

## OVULATION RATE, METABOLITE AND HORMONAL PROFILES OF EWES IN LOW BODY CONDITION STIMULATED WITH HIGH-ENERGY DIET DURING THE LATE-LUTEAL PHASE OF THE ESTROUS CYCLE

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### ABSTRACT

The aims of this study were to determine the effects of short-term feeding with a high-energy diet on the ovulatory capacity, metabolite, and hormonal profiles of ewes in low body condition score (BCS), and to investigate the relationship between these variables in the ovulated ewes. Sixteen ewes that had ovulated (day 0) after synchronized estrus were allotted into two experimental groups. The control group received a control diet (CD) throughout the experimental period, while the supplemental group was offered a high-energy diet (H-ED) for a 5-day period from days 10–14 after ovulation. The follicles were scanned by ultrasonography, and the blood was sampled to measure the metabolites and progesterone. On day 14, the number of large-sized follicles was greater in the H-ED than in the control ewes. On days 11, 13 and 14, the concentrations of glucose were found to be higher for the H-ED group compared to the CD group. Ovulation rate was greater in the H-ED than in the control ewes. Ovulation rate tended to be positively correlated with the number of ovulatory follicles. Thus, these data emphasize that use of a high-energy diet improves the glucose concentration, follicular growth, and subsequent ovulation rate in low-BCS ewes.

**Key words:** diet treatment, ewes, follicular population, glucose, ovulation rate.

### INTRODUCTION

The body condition of the ewe before mating, at mating, and after the mating period has long been shown to influence the subsequent reproductive function and productive performance (Aliyari *et al.*, 2012; Vatankhah *et al.* 2012). In fact, reproductive performances of ewes are normally correlated with body condition score (BCS) and live weight changes (Sezenler *et al.*, 2011). Low levels of ewe body condition are known to result in the development of a low number of large follicles compared with ewes in high levels of body condition, and these differences are then reflected in the ovulation rates and the reproductive performances of the animals (Rhind and McNeilly, 1998). Indeed, ovulation rate can be a major determinant of reproductive capability in ewes. Alternatively, ovulation rate can be manipulated by the administration of exogenous hormones or nutritional adjustment during the critical period of follicular growth and development (Hunter *et al.*, 2004). However, using dietary supplement to manipulate the ovulation rate provides a useful strategy that may be inexpensive and avoids the use of chemicals and hormones (King *et al.*, 2010). In brief, the nutritional status during the specific period of the estrous cycle can exert quantitative effects on small follicular population and qualitative effects on the dominant follicles (DF) in small ruminants (Scaramuzzi *et al.*, 2006; Zabuli *et al.*, 2010). In previous studies, it has been demonstrated that high nutrient

supplementation (energy or protein) is adequate to increase the ovulation rate and to enhance the reproductive performance in ewes (Scaramuzzi *et al.*, 2006; Senosy *et al.*, 2013). This short-term feeding stage is a critical period in the luteal phase of the estrous cycle (Somchit *et al.*, 2007) or during the 6-day period before functional luteolysis (King *et al.*, 2010). Thus, enhancing the reproductive function of ewes provides the potential for increasing the productivity in sheep meat production systems (King *et al.*, 2010).

The information gained from some strategic metabolic indicators and body condition can, however, possibly provide a more substantial basis regarding the knowledge of the metabolic status and reproductive management of the ewes and, consequently, diets can be adjusted together with improved reproduction (Munir *et al.*, 2007). We hypothesized that short-term feeding with a high-energy diet could be made to influence the ovarian function and the subsequent ovulation rate of low-BCS ewes. Therefore, the objectives of the present study were the following: (1) to determine the effects of short-term feeding (5-day period) with a high-energy diet on the ovarian follicular population, ovulatory capacity, hormonal profile, and blood metabolites of low-BCS ewes during the late-luteal phase of the estrous cycle; and (2) to investigate the relationship between these variables in the ovulated ewes.

## MATERIALS AND METHODS

**Experimental location:** This experiment was conducted at a goat and sheep farm, in the Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University, Thailand (Latitude: 18°45' N / Longitude: 98°55' E). The climate was tropical with distinct differences between dry weather (October–April) and wet weather (May–September). The average annual rainfall based on data from the Northern Meteorological Center, Thai Meteorological Department, Thailand, for the period from 2013–2014 was 1263 mm. The ambient temperature and the relative humidity during the experiment (February–May 2014) ranged, respectively, from 21.0–34.9°C and from 44.1–83.9%.

