Revista Brasileira de Zootecnia

Effects of flushing with rehydrated corn grain silage on follicular development in tropical Santa Inês ewes

Lucas Machado Figueira[1,2] (iD), **Letícia Rodrigues Faria**[1] (iD), **João Pedro Araújo Campos**[1] (iD), **Débora Regina da Silva**[3] (iD), **Iraides Ferreira Furusho-Garcia**[1] (iD), **Rafael Fernandes Leite**[4] (iD), **Renato Ribeiro de Lima**[5] (iD), **Nadja Gomes Alves**[1,3*] (iD)

[1] Universidade Federal de Lavras, Departamento de Zootecnia, Lavras, MG, Brasil.
[2] Universidade Federal Fluminense, Faculdade de Medicina Veterinária, Niterói, RJ, Brasil.
[3] Universidade Federal de Lavras, Departamento de Medicina Veterinária, Lavras, MG, Brasil.
[4] Universidade Federal de São João del-Rei, Departamento de Zootecnia, São João del-Rei, MG, Brasil.
[5] Universidade Federal de Lavras, Departamento de Estatística, Lavras, MG, Brasil.

ABSTRACT - The objective of this study was to evaluate the effect of replacing ground corn (402 g kg⁻¹ of dry matter) with rehydrated corn grain silage (RCGS; 425 g kg⁻¹ of dry matter) in a flushing diet on follicular development in tropical Santa Inês ewes. Fifteen ewes were randomly assigned to one of two treatment groups: a diet with ground corn (control, n = 7) or a diet with RCGS (n = 8). The first day of the diets was designated d0, and the diets were fed for 30 days, up to two days after the end of the estrus synchronization protocol. The estrus synchronization protocol (intravaginal progestogen sponge for 11 days plus 300 IU eCG and 37.5 μg d-cloprostenol IM two days before sponge removal) started on d17 of the diets. The number and diameters of ovarian follicles ≥3 mm were assessed by ultrasound on the day before the diets were provided (d−1), on d14, and then daily from two days before sponge removal until ovulation or up to the eighth day after sponge removal. Blood samples were collected on days −1, 0, 6, 12, 18, 24, and 30 for glucose and urea analyses. There was no effect of the treatments on dry matter intake, plasma glucose, and urea nitrogen concentrations, or on the percentage of ewes that ovulated and on the number of ovulations. The number of follicles ≥3 mm did not differ between treatment groups; the number increased between d−1 and d14 and did not differ between d14 and d28. The diameter of ovulatory follicles at sponge removal, the interval from sponge removal to estrus, and the growth rate of the ovulatory follicles were greater in the RCGS group than in the control group, resulting in larger follicles at ovulation. Replacement of ground corn by RCGS in the flushing diet does not increase the number of ovulations but results in delayed onset of estrus and ovulation of larger follicles.

Keywords: blood metabolites, energy, follicular diameter, ovulation

1. Introduction

The reproductive performance of small ruminants has been positively associated with energy balance (Scaramuzzi et al., 2006). An increase in energy intake around breeding can have a positive impact on reproduction of these animals. Flushing promotes an increase in concentrations of blood metabolites such as glucose, which plays an important role in folliculogenesis and ovulation (Scaramuzzi et al., 2006; Zabuli et al., 2010; Habibizad et al., 2015).

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The results obtained in flushing programs may vary according to differences in the body condition scores (BCS) of the ewes (Gottardi et al., 2014), the feeds included in their diets (Rekik et al., 2012), and the length of the flushing period (Habibizad et al., 2015). Inclusion of different ingredients in the diet results in variation in the ruminal fermentation profiles and post ruminal digestion products (Roche et al., 2011). Furthermore, the addition of ingredients that provide highly fermentable energy content in the rumen may result in more efficient use of dietary protein (Wilkerson et al., 1997) and reduce the plasma urea nitrogen (PUN) concentration. The PUN concentration is related to the ammonia and urea concentrations in the follicular and uterine fluid (Hammon et al., 2005) and has been shown to negatively affect oocyte (Sinclair et al., 2000) and embryonic development if in excess of 19.0 mg dL^{-1} (McEvoy et al., 1997).

