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Effect of long-term or short-term supplementation of high energy or high energy-protein diets on ovarian follicles and blood metabolites and hormones in ewes

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ABSTRACT

This study was conducted to compare the effect of high energy or high energy-protein diets in long-term (16 days, flushing) or short-term (6 days, focus feeding) supplementation period on ovarian performance and serum metabolites and hormones of ewes. For this purpose 40 cyclic Naeini ewes (40 ± 1 kg BW and 2–3 years of age) were randomly assigned to experimental groups: 1—Control (CON), 2—long-term high energy diet (LE), 3—short-term high energy diet (SE), 4—long-term high energy-protein diet (LEP), and 5—short-term high energy-protein diet (SEP). Ewes were housed in the individual pens with free access to food and water. The estrous cycle of ewes was synchronized with insertion of intravaginal progesterone sponges for a 12-day period. Follicular development was observed by ultrasound four days before to one day after sponge removing (−4 to +1) and blood was sampled once a day during this time. Results showed that LE and SE groups had more large-size (>5 mm) follicles ($P < 0.05$) than those the other group on day +1. Double ovulation rate was high in ewes fed with high energy diets. The LE and SE groups had higher ($P < 0.05$) serum glucose, cholesterol and insulin, but lower urea concentration compared to the other groups. Feeding high energy caused low serum oestradiol before the sponge removing. However, the LE and SE groups had the highest ($P < 0.05$) oestradiol concentration before the estrus time. Results of the present study revealed that change in dietary energy levels for a short-term or long-term period just before ovulation could improve the blood metabolites and reproductive performance of the ewes.

1. Introduction

Nutrition level is one of the most important factors which can influence reproductive performance of ruminant animals, especially small ruminants (Webb et al., 2004; Scaramuzzi et al., 2006; Zare-Shahneh et al., 2008; Ying et al., 2011). The breeding season of ewes in most countries begins in the late summer or in early autumn (Ocak et al., 2006; Koyuncu and Canbolat, 2009; Sen et al., 2013) and during this time, the quality of rangeland forage is usually low (Ucar et al., 2005; Ocak et al., 2006; Sen et al., 2013). So, flushing is a known management program for increasing sheep reproductive performance during the breeding season (Nottle et al., 1997; Godfrey et al., 2003; Abu El-Ella, 2006). It has been shown that nutrition has stimulatory effect on follicle numbers and feeding high energy and/or protein diets may increase ovulation rate in ewes with different schemes of time and duration (Abu El-Ella, 2006; Ocak et al., 2006; Koyuncu and Canbolat, 2009). For example, it is reported that ewes fed lupin grain, a high energy and protein supplement, had higher plasma glucose (Kosior-Korzecka and Bobowiec, 2003), plasma insulin (Muñoz-Gutiérrez et al., 2002) and ovulation rate (Stewart and Oldham, 1986).

The relationship between nutrition and reproduction is complex and responses are often quite variable or inconsistent (O'Callaghan et al., 2000; Boland et al., 2001; Somchit, 2011; Safari et al., 2012). Some factors such as timing and duration of flushing, genotypes, and amount and quality of dietary supplements may affect the reproductive performance of small ruminants (Sormunen-Cristian and Jauhiainen, 2002; Acero-Camelo et al., 2008; Sabra and Hassan, 2008). On the other hand, O'Callaghan et al. (2000) demonstrated that dietary change has an immediate effect on systemic hormone and metabolite concentrations of ewes. Many other reports verified a significant relationship between ovarian performance and concentrations of glucose, insulin and leptin in ewes fed high levels of nutrition (Downing et al., 1995; Viñoles et al., 2005; Ying

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Table 1
Ingredient and calculated chemical composition of the experimental diets.

Item	Experimental diets		
Ingredient, %	Maintenance	High energy	High energy-protein
Alfalfa hay	23	23	23
Wheat straw	35.5	12	12
Corn	15	49	44.8
Barley	9.5	9.5	9.5
Soybean meal	3	2	6.7
Wheat bran	12	2	2
Vitamin–mineral	0.4	0.4	0.4
CaHP4	0.5	0.5	0.2
CaCO3	0.5	0.5	0.8
Salt	0.6	0.6	0.6
Composition			
DE Mcal/kg	2.4	3.00	3.00
CP (%)	10.5	10.5	12.5
Ca (g/day)	7	7	7
P (g/day)	4	4	4

et al., 2011; Senosy et al., 2013). Actually, it is well defined that glucose is the major source of energy and metabolic substrate for the ovary, follicle development and the primary metabolic fuel used by the central nervous system (Rabiee and Lean, 2000; Viñoles et al., 2009; Scaramuzzi et al., 2010; Sutton-McDowall et al., 2010). Moreover, Somchit (2011) reported that insulin plays a critical role in the process of follicular development because it has a general regulation of glucose uptake by ovarian cells. Viñoles et al. (2005) who tested the short-term nutritional supplementation of ewes reported that follicle development was associated with concentrations of glucose, insulin and leptin which act directly at the ovarian level. They suggested that the mechanism by which short-term nutritional supplementation affects follicle development does not involve plasma FSH concentrations.