**Animals, estrous synchronization, and management:** Sixteen mature crossbred ewes (Merino × native; 12–24 months of age) with low levels of BCS (BCS = 2 unit) and with an average body weight of  $30.6 \pm 0.5$  kg were used to synchronize the estrous cycles. The estrous periods of 16 mature crossbred ewes were synchronized with two injections of prostaglandin  $F_{2\alpha}$  (PGF<sub>2 $\alpha$</sub> ; Lutalyse, Pfizer Animal Health, New York, USA), given 10 days apart. Using transrectal ultrasonography, the ovaries were determined daily, from the day of the second PGF<sub>2 $\alpha$</sub>  injection until the ovulation. The day of ovulation (day 0) was defined as the day on which a large, previously identified ovarian follicle ( $\geq 5.0$  mm in diameter) was no longer seen (Toosi *et al.*, 2009). All ewes were housed in individual pens (1.5 m × 2.2 m) on slatted floors, with a section of the pens provided with a sheltered area for protection from sun and rain. All ewes were kept under natural photoperiod and were pre-fed a maintenance diet for a 7-day period (the adaptation period). In each pen, the animals had *ad libitum* access to clean water and mineral block.

**Experimental design and diets:** Sixteen ewes that had ovulated after the second PGF<sub>2 $\alpha$</sub>  injection were randomly assigned to two groups and kept in different pens in separate groups according to the type of diets. In the control group (n = 8), ewes were offered a control diet (CD) throughout the experimental period. Prior to the initiation of experimental dietary period in the supplemental group (n = 8), ewes were fed a CD from days 0–9 after ovulation and then ewes were offered a high-energy diet (H-ED) for 5 days (short-term period) from days 10–14 after ovulation. The CD consisted of 70% Napier grass (*Pennisetum purpureum* Schumach.) and 30% concentrate. The H-ED consisted of 30% Napier grass and 70% concentrate. The digestible energy of a control diet and a high-energy diet was approximately 2395 and 3118 kcal/kg of dry matter (DM), respectively. The ingredient composition and the calculated nutrient content are demonstrated in Table 1. The experimental diets were fed daily as two meals at 08:00 h and 15:00 h.

The animals were weighed at the beginning (day 10) and after the end (day 14) of the feeding treatment periods. Additionally, the ewes were fed the experimental diets from days 10–14 after ovulation, which corresponded to the late-luteal phase of the estrous cycle (Contreras-Solis *et al.*, 2008).

**Ultrasonography examination, ovarian follicular populations, and detection of ovulation:** The ovaries were scanned daily using transrectal ultrasonography with a 7.5 MHz rectal transducer (Toshiba Just Vision 200, Japan) from the start of the dietary treatments (day 10) until the second ovulation. The relative position and dimension of all follicles  $\geq 2.0$  mm in diameter and all corpora lutea (CLs) were also sketched on ovarian charts. In combination with ultrasound records, the visible follicles were classified by size as total follicles ( $\geq 2$  mm in diameter), small follicles (2.0–3.4 mm in diameter), medium follicles (3.5–4.4 mm in diameter), and large or ovulatory follicles ( $\geq 4.5$  mm in diameter), as described previously (Contreras-Solis *et al.*, 2008). At the start and at the end of the nutritional treatments, the numbers of follicles in the different size categories were also analyzed. Following the nutritional treatments, ovulation of a preovulatory follicle was considered to occur when a follicle, which was greater than 5.0 mm in diameter and observed in the previous scanning, had disappeared (Toosi *et al.*, 2009). Additionally, all the ewes were continuously examined by ultrasonography for 6 days after the dietary treatments in order to estimate the number of ovulations and to monitor the formation of luteal structures (Bartlewski *et al.*, 2004).

On the basis of these measurements, the average number of follicles (total number of follicles in the different size categories per total number of ewes in each group), the number of ovulating ewes (total number of ovulating ewes per total number of ewes in each group), the number of multiovulating ewes (total number of ewes recording multiple ovulations per total number of ewes in each group), the ovulation rate (total number of CL per total number of ovulating ewes in each group), and the mean ovulation rate (total number of CL per total number of all ewes in each group) were measured in each of the control and supplemental groups.

**Blood sampling:** Jugular vein blood samples (10 mL) were collected into venipuncture on days 9–15 after the first ovulation to measure the glucose, blood urea nitrogen (BUN), and progesterone (P<sub>4</sub>) concentrations. Each sample was separated into two aliquots of 5 mL. From one aliquot, the blood sample was placed into the gray-top tube with the ethylenediaminetetraacetic acid (EDTA) and sodium fluoride, which is used for plasma determination. The other aliquot was placed into a tube without anticoagulants, which is used for serum evaluation. After collecting the whole blood, the samples were centrifuged at room temperature at  $1200 \times g$  for at

least 10 min. The blood plasma and the serum were kept at  $-20^{\circ}\text{C}$  until the glucose, BUN, and  $\text{P}_4$  concentrations were measured.