The use of rehydrated corn grain silage (RCGS) enhances ruminal digestibility of starch (Ferraretto et al., 2015), which reduces the acetate:propionate ratio. Propionate is the main substrate for hepatic gluconeogenesis (Bergman, 1990; Brockman, 1990). Scaramuzzi and Martin (2008) suggested that the energy derived from glucose plays a more relevant role in ovarian function than other digestion products. The increase in glucose uptake by follicular cells may be critical for follicle growth and prevention of atresia, which will result in an increased number of ovulations (Scaramuzzi et al., 2006; Zabuli et al., 2010; Habibizad et al., 2015).

Therefore, we hypothesized that flushing with RCGS as a replacement for ground corn would positively affect folliculogenesis and the number of ovulations in ewes. The objective of this study was to evaluate the effect of flushing with ground corn or with RCGS on follicular development in tropical Santa Inês ewes.

2. Material and Methods

All the procedures involving animals in this study were approved by the Animal Ethics Committee (case no. 065/2016). The experiment was conducted in Lavras, Minas Gerais, Brazil (21°14'43" S, 44°59'58" W, 924 m asl), from July 2016 to February 2017.

Fifteen non-pregnant tropical Santa Inês ewes (nulliparous = 6, multiparous = 9), with 41.93±13.94 kg (mean±SD) body weight and 3.11±0.39 BCS (scale from 1 to 5, in which 1 = emaciated and 5 = obese; Gordon, 1997) were selected for the study. The ewes were randomly assigned to one of two treatment groups, which consisted of flushing with either ground corn (402 g kg^{-1} of dry matter; control, n = 7) or RCGS (425 g kg^{-1} of dry matter; n = 8) for a 30-day period (d0 = first day of the experimental diets). To adjust the flushing diets (Table 1), samples of the ingredients were collected and analyzed at the beginning of the experiment, and the diets were formulated for adult sheep with an average body weight of 50 kg (NRC, 2007). After 30 days of feeding the flushing diets, all the ewes were offered corn silage and commercial concentrate to meet their nutritional requirements (NRC, 2007).

The animals were allocated to individual stalls with free access to water and received a total mixed ration twice a day (08:00 and 17:00 h). The orts from each animal were removed and weighed daily to adjust the daily intake, allowing at least 5% orts. The daily dry matter intake (DMI) and intake of neutral detergent fiber (NDF), crude protein (CP), ash, ether extract (EE), and nonfibrous carbohydrates (NFC) were analyzed.

The ewes were weighed the day before the experimental diets were provided (d−1) and on d30 to evaluate the average daily gain (ADG). On those same days, the BCS was determined by averaging the scores given by two independent evaluators.

Daily samples of the orts from each animal were pooled every week to create composite samples. The chemical composition of the feed ingredients and of the composite samples of the orts for each animal was analyzed according to the standard analytical procedures of the Instituto Nacional de Ciência e Tecnologia de Ciência Animal (INCT-CA) (Detmann et al., 2012). The samples were oven-dried at 55 °C for 72 h and then ground in a Wiley type mill (Arthur H. Thomas, Philadelphia, PA, USA) to pass through a 1-mm screen. The samples were analyzed for dry matter (DM) (oven dried at 105 °C

for 24 h; INCT-CA, no. G-003/1), ash (furnace incineration at 600 °C for 5 h; INCT-CA, no. M-001/1), nitrogen (micro Kjeldahl; INCT-CA, no. N-001/1), EE (Randall; INCT-CA, no. L-005/1), and NDF (TECNAL TE-149 analyzer, with addition of α-amylase and sodium sulfite; INCT-CA, no. F-001/1). Non-fibrous carbohydrates were calculated according to the NRC (2007) using the formula NFC = 100 − (CP + EE + NDF + ash).

The hormonal protocol for estrus synchronization was initiated 17 days after the start of the flushing and completed two days before the end of the supply of the diets. An intravaginal sponge impregnated with progestagen [60 mg of medroxyprogesterone acetate (MPA), Progespon®, Zoetis, SP, BR] was used for 11 days, with application of 37.5 µg of d-cloprostenol IM (Prolise®, Arsa, AR, USA) and 300 IU of eCG (Novormon®, Zoetis, SP, BR) on d26, followed by removal of the implant on d28.

The animals were evaluated after the end of the synchronization protocol for signs of estrus by a teaser ram twice daily (07:00 and 17:00 h) for 15 min each time until five days after removal of the intravaginal sponge. Ewes were considered in estrus when they allowed the teaser ram to mount consistently. Females in estrus were mated at 12-h intervals until no mounting acceptance, with a ram previously evaluated by andrological examination and found to be suitable for reproduction and to exhibit good libido. The length of estrus was considered the interval between the first mount and last time that the ewe stood to be mounted.

