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Short Communication

Focused nutrition during oestrus synchronization in Pelibuey ewes with different body conditions

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Abstract

The aim of this study was to evaluate ovarian follicular activity, ovulation percentage, and body weight change in response to focused nutrition during synchronization of oestrus with an intravaginal device (CIDR®) (9 days) in Pelibuey ewes with high and low body condition. Seventy-two non-pregnant ewes without offspring were randomly distributed to one of four treatments, namely T1: (n = 18) ewes without focused nutrition and low body condition; T2: (n = 18) ewes without focused nutrition and high body condition; T3: (n = 18) ewes with focused nutrition and low body condition; and T4: (n = 18) ewes with focused nutrition and high body condition. While the CIDR device was in place, the ewes with focused nutrition received a supplement of 1.5 kg ewe/day, which contained 2.9 MCal metabolizable energy, 16% crude protein, 88.5% dry matter, 11.8% crude fibre, and 6.7% of ash. The concentration of progesterone (P_4) was measured to determine ovulation percentage. The follicular population was quantified (2–3 mm, 4–5 mm, and >6 mm in diameter). The number of follicles between 2–3 mm and 4–5 mm in diameter was similar among treatments. The number of follicles >6 mm in diameter was higher in T4 ewes (2.2 ± 0.2) compared with T1, T2, and T3. The response to oestrus, the onset of oestrus, and the ovulation percentage were similar among treatments. Focused nutrition increased the number of ovarian follicles >6 mm in diameter in ewes with high body condition.

Keywords: body weight change, ovarian activity, ovarian follicles, supplementation, ultrasonography
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Introduction

Progestogen-based protocols are associated with prostaglandins (Abecia *et al*., 2011). A decrease has been indicated in the duration of progestogen treatment (Oliveira da Silva *et al*., 2020), which was traditionally used for 12 to 14 days, because longer treatment periods can interrupt ovarian follicular development (Shabankareh *et al*., 2012). The physiological processes of reproduction that are affected by nutrition during ovarian follicular development include ovulation rate and prolificacy, particularly in extensive sheep production systems (Martin *et al*., 2004).

The reproductive efficiency of Pelibuey ewes changes, depending on dietary nutritional content. The response is observed in the number of eggs produced in each ovulatory cycle (Chay-Canul *et al*., 2016). The provision of more than adequate nutrition for animals to gain weight before mating was an old technique in livestock farming called 'flushing' (Scaramuzzi *et al*., 2006). Focused nutrition increases ovarian follicular development and ovulation in sheep. There are responses in the long-term increase in body weight, the 'static effect'; and responses to short-term and timely supplements (for example four days in the final stages of the oestrous cycle), i.e., an 'acute effect' (Scaramuzzi *et al*., 2006). The static and acute effects of nutrition modify

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ovarian folliculogenesis (Viñoles *et al*., 2005), apparently through direct action on the ovary by leptin, insulin-like growth factor 1, insulin, and glucose (Scaramuzzi *et al*., 2006).

Currently, strategic feeding consists of short-term supplementation periods of five to seven days, during days 9 to 13 of the oestrus cycle, with diets high in protein and energy to change the metabolic and nutritional status of the ewes and increase their ovarian potential (Scaramuzzi *et al*., 2013). Thus, the objective of the present study was to evaluate ovarian follicular activity, ovulation percentage, and body weight change in response to focused nutrition during the synchronization of oestrus using an intravaginal device in Pelibuey ewes of high and low body condition.

Materials and Methods

The experimental protocol was carried out according to the Standards for Ethics, Biosecurity, and Animal Welfare mandated by Colegio de Postgraduados, Campus Montecillo, México, and to the Official Mexican Standards NOM-024-ZOO-1995 and NOM-051-ZOO-1995 (SAGARPA, 2017). The study was performed with adult Pelibuey ewes in the Laboratorio de Reproducción de Ovinos y Caprinos (LaROCa) at Colegio de Postgraduados, Campus Montecillo, Texcoco, Mexico State, located at 19°29' N and 98°53' W, at an altitude of 2250 m. Seventy-two non-pregnant ewes without offspring with an average age and weight of 3.2 ± 0.97 years and 47.85 ± 6.83 kg were used. A complete design and a 2 x 2 factorial arrangement were used, where the combination of the two levels of each factor generated the treatments, namely A: with or without focused nutrition, and B: high body condition (4 to 4.5 units) or low body condition (1.5 to 2 units) on a scale of 1 to 5 (Russel *et al*., 1969). Subsequently, the ewes were randomly assigned to one of the four experimental treatments, namely T1: (n = 18) ewes without focused nutrition and low body condition; T2: (n = 18) ewes without focused nutrition and high body condition; T3: (n = 18) ewes with focused nutrition and low body condition; and T4: (n = 18) ewes with focused nutrition and high body condition.