**Glucose, BUN, and  $\text{P}_4$  assays:** The plasma samples were obtained in the gray-top tube and sent to the laboratory for glucose analysis by the hexokinase method (Aekplakorn *et al.*, 2007). The BUN was determined by the enzyme kinetic method. The serum  $\text{P}_4$  concentrations were measured in duplicate using the competitive enzyme-linked immunosorbent assay (competitive ELISA; adapted from Brown *et al.*, 2005). For the  $\text{P}_4$  analysis, the assay sensitivity and the intra-assay coefficient of variation were 0.203 ng/mL and 4.1%, respectively.

**Statistical analyses:** The data are presented as mean $\pm$ SEM. The body weight, number of follicles, diameter of the largest of the follicles, ovulation rate, mean ovulation rate, glucose, BUN, and  $\text{P}_4$  concentrations were analyzed with ANOVA using the general linear model (GLM) procedure of SAS (SAS Institute Inc, Cary, NC, USA). The differences between the means were evaluated by Student's t-test (Steel *et al.*, 1997). The proportion of ewes undergoing ovulation was analyzed using chi-square analysis (Steel *et al.*, 1997). Differences with  $P < 0.05$  were considered significant, and those with  $0.05 < P < 0.10$  were considered a tendency (Lima *et al.*, 2013). Simple linear correlations between specific variables were determined by using PROC CORR of SAS (Steel *et al.*, 1997), as previously described (Moonmanee *et al.*, 2013).

## RESULTS AND DISCUSSION

At the start of the nutritional treatments, the CD and H-ED ewes had similar ( $P > 0.05$ ) body weight ( $31.1 \pm 0.8$  kg vs.  $30.1 \pm 0.5$  kg). Similarly, there was no difference ( $P > 0.05$ ) between the CD and H-ED groups regarding live weight after the end of the feeding period ( $31.2 \pm 0.8$  kg vs.  $30.3 \pm 0.5$  kg). These results are consistent with the body weight change in terms of short-term supplementation in ewes reported by Somchit *et al.* (2007), who found that short-term nutritional supplementation with lupin grain during the luteal phase of the estrous cycle increased the ovarian activity without impacting live weight. In fact, administration of short-term nutritional supplementation was reported to increase folliculogenesis in ewes, with no change in live weight or body condition (Scaramuzzi *et al.*, 2006; Zouaidi *et al.*, 2009).

At the start of the nutritional treatments (day 10), there were no differences in the numbers of small (2.0–3.4 mm in diameter), medium (3.5–4.4 mm in diameter), large ( $\geq 4.5$  mm in diameter), and total ( $\geq 2.0$  mm in diameter) follicles between the ewes receiving the

CD and H-ED treatments. At the end of the nutritional treatments (day 14), although there was no difference in small and medium follicles between the CD and H-ED ewes, the number of large follicles ( $P < 0.05$ ) was greater in the H-ED group than in the CD group ( $2.0 \pm 0.3$  vs.  $1.1 \pm 0.3$ ). Additionally, the number of total follicles ( $P = 0.08$ ) tended to be greater in the H-ED group than in the control ewes ( $2.8 \pm 0.2$  vs.  $2.1 \pm 0.3$ ). At the end of the feeding treatments, the diameter of the largest follicle in ewes receiving the H-ED treatment had a greater ( $P < 0.05$ ) size compared with the ewes receiving the CD treatment ( $6.2 \pm 0.18$  mm vs.  $5.7 \pm 0.17$  mm). In ewes receiving the CD treatment, the numbers of medium, large, as well as total follicles did not differ statistically between day 10 and day 14 (Fig. 1A). In contrast, the number of follicles 2.0–3.4 mm in diameter was greater at the start (day 10) than at the end (day 14) of the dietary treatments (Fig. 1A). In ewes being offered the H-ED treatment, the number of follicles 2.0–3.4 mm in diameter was greater ( $P < 0.05$ ), but the number of large follicles was less ( $P < 0.05$ ) at the start (day 10) than at the end (day 14) of the nutritional treatments (Fig. 1B). The number of medium follicles did not differ between days 10 and 14 (Fig. 1B). Moreover, the number of total follicles was higher ( $P < 0.05$ ) on day 14 than on day 10 (Fig. 1B). In addition, the number of small follicles on day 10 was positively correlated with the number of large follicles on day 14 ( $R^2 = 0.802$ ;  $P < 0.05$ ).

