P < 0.05$) of nutrient intake, as following: dry matter intake (714.45 vs 514.23 g/animal/day), organic matter (695.65 vs 454.63 g/day), crude protein (82.91 vs 52.98 g/day), neutral detergent fiber corrected for ash and protein (347.67 vs 232.49 g/day) and acid detergent fiber (176.01 vs 117.89 g/day), respectively. However, the Oil group showed a higher intake ($P < 0.05$) of ether extract (32.8 vs. 113.6 g/day), non-fiber carbohydrates (240.12 vs 384.72 g/day), and total carbohydrates (268.98 vs 384.74 g/day), respectively, for control and Oil groups.

Table 2 –
Mean ± standard error of means of the heart rate, respiratory rate, and rectal temperature of Canindé goats in the control and oil groups at different times of the day.

Physiological variable	Time of the day	Control	Licuri oil
Heart rate (beat/min)	Forenoon	83.40 ± 2.56^{b}	84.81 ± 2.88
	Afternoon	95.03 ± 2.26^{Aa}	88.07 ± 2.58^{B}
Respiratory rate (mov/min)	Forenoon	30.74 ± 2.73^{Ab}	24.59 ± 1.16^{Bb}
	Afternoon	39.18 ± 2.34^{Aa}	34.44 ± 3.73^{Ba}
Rectal temperature (°C)	Forenoon	38.71 ± 0.04	38.70 ± 0.04^{b}
	Afternoon	38.82 ± 0.02	38.85 ± 0.03^{a}

Means followed by uppercase letters (A, B) indicate group (Control or Oil) difference ($P < 0.05$) in the same time of the day. Means followed by lowercase letters (a, b) indicate time of the day difference ($P < 0.05$) in the same group.

The concentrations of cholesterol, NEFA and BHB from day 0 to day 9 after sponge insertion are presented in Fig. 2. The concentration of serum cholesterol was significantly higher ($P < 0.05$) in the Oil group on days 7 and 9 (considering as day zero the day the intravaginal sponge was inserted) (Fig. 2a). Similarly, the NEFA concentrations were higher ($P < 0.05$) in the Oil group on days 7 and 9 compared to the control group (Fig. 2b). During the experimental period, no differences were observed ($P > 0.05$) between the oil and control groups for the BHB concentrations (Fig. 2c).

In general, 80.5 % (29/36) of the females detected in estrus, showed ovulations. Furthermore, 19.4 % (7/36) of the total females did not show estrous behavior, but from these the 71.4 % (5/7) of goats that was not detected in estrus had ovulations without estrous symptoms. In the first 32 h after sponges withdrawal, 79 % (23/29) of the goats were already detected in estrus. No significant differences were recorded between oil and control groups in estrus detection percentage (72.2 vs 88.8 %), mean intervals from sponge withdrawal to estrus detection (25.8 ± 2.6 vs 32.0 ± 3.4 h), estrus duration (39.1 ± 5.0 vs 29.7 ± 3.7 h), ovulations (1.77 ± 0.20 vs 1.83 ± 0.12) and the size of preovulatory follicle (6.40 ± 0.15 vs 6.50 ± 0.28), respectively for control and oil groups.

The number of small, medium and large follicles on days 9, 10 and 11 are presented in Fig. 3. The number of small follicles was significantly higher ($P < 0.05$) in the Oil group on days 9 and 10 (considering as day