All ewes were dewormed, had their hooves trimmed, and were given vitamins. They were kept in pens provided with shade, a feeder, an automatic water dispenser, and dirt floor. Ewe weights were recorded every week to monitor changes during the experiment. An intravaginal device (CIDR) (0.3 g P_4) (Pfizer, Mexico) was used to synchronize oestrus. The CIDR remained for nine days, and 48 hours before its removal, ewes were injected with 1 mL of synthetic prostaglandin (250 µg cloprostenol sodium, Celosil®, MSD Animal Health, Mexico) to analyse any corpus luteum.

After CIDR removal, all ewes were exposed to a ram for 30 min every four hours for 72 hours. The ram was equipped with an insemination prevention apron. The ewes that responded to oestrus were housed in a different pen and the onset of oestrus was recorded. All ewes in oestrus were identified for subsequent blood sampling and ultrasonography. During the insertion of the CIDR, T1 and T2 received a basal diet of 1.5 kg ewe/day (9 days) containing ground oat hay (70%) mixed with a commercial concentrate containing 15% crude protein and 2.6 MCal of metabolizable energy (30%), mineral salts, and free access water. In turn, T3 and T4 received a basal diet of 1.5 kg ewe/day (9 days) containing 2.9 MCal of metabolizable energy, 16% crude protein, 88.5% dry matter (DM), 11.8% crude fibre, and 6.7% ash. The composition of the focused nutrition diet was based on oat hay (40%), ground sorghum (16%), ground corn (16%), soybean paste (8%), wheat bran (6%), molasses (6%), corn gluten (4%), orthophosphate (1%), CaCO3 (1%), mineral premix (1%; calcium: 14%, magnesium: 1%, zinc: 5000 mg, selenium: 40 mg), sodium chloride (0.5%) and vegetable oil (0.5%). This supplementation during oestrus synchronization constituted the focused nutritional strategy (Viñoles *et al*., 2009) in the current study.

All ewes had a blood sample taken via jugular vein puncture at various times: i) at 12 hours before CIDR removal, ii) after oestrus presentation, iii) at 9 days after CIDR removal, and iv) at 11 days after CIDR removal to determine ovulation in response to treatments. All blood samples had plasma separated by centrifugation for 15 min at 600 x *g* and were then stored at -20 °C before determining the P_4 concentration using a radioimmunoassay. After performing blood sampling at four times, three ewes from each treatment were randomly selected to observe the ovarian follicular population with a 7.5 MHz transrectal transducer integrated in an ultrasound machine (Sonoace Pico, Madison, WI, USA). Ovarian follicles were quantified and classified according to their diameters of 2–3 mm, 4–5 mm, and >6 mm (Denadai *et al*., 2017).

The recorded variables were ovarian follicular development, ovulation percentage, ewes in oestrus, oestrus onset, and body weight change. Statistical Analysis Systems® software (SAS Institute Inc, 2012) was used for data analysis. The number of ovarian follicles was analysed using the GLM procedure. The response to oestrus and ovulation was analysed using PROC LOGISTIC. For oestrus onset, the Shapiro and Wilks test was performed to observe univariate normality and justify the analyses with the survival curve method of Kaplan Meier, and the log-rank test with the LIFETEST procedure. The change in body weight of ewes was evaluated with an analysis of variance using the PROC MIXED procedure (Littell *et al*., 1998). LSMEANS was used to calculate the least-squares means of the fixed effects and ADJUST was used to fit the multiple comparison tests of means in pairs (Tukey) (α = 0.05), with a mixed model in a 2 x 2 factorial arrangement, to observe the main effects and interactions in the evaluation period (week).