To the best of our knowledge the effect of long-term or short-term feeding of high energy or high energy-protein diets before the ovulation time on reproductive performance of sheep were not compared in scientific papers. Therefore, the objective of this study was to test whether a 16-day or 6-day nutritional high energy or high energy-protein supplements has stimulatory effect on follicle development, ovulation rate, some blood metabolites, insulin and oestradiol in cyclic ewes.

2. Materials and methods

2.1. Animals and diets

The study was carried out at the small ruminants' research station of the College of Agriculture, Isfahan University of Technology, Iran. Naeini ewes ($n = 40$, 2–3 years of age) with similar live weight (40 ± 1 kg,) and body condition scores (2.62 ± 0.18) were assigned to five experimental groups. This fat-tailed sheep is essentially uniparous and has been reared in arid and semi-arid regions of central Iran. The ewes were housed in the individual pens (1.12 m × 1.46 m) with free access to food and water. During the experiment, the animals were maintained under 13 h light per day and all ewes received two meals of equal amounts at 08:00 and 17:00.

All animals were fed similar maintenance diet formulated based on Cornell Net Carbohydrate and Protein System of Sheep (CNCPS-S) for a 3-week period adaptation. Then the treatment groups received high energy or high energy-protein diet for long-term or short-term period, while control group received maintenance diet throughout the experimental period (Table 1).

2.2. Experimental procedure

The estrus cycle was synchronized with the insertion of intravaginal progesterone sponge containing 30 mg fluorogestone acetate (Bioniche, Animal Health, PTY, Armidale, NSW, Australia). After 12 days, the sponges were removed and estrous behavior was detected by the rams equipped with anti-mating aprons every 12 h during the next 3 days. High energy and high energy-protein diets were fed in a long-term or short-term period. The long-term period lasted 16 days (15 days before to 1 day after sponge removing) and short-term period lasted 6 days (5 days before to 1 day after sponge removing). Finally five experimental groups were: 1—Control (CON), 2—long-term high energy diet (LE), 3—short-term high energy diet (SE), 4—long-term high energy-protein diet (LEP), and 5—short-term high energy-protein diet (SEP) (Fig. 1).

2.3. Ultrasound examination and blood sampling

In order to estimate the effect of diet treatments on the follicular population, ovaries were examined daily by ultrasonography. Ultrasound examination began four days before of sponge removal and continued for 6 days (days −4, −3, −2, −1, 0, +1). Transrectal ultrasound examinations were conducted using a real-time B-mode diagnostic scanner equipped with a transrectal 7.5 MHz linear array transducer (Shenzhen Emperor Electronic Technology Co., Ltd., EMP China). The total number, diameter and position of all follicles ≥2 mm were assessed in both ovaries every day. In each observation, the relative locations of all follicles were noted on an ovarian map to follow the sequential follicular development. Visible follicles on the surface of the ovaries were classified, based on their diameter: (i) small-size (2–3.5 mm), (ii) medium-size (>3.5–5 mm) and (iii) large-size (>5 mm) (Karami Shabankareh et al., 2010). The first ultrasonic detection of follicles with 2 mm in diameter was considered as the day of appearance. When this follicular diameter was more than 2 mm at first detection, the previous day was considered as the day of emergence. Single and double ovulation status was assessed using ultrasonography and the number of corpora lutea were determined 10 days after sponge removing.