In the present study, the short-term feeding with H-ED for the low-BCS ewes increased the number of large follicles and, subsequently, the total number of follicles. Similar to the findings reported by Scaramuzzi *et al.* (2006), it was observed that ewes offered with lupin grain (high-energy diet) had increased number of large-sized follicles. Similarly, the ewes receiving the short-term treatment with soya-maize diet or intravenous glucose demonstrated an increase in the total number of follicles (Zouaidi *et al.*, 2009). On the other hand, Senosy *et al.* (2013) reported no effect of the supplementing high-energy diet in ewes as regards the numbers of medium-sized (3–5 mm in diameter) and large-sized ( $> 5$  mm in diameter) follicles after ovulation. Differences in the two studies may be correlated in terms of the different effects of the short-term regime and the stimulus used. These results are consistent with the ovarian follicular response to short-term nutritional supplementation of low-BCS ewes during the luteal phase reported by Viñoles *et al.* (2005), who found that enhancement in the blood metabolites (glucose, insulin, and leptin) on the day before the ovulatory wave emergence was related to the increase in the number of follicles that grow up to 2–3 mm in diameter and influences the DF to grow for a longer period.

As such, these data support the idea that short-term effects of dietary stimulation are probably mediated directly at the follicular level to modify gonadotropin-

induced follicular growth and development (Scaramuzzi *et al.*, 2010). It is strongly implied that dietary supplementation with H-ED treatment acts directly in small growing follicles (Scaramuzzi *et al.*, 2015) to encourage growth, leading to greater number of the largest preovulatory follicle in low-BCS ewes. Taken together, the present finding supports a previous study in cattle (Armstrong *et al.*, 2001) and a previous review which states that diet is also positively correlated with the growth rate and diameter of preovulatory follicles (Webb *et al.*, 2004). It appears that severe acute dietary supplementation has an immediate effect not only on the follicle growth rate and the follicle diameter, but also on the ability of the small growing follicles to grow as it compromises the same. Although follicular growth evaluation was not part of our study, it is evident that the increased rate observed in follicular growth was strongly indicated by the increase observed in the number of follicles (Gallet *et al.*, 2011).

The plasma concentration of glucose was higher ( $P < 0.05$ ) for low-BCS ewes fed the H-ED compared with ewes fed the CD on days 11, 13, and 14 of the nutritional period (Fig. 2A), but this difference was not statistically significant on days 10 and 12 for the duration of the sampling period (Fig. 2A). There were no significant treatment effects on the plasma concentration of BUN (Fig. 2B;  $P > 0.05$ ). Nutritional treatments did not affect the overall serum concentration of  $P_4$  throughout the experimental period (Fig. 3;  $P > 0.05$ ). In our study, the  $P_4$  levels corroborated that all the ewes were undergoing normal estrous cycle.

In the present study, the plasma value of glucose of the supplemented low-BCS ewes was similar to those demonstrated for ewes in low body condition (Viñoles *et al.*, 2005) and ewes that were offered high-energy diets (Senosy *et al.*, 2013). In agreement with our hypothesis, the glucose concentration of low-BCS ewes receiving the H-ED treatment was greater than that of ewes receiving the CD treatment. Compared with the low-BCS ewes in the H-ED group, the present result indicated that the low-BCS ewes in the CD group produced substantially less glucose throughout the experimental period. Evident undernutrition with a BCS of 2, particularly of 1.25 in ewes, was demonstrated by low concentration of plasma glucose (Caldeira *et al.*, 2007). On the organ level, the ovary can respond directly to metabolic inputs, independent of the gonadotropin drive (Chagas *et al.*, 2007). In ewes, nutritional alterations can encourage the capability of antral follicle (gonadotropin-dependent follicle) to utilise the small amounts of follicle-stimulating hormone (FSH) in the final stages of follicular growth, which are the most responsive to low FSH levels (Garcia-Garcia, 2012). On a cellular level, glucose consumed by ovarian follicular cells (theca and granulosa) can be used for major energy production, cellular homeostasis, nuclear maturation, and substrates

for matrix production to maintain growing-follicle development (Sutton-McDowall *et al.*, 2010; Ying *et al.*, 2011). However, intrafollicular glucose was not determined in the present study, and this information would be required to test such a hypothesis.

