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Lamb weight gain and reproductive performance of post-partum ewes supplemented with palm kernel cake and sexual stimulated by a ram

Lorenzo Buenabad-Carrasco[1], Juan Sicairos-Díaz[2], Paulina Vázquez-Mendoza[3], Brooke Latack[4], Raymundo Rodríguez De Lara[5] and Juan González Maldonado[2*]

[1]Faculty of Animal Sciences and Ecology, Chihuahua University, Chihuahua, Mexico. [2]Institute of Agricultural Sciences, Baja California University, Highway Delta S/N C.P. 21705 Ejido Nuevo Leon, Baja California, Mexico. [3]"Costa Chica" Regional Center of Superior Education, Guerrero University, Guerrero, Mexico. [4]Cooperative Extension, Division of Agriculture and Natural Resources, University of California, California, USA. [5]Animal Production Postgraduate Program, Chapingo University, Estado de Mexico, Mexico. *Author for correspondence. E-mail: juan.gonzalez.maldonado@uabc.edu.mx

ABSTRACT. The objective of this study was to evaluate the effect of palm kernel cake and ram effect on reproductive activity of postpartum and lactating ewes. Twenty multiparous ewes, five to eleven days postpartum, were separated into four different treatments: control, control-ram, palm and palm-ram. Body weight, difference in body weight recorded between days 0 and 42 of the experiment, diameter of the largest follicle at day zero of the study, number of ewes bearing a corpus luteum and pregnancy rate were measured in ewes. Average daily weight gain was measured in lambs. There was no effect ($p \geq 0.05$) of palm kernel supplementation, ram stimulation, or their interactions on the weights of ewes and lambs. A greater number of ewes with corpus luteum ($p < 0.05$) were observed in ewes stimulated by rams than those not stimulated. The number of pregnant ewes was not affected by ram effect or palm kernel supplementation ($p \geq 0.05$). In conclusion, the use of the ram effect increases the incidence of corpus luteum in postpartum and lactating ewes. In addition, palm kernel cake in the diet does not affect body weight of postpartum ewes and lambs.

Keywords: anestrus; corpus luteum; pregnancy; male effect.

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Introduction

The postpartum period and lamb suckling are well-recognized factors that naturally downregulate the reproductive activity of ewes by limiting GnRH and gonadotropins access to ovarian cells, which reduce ovulation incidence and corpus luteum formation rate (Wise, 1990; Smart, Singh, Smith, & Dobson, 1994). Lamb suckling during the postpartum period induces anestrus in ewes by increasing endogenous opioid peptide concentrations (Gordon, Renfree, Short, & Clarke, 1987). They promote prolactin production by a mechanism mediated by salsolinol (Misztal, Tomaszewska-Zaremba, Górski, & Romanowicz, 2010). It is well known that endogenous opioid peptides downregulate GnRH secretion by limiting the release of kisspeptin (Uenoyama, Tsuchida, Nagae, Inoue, & Tsukamura, 2022), but the role of prolactin on GnRH secretion is controversial (Peclaris, 1988; Pijoan & Williams, 1985). The cessation of reproductive activity after lambing allows the ewe to recover from lambing and nurse their lambs. However, a more rapid onset of reproductive activity after lambing enables earlier pregnancy success after lactation.

The application of progesterone, gonadotropins, early weaning and restricted suckling are the most common methods used to break postpartum anestrus and reactivate the reproductive axis of the ewe after lambing (Ascari, Alves, Alves, Garcia, & Junqueira, 2016). Early weaning might be the most effective way to rapidly and easily overcome postpartum anestrus because it eliminates the effect of lactation and the presence of the lamb (Mallampati, Pope, & Casida, 1958). However, there is always a concern about lower productive performance of early weaning lambs compared to those weaned at an older age (Wang et al., 2019). Hormone therapy is effective in increasing fertility in lactating ewes to a similar level of non-lactating ewes (Ronquillo et al., 2008), but is costly and some therapies might have detrimental effects on animal health and welfare (Martinez-Ros et al., 2018), which makes them undesirable.