Blood samples were collected by jugular venipuncture daily during the days −4 to +1. Then the samples were centrifuged at 2500 rpm for 20 min and serum was stored at −20 °C until assayed for glucose, cholesterol, urea nitrogen, insulin and oestradiol (E2) concentrations. Serum glucose and cholesterol concentrations were determined by ELISA Reader (ELx 808-Ultramicroplate Reader Bio-Tek Instruments Inc., U.S.A) using commercial kits (Zeist Chimi, Cat No. 10-505 ZiestChem Diagnostics Tehran, Iran and Pars Azmun, Karaj, Iran), respectively. Urea nitrogen concentrations were measured by Spectrophotometer (UV–vis Recording Spectrophotometer Shimadzu-UV2100, Japan) using commercial kits (Pars Azmun, Karaj, Iran). Insulin concentrations were determined using commercially available sheep insulin ELISA kits (Hangzhou Eastbiopharm Co., Ltd., Cat. No.: CK-E90956, Hangzhou, China). According to the manufacturer, the sensitivity of the assay was 0.13 mIU/L. The intra-assay CV was <10% and the inter-assay CV was <12%. Serum E2 concentrations were determined using commercially available sheep E2 ELISA kits (Hangzhou Eastbiopharm Co., Ltd., Cat. No.: CK-E91162, Hangzhou, China). According to the manufacturer, the sensitivity of the assay was 0.92 ng/L. The intra-assay CV was <10% and the inter-assay CV was <12%.

2.4. Statistical analyses

The experiment was performed using a completely randomized design with the GLM procedure of SAS (Statistical Analysis Systems Institute, 1998). The model included the fixed effect of treatment and the random effect of ewe within group. For the anal-

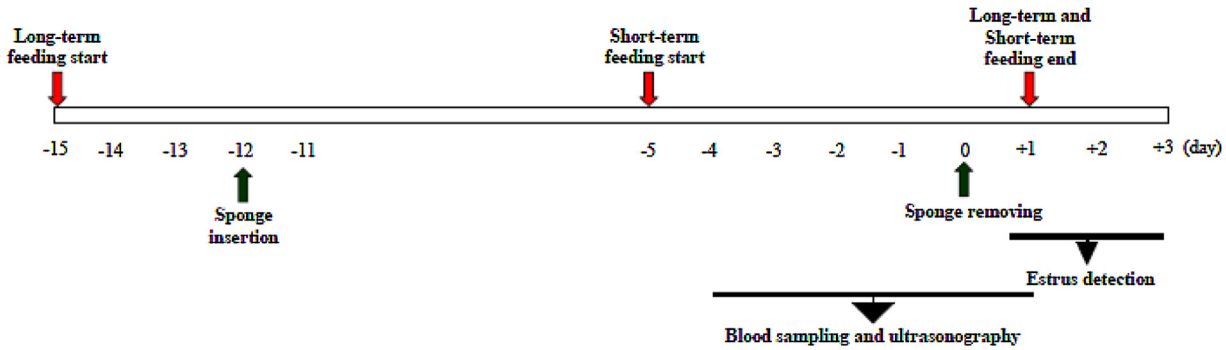

Fig. 1. Schematic representation of the experimental design.

ysis of repeated measurements, the mixed procedure of the SAS was used. The covariance structure was modelled using the random effect of ewe within group plus autoregressive order 1, to account for the correlation between sequential measurements within the same animal (Littell et al., 2000). Data of estrous response and CL variables were analyzed using PROC GENMOD. Mean values were compared by the Duncan's multiple range test and/or orthogonal contrasts. Probability values of less than 0.05 ($P < 0.05$) were considered significant. Results are expressed as means ± S.E.

3. Results

3.1. Folliculogenesis and ovulation rate

The populations of small, medium, and large-size follicles at the different times before and after sponge removing (days −4 to +1) are presented in Fig. 2. The results showed the treatments affected the number of small follicles. Ewes which received LE diet had a higher number ($P < 0.05$) of small follicles. However, there was no significant difference between the LE and SE diets on days −2, −1, 0, +1 ($P > 0.05$). Our observation revealed that the treatments had no effect on medium-size follicles population on days −4 and −3. Ewes fed LEP had the lowest number of medium-size follicles near the time of sponge removing. Orthogonal contrasts showed that high-energy groups (LE & SE) had more ($P < 0.05$) medium-size follicles compared to the control group one day after sponge removing (day +1). There was no significant difference between the long-term and short-term feeding program for medium-size follicle population. The ultrasound scanning revealed that the emergence of large-size follicles occurred on days 0 and +1. On the other hand, no large-size follicles were observed before sponge removing. Based on the orthogonal contrasts, ewes fed high energy diets (LE & SE) had more ($P < 0.05$) large-size follicles than those the control group and high energy-protein (LEP & SEP) groups. There was no significant difference between the effects of long-term or short-term feeding program on large-size follicle population ($P > 0.05$).