Ultrasonographic monitoring of the ovaries (ALOKA SSD 500, with a transrectal transducer UST-660 7.5-MHz, JHS, JP) was performed on d−1, d14, and daily after application of d-cloprostenol and eCG (d26) until ovulation was confirmed or until eight days after sponge removal if ovulation had not occurred. The examinations were performed by the same technician between 08:00 and 11:00 h to determine the number of follicles ≥3 mm in diameter and the diameter of the preovulatory follicles. The growth rate was calculated for the ovulatory follicles, which was considered to be the difference between the maximum and minimum diameter divided by the duration of the growth phase (Alves et al., 2011). Ovulation was determined by the disappearance of follicles ≥5 mm and subsequent detection of corpus luteum. The interval from sponge removal to ovulation was estimated considering the time of ovulation as the mean interval between the last sighting and the disappearance of the first ovulatory follicle (≥5 mm).

Table 1 - Ingredients and chemical composition of the flushing diets based on ground corn (control; n = 7) or rehydrated corn grain silage (RCGS; n = 8)

Item	Control	RCGS
Ingredient (g kg^{-1} DM)		
Corn silage	396	396
Ground corn	402	-
Rehydrated corn grain silage	-	425
Soybean meal	182	159
Mineral mix[1]	20	20
Chemical composition		
Dry matter (g kg^{-1})	521	473
Crude protein (g kg^{-1} DM)	125	125
Neutral detergente fiber (g kg^{-1} DM)	312	293
Ash (g kg^{-1} DM)	35	35
Ether extract (g kg^{-1} DM)	31	31
Non-fibrous carbohydrates[2] (g kg^{-1} DM)	497	516

DM - dry matter.
[1] Guaranteed analysis per kg of product: 80 g of P, 18 g of Mg, 150 g of Na, 15 g of S, 125 mg of Ca, 65 mg of Co, 95 mg of I, 1500 mg of Mn, 30 mg of Se, 350 mg of Zn, 800 mg of F, 30,000 IU of vitamin A, 3,000 IU of vitamin D, and 60 IU of vitamin E.
[2] According to NRC (2001): NFC = 100 - (CP + EE + NDF + ash).

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Blood samples were collected on days −1, 0, 6, 12, 18, 24, and 30 approximately 4 h after feeding the experimental diets. Samples (4 mL) were collected by jugular venipuncture in tubes containing sodium fluoride and EDTA (Vacutainer®, BD, SP, BR), which were immediately stored in an ice-filled insulated container and centrifuged ($1,500 \times g$ for 15 min). Plasma was stored (−20 °C) in microtubes (1.5 mL). Plasma glucose and urea concentrations were analyzed in a spectrophotometer (Kasuaki UV/VIS-IL592; IonLaB®, PR, BR) using a commercial enzymatic colorimetric kit (Glucose Liquiform and Urea CE®, LABTEST, MG, BR). The samples were analyzed in duplicate, and the mean value was considered if the relative difference between them was <5%. The sensitivity was 1.77 and 0.94 mg/dL for glucose and urea, respectively. The intra-assay coefficient of variation was 2.8% for glucose and 2.7% for urea. The results of the urea analyses were transformed to PUN by multiplying the urea concentration value by 0.4666.

All analyses were performed using generalized linear models with SAS (Statistical Analysis System, version 9.3). The initial weight was used as a covariate in all statistical analyses with the following exceptions: the glucose and PUN analyses, in which the concentrations of respective metabolites on d−1 were used as covariates, and analysis of the number of follicles ≥3 mm, in which no covariates were used.

The DMI, intake of nutrients, plasma glucose concentration, and PUN concentration were analyzed over time using the MIXED procedure considering the category (nulliparous or multiparous), treatment, week, and treatment by week interaction as fixed effects and the animal as a random effect. The BCS and weight gains were analyzed using PROC GLM considering treatment as a fixed effect.

The percentages of ewes in estrus, percentages of ewes that had ovulation detected, length of estrus, interval from sponge removal to estrus, intervals from sponge removal to ovulation and from estrus to ovulation, and number of ovulations were analyzed using the PROC GENMOD, considering treatment as a fixed effect. The number of follicles ≥3 mm was analyzed over time by PROC GLIMMIX considering treatment, day, and treatment by day interaction as fixed effects and animal as a random effect. The variables diameter of the ovulatory follicle at sponge removal, growth rate, and diameter of the ovulatory follicles were analyzed using the PROC GLM, considering treatment and ewe nested in treatment as fixed effects.