The proportion of ewes that ovulated after short-term dietary supplementation did not differ ( $P > 0.05$ ) between the CD and H-ED groups (Table 2). However, the percentage of ewes with multiple ovulations was higher ( $P < 0.05$ ) in the H-ED than in the CD ewes (Table 2). In the ewes that were offered the control diet, the majority of the characteristic ovulations were single (80.0%); only 20.0% of the ovulating ewes had twin ovulations. In the ewes that received the H-ED treatment, incidences of ovulations were observed, with the majority of the ewes having twin (83.3%) and some of them having single (16.7%) ovulations. In addition, none of the ovulating ewes exhibited triplet ovulations. Although the mean ovulation rate did not differ between the ewes that were offered the CD treatment and the ewes that received the H-ED treatment, the ovulation rate was greater ( $P < 0.05$ ) in the H-ED ewes than in the control ewes (Table 2).

In our study, the effect of dietary supplementation on the ovulation rate was significant with 0.6 extra ovulations per ewe achieved through the H-ED treatment. Moreover, the ovulation rate in the low-BCS ewes that were offered the H-ED treatment was 1.8 ovulations per ewe. Similar to the findings published by Letelier *et al.* (2008), it was suggested that the use of diets containing high starch, which would increase the folliculogenesis and the ovulation rate in Maiden ewes (1.4 ovulations per ewe). In contrast, short-term supplementation with corn grain and soybean meal from days 9–14 of the estrous cycle was not found to affect the ovulation rate (1.1 ovulations per ewe) in Corriedale ewes (Viñoles *et al.*, 2005).

As far as ovulated ewes in the group of the CD treatment are concerned, upon evaluation, it was found that the diameter of the largest preovulatory follicle (DLPF) was positively correlated ( $P < 0.05$ ) with the ovulation rate (OR; Table 3). As for ovulated ewes in the group of the H-ED treatment, upon assessment, it was observed that their plasma glucose level was positively correlated ( $P < 0.05$ ) with their number of ovulatory follicles (NOFs; Table 4). The number of total follicles (TFs) was positively associated ( $P < 0.05$ ) with the DLPF (Table 4). Additionally, the OR tended to be positively related ( $P = 0.08$ ) with the NOFs (Table 4). The present study confirmed the hypothesis that higher glucose concentrations increase the follicular growth rate, as indicated by the increase in the number of the ovulatory follicles before ovulation. On the other hand, these relationships were not observed in ewes that received the CD treatment. The positive correlation between nutrition and ovulatory capacity has been demonstrated in ewes

infused intravenously with glucose for the short-term period of days 8–12 of the estrous cycle. This model increased the glucose concentration in the serum and, subsequently, increased the ovulation rate (Downing *et al.*, 1995; Gil, 2003). In the present study, the higher number of ovulatory follicles in the group of the H-ED ewes was found to correlate with the greater ovulation rate. This result is supported by a previous study which

demonstrated that multiple ovulations are caused by an increase in the number of ovulatory follicles available for further development (Scaramuzzi *et al.*, 1993). Hence, overall, our results indicate that the higher ovulation rate found in the supplemented ewes may be associated with an increased developmental competence of the ovulatory follicles.