The results of estrus detection and single or double ovulations are presented in Table 2. Estrus response was not affected by the treatments and 24–72 h after sponge removing all animals showed estrus behaviour. Feeding high energy (LE & SE) diets increased the number and percentage of ewes with double ovulation rate (4/8, 50%). Whereas, ewes fed high energy-protein (LEP & SEP) diets had the lowest double ovulation rate (1/8, 12.5%).

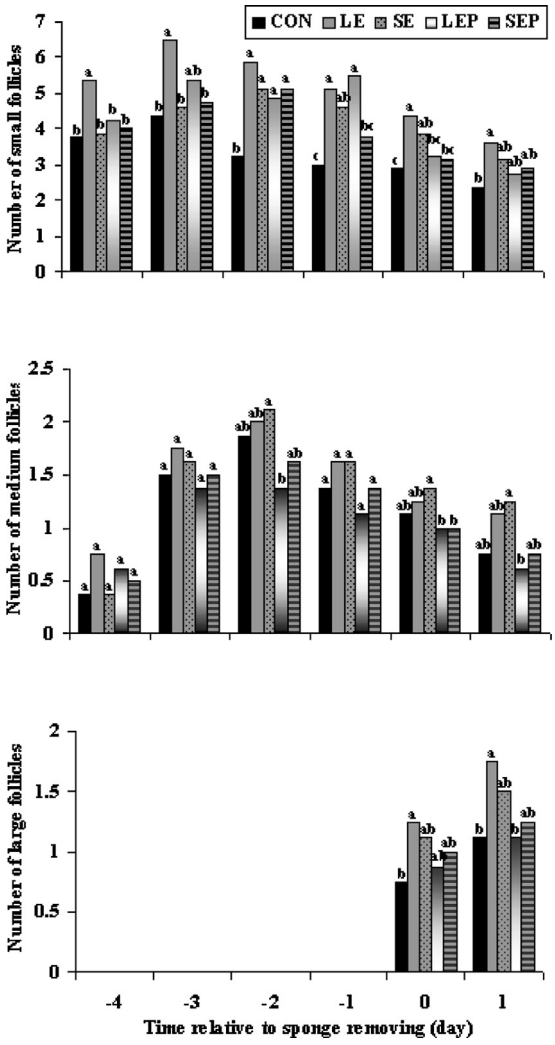

Fig. 2. Effect of long-term and short-term feeding of high energy and high energy-protein diets on follicles with different sizes in Naeini ewes. CON = control, LE = long-term feeding of high energy diet, SE = short-term feeding of high energy diet, LEP = long-term feeding of high energy-protein diet, SEP = short-term feeding of high energy-protein diet. The bar with different small letter (a–c) had significant difference ($P < 0.05$).

3.2. Serum glucose, cholesterol and urea nitrogen concentrations

The results of serum glucose, cholesterol and urea nitrogen concentrations are presented in Tables 3–5, respectively.

Our data showed that ewes fed high energy diets (LE & SE) had higher ($P < 0.05$) serum glucose concentration than those of the other groups. Moreover, SE group had the highest and LEP group had the lowest serum glucose ($P < 0.05$) on days 0 and +1. Interestingly, the short-term feeding of high energy and high

Table 2
Effect of long-term and short-term feeding of high energy and high energy-protein diets on estrous response and incidence of single and double ovulation on ovary in Naeini ewes.

	Treatments[a]				
Event	CON	LE	SE	LEP	SEP
Estrous response% (*n*)	100 (8/8)	100 (8/8)	100 (8/8)	100 (8/8)	100 (8/8)
Single ovulation% (*n*)	75 (6/8)	50 (4/8)	50 (4/8)	87.5 (7/8)	87.5 (7/8)
Double ovulation% (*n*)	25 (2/8)	50 (4/8)	50 (4/8)	12.5 (1/8)	12.5 (1/8)

[a] CON = control, LE = long-term feeding of high energy diet, SE = short-term feeding of high energy diet, LEP = long-term feeding of high energy-protein diet, SEP = short-term feeding of high energy-protein diet.

Table 3
Effect of long-term and short-term feeding of high energy and high energy-protein diets on serum glucose concentration (Mean ± SE) during the different synchronized days of Naeini ewes (mg/dl).