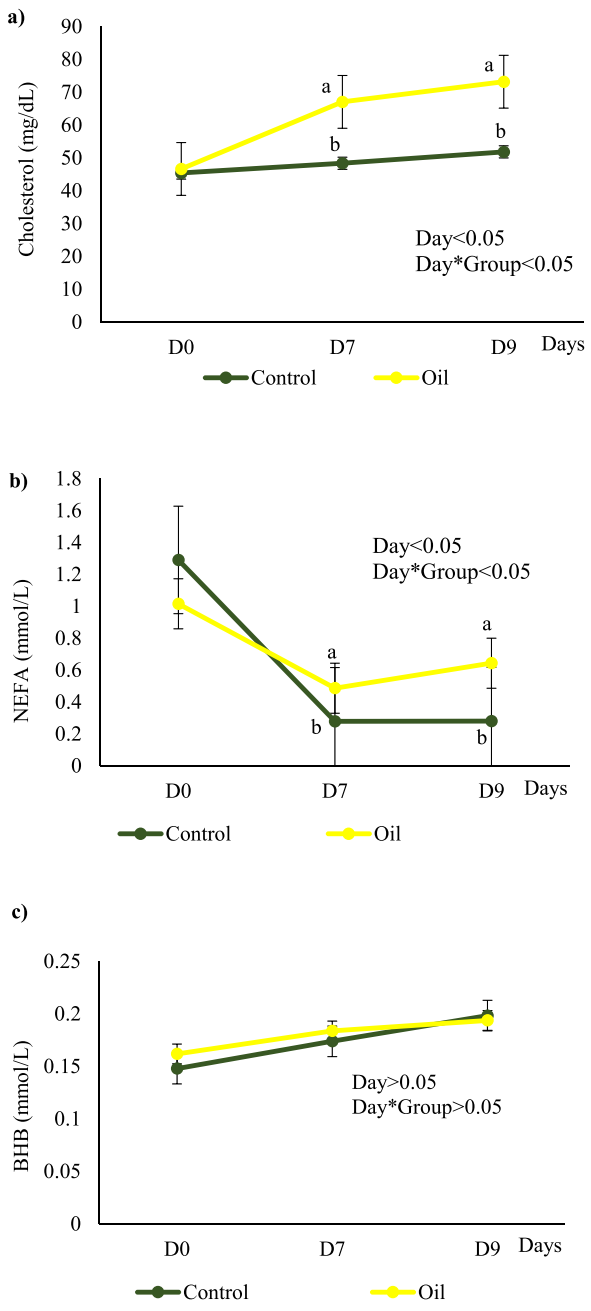

Fig. 2. Mean values of a) cholesterol (mg/dL), b) non-esterified fatty acids (NEFA) (mmol/L), and c) β- hydroxybutyrate (BHB) (mmol/L) in Canindé goats supplemented with Licuri oil.