Results and Discussion

Focused nutrition and body condition did not influence (P >0.05) the number of ovarian follicles 2–3 mm and 4–5 mm in diameter among ewes in the treatments, except for T4, which developed the highest (P <0.05) (2.2 ± 0.2) number of ovarian follicles >6 mm in diameter compared with other treatments (P >0.05) (T1: 1.2 ± 0.2, T2: 1.3 ± 0.2, and T3: 1.6 ± 0.2). The relationship between body condition and focused nutrition over short periods is still controversial. Morley *et al*. (1978) reported that ewes with body condition 2 (on a scale of 1 to 5 units) (Russel *et al*., 1969) responded better to focused nutrition over short periods, compared to those with a higher body condition. These results differ from the present study in the number of follicles 2–3 mm and 4–5 mm in diameter. Somchit *et al.* (2007) fed oat hay with daily supplements of 500 g of *Lupinus luteus* to Welsh Mountain ewes and did not find differences in the number of follicles between 2.0 and 2.4 mm, 2.5 and 3.5 mm, and >3.5 mm in diameter. However, supplementation with 250 g of *Lupinus angustifolius* to Polish Mountain ewes twice daily altered the duration of the follicular growth phase (penultimate wave) and advanced the appearance of the last wave of the oestrous cycle, but still failed to increase the number of ovulatory follicles (Murawski *et al*., 2020). Leury *et al*. (1990) observed that ewes with high body condition responded better to focused nutrition compared to those with a low body condition. This corresponded to the results in the present study for T4 during the synchronization period, which was reflected in the number of follicles >6 mm in diameter.

Dietary deficiencies, because of nutrients or metabolic intermediaries, affect folliculogenesis along the hypothalamic-pituitary-ovary axis (Scaramuzzi *et al*., 2011), although the number of ovarian follicles does not increase in response to supplementation (Somchit *et al*., 2007). Thus, in ewes without offspring (as in the present study), the response was due to nutrition during the short periods in the synchronization of oestrus, because there are no external factors that affected the response, such as suckling or lactation. In the present study, the number of follicles >6 mm in diameter in T1 and T2 was different. It is possible that body condition in ewes increases the number of follicles of greater diameter, and this response was attributed to the nutrients provided by focused nutrition. In periods of short-term, focused nutrition with a diet high in energy and protein translates into a higher ovulation rate without changes in body weight or body condition (an intermediate effect of nutrition; Viñoles, 2003), or a static body condition. Immediate (short-term supplementation) in ewes with a high body condition (4 units) (Russel *et al*., 1969) respond better to supplementation (Viñoles *et al*., 2010), compared to those with a low body condition (2 units) (Russel *et al*., 1969).

It is apparent that when nutrient supply is limiting and when feed demand is high, animals, including ewes, utilize their body reserves to meet their requirements (Keynon *et al*., 2014). Various internal and external factors influence the function of the ovarian follicle (Dupont *et al*., 2014), including nutrition, body condition, and the neuroendocrine system. The presence of luteal structures does not appear to affect the lifespan of follicles that reach a diameter >5 mm or the number of ovulations per ovary in cyclic ewes. Therefore, the largest follicle could be in the active growth phase five days after the insertion of the CIDR device in ewes (Martínez-Ros *et al*., 2019). The occurrence of ovulation (a progesterone concentration greater than 1 ng mL^{-1} in a single sample, or that exceeding 0.5 ng mL^{-1} in two consecutive samples) was similar (P >0.05) among the ewes (T1: 94.4%, T2: 100%, T3: 94.4%, and T4: 94.4%).

Follicular development in ewes during the oestrous cycle occurs in a pattern-like wave, where groups of follicles begin their growth in response to an increase in follicle stimulating hormone, but only some are selected as dominant and ovulate if their dominance coincides with the lysis of the corpus luteum and the reduction of progesterone (Rosales-Torres *et al*., 2012). Evidence exists that the ovulatory rate increases with better nutrition (flushing) over 10 days before mating, and even over shorter periods, from four to eight days before ovulation, which coincides with the ovarian follicular wave (Viñoles *et al*., 2009). The oestrus synchronization in the present study was carried out between April and June. Thus, it is possible that environmental factors such as reproductive season, geographic region, photoperiod, nutritional level, and physiological state influenced the reproductive responses of the ewes (Hashem *et al*., 2011).