Days	Treatments*				
	CON	LE	SE	LEP	SEP
−4	53.68 ± 2.19[b,**]	58.13 ± 2.98 [a]	53.11 ± 2.58 [bc]	50.81 ± 2.44 [c]	54.56 ± 2.64 [b]
−3	51.02 ± 2.37[d]	63.40 ± 1.78 [a]	59.68 ± 2.71 [b]	53.15 ± 1.93 [d]	56.58 ± 2.72 [c]
−2	54.16 ± 3.19[c]	60.32 ± 2.91 [b]	64.96 ± 3.21 [a]	52.72 ± 2.56 [c]	57.22 ± 2.81 [bc]
−1	55.93 ± 2.37 [b]	59.88 ± 1.84 [a]	62.02 ± 2.78 [a]	46.58 ± 2.80 [d]	50.78 ± 3.08 [c]
0	47.15 ± 2.73 [c]	55.48 ± 2.33 [b]	59.28 ± 2.09 [a]	44.22 ± 3.81 [d]	47.95 ± 1.90 [c]
1	48.27 ± 2.82 [c]	53.36 ± 1.82 [b]	56.61 ± 2.24 [a]	43.75 ± 3.23 [d]	45.96 ± 2.84 [cd]

P-value

Orthogonal contrasts	−4	−3	−2	−1	0	1
LE&SE vs CON	0.0919	0.0001	0.0001	0.0001	0.0001	0.0001
LEP&SEP vs CON	0.3772	0.0006	0.3879	0.0001	0.3637	0.0051
LE&SE vs LEP&SEP	0.0028	0.0001	0.0001	0.0001	0.0001	0.0001
LE&LEP vs SE&SEP	0.4896	0.8690	0.0075	0.0016	0.0003	0.0060

* CON = control, LE = long-term feeding of high energy diet, SE = short-term feeding of high energy diet, LEP = long-term feeding of high energy-protein diet, SEP = short-term feeding of high energy-protein diet.
** Different letters in the same row indicate significant differences ($P < 0.05$).

Table 4
Effect of long-term and short-term feeding of high energy and high energy-protein diets on serum cholesterol concentration (Mean ± SE) during the different synchronized days of Naeini ewes (mg/dl).

Days	Treatments*				
	CON	LE	SE	LEP	SEP
−4	43.76 ± 2.28[b,**]	51.86 ± 3.30[a]	44.12 ± 1.53 [b]	45.71 ± 3.67[b]	43.43 ± 1.97 [b]
−3	51.26 ± 2.19[c]	58.61 ± 3.00 [a]	55.48 ± 2.24 [b]	49.92 ± 3.57 [c]	46.52 ± 2.08 [d]
−2	52.17 ± 1.67 [b]	62.97 ± 3.11[a]	60.21 ± 2.57 [a]	48.95 ± 3.19 [c]	44.23 ± 3.09 [d]
−1	46.27 ± 1.76 [b]	57.21 ± 3.04 [a]	55.65 ± 2.30 [a]	45.80 ± 2.70 [b]	41.75 ± 1.97 [c]
0	45.11 ± 2.29 [c]	51.16 ± 2.56 [a]	48.13 ± 3.18 [b]	42.33 ± 2.15[d]	39.81 ± 1.81 [e]
1	43.55 ± 2.27 [b]	47.57 ± 2.26 [a]	46.31 ± 3.55 [a]	40.32 ± 1.88 [c]	38.12 ± 2.64 [c]

P-value

Orthogonal contrasts	−4	−3	−2	−1	0	1
LE&SE vs CON	0.0009	0.0001	0.0001	0.0001	0.0001	0.0046
LEP&SEP vs CON	0.4887	0.0131	0.0001	0.0218	0.0005	0.0005
LE&SE vs LEP&SEP	0.0010	0.0001	0.0001	0.0001	0.0001	0.0001
LE&LEP vs SE&SEP	0.0001	0.0015	0.0006	0.0022	0.0029	0.0668

* CON = control, LE = long-term feeding of high energy diet, SE = short-term feeding of high energy diet, LEP = long-term feeding of high energy-protein diet, SEP = short-term feeding of high energy-protein diet.
** Different letters in the same row indicate significant differences ($P < 0.05$).

energy-protein diets (SE & SEP) compared to the long-term feeding (LE & LEP) increased ($P > 0.05$) serum glucose of ewes on days 0 and +1. At different times before and after sponge removing (days −4 to +1), LE group had the highest ($P > 0.05$) serum cholesterol concentration. Orthogonal contrasts showed that feeding high energy (LE & SE) compared to the high energy-protein (LEP & SEP) diets increased ($P < 0.05$) serum cholesterol concentration. There was a significant difference ($P < 0.05$) between the long-term and short-term feeding programs for serum cholesterol concentrations. The ewes which received high energy diets (LE & SE) had lower ($P < 0.05$) serum urea nitrogen compared to the other groups, especially one day after sponge removing (+1). Orthogonal contrasts revealed that feeding high energy-protein diets (LEP & SEP) increased ($P < 0.05$) serum urea nitrogen compared to the control and high energy (LE & SE) diets.

3.3. Serum insulin and oestradiol concentrations

The data of serum insulin and oestradiol concentrations are shown in Tables 6 and 7, respectively.

Table 5
Effect of long-term and short-term feeding of high energy and high energy-protein diets on serum urea nitrogen concentration (Mean ± SE) during the different synchronized days of Naeini ewes (mg/dl).

Days	Treatments*				
	CON	LE	SE	LEP	SEP
−4	15.71 ± 1.97b, **	14.23 ± 1.48 b	15.93 ± 1.70 b	18.91 ± 2.24a	15.56 ± 1.80 b
−3	16.88 ± 1.30 bc	15.23 ± 1.36 c	15.46 ± 1.78 bc	20.73 ± 2.01 a	17.17 ± 1.48 b
−2	19.58 ± 1.25 a	16.80 ± 1.41bc	16.38 ± 1.94 c	19.73 ± 1.31a	18.26 ± 1.83ab
−1	18.98 ± 1.82 b	18.50 ± 1.63 b	18.96 ± 1.87 b	21.15 ± 1.81a	22.42 ± 1.83a
0	20.41 ± 2.26 bc	19.15 ± 2.54 bc	18.32 ± 1.61c	23.41 ± 1.81a	21.22 ± 1.66 b
1	20.86 ± 1.96 b	18.77 ± 2.35 c	18.63 ± 1.72 c	22.12 ± 1.71ab	23.67 ± 1.73 a

P-value

Orthogonal contrasts	−4	−3	−2	−1	0	1
LE&SE vs CON	0.4431	0.0345	0.0001	0.7444	0.0627	0.0135
LEP&SEP vs CON	0.0667	0.0055	0.3960	0.0010	0.0355	0.0191
LE&SE vs LEP&SEP	0.0024	0.0001	0.0001	0.0001	0.0001	0.0001
LE&LEP vs SE&SEP	0.2181	0.0060	0.0998	0.1811	0.0414	0.3040

* CON = control, LE = long-term feeding of high energy diet, SE = short-term feeding of high energy diet, LEP = long-term feeding of high energy-protein diet, SEP = short-term feeding of high energy-protein diet.
** Different letters in the same row indicate significant differences ($P < 0.05$).

Table 6
Effect of flushing and focus feeding diets on serum insulin concentration (Mean ± SE) during the different synchronized days of Naeini ewes (mIU/L).

Days	Treatments*				
	CON	LE	SE	LEP	SEP
−4	8.06 ± 0.78 b, **	11.67 ± 0.64 a	8.17 ± 0.58 b	7.57 ± 1.13 b	8.21 ± 0.69 b
−3	10.15 ± 0.64 d	14.21 ± 0.83 a	12.82 ± 0.77 b	9.65 ± 1.40 d	11.43 ± 0.65 c
−2	9.22 ± 0.74 d	13.95 ± 0.89 b	14.93 ± 0.89 a	9.11 ± 1.34 d	11.56 ± 0.73 c
−1	8.92 ± 1.04 c	14.62 ± 0.77 a	14.62 ± 0.66 a	10.17 ± 0.95 b	10.25 ± 1.34 b
0	11.27 ± 0.77 c	15.28 ± 1.12 b	16.41 ± 0.66 a	10.51 ± 1.06 c	10.97 ± 0.86 c
1	11.41 ± 0.80 b	14.87 ± 1.06 a	15.48 ± 0.89 a	8.12 ± 1.19 c	8.72 ± 0.87 c

P-value

Orthogonal contrasts	−4	−3	−2	−1	0	1
LE&SE vs CON	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
LEP&SEP vs CON	0.6260	0.3239	0.0107	0.0048	0.1881	0.0001
LE&SE vs LEP&SEP	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
LE&LEP vs SE&SEP	0.0001	0.5376	0.0001	0.9150	0.0191	0.0881

* CON = control, LE = long-term feeding of high energy diet, SE = short-term feeding of high energy diet, LEP = long-term feeding of high energy-protein diet, SEP = short-term feeding of high energy-protein diet.
** Different letters in the same row indicate significant differences ($P < 0.05$).

Table 7
Effect of flushing and focus feeding diets on serum oestradiol concentration (Mean ± SE) during the different synchronized days of Naeini ewes (ng/L).

Days	Treatments*				
	CON	LE	SE	LEP	SEP
-4	1.79 ± 0.10 b, **	1.52 ± 0.12 c	1.80 ± 0.08 b	1.97 ± 0.24 a	1.79 ± 0.14 b
-3	1.57 ± 0.10 c	1.68 ± 0.12bc	1.73 ± 0.10 b	1.88 ± 0.21 a	1.66 ± 0.15bc
-2	1.96 ± 0.12 a	1.74 ± 0.12 b	1.53 ± 0.06 c	2.06 ± 0.20 a	1.59 ± 0.14 bc
-1	2.21 ± 0.17 a	1.89 ± 0.15 b	1.40 ± 0.09 c	2.34 ± 0.15 a	1.94 ± 0.11 b
0	2.70 ± 0.16 a	2.59 ± 0.16ab	2.36 ± 0.16 c	2.45 ± 0.13 bc	2.34 ± 0.13 c
1	3.11 ± 0.13 b	3.55 ± 0.20 a	3.48 ± 0.21a	3.00 ± 0.15 b	2.95 ± 0.15 b

P-value

Orthogonal contrasts	−4	−3	−2	−1	0	1
LE&SE vs CON	0.0585	0.0431	0.0001	0.0001	0.0020	0.0001
LEP&SEP vs CON	0.1632	0.0035	0.0302	0.2398	0.0001	0.0903
LE&SE vs LEP&SEP	0.0002	0.2134	0.0003	0.0001	0.1154	0.0001
LE&LEP vs SE&SEP	0.3828	0.1015	0.0001	0.0001	0.0031	0.3545

* CON = control, LE = long-term feeding of high energy diet, SE = short-term feeding of high energy diet, LEP = long-term feeding of high energy-protein diet, SEP = short-term feeding of high energy-protein diet.
** Different letters in the same row indicate significant differences ($P < 0.05$).

Results showed that serum insulin was high ($P < 0.05$) in ewes fed high energy diets (LE & SE) at different times before and after sponge removing. High energy-protein (LEP & SEP) groups had lower serum insulin concentration than the control group, especially one day after sponge removing. The concentration of serum oestradiol was elevated from day −4 to +1 in all experimental groups. However, ewes fed high energy diets (LE & SE) had lower ($P < 0.05$) serum oestradiol before the sponge removing.

4. Discussion

Feeding high energy diets in a long-term or short-term program improved reproductive performance of cyclic ewes. This result was in agreement with the findings of Zabuli et al. (2010) who demonstrated a marked improvement of ovarian performance and blood glucose and insulin in cycling goats after feeding high energy diet.

4.1. Folliculogenesis and ovulation rate

Scaramuzzi and Martin (2008) demonstrated that the component of the diet that is probably the most important with respect to ovarian function is "energy" particularly that derived from glucose. Our results also showed that feeding high-energy diets (LE & SE) increased the number of follicles in all follicle classes. This effect was more obvious for large-size follicles near to the time of sponge removal. The finding was in accordance with Letelier et al. (2008) and Senosy et al. (2013) who reported that the average number of small follicles and the number of follicles entering in the terminal growth phase was greater in ewes receiving short-term energy diet. Scaramuzzi et al. (2006) demonstrated that glucose infused for 3 or 5 days stimulated folliculogenesis of ewes by increasing the number of large follicles but without any effect on the number of small- and medium-sized follicles. Muñoz-Gutiérrez et al. (2002) clarified that dietary energy can directly stimulate folliculogenesis in recruited and selected follicles, and this effect may be mediated by the changes in systemic leptin concentrations and the hexosamine energy-sensing pathway in the follicle. An alternative hypothesis favored by us is that nutrition acts locally on the ovary to perturb the negative feedback control systems that regulate folliculogenesis (Somchit et al., 2007). Interestingly, ewes fed high energy-protein diets (LEP & SEP) had lower number of medium- and large-size follicles than those fed high energy diets (LE & SE). This result could be related to the difference of serum urea nitrogen and its adverse effect on ovine folliculogenesis (Rooke et al., 2004).

Our data provide some evidence that nutritional program increased double ovulation in response to long-term and short-term feeding of high energy diets. However, the differences were not significant because of the low number of animals involved. In accordance with the previous reports (Viñoles et al., 2005; Senosy et al., 2013) high energy diets (LE & SE) caused 50% double ovulation rate in ewes. Scaramuzzi et al. (2006) reviewed the effects of supplementary nutrition on ovulation rate and concluded that energy balance leads to increased leptin and insulin concentrations in blood and increased glucose uptake; these changes appear to affect the ovary directly and are associated with increased ovulation rate in sheep.

4.2. Serum glucose, cholesterol and urea nitrogen

The results showed that ewes fed high energy diets (LE & SE) had higher serum glucose and cholesterol during different times of experiment. However, in agreement with Viñoles et al. (2005) short-term feeding of high energy diet (SE) had a delay with regard to elevation of serum glucose levels. They reported that the concentrations of glucose reached peak values 2 or 3 days after the start of a high level of feeding in ewes. Significant improvement in blood metabolites during the transient high energy could be attributed to the positive energy balance of animals (Scaramuzzi et al., 2006). This condition may increase the folliculogenesis, which supports our finding for large-size follicles and double ovulation rate.

It is well known that energy intake is positively related to serum cholesterol concentrations and steroid hormones synthesis in animals (Mahmoud et al., 2012; Senosy et al., 2013). On the other hand, Rabiee and Lean (2000) demonstrated that glucose may promote cholesterol uptake into the ovarian cells and vice versa. It seems the metabolic responses to long-term and short-term high energy feeding in the present study are similar to previous findings (Rabiee and Lean, 2000; Mahmoud et al., 2012; Senosy et al., 2013).

Urea is the product of protein catabolism in the liver and is elevated in negative energy balance condition and in animals fed high dietary crude protein (Butler, 1998; Scaramuzzi et al., 2006; Sensoy et al., 2013). Results of the present study showed that ewes fed high energy-protein diets (LEP & SEP) had higher serum urea nitrogen than those the other groups. Moreover, ewes fed high energy diets (LE & SE) had lower urea nitrogen compared to the control group. This observation may support our results for folliculogenesis and ovulation rates. It is reported that blood urea nitrogen had adverse effect on the oocyte growth and metabolism of the oocyte-supporting granulosa cells (Rooke et al., 2004).

4.3. Serum insulin and oestradiol concentrations

In addition to stimulating folliculogenesis, the nutritional supplements had a range of effects on circulating hormones (Somchit et al., 2007). It is well known that insulin regulates the transport of glucose across the blood follicle barrier and its utilization by the follicular cells in different species (Cosgrove and Foxcroft, 1996; Khan et al., 2012). Similar to Branca et al. (2000) our data showed that the serum insulin concentrations were high in ewes fed high energy diets (LE & SE). On the other hand, serum glucose and insulin were low in ewes fed high energy-protein diets (LEP & SEP) compared to the control group. This is in contrast with previous reports (Downing et al., 1995; Somchit et al., 2007) that showed feeding lupin grain as a high energy-protein diet increased glucose availability and plasma insulin concentration of ewes. These discrepancies could be due to the source of protein in the diets (Branca et al., 2000). Scaramuzzi et al. (2006) demonstrated that an increase in insulin-mediated glucose uptake by follicular cells may be critical for the growth of follicles and the prevention of atresia, thereby increasing the pool of ovulatory follicles which supports our findings about the folliculogenesis and double ovulation rate.

The effect of nutrition on follicular oestradiol secretion is controversial. However, our data support some accepted opinion (Williams et al., 2001; Kosior-Korzecka and Bobowiec, 2003). In the present study, ewes fed high energy diets (LE & SE) had lower oestradiol concentrations during the days before sponge removal. While, one day after sponge removing ewes fed high energy diets had the highest serum oestradiol. Muñoz-Gutiérrez et al. (2004) reported that glucose and metabolic hormones act directly at the ovarian level to regulate steroidogenesis. Decrease in oestradiol level causes the successive follicles to escape the suppressive influence of this oestrogen and reach the stadium of ovulation (Kosior-Korzecka and Bobowiec, 2003). Moreover, it is reported that the positive energy balance is also associated with alterations in the hepatic metabolism of steroids (Scaramuzzi et al., 2006).

5. Conclusion

Results of the present study showed that long-term or short-term feeding program affected the number of different size follicles, double ovulation rate and serum metabolites and hormones of cyclic ewes. The exact mechanism controlling the effects of nutritional supplementation on folliculogenesis and the growth of dominant follicles is unclear. However, our findings clearly indicated that due to changes in blood levels of glucose, insulin and oestradiol the number of large-size follicles and ovulation rate were increased in ewes fed high energy diets and there were no significant differences between the two energy supplemented (LE & SE) groups. Therefore, the period of diet treatment on stages of follicular development can be reduced to 6 days. Overall, our observations

suggest that there is a better response to the immediate effect of nutrition in high energy diet than high energy-protein diet.

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