Restricting the time of ewe-lamb interaction or suckling restriction, though time consuming, has proven to be an effective strategy to reduce the length of postpartum anestrus (Morales-Teran, Pro-Martinez, Figueroa-Sandoval, Sanchez-del-real, & Gallegos-Sanchez, 2004; Oliveira et al., 2013). The male effect is also effective in shortening the duration of anestrus in ewes (Ferreira-Silva et al., 2017), but there is a suggestion that a minimum time of separation between lambs and ewes is required to improve reproductive response (Pevsner, Rodrguez-Iglesias, & Ciccioli, 2010). However, others have not mentioned such a requirement (Fraire-Cordero et al., 2018). Therefore, the male effect as strategy to break the postpartum anestrus requires more research.

The nutritional approach to manipulate animal reproduction is well known (D'Occhio, Baruselli, & Campanile, 2019). The effect of nutritional inputs on reproductive traits depends on several factors such as the nutritional profile of the ingredients and the amount of nutrients effectively used by the animal. Desirable characteristics of any feed ingredient used in animal diets are low cost and few-to-no undesirable effects on animal health, welfare and performance. Palm kernel cake used in sheep production reduces the cost of feeding (Ribeiro et al., 2018), and has been reported to improve semen quality in water buffalos (Santos et al., 2014), but there is a lack of research evaluating its effect on reproductive performance in ewes during the postpartum period.

Therefore, the objective of the present study was to evaluate the effect of palm kernel cake and ram effect on reproductive activity of postpartum and lactating ewes.

Material and methods

Animals and experimental procedure

Animal were treated according to the guidelines of the Canadian Council on Animal Care in Sciences (CCAC, 2009). Twenty multiparous ewes (crossbred Dorper × Pelibuey × Katahdin), five to eleven days postpartum, were randomly separated into four different treatments: control, control-ram, palm and palm-ram. The ewes in the control treatment (n = 5, 52.6 ± 3.44 kg) were not supplemented with palm kernel cake and were not stimulated by a ram. The ewes in the control-ram treatment (n = 5, 57.26 ± 4.52 kg) were stimulated by a ram without supplementation of palm kernel. The ewes in the palm treatment (n = 5, 54.38 ± 6.92 kg) were supplemented with palm kernel cake (20% of the diet on a dry mater basis) and were not stimulated by a ram. The ewes in the palm-ram treatment (n = 5, 50.9 ± 1.91 kg) were supplemented with palm kernel cake and were stimulated by a ram. Palm kernel was supplemented from days 0 to 42.

The sexual stimulation of the ewes was carried out by allowing the interaction between one of two mature rams (crossbred Dorper × Pelibuey × Katahdin) wearing an apron and ewes from control-ram and palm-ram. The ewes were stimulated by rams at mornings (6 - 7 am) and evenings (5 - 7 pm) for periods of five minutes for 27 days starting on day zero of the experiment.

The lambs (n = 33) were separated from their mothers at day -7 of the experiment and were allocated into two different groups according to their dam's dietary treatment: control and control-ram (n = 16, 4.62 ± 0.74 kg) and palm and palm-ram (n = 17, 4.89 ± 1.03 kg). Interaction between lambs and ewes was only allowed mornings (6 - 7 am) and evenings (5 - 7 pm) for periods of 15 minutes from day -7 to 42 of the experiment.

Nutrition and feeding

Ewes were fed 2 kg day^{-1} of the experimental diet (Table 1) formulated to supply similar amount of nutrients to all treatments according to the nutrient requirements for lactating ewes (National Research Council [NRC], 2007). The total feed was provided in two meals per day in the morning (6 - 7 am) and afternoon (2 - 3 pm). All ewes were fed with the same diet (without palm kernel supplementation) from day -7 to 0 of the experiment.

Lambs were fed once a day in the morning (6 - 7 am) from day -7 to 42 of the experiment with a diet containing 20% wheat straw and 80% concentrate (69.28% ground wheat grain, 28.57% soybean meal and 2.14% ground limestone) as fed. Water was available ad libitum to ewes and lambs.