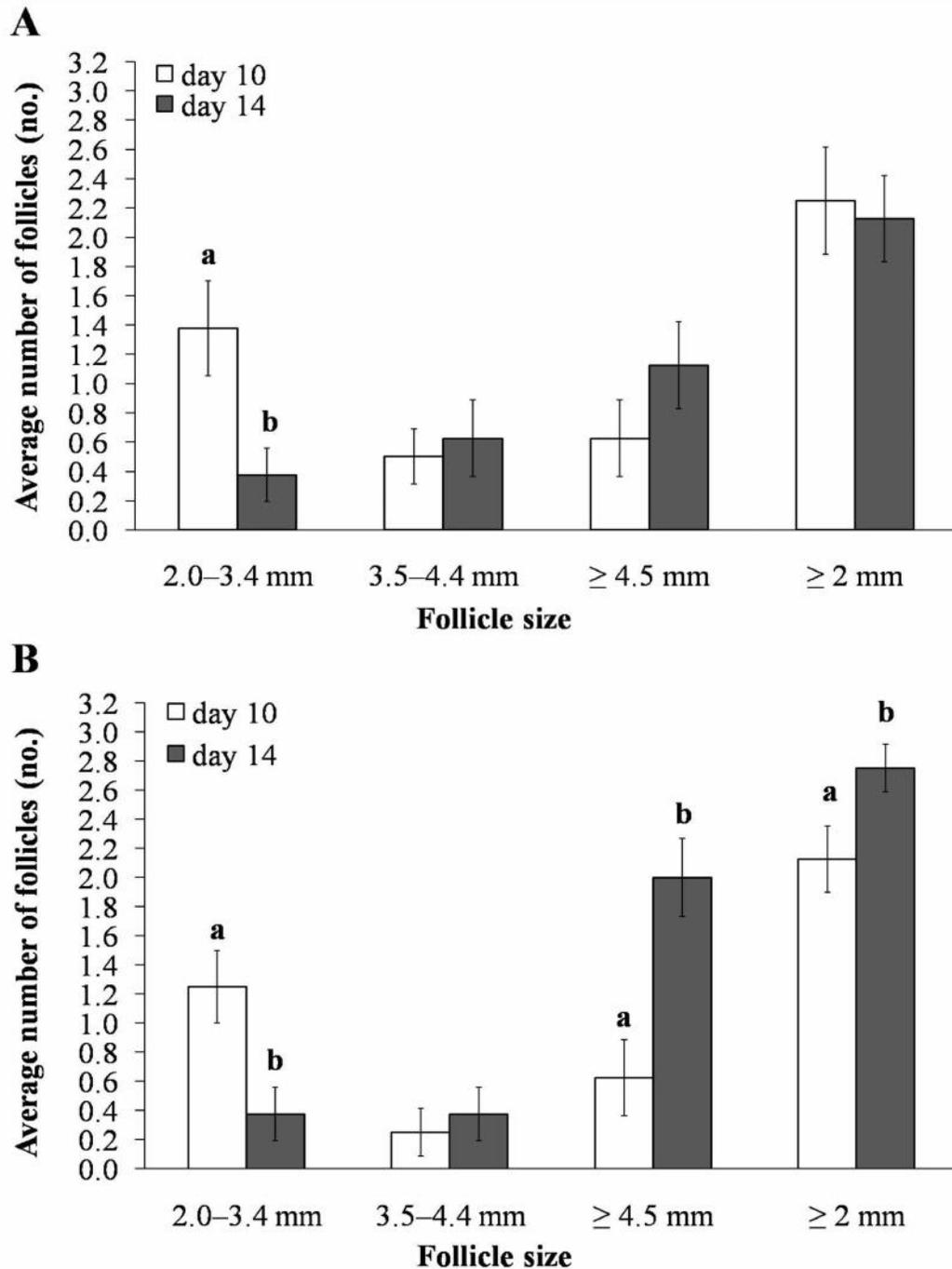


Fig. 1. The ovarian follicular populations of low-BCS ewes stimulated with the control diet (CD; A) or the high-energy diet (H-ED; B) at the start (day 10) and at the end (day 14) of the nutritional treatments