All means were evaluated using the least square means methods, and the data were reported as the least square means ± SEM. Statistical significance was considered at 5%.

3. Results

The DMI (kg day⁻¹), DMI in relation to body weight (g kg⁻¹ BW), and intake of NDF, CP, ash, EE, and NFC (g day⁻¹) did not differ (P>0.05) between treatment groups (Table 2). The DMI of the animals attained a maximum value on d16 of flushing and then began to reduce in both treatment groups (Figure 1). The ADG and BCS were positive but did not differ (P>0.05) between treatment groups. The ADG was 148.3±26.5 g day⁻¹ (control group) and 96.5±24.7 g day⁻¹ (RCGS group), and the gain in BCS was 0.4±0.1 (control group) and 0.2±0.6 (RCGS group).

The plasma glucose concentrations did not differ (P>0.05) between the control (72.4±5.1 mg dL⁻¹) and RCGS (79.8±4.8 mg dL⁻¹) groups and showed cubic response over time (P<0.01). A first peak in glucose concentrations was observed on d6. A decrease was then observed until d24, which preceded a second peak on d30. There was no effect of the treatment by week interaction (P>0.05; Figure 2). The PUN concentration did not differ (P>0.05) between the control (16.2±0.6 mg dL⁻¹) and RCGS (15.3±0.6 mg dL⁻¹) groups and had a quadratic response over time (P<0.001) reaching the maximum concentration of 19.5 mg dL⁻¹ on d18 (Figure 2). There was no effect of the treatment by week interaction (P>0.05; Figure 2).

The numbers of follicles ≥3 mm did not differ (P>0.05) between treatment groups and were 1.1±0.3, 2.6±0.2, and 3.3±0.2 on days −1, 14, and 28, respectively. There was an increase (P<0.05) in the number of follicles ≥3 mm between d−1 and d14 and between d−1 and d28, but there was no difference (P>0.05) between d14 and d28 (Figure 3).

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The percentage of ewes in estrus did not differ (P>0.05) between treatment groups (Table 3). The interval between sponge removal and estrus was shorter (P<0.01) in the control group than in the RCGS group, but the intervals from sponge removal to ovulation and from estrus to ovulation did not

Table 2 - Daily intake of dry matter (DM), neutral detergent fiber (NDF), crude protein (CP), ash, ether extract (EE), and nonfibrous carbohydrates (NFC) by tropical Santa Inês ewes during the flushing period

Variable	Control (n = 7)	RCGS (n = 8)	P-value
DM (kg day^{-1})	1.10±0.07	1.00±0.07	0.33
DM (g kg^{-1} BW)	26.57±1.70	24.2±1.53	0.30
NDF (g day^{-1})	330.40±22.31	288.30±20.13	0.16
CP (g day^{-1})	138.20±8.90	125.10±8.03	0.27
Ash (g day^{-1})	36.69±2.79	33.34±2.53	0.37
EE (g day^{-1})	33.57±2.26	30.45±2.07	0.31
NFC (g day^{-1})	551.60±41.12	510.00±37.49	0.45

RCGS - rehydrated corn grain silage; BW - body weight.
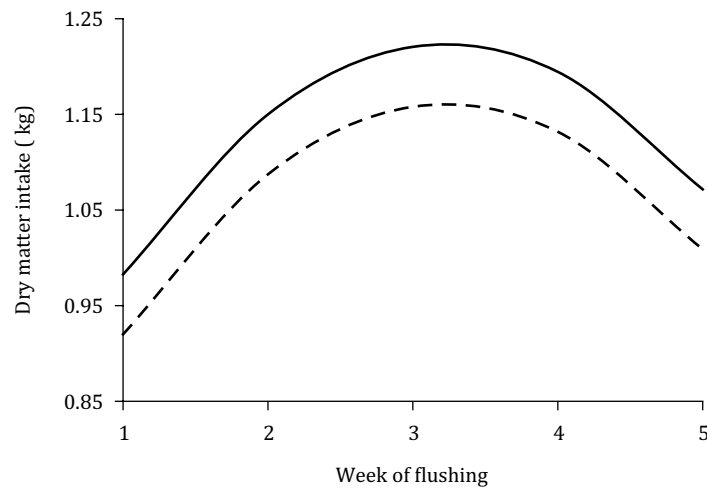Values are lsmeans±SEM; P>0.05.