Reproductive management

Ewes were injected intramuscularly with 12.5 mg of dinoprost (Lutalyse®, Zoetis) at day 28 of the experiment (the day following the final day of ram stimulation). The ewes were monitored for signs of estrus for nine days after prostaglandin injection. The ewes not detected in estrus received a second injection of prostaglandin at day 37 of the experiment and were observed for signs of estrus for nine days. Ewes were declared in estrus when they stood to be mounted by a ram wearing an apron. Estrus detection was carried out every 6h for the first five days after prostaglandin injection and every 12h for the next four days. All ewes that showed estrus were inseminated via cervical insemination with 200×10^{-6} sperm cells of fresh semen from a single ram 12h after estrus detection.

Table 1. Ingredient and nutrient composition of diets fed to experimental groups of postpartum ewes.

	Treatment	
	Control and control-ram (%)	Palm and palm-ram (%)
Ingredient composition, % DM		
Wheat straw	45.8	45.7
Ground wheat grain	40	20
Soybean meal	12	12
Palm kernel cake	0	20
Ground limestone	1.2	1.3
Salt	0.5	0.5
Minerals	0.5	0.5
Nutrient composition, DM basis		
Metabolizable energy (Mcal)	2.34	2.31
Protein crude (%)	13.45	13.45
Neutral detergent fiber (%)	43.78	54.24
Acid detergent fiber (%)	29.68	37.17
Calcium (%)	0.53	0.61
Phosphorus (%)	0.28	0.32
Cooper (ppm)	5.03	8.63

Response variables

Ewe and lamb body weight was recorded at seven day intervals from day -7 to 42 of the experiment. The amount of feed offered and rejected by each group of lambs was measured daily during the same period of time.

Average daily gain was recorded for lambs. Body weight, difference in body weight recorded between days 0 and 42 of the experiment, diameter of the largest follicle at day zero of the study, number of ewes bearing a corpus luteum and pregnancy rate were measured in ewes. The diameter of the largest ovarian follicle was calculated by averaging the horizontal and vertical measurements of the follicle at day zero of the experiment. The number of animals bearing a corpus luteum was determined at day 0, 9, 18 and 27 of the experiment. Pregnancy diagnoses were performed 40 days after artificial insemination. Ovarian structure measurements and pregnancy detection were carried out by trans-rectal ultrasonography (Aloka SSD-500, with 7.5 MHz linear transducer; Aloka Ltd, Tokyo, Japan).

Statistical analysis

Ewe body weight data was analyzed by PROC MIXED using a factorial design with repeated measures and considering two factors, supplementation with two levels (control and palm kernel) and ram stimulation with two levels (control and ram stimulation). The same experimental design, but without repeated measures, was used for weight change in ewes from day 0 to 42 of the experiment and diameter of the largest ovarian follicle by PROC GLM. Average daily weight gain in lambs was analyzed by repeated measures using PROC MIXED. Two lambs suckling ewes supplemented with palm kernel died and their data was not included in the statistical analysis. The number of ewes bearing a corpus luteum on day 27 and pregnancy rate was analyzed by Fisher Exact Test using PROC FREQ, but the effects of ram and palm kernel supplementation were analyzed separately because of the small sample size. The means from continuous data were compared using Tukey test. A $p < 0.05$ was considered as significant. The statistical package used at all times was SAS University Edition (2020).

Results and discussion

The results regarding the effect of treatments on body weight, body weight change in ewes, and the average daily weigh gain in lambs are depicted in Tables 2, 3 and Figure 1. The ewes from all treatments experienced weight loss ranging from 0.66 to 1.84 kg from day zero to 42 of the experimental period. The average daily gain in lambs suckled from non-supplemented ewes and ewes supplemented with palm kernel cake was similar (0.21 ± 0.01 vs 0.22 ± 0.01 kg day^{-1}). In general, there was no effect ($p \geq 0.05$) of the evaluated factors (palm kernel supplementation and ram stimulation) or their interactions on the weights of ewes and lambs. Daily feed intake from lambs was not statistically analyzed due to the lack of repetition. However, the raw data from the daily feed intake is depicted in Figure 2.