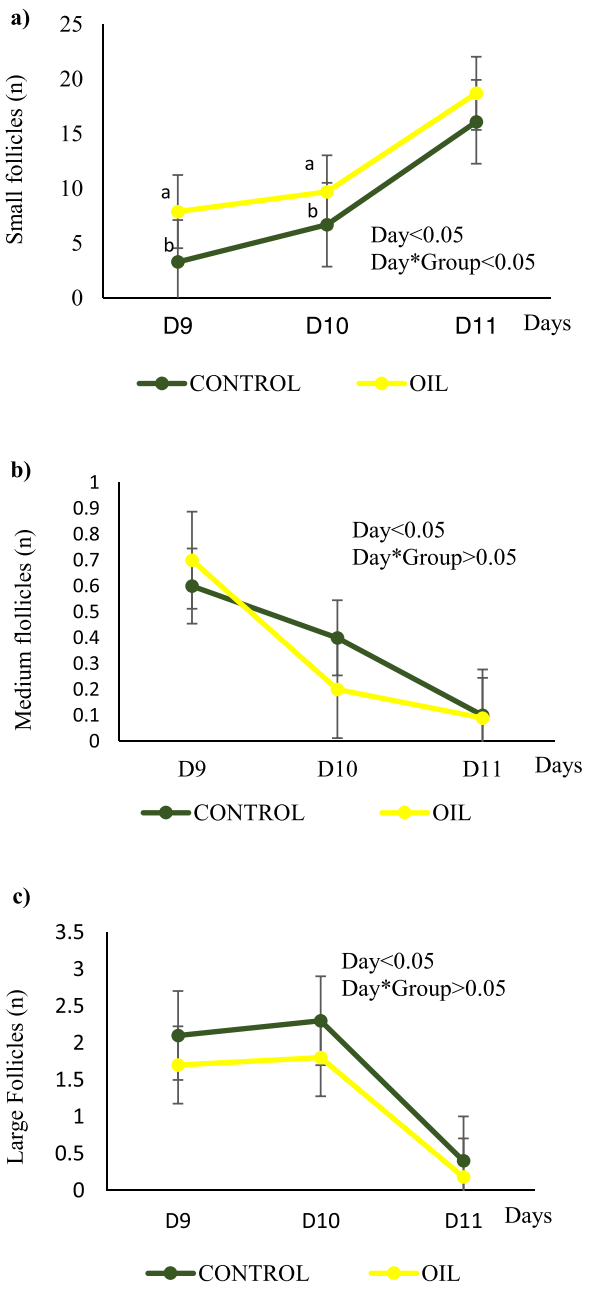

Fig. 3. Mean of the number of a) small follicles (2–3 mm), b) medium follicles (>3 to <4 mm), and c) large follicles (>4 mm) in Canindé goats subjected to supplementation with Licuri oil.

zero the day the intravaginal sponge was inserted) compared to the control group (Fig. 3a). The number of medium follicles decreased over the days ($P < 0.05$), with no clear difference between experimental groups ($P > 0.05$) (Fig. 3b). The number of large follicles decreased between days 10 and 11 ($P < 0.05$). However, there were no significant differences ($P > 0.05$) between experimental groups (Fig. 3c).

4. Discussion

There is no evidence of the effect of using licuri oil in goat supplementation on reproductive parameters. To the authors' knowledge, this is the first study to demonstrate the use of Licuri oil in short-term supplementation associated with an estrus synchronization protocol in goats. The group supplemented with Licuri oil showed better thermal comfort, lower DMI and higher concentrations of cholesterol and NEFA. The increase in NEFA concentrations is due to the mobilization of fats or body reserves, however this increase did not affect estrus behavior nor ovulations, since these results were similar between control and Oil groups. High concentrations of NEFA are detrimental to follicular development and reduces the granulosa, theca and cumulus cells growth (Vanholder et al. 2005, Baddela et al., 2020). However, steroidogenesis may be not be affected.

In the present study, the higher respiratory rate in the afternoon shift can be explained by the increase in air temperature and the decrease in relative air humidity. The respiratory rate of the animal increases to maintain the body temperature, thus dissipating more heat through evaporative cooling (Jesus et al. 2010). The Canindé goats supplemented with Licuri oil probably made less effort to maintain the homeothermic condition by dissipating heat through respiration since they

showed significantly lower values of respiratory rate and heart rate in the afternoon shift compared to the control group. According to Bagaldo, et al. (2019), this result occurs due to the use of plant oil in the diet, including Licuri oil, which decreases the heat produced by the nutrient metabolism, providing higher thermal comfort. Furthermore, lipids contain about 2.25 times more energy than carbohydrates and are known as 'cold nutrients` since, during their digestion and use by the organism, these components produce less heat than carbohydrates and proteins (Ivan et al., 2013). Thus, in regions with higher temperatures, lipids help decrease the thermal stress caused to the animal (Lu, 1989). In our study, rectal temperature in oil group remained within the range considered normal for goats, from 38.5 ºC to 40 ºC (Jesus et al., 2010), suggesting that the animals did not manifest thermal stress.

In this study, the goats supplemented with Licuri oil showed lower DMI than the control group. This lower DMI in diets with high ether extract contents can be explained by the fact that fat adheres to fiber particles in the rumen, hindering the action of microorganisms, especially cellulolytic ones, thus decreasing the digestion of fiber particles and the time of passage through the gastrointestinal tract (NRC, 2001). This scenario could help explain the DMI and $NDF_{ap}I$ reduction in the diet with 3% Licuri oil. Furthermore, the ether extract intake increased, suggesting that the higher energy density of the diet also influenced the DMI reduction, which may have decreased by a post-absorptive satiety mechanism (Cavalcanti et al., 2022).

The water intake recorded in the present study was below the average (4.42 liters) for Canindé goats (Ribeiro et al. 2006). This result can be explained by the presence of cactus in the diet, since juicy forage, characterized by showing high nutrient concentrations and low dry matter contents are sources of water to animals (Costa et al., 2009).