Figure 1 - Dry matter intake (kg day^{-1}) by tropical Santa Inês ewes fed flushing diets with ground corn (control; ———) or rehydrated corn grain silage (RCGS; - - - -) for 30 days.

$\hat{y} = 68.9897 + 3.8116x - 0.03276x^2 + 0.007295x^3$ (P = 0.0078)

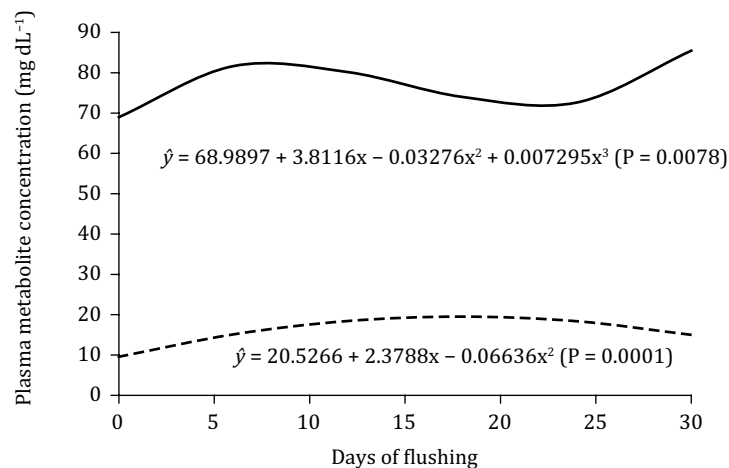$\hat{y} = 20.5266 + 2.3788x - 0.06636x^2$ (P = 0.0001)

Figure 2 - Plasma urea nitrogen (- - - -) and glucose (———) concentrations in tropical Santa Inês ewes fed flushing diets for 30 days.

differ between treatment groups (P>0.05; Table 3). Among ewes that showed estrus, 100% in control and 60% in RCGS expressed signs of estrus within 72 h of sponge removal (period considered desirable for synchronization). The length of estrus did not differ between treatment groups (P>0.05; Table 3).

The percentage of ewes that ovulated and the number of ovulations did not differ (P>0.05) between treatment groups (Table 3). The diameter of ovulatory follicles at sponge removal (d28) and the growth rate of the ovulatory follicles were greater in the RCGS group than in the control group, resulting in ovulatory follicles with a larger diameter in the RCGS group (P<0.01; Table 3).
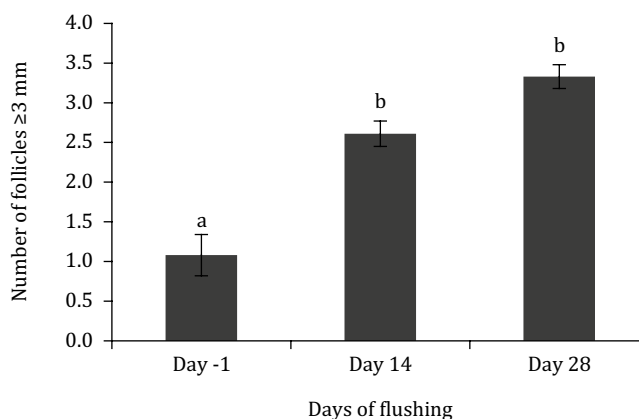
Means with different letters are significantly different (P<0.05).
Error bars represent SEM.

Figure 3 - Number of follicles ≥3 mm in tropical Santa Inês ewes on the day before flushing (d−1) and on days 14 and 28.

Table 3 - Effect of flushing with ground corn (control; n = 7) or rehydrated corn grain silage (RCGS; n = 8) on the reproductive variables of tropical Santa Inês ewes

Variable	Control	RCGS	P-value
Estrus expression (%)	57(4/7)	63(5/8)	0.80
Ovulation (%)	86(6/7)	75(6/8)	0.50
Length of estrus (h)	43.6±10.6	37.1±9.5	0.65
Interval from sponge removal to estrus (h)	26.5±8.5b	62.8±7.5a	0.002
Interval from sponge removal to ovulation (h)	71.2±14.2	86.8±14.2	0.47
Interval from estrus to ovulation (h)	41.2±11.2	36.6±10.0	0.76
Number of ovulations (n)	2.7±0.2	1.6±0.3	0.25
Diameter of the ovulatory follicles at sponge removal (mm)	5.2±1.1b	6.3±1.0a	0.01
Growth rate of ovulatory follicles (mm day^{-1})	0.5±0.1b	0.7±0.1a	0.04
Ovulatory follicle diameter (mm)	6.2±0.5b	8.2±0.7a	<0.01

Values are the lsmeans ± SEM.
a,b - Means followed by different letters in the rows differ from each other (P<0.05).