Table 2. Body weight (kg, least square mean ± EE) of lactating ewes supplemented with palm kernel and stimulated by a ram.

	Control	Palm kernel cake	
Control	52.13 ± 1.70	55.14 ± 1.71	53.64 ± 1.20
Ram	55.18 ± 1.88	50.96 ± 1.89	53.07 ± 1.33
	53.65 ± 1.27	53.05 ± 1.27	

Number with different superscript between rows and columns are statistically different (p < 0.05).

Table 3. Body weight change (kg, least square mean ± EE) of lactating ewes supplemented with palm kernel and stimulated by a ram.

	Control	Palm kernel cake	
Control	-1.72 ± 0.68	-0.66 ± 0.68	-1.19 ± 0.48
Ram	-1.56 ± 0.68	-1.84 ± 0.68	-1.70 ± 0.48
	-1.64 ± 0.48	-1.25 ± 0.48	

Number with different superscript between rows and columns are statistically different (p < 0.05).

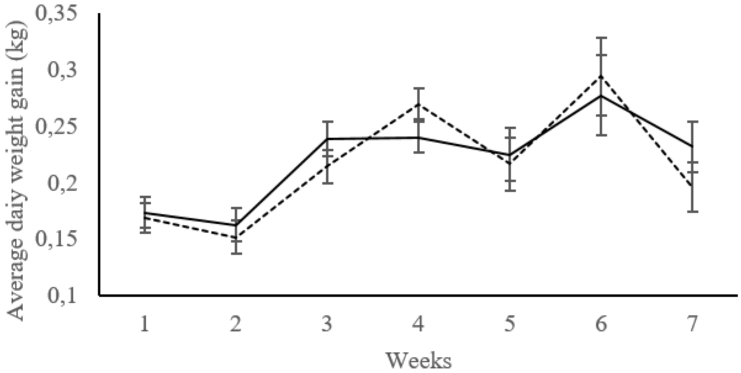

Figure 1. Average daily weight gain (kg) measured every week in lambs suckling ewes from control (dotted line) and palm kernel supplemented groups (solid lined). The experimental period lasted 42 days; from day zero to week one, the ewes were fed the same diet. Supplementation period: week 2 - 7.

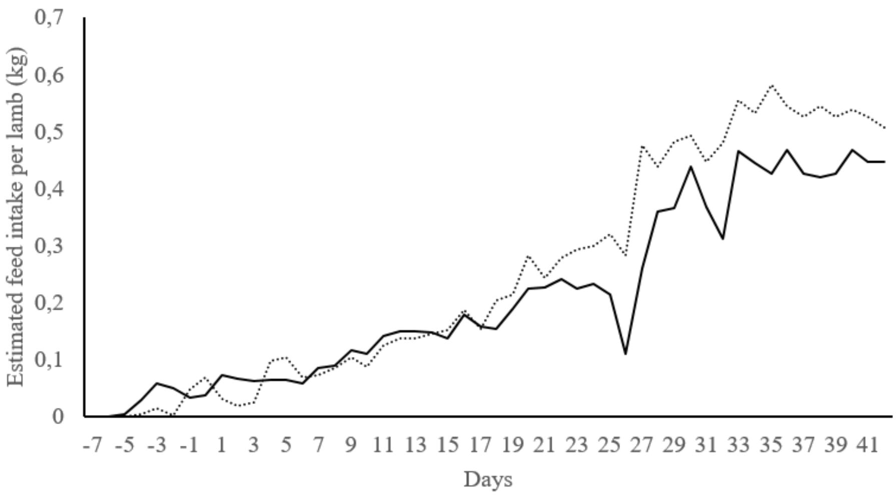

Figure 2. Estimated feed intake per lamb (kg) suckling ewes from control (dotted line) and palm kernel supplemented groups (solid lined). The experimental period of ewes lasted 42 days (day zero to 42).