Through ultrasound examinations, ovulations were observed in the 71.4 % of animals that did not show estrus. Variations in the social environment can inhibit or stimulate ovulation (Chemineau et al., 1992; Hogan et al., 2004). Therefore, the fact that some animals did not show estrus can be explained by the stress caused by the confinement of adult goats, which were raised under the semi-extensive system in the Caatinga area. Furthermore, at the beginning of rainfall, during the transition from the dry to the rainy period, there were intermediate situations such as estrus without ovulation (anovulatory estrus) or ovulations unaccompanied by estrous behavior (silent ovulations) (Chemineau et al., 1992). However, in goats, this event is less common compared to females of other species since, usually, the beginning of rainfall induces the onset of estrus accompanied by ovulation (Lopes Júnior et al., 2001).

In our study, numerically higher percentage of goats (88.8 %) was detected in estrus in Oil group. Furthermore, onset of estrus was recorded sooner and the duration of estrus was higher in Oil group compared to control group. However, differences were not significant and these results could be related to a better reaction of animals that received Licuri oil. Further research is needed to support these results.

In the present study, the concentration of cholesterol was higher than in control group. These results corroborate with Senosy et al. (2013), who reported an increase in the production of metabolites such as IGF-I, leptin, insulin, glucose and cholesterol in ewes supplemented with a high-energy diet with concentrate mixture, alfalfa hay and wheat straw that was compared to the group that received a maintenance diet. The cholesterol is precursor of steroid hormones that may reflect on the reproductive performance of ruminants due to indirect actions on the ovary. The levels of BHB were within the expected range (<0.4 mmol/L) for goats (Souto et al., 2013). Despite similar values of BHB between experimental groups, NEFA showed higher concentrations in Oil group, indicating that there was mobilization of fats reserves for energy production in the Oil group.

In this study, the number of small follicles increased, unlike the number of large follicles, which decreased as the days went by, probably because of the influence of the dominant follicle on the recruitment of new follicles. In the absence of the dominant follicle, FSH increases the population of small follicles (Scaramuzz et al., 2011; Rubianes and Menchaca, 2003). On day 11 (day 0: sponge insertion), we observed the ovulation of large follicles, a reduction in follicular dominance, and new recruitment of small follicles. Furthermore, short-term supplementation also increased the number of follicles recruited in ewes (Senosy et al., 2013).

The lack of effects of oil supplementation on the follicular dynamics can be explained by the fact that saturated fatty acids have a minor role in ovarian activity compared to unsaturated fatty acids such as linoleic acid, which has a stimulatory effect on the synthesis of prostaglandins (Staples et al., 1998).

Results in follicular dynamics are due to short-term energy supplementation followed by estrus synchronization, increase the size of preovulatory follicles (Letelier et al., 2008). These results are within the range of 6–9 mm for the preovulatory follicle diameter of small ruminants (Uribe-Velásquez et al., 2015), indicating that short-term supplementation associated with estrus synchronization favored normal follicle growth in Canindé goats.

In the present study, the type of supplementation had no effect on the number of ovulations. However, the addition of 220 g of corn per animal/day during short-term supplementation increased the ovulatory rate of native and Boer goats by 43 % in the supplemented group compared to the non-supplemented groups (Nogueira et al., 2016, Nogueira et al., 2017). The group supplemented with Licuri oil showed the highest follicular recruitment without, however, affecting the number corpora lutea (ovulations). This inconsistent effect of short-term supplementation on the number of ovulations suggests that an increase in the latter may depend on the follicular status at the beginning of nutritional treatment (Viñoles et al., 2005).

The hypothesis that supplementation with Licuri oil could improve the follicular performance and the number of ovulations in Canindé goats was partially accepted. Ovarian activity was stimulated, with an increase in the number of small follicles, but no increase in the number of ovulations compared to the goats supplemented only with the corn and soybean concentrate (control group). The hypothesis that Licuri oil could improve the physiological responses of Canindé goats was accepted. The animals supplemented with Licuri during the afternoon showed the lowest heart and respiratory rates, since temperatures during the afternoon are usually higher than in the morning, thus increasing these physiological variables.

Licuri oil favored a reduction in dry matter intake and can be used to reduce the concentrated feed supplementation without reducing the manifestation of estrus or the number of ovulations in Canindé goats. The goats supplemented with Licuri oil showed better thermal comfort during the afternoon. Supplementation with licuri oil provided a marginal increase in the ovarian activity of the goats.

Declaration of Competing Interest

The authors have no conflict of interest.

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