4. Discussion

The present study showed that the substitution of one energy ingredient for another with better ruminal starch digestibility, i.e., RCGS, in flushing diets increased the diameter of the ovulatory follicles at sponge removal, prolonged the interval from sponge removal to estrus, and increased the growth rate of the ovulatory follicles, resulting in greater follicular diameter at ovulation. The DMI was not affected by the starch source in this study, corroborating Callison et al. (2001) and Oba and Allen

(2003), who reported that a supply of corn with greater ruminal digestibility of starch did not affect the DMI. According to Oba and Allen (2003), the effects of rumen digestibility of starch on DMI are inconsistent, and a threshold seems to exist, after which propionate affects the DMI. The reduction in DMI after d16 of flushing might be related to the greater concentration of propionate available for gluconeogenesis. When the propionate flux exceeds gluconeogenesis capacity, oxidation of propionate may occur (Bradford and Allen, 2007), which increases hepatic ATP production and thus triggers mechanisms of satiety and intake inhibition (Allen et al., 2009).

Nutrition can have acute, dynamic, and static effects on reproduction (Scaramuzzi et al., 2006). In the present study, there was a dynamic effect, because the increase in the number of follicles on d14 was associated with BW gain throughout the flushing period in both treatment groups. This result also suggests that the diets tested may have beneficial effects on short-term flushing (Zabuli et al., 2010; Habibizad et al., 2015) in ewes with moderate BCS. The increase in the number of follicles in ewes with better nutritional status is consistent with previous reports (O´Callaghan et al., 2000; Viñoles et al., 2009) and may be related to an increase in glucose supply to follicular cells (Zabuli et al., 2010; Habibizad et al., 2015). The ADG, along with the increased plasma glucose concentration, suggests that the animals had a positive energy balance. As reported by Scaramuzzi et al. (2006), a positive energy balance is associated with an increase in plasma glucose concentrations and stimulation of the IGF (insulin-like growth factor) system, which, in turn, are positively related to folliculogenesis and number of follicles. Furthermore, increased DMI is associated with alterations in the hepatic metabolism of steroids (Parr et al., 1987; Parr et al., 1993), which can decrease the negative feedback between the ovary and the hypothalamo-pituitary system, thus stimulating folliculogenesis. These possible mechanisms could lead to an increase in the number of follicles over time. Because propionate is the primary precursor for gluconeogenesis (Huntington et al., 2006) and there was no difference in the DMI, we can assume that the contribution of propionate to gluconeogenesis was similar in both treatment groups. In the present study, the glucose concentrations were close to the upper limit of the reference range of 50 to 80 mg dL^{-1} for sheep (Kaneko, 1997), indicating that gluconeogenesis was regulated over time to meet the requirements of the animals (Huntington, 1997).

The corn rehydration and ensiling processes may increase rumen digestibility of starch (Ferraretto et al., 2013). Diets that provide a greater amount of fermentable energy in the rumen reduce the ruminal ammonia (Dias et al., 2018) and, consequently, the PUN concentrations. However, in the present study, no reduction in PUN concentration was found in the group fed RCGS, which might be related to the lack of difference in the CP intake between treatment groups. The CP content in the diets met the recommendations of the NRC (2007), which contributed to PUN values below those considered harmful to sheep reproduction (Bishonga et al., 1996; McEvoy et al., 1997; Branca et al., 2000).

We observed that the number of ewes that ovulated was higher than the number of ewes that showed estrus, suggesting the occurrence of silent ovulations in both treatment groups. Because the main hormonal stimulus for estrus is estradiol, we can reasonably assume that increased hepatic clearance of estradiol (Adams et al., 1994) in overfed animals may have limited estrus. In addition, the use of intravaginal MPA implants may cause vaginitis (Martins et al., 2009) and, thus, adversely affect sexual attractiveness and detection of estrus by rams (Gatti and Ungerfeld, 2012).