The ovarian status was similar among experimental groups at day 0. There were no corpus luteum observed and the diameter of the largest follicle was not different among groups (Table 4). The number of ewes bearing a corpus luteum for each experimental group is depicted in Figure 3. In general, a greater number of ewes with a corpus luteum (p < 0.05) was observed in ewes stimulated by rams (9 / 10) than those not stimulated (3 / 10). A similar number of ewes bearing a corpus luteum (p ≥ 0.05) was observed between those supplemented with palm kernel cake (7 / 10) and those in control group (5 / 10) at day 27. The number of pregnant ewes was similar (p ≥ 0.05) between groups stimulated (6 / 10) and those not stimulated by a ram (4 / 10). In addition, no differences in the number of pregnant ewes (p ≥ 0.05) were found between those supplemented (3 / 10) and not-supplemented with palm kernel cake (7 / 10).

Table 4. Diameter of the largest ovarian follicle (mm, least square mean ± EE) of lactating ewes supplemented with palm kernel and stimulated by a ram.

	Control	Palm kernel cake	
Control	2.86 ± 0.37	2.87 ± 0.37	2.86 ± 0.26
Ram	2.35 ± 0.37	3.75 ± 0.37	3.05 ± 0.26
	2.60 ± 0.26	3.31 ± 0.26	

Number with different superscript between rows and columns are statistically different ($p < 0.05$).

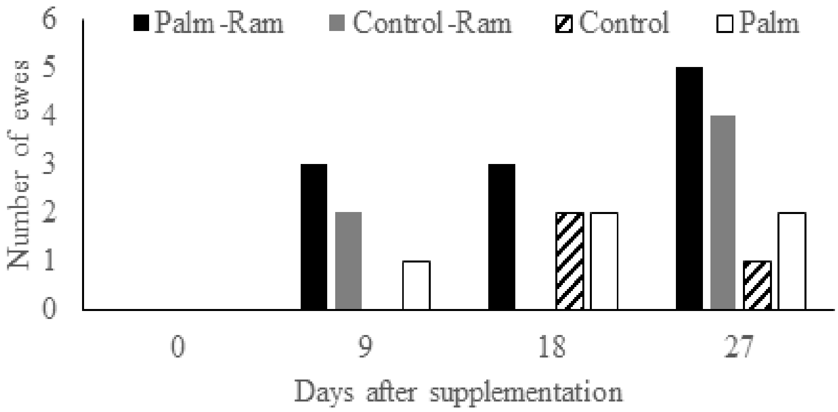

Figure 3. Number of ewes bearing a corpus luteum at nine-day intervals during the 27 days study period with and without palm kernel supplementation and with and without ram stimulation.

The postpartum period is a dynamic period where ewes experience weight loss due to metabolic and anatomical adjustments taking place after lambing (Pedernera et al., 2018). There is scientific evidence proving that weight loss around reproductive events such as mating can jeopardize female fertility. Weight loss between 3.9 to 6.8 kg due to nutritional restrictions decreases the number of follicles (⩽ 3 mm) and embryo quality in sheep (O'Callaghan, Yaakub, Hyttel, Spicer, & Boland, 2004; Abecia et al., 2014). Similarly, a higher incidence of degenerated embryos was observed in postpartum cows suffering greater weight loss compared to those with less severe weight loss (Carvalho et al., 2014). Postpartum weight loss indicates the presence of a negative energy balance in the animal, mainly because nutrients from feed intake are redirected to sustain milk production after parturition, which causes a hormonal imbalance in the animal, such as the observed reduction of leptin blood concentrations in sheep and cattle (Temizel et al., 2018; Młynek, Strączek, & Głowińska, 2022).

Leptin is a hormone that signals the nutritional status of the body to the brain. Ewes losing weight have lower blood concentration of leptin and gonadotropin than those with normal body weights (Towhidi, Khazali, & Zhandi, 2007). In addition, it is known that leptin directly stimulates embryo growth (Panda et al., 2017), probably by acting as a survival factor (Kšinanová et al., 2017). Therefore, it might be suspected that fertility is compromised in ewes suffering weight loss by a mechanism that induces low leptin blood concentrations. However, it has been reported that pregnancy rate of ewes with a breed profile and weight (Katahdin x Pelibuey, 50 kg) similar to the animals used in the present study were not compromised by weight loss (2.6 to 5.4 kg) induced by feed restriction around mating (Macías-Cruz et al., 2017). The difference in results (O'Callaghan et al., 2004; Abecia et al., 2014; Macías-Cruz et al., 2017) might be explained by the "genetic dimension", which indicates that the susceptibility of reproduction to metabolic and nutritional inputs varies among genotypes (Blache & Bickell, 2011). Thus, it can be suggested that differences in body weight and body weight loss among experimental groups in the present study are not a concern to reproductive performance.