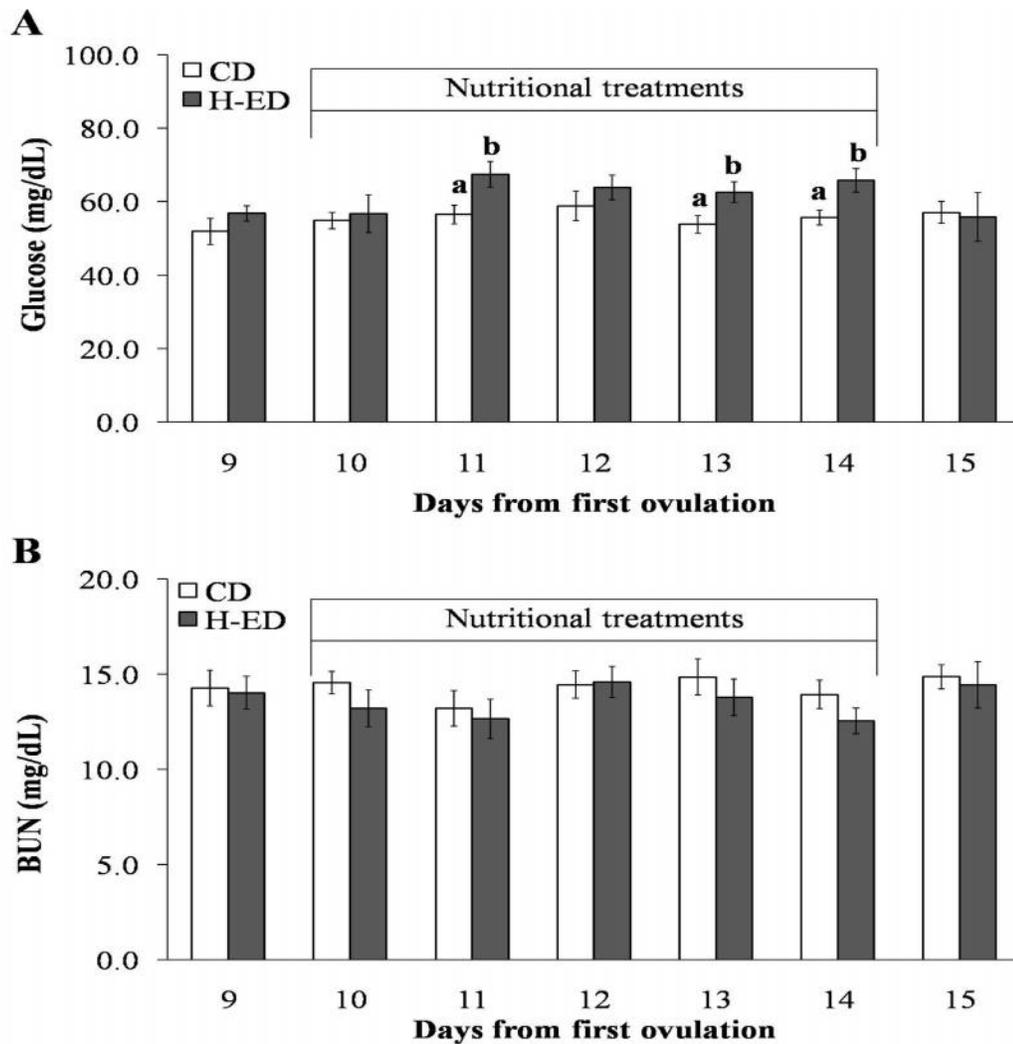


Fig. 2. The plasma concentrations of glucose (A) and blood urea nitrogen (BUN; B) of low-BCS ewes stimulated with the control diet (CD) or the high-energy diet (H-ED) during the late-luteal phase of the estrous cycle

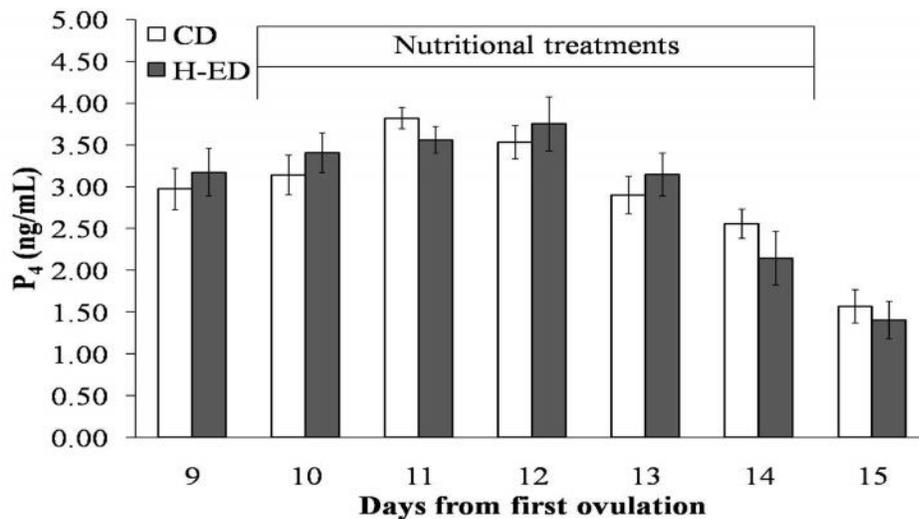


Fig. 3. The serum concentration of progesterone (P<sub>4</sub>) of low-BCS ewes stimulated with the control diet (CD) or the high-energy diet (H-ED) during the late-luteal phase of the estrous cycle

**Table 1. Ingredient composition and calculated nutrient of control diet (CD) and high-energy diet (H-ED) fed to low-BCS ewes.**

Items	Experimental diets	
	CD	H-ED
<b>Ingredients (%)</b>		
Napier grass	70.00	30.00
Rice bran	11.00	40.00
Corn meal	1.60	5.00
Soybean meal	5.80	5.00
Molasses	9.60	18.00
Dicalcium phosphate	1.00	1.00
Mineral premix <sup>†</sup>	1.00	1.00
<b>Calculated values</b>		
Dry matter (DM, %)	78.29	78.28
Crude protein (% of DM)	12.84	12.95
Crude fiber (% of DM)	17.68	10.15
Ether extract (% of DM)	6.39	8.63
Neutral detergent fiber (% of DM)	40.60	35.43
Digestible energy (kcal/kg of DM)	2395	3118

<sup>†</sup>supplied per kg of diet: Mn, 54 mg; Fe, 142 mg; Cu, 10 mg; Zn, 29 mg; Na, 39 mg; I, 0.19 mg; K, 0.009 mg; and Co, 0.011 mg.