Focused nutrition and body condition and the interaction between them did not influence (P >0.05) (log-rank 0.9865) the response to oestrus (T1: 72.2%, T2: 72.2%, T3: 72.2%, and T4: 77.8%) and onset of oestrus (T1: 41.4 ± 2.7 h, T2: 42.2 ± 2.7 h, T3: 41.9 ± 2.2 h, and T4: 42.6 ± 2.8 h). The data for the initiation of oestrus did not show univariate normality (P >0.05). One study reported that 70% of the ewes had oestrous with a short-term protocol and more than 80% with a long-term protocol. However, the long-term protocol showed better results of positive oestrus in ewes (Oliveira da Silva *et al*., 2020). According to researchers, the CIDR should remain in the vagina between 10 and 14 days to confirm the length of the luteal phase of the natural oestrus cycle (Hosseinipanah *et al*., 2014), because P_4 and its analogues simulate the action of natural P_4 produced by the corpus luteum after ovulation (Abecia *et al*., 2012). In addition, it is possible to relate the effects of nutrition to reproductive events in the energy balance, which was observed when the nutritional requirements exceeded the consumption of nutrients, because animals use their energy reserves to cover the deficit. Therefore, the mechanisms of nutritional effects for folliculogenesis are probably not related to the supply of nutrients but correspond to the effects of specific nutrients initiating the link between reproduction and favourable environmental conditions for reproduction (Somchit-Assavacheep, 2011).

Focused nutrition and body condition promoted (*P* <0.05) changes in body weight of ewes during the experiment. Lower weight was recorded in T1 and a higher weight in T4. Body weight increased (*P* <0.05) from day 15 in all treatments, and all remained similar (*P* >0.05) until the last two weeks of the experiment (Table 1). In addition, the highest weight was recorded in those ewes fed focused nutrition, with high body condition (T4) and the weight change was observed after 21 days of treatment. This result is attributed to the effect of focused nutrition on empty ewes with a low body condition because the addition of a feed ration favours weight and body condition (Khalifa *et al*., 2013).

Table 1 Changes in body weight of Pelibuey ewes with and without focused nutrition and different body condition

Treatments (T)	Experimental period (d)						$\bar{x}$
	-15	-7	0	7	15	21	
T1: ewes without focused nutrition and with low body condition	41.4^{a}_{C}	43.3^{a}_{B}	42.3^{b}_{BC}	42.4^{a}_{BC}	46.7^{a}_{A}	46.8^{b}_{A}	43.8^{a}
T2: ewes without focused nutrition and with high body condition	48.0^{a}_{BC}	48.9^{ab}_{B}	49.3^{ab}_{B}	47.2^{ab}_{C}	53.1^{ab}_{A}	52.0^{ab}_{A}	49.7^{c}
T3: ewes with focused nutrition and low body condition	45.7^{a}_{B}	45.4^{a}_{B}	45.4^{bc}_{B}	45.7^{b}_{B}	49.6^{a}_{A}	48.9^{a}_{A}	46.8^{b}
T4: ewes with focused nutrition and high body condition	56.3^{b}_{B}	53.3^{b}_{CD}	53.9^{c}_{C}	52.0^{b}_{D}	58.6^{b}_{A}	58.6^{b}_{A}	55.5^{d}
P-value	0.0437						

[a, b, c] Values with different superscripts in the same column are different (*P* <0.05)
[A, B, C, D] Values with different subscripts in the same row are different (*P* <0.05), 0: day that focused nutrition started

Conclusions

Focused nutrition and body condition did not affect the number of ovarian follicles 2–3 mm and 4–5 mm in diameter during the synchronization of oestrus with progestogens and prostaglandins in the ewes. Focused nutrition increased the number of ovarian follicles >6 mm in diameter in ewes with high body condition. The response to oestrus, the onset of oestrus, and the percentage of ovulation were not affected by body condition or focused nutrition in ewes synchronized with a CIDR intravaginal device for a nine-day protocol. Pelibuey ewes with high body condition (T2 and T4) showed changes in body weight in response to focused nutrition during oestrogen synchronization with progestogens and prostaglandins.

Authors' Contributions

All authors participated in collating the results, carrying out statistical analyses, interpreting the study, and writing and correcting the manuscript.

Conflict of Interest Declaration

The authors declare that they have no conflicts of interest. They confirm that this manuscript has been read and approved by all authors and that the order of authors listed in the manuscript has been approved by all of them.

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