The absence of ovulation and a corpus luteum is common during the postpartum period in ewes. The formation of a corpus luteum after lambing marks the end of the postpartum anestrus. In order for this to happen, a preovulatory LH must occur in the presence of a susceptible follicle. The preovulatory follicle can be disregarded as susceptible to the action of GnRH/LH. The size of the preovulatory follicle range from 3.8 – 7.4 mm in diameter (Cahill, Mariana, & Mauleon, 1979; Ferreira-Silva et al., 2018) and ovulations have been observed in ewes bearing follicles with a diameter of 5 mm after exogenous GnRH injection (Rubianes et al., 1997). However, despite the presence of ovarian follicles ranging from 3 - 8 millimeters as early as 10 days postpartum, as in some groups of the present study, the first ovulation occurred between 26 to 59 days after lambing in Pelibuey ewes (Gonzalez, Murphy, de Alba, & Manns, 1987). This might be explained by the fact that GnRH frequency and amplitude are reduced during the postpartum period in lactating ewes (Hernández-Hernádez et al., 2021; Smart et al., 1994), but as the postpartum period progresses, the gonadotropin secretion is gradually restored (Wise, 1990).

The lack of difference in follicles size among all experimental groups and the absence of a corpus luteum at day zero of the experimental period allow us to suggest that the animals were in a similar reproductive status (anestrus). However, the number of ewes bearing a corpus luteum was higher in those stimulated by a ram than in those not stimulated, but the incidence of estrus after prostaglandin injection was similar among groups.

The expression of estrus after prostaglandin injection can only be explained by an induced corpus luteum regression (Fierro, Gil, Viñoles, & Olivera-Muzante, 2013), but such ovarian structures were not present in most of the non-ram stimulated groups. The similar incidence of estrus observed in all groups can be attributed to the ram effect (sudden introduction of the ram to ewes). The ram effect is known to stimulate follicular estradiol production by increasing LH pulsatile secretion, which eventually culminates in estrus behavior display, LH surge and ovulation (Fabre-Nys, Kendrick, & Scaramuzzi, 2015; Fabre-Nys et al., 2016). In agreement with our results, others have reported that the ram stimulation for 21 - 35 days, beginning at 8 or 35 days after lambing, increases progesterone concentrations (indication of the presence of corpus luteum) in lactating ewes (Ferreira-Silva et al., 2017; Fraire-Cordero et al., 2018). All experimental ewes showed estrus behavior and pregnancy rate was not affected by ram stimulation. A lower pregnancy rate (28.5%) has been reported in lactating ewes synchronized using only prostaglandins in comparison to those synchronized using eCG and progestogens (35-43%) (Ronquillo et al., 2008). However, the results of the present study are similar to those obtained when the male effect was carried out before an estrus synchronization program (60-62%) (Fraire-Cordero et al., 2018). Therefore, it can be stated that ram exposure to postpartum ewes, even for a short period of time, in combination with prostaglandins was equally effective to induce estrus behavior, without affecting pregnancy rate, in comparison to ewes stimulated for a longer period (36 days).

The palm kernel cake did not affect the body weight of postpartum ewes or the average daily gain of their lambs. The average daily weight gain and milk composition respond quadratically to the dietary inclusion of palm kernel cake (de Carvalho Rodrigue et al., 2021; Ferreira et al., 2021). In general, the benefit of adding palm kernel to the ration is dependent on the cost reduction of the diet (Ribeiro et al., 2018).

Conclusion

The use of the ram effect increases the incidence of corpus luteum in postpartum and lactating ewes. In addition, palm kernel cake in the diet does not affect body weight of postpartum ewes and lambs.

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