The longer interval from sponge removal to estrus in the animals fed RCGS could not be explained by nutritional status, because there was no difference between treatment groups regarding DMI, ADG, BCS, and plasma glucose concentration. It is possible that other hormones and metabolites not measured in this study are involved with the longer interval to estrus behavior after sponge removal in the RCGS group. The blood estradiol concentration and estrus expression may be affected by changes in the regulation of steroidogenesis mediated by glucose and metabolic hormones (Muñoz-Gutiérrez et al., 2004). Insulin is a mediator of glucose uptake by follicular cells (Somchit-Assavacheep et al., 2013) and acts in conjunction with IGF-1 to increase the sensitivity of granulosa cells to follicle-stimulating hormone (FSH) for steroidogenesis (Webb et al., 2004). Thus, more studies are necessary to determine the effects of RCGS on hormones and metabolites related to steroidogenesis and follicular development.

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A high percentage of ewes in estrus within 72 h after sponge removal is desirable in estrus synchronization protocols for artificial insemination (AI) or embryo transfer (Godfrey et al., 1999). However, it was only in the control group that all ewes expressed estrus within this interval. The eCG was administered 48 h before sponge removal, similar to the study of Ali (2007), and, interestingly, the interval from sponge removal to ovulation in the control group was similar in both studies. A shorter interval to estrus and ovulation and earlier development of large follicles might be beneficial for fixed-time AI (Ali, 2007). Therefore, a practical consideration is that it may be possible to achieve a good pregnancy rate with the use of fixed-time AI in ewes receiving flushing with ground corn.

The length of estrus observed was similar to that reported by Ascari et al. (2013) and Teixeira et al. (2016) in Santa Inês sheep after estrus synchronization with progestagens. The greater variability observed may be related to the presence of animals with single or multiple ovulations (double or triple). Figueira et al. (2015) reported longer estrus length in Santa Inês sheep with multiple ovulations than in those with single ovulations, which is consistent with our observations.

The data on DMI, ADG, BCS, and plasma glucose suggest that the nutritional status was similar in ewes from both treatment groups, which may be related to the absence of statistical difference in the number of ovulations. Furthermore, some variables, such as the number of ovulations, may have high natural variability, which could contribute to similarity in the result between treatments. The number of ovulations observed in ewes fed the control diet was similar to that reported in Santa Inês ewes fed flushing diets for 28 days (Saunders et al., 2010; Lazarin et al., 2012). In contrast, the number of ovulations observed in ewes fed the RCGS was similar to that reported in Santa Inês ewes fed a maintenance diet (Cavalcanti et al., 2012; Lazarin et al., 2012).

The ovulatory follicle diameter in the RCGS treatment group was larger than the follicle diameters reported in other studies with Santa Inês ewes (Lazarin et al., 2012; Ascari et al., 2013; Teixeira et al., 2016). At the time of sponge removal, the follicle diameter of the animals in the RCGS group was 1.1 mm greater than that of ewes in the control group. In addition, the onset of estrus was delayed, which allowed a longer period of ovulatory follicle growth and ovulation of larger follicles. It is known that the maximum diameter attained by follicles depends on their lifespan (Bartlewski et al., 2011). The ovulation of large dominant follicles with extended lifespan was also reported by Viñoles et al. (2001) after estrus synchronization protocols with long-term progestagen exposure, similar to the protocol of the present study. The largest first-wave follicle is dominant in the ewe, and the decline in progesterone concentrations to subluteal levels over long-term protocols can prolong its lifespan and extend this dominance (Viñoles et al., 1999). However, these authors observed the occurrence of persistent follicles only in those ewes that did not receive eCG. Therefore, the reason for persistent follicle growth in the RCGS group is uncertain. The combination of estrus synchronization and a high-energy diet may contribute to an increase in the ovulatory follicle diameter (Senosy et al., 2017).

5. Conclusions

Replacing ground corn with rehydrated corn grain silage in flushing diets can alter follicular development, resulting in delayed onset of estrus and ovulation of larger follicles. This does not benefit the number of ovulations. The increase in the number of antral follicles after 14 days of flushing with ground corn or rehydrated corn grain silage suggests the possibility of reducing the period of overfeeding.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: L.M. Figueira, I.F. Furusho-Garcia, R.F. Leite and N.G. Alves. Data curation: L.M. Figueira, L.R. Faria, J.P.A. Campos, D.R. Silva and N.G. Alves. Formal analysis: L.M. Figueira, R.R. Lima

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