**Table 2. Proportion of ewes with ovulation and multiple ovulations, ovulation rate, and mean ovulation rate in group of low-BCS ewes receiving control diet (CD) or high-energy diet (H-ED) during the late-luteal phase of the estrous cycle.**

Items	Experimental diets		P value
	CD	H-ED	
Number of ewes	8	8	
Number of ovulating ewes (%)	5 (62.5)	6 (75.0)	0.60
Number of multiovulating ewes (%) <sup>†</sup>	1 (20.0)	5 (83.3)	0.03
Ovulation rate <sup>‡</sup>	1.2 ± 0.20	1.8 ± 0.17	0.04
Mean ovulation rate <sup>§</sup>	0.8 ± 0.16	1.4 ± 0.14	0.15

<sup>†</sup>The number of ewes that recorded multiple ovulations (2+ ovulations).

<sup>‡</sup>Ovulation rate calculated for the ovulating ewes in each group.

<sup>§</sup>Mean ovulation rates calculated for all the ewes in each group.

**Table 3. Correlation coefficients between variables evaluated for ovulated ewes in low body condition that were fed control diet (CD; n = 5).**

Items	Glucose	BUN	P <sub>4</sub>	TFs	NOFs	DLPF	OR
Glucose (mg/dL)		-0.154	0.649	0.620	0.080	0.068	0.196
		NS	NS	NS	NS	NS	NS
BUN (mg/dL)	-0.154		0.086	-0.310	-0.721	0.068	-0.294
	NS		NS	NS	NS	NS	NS
P <sub>4</sub> (ng/mL)	0.649	0.086		0.575	0.336	0.109	0.440
	NS	NS		NS	NS	NS	NS
TFs (no.)	0.620	-0.310	0.575		0.646	0.735	0.791
	NS	NS	NS		NS	NS	NS
NOFs (no.)	0.080	-0.721	0.336	0.646		0.380	0.408
	NS	NS	NS	NS		NS	NS
DLPF (mm.)	0.068	-0.294	0.109	0.735	0.380		0.930
	NS	NS	NS	NS	NS		P<0.05
OR (ovulations per ewe)	0.196	0.068	0.440	0.791	0.408	0.930	
	NS	NS	NS	NS	NS	P<0.05	

Abbreviations: BUN, blood urea nitrogen; P<sub>4</sub>, progesterone; TFs, total follicles; NOFs, number of ovulatory follicles; DLPF, diameter of largest preovulatory follicle; OR, ovulation rate; NS, not significant.

**Table 4. Correlation coefficients between variables evaluated for ovulated ewes in low body condition that were fed high-energy diet (H-ED; n = 6).**

Items	Glucose	BUN	P <sub>4</sub>	TFs	NOFs	DLPF	OR
Glucose (mg/dL)		-0.344 NS	-0.642 NS	0.478 NS	0.820 P<0.05	0.545 NS	0.504 NS
BUN (mg/dL)	-0.344 NS		-0.086 NS	-0.636 NS	-0.123 NS	-0.688 NS	-0.193 NS
P <sub>4</sub> (ng/mL)	-0.642 NS	-0.086 NS		0.290 NS	-0.293 NS	0.011 NS	-0.229 NS
TFs (no.)	0.478 NS	-0.636 NS	0.290 NS		0.686 NS	0.839 P<0.05	0.632 NS
NOFs (no.)	0.820 P<0.05	-0.123 NS	-0.293 NS	0.686 NS		0.545 NS	0.759 P=0.08
DLPF (mm.)	0.545 NS	-0.688 NS	0.011 NS	0.839 P<0.05	0.545 NS		0.587 NS
OR (ovulations per ewe)	0.504 NS	-0.193 NS	-0.229 NS	0.632 NS	0.759 P=0.08	0.587 NS	

Abbreviations: BUN, blood urea nitrogen; P<sub>4</sub>, progesterone; TFs, total follicles; NOFs, number of ovulatory follicles; DLPF, diameter of largest preovulatory follicle; OR, ovulation rate; NS, not significant.

**Conclusion:** The data highlight that use of a high-energy diet improves glucose concentration, ovarian follicular growth, and subsequent ovulation rate in low-BCS ewes, and that a high-energy diet can be applied in short-term feeding practices.

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