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Effect of feeding greater amounts of dietary energy for a short-term with or without eCG injection on reproductive performance, serum metabolites and hormones in ewes

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ABSTRACT

This study was conducted to compare the effect of transient high-energy diet in a short-term period with or without eCG injection on ovarian follicle development, twining rate, serum metabolites and hormones in ewes. A total of 45 estrous cyclic Naeini ewes were randomly assigned to three experimental groups: 1—Control (control), 2—High energy short-term feeding (HE), and 3—high energy short-term feeding + eCG injection (HEe). Ewes were housed in individual pens with free access to feed and water. The stage of the estrous cycle of all ewes was synchronized by insertion of intravaginal progesterone sponges. Focus feeding started from 4 days before until 1 day after sponge removal. Follicle development was monitored from 4 days before until 1 day after sponge removal and blood samples were taken during this time. Results showed that ewes fed high energy diets (HE and HEe) had greater ($P < 0.05$) large follicle numbers compared with the control group. Feeding high energy diets increased ($P < 0.05$) serum glucose, cholesterol and insulin, but had lesser ($P < 0.05$) serum urea nitrogen concentrations near the time of ovulation. After the start of experiment, ewes fed high energy diets had less ($P < 0.05$) serum estradiol. However, 1 day after sponge removal, serum estradiol in HE and HEe groups increased ($P < 0.05$). It was concluded that short-term (6-day) changes in amount of dietary energy with or without eCG injection increased twin births and had beneficial effects on the blood metabolites and hormone concentrations in Naeini ewes.

1. Introduction

One of the most important environmental factors associated with the reproductive performance of sheep is nutrition (Scaramuzzi et al., 2006; Somchit et al., 2013; Vinoles et al., 2014). The stimulatory effect of nutrition on folliculogenesis and ovulation rate may be exerted through long-term or short-term nutritional supplementations. Long-term supplementation (flushing) is a practice that has been used in ruminant production systems since at least the beginning of the 19th century (Scaramuzzi and Martin, 2008). There is some evidence that a shorter period of increased metabolic status could stimulate the follicular growth and ovulation rate in sheep and goats (Blache and Martin, 2009; Zabuli et al., 2010). Therefore, some short-term supplementation (focus feeding) has been developed for improving the reproductive performance of small ruminants. Administration of gonadotropins such as eCG prior to removal of a progestin-sponge can stimulate follicular growth and increase the ovulation rate and fertility in both anestrous and estrous

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cycling sheep (Dogan and Nur, 2006; Ali, 2007; Koyuncu and Ozis Alticekic, 2010). However, no reports are available on the effects of the transient feeding of high-energy diets with or without eCG treatment on reproductive performance of sheep breeds kept under the conditions of arid and semi-arid areas.

Short-term nutritional supplementation with energy yielding substrates increases the number of larger follicles, ovulation rate (Gallet et al., 2011; Berlinguer et al., 2012), litter size (Vinoles et al., 2009) and decreases follicular atresia (Somchit et al., 2007; Santos et al., 2009; Ying et al., 2011). Munoz-Gutierrez et al. (2002) reported that high-energy short-term feeding of ewes for a transient period before progestin-sponge removal stimulated folliculogenesis. Koyuncu and Canbolat (2009) concluded that increasing the amount of dietary energy supplementation before mating time had some beneficial effects on lambing rate and litter size in ewes. Vinoles et al. (2005) noted that short-term nutritional supplementation during the luteal phase of the estrous cycle increased glucose and metabolic hormones, and the development of small follicles. However, there was no effect on ovulation rate of ewes.

The most widely used hormone for improving sheep fertility is eCG (Barrett et al., 2004; Ali, 2007). Zare Shahneh et al. (2006) demonstrated that injection of eCG, increased twinning and lambing rates in Iranian fat-tailed ewes. Zeleke et al. (2005), administered eCG 24 h prior of sponge removal, reported that the reproductive performance of ewes increased. In addition, there is also some evidence suggesting that eCG can increase the twinning rate in breeds characterized by small litter size (Boscos et al., 2002; Ali, 2007).

Naeini ewes which are maintained in arid and semi-arid areas of central Iran had an important effect on rural livelihood. These sheep are seasonal breeders and ewes usually give birth one lamb per breeding season. Therefore, this fat-tailed Iranian sheep may need nutritional consideration and hormone therapy before mating to improve reproductive performance. The objective of the present study was to determine the effect of short-term feeding of a high-energy diet before the ovulation time with or without eCG injection on follicular development, twin births and some blood parameters in ewes with poor reproductive performance.

2. Materials and methods

2.1. Animals and experimental procedures

This study was conducted from November 2013 to May 2014 at the Small Ruminant Research Center (SRRC) of Isfahan University of Technology, Isfahan, Iran (32°35′N latitude and 52°33.5′E longitude). A total of 45 estrous cycling Naeini ewes (2–3 years of age, 42 ± 1.4 kg BW) were randomly assigned to three experimental groups. Treatments were: (1) control, (2) high energy short-term feeding (HE), and (3) high energy feeding + eCG injection (HEe). The control group was fed a maintenance diet and the short-term feed groups were fed a high energy diet (Table 1). The animals had no previous evidence of reproductive or health problems. One month before the onset of the experimental treatments, the ewes were housed in individual pens (1.12 × 1.46 m) with free access to feed and water. The stage of the estrous cycle of all ewes was synchronized by use of intravaginal progestin sponges (30 mg fluorogestone acetate, Bioniche, Animal Health, PTY, Armidale, NSW, Australia) for a 12-day period. Short term feeding of high energy diet was started from 4 days before until 1 day after sponge removal. The injection of eCG occurred 1 day before sponge removal (400 IU, Bioniche Animal Health (A/Asia), Pty Ltd, ABN, 64006949480, Australia). Follicle development was monitored from 4 days before until 1 day after sponge removal and blood samples were collected daily during this time interval. The diets were balanced using CNCPS-S (Cornell Net Carbohydrate and Protein System of Sheep). Ewes were checked for signs of estrus from 12 h after sponge removal, using nine intact rams. The schematic of the experimental design is depicted in Fig. 1. A ewe was considered in estrus only when she allowed a ram to mount and this was registered as the onset time of estrus. Ewes in estrus were mated twice a day (morning and evening) with Naeini rams that were rotated after every four matings. The ewes which did not return to estrus for an interval equivalent to at least three consecutive estrous cycles were considered pregnant. The reproductive variables measured in experimental groups were: time to onset of estrus (h), estrous response (%), fertility [(number of lambed ewes/number of mated ewes) × 100], fecundity rate (number of lambs born/number of mated ewes) and prolificacy rate (number of lambs born/number of ewes lambed).

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

Item	Experimental diets	
Ingredient (%)	Maintenance	High energy
Alfalfa hay	23	23
Wheat straw	35.5	12
Corn	15	49
Barley	9.5	9.5
Soybean meal	3	2
Wheat bran	12	2
Vitamin-Mineral	0.4	0.4
$CaHP_4$	0.5	0.5
$CaCO_3$	0.5	0.5
Salt	0.6	0.6
Composition		
DE Mcal/kg	2.4	3.00
CP (%)	10.5	10.5
Ca (g/day)	7	7
P (g/day)	4	4

2.2. Ultrasonography examination

To evaluate the effect of diet treatments on the follicular development, ovaries were examined by ultrasonography on a daily basis. Transrectal ultrasonography was conducted from 4 days before until 1 day after sponge removal (Days −4, −3, −2, −1, 0 and +1) 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

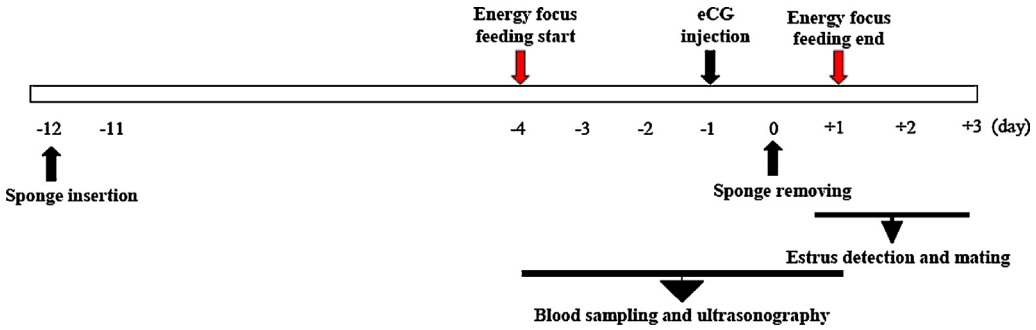

Fig. 1. Schematic representation of the experimental design.

ovaries every day. In each observation, the relative location of all follicles was noted on an ovarian map to follow the sequential follicular development. Visible follicles on the surface of the ovaries were classified based on 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 the diameter of 2 mm was considered as the day of appearance. When this follicular diameter was more than 2 mm at the first detection, the previous day was considered as the day of emergence. Number (1 or 2) of ovulations was assessed using ultrasonography and the number of corpora lutea was determined 10 days after sponge removal.

2.3. Blood sampling and variables

Blood samples were collected by jugular venipuncture at 9:00 a.m. (before the morning feeding) and the sampling was repeated daily during Days −4 to +1. The sample tubes were placed into icy water immediately after collection, and then transported to the laboratory within 10 min. In the laboratory, the samples were centrifuged at 2500 rpm for 15 min and serum was harvested and stored at −20 °C until assayed for glucose, cholesterol, urea nitrogen, insulin and estradiol (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 Ziest Chem Diagnostics Tehran, Iran and Pars Azmun, Karaj, Iran, respectively). Serum 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 estradiol 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 each group. The covariance structure was modelled using the random effect of ewe within groups plus an autoregressive order 1 to account for the correlation between sequential measurements within the same animal (Littell et al., 2000). Data for estrous response and corpora lutea number, number of lambs born, fecundity and prolificacy rates were analyzed using PROC GENMOD. For the analysis of repeated measurements, the mixed procedure of SAS was used. Mean values were compared by the Duncan's multiple range test. Probability values of less than 0.05 ($P < 0.05$) were considered significant.

3. Results and discussion

3.1. Follicular characteristics

The populations of small, medium and large-size follicles at different times before and after sponge removal (Days −4 to +1) are depicted in Fig. 2. At the start of the short-term feeding program and hormone injection, no significant difference was detected between the three experimental groups, either in the number of follicles classified or in the total number of follicles. Ultrasonic assessments revealed that the selection of large size follicles occurred on Days 0 and +1. The population of small and medium-sized follicles was reduced at this time. After sponge removal, mean number of different size follicles was the least in the control group ($P < 0.05$) and no significant effect was observed between the two short term feeding groups with or without eCG injection ($P > 0.05$). This finding indicated eCG had no effect on follicular populations in Naeini ewes which was unexpected and is difficult to explain. In a previous study, Salehi et al. (2010) used eCG 48 h before and at the time of CIDR removal and found that injection of eCG had no superovulatory effect on ewes. In the present study more selection of large follicles could be due to the effect of short-term energy feeding and it appears that injection of eCG may have a different effect based on feeding program and the time and method

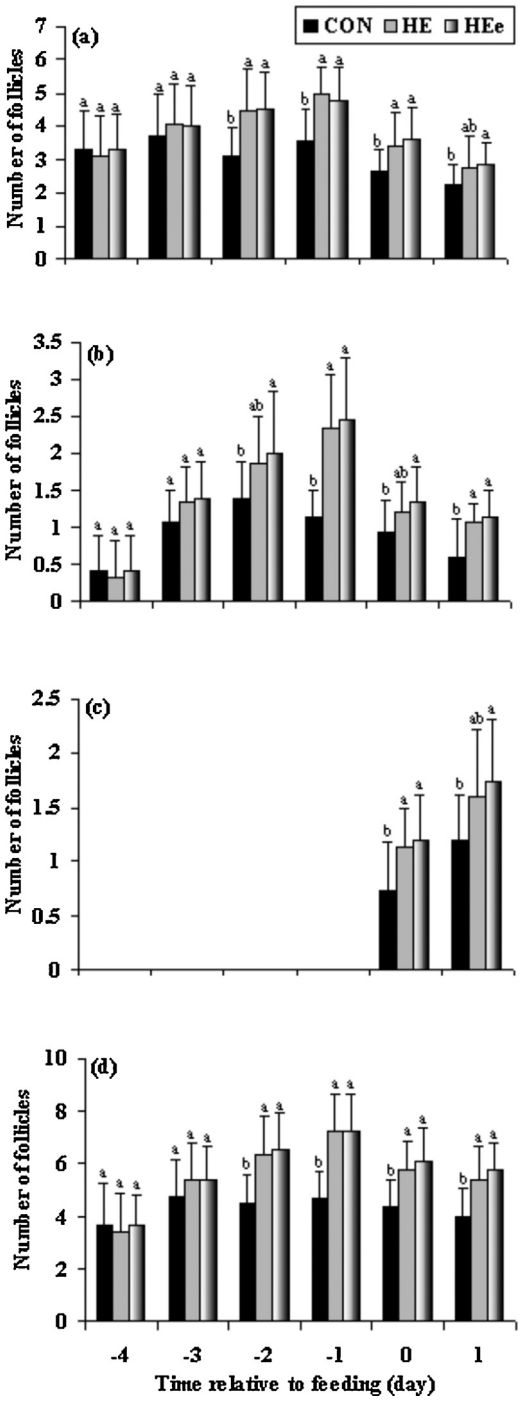

Fig. 2. Number of ovarian follicles classified based on the size (a = small; b = medium; c = large and d = total) in Naeni ewes. (CON = Control, HE = High energy short-term feeding, HEe = High energy short-term feeding + eCG injection).

of injection (Boscos et al., 2002; Emsen and Yaprak, 2006; Ali, 2007).

There is some evidence for the relationship between ovarian activity and blood concentrations of glucose and insulin in ewes (Vinoles et al., 2005; Somchit et al., 2007).

Table 2
Effect of short-term feeding of greater amounts of energy with or without eCG injection on estrous response, ovulation rate and reproductive performance in Naeni ewes.

Variables	Treatments[a]		
	CON	HE	HEe
Onset of estrus (h)	42.19a	31.24b	27.56b
Estrus response (%)	15/15 (100)	15/15 (100)	15/15 (100)
Single Ovulation (%)	14/15 (93.3)	10/15 (66.6)	9/15 (60.0)
Double Ovulation (%)	1/15 (6.6)	5/15 (33.3)	6/15 (40.0)
Mating of ewes (%)	15/15 (100)	15/15 (100)	15/15 (100)
Parturition of ewes (%)	12/15 (80)	14/15 (93.3)	13/15 (86.7)
Fertility (%)	12/15 (80)	14/15 (93.3)	13/15 (86.7)
Single births (%)	12/15 (80.0)	11/15 (73.3)	9/15 (60.0)
Twin births (%)	0/15 (0)	3/15 (20)	4/15 (26.7)
Fecundity	0.8	1.13	1.13
Prolificacy	1	1.21	1.31

[a] CON = Control, HE = High energy short-term feeding, HEe = High energy short-term feeding + eCG injection.

A potential mechanism of short-term nutritional effect on folliculogenesis is that nutritional supplementation may increase the number of follicles that did not undergo atresia (Ying et al., 2011). In agreement with the previous finding, present results confirm that short-term dietary supplementation increased the serum glucose and insulin, and the large follicle number as well. In an assay, intravenous infusion of glucose for 5 days promoted folliculogenesis by increasing the number of large follicles in ewes (Munoz-Gutierrez et al., 2002).

3.2. Estrus, ovulation, fecundity and prolificacy

The data for estrous response, ovulation rate and reproductive performance are presented in Table 2. Results showed that the ewes of the control group needed a longer time ($P < 0.05$) to express estrous behavior after sponge removal compared to the short-term fed groups. Injection of eCG decreased this time but the difference was not significant and there was no significant difference between the two short-term fed groups. The present results revealed that estrous synchronization using the progestin sponge could be effective in Naeini ewes during the breeding season. The earlier observation of estrous behavior in ewes fed high energy diets (HE and HEe) may be related to the large number of preovulatory follicles at the time of sponge removal (Fig. 2). Contrary to the present finding, previous studies have shown that diet with variable amounts of energy or use of a "flushing" program in an attempt to enhance reproduction had no effect on the time to onset of estrus in ewes (McEvoy et al., 1995; Naqvi et al., 2002). This inconsistency in findings could be due to the differences in breed, age, nutritional conditions and estrous synchronization protocols (Boscos et al., 2002).

The other reproductive variables of ewes were not affected by the treatments. However, there were numerical differences between the experimental groups. For example, the ultrasonographic observation of corpus luteum formation showed that short-term feeding to enhance energy in diets increased the number of ewes having two ovulations (33% and 40% compared with 6.6%). These groups also had greater numbers of twin births (20% and 26.7% compared

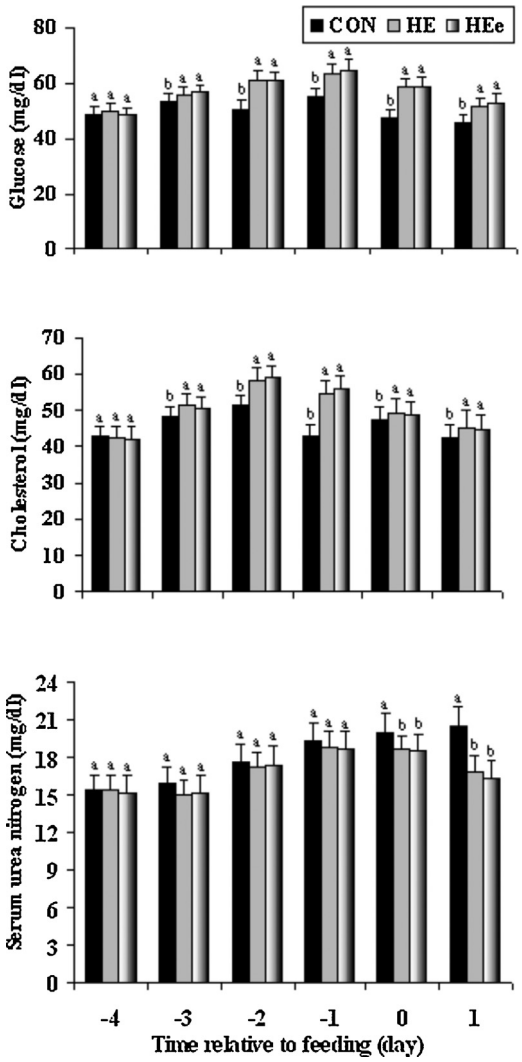

Fig. 3. Serum glucose, cholesterol and urea nitrogen concentrations in Naeni ewes (CON = Control, HE = High energy short-term feeding, HEe = High energy short-term feeding + eCG injection).

with 0%), fecundity (1.13 and 1.13 compared with 0.8) and prolificacy (1.21 and 1.31 compared with 1) than the control group. Injection of eCG with short-term energy feeding increased the twin births. However, the number of lambs born per mated ewe was similar in the two short-term feeding groups (1.13). There is limited information about twining rate responses to the transient feeding of greater energy content diets, with or without the eCG treatment in ewes.

3.3. Serum glucose, cholesterol and urea nitrogen concentrations

Serum concentrations of glucose, cholesterol and urea nitrogen are depicted in Fig. 3. Results showed that 1 day after the short-term feeding program started, blood glucose concentrations were affected by the treatments ($P < 0.05$). The was a greater concentration of glucose observed 1 day before sponge removal (Day −1) for all treatments and there were also greater populations of small and medium sized follicles (Fig. 2). There was no significant difference between the two focus feeding groups (HE and HEe) in this respect ($P > 0.05$). Serum cholesterol concentration was affected by the treatments 1 day after starting the short-term feeding program. During this time, ewes fed the greater energy diets had greater ($P < 0.05$) cholesterol concentrations compared with the control group. The treatments had no significant effect on serum urea nitrogen on the first days of experimental periods. During the final 2 days of sampling (Days 0 and 1), the short-term feeding groups had less serum urea nitrogen compared with the control group. At this time there was no significant difference between the HE and HEe groups in this respect ($P < 0.05$).

Previous reports clarified that blood glucose and insulin concentrations regulate glucose availability at in ovarian follicles and folliculogenesis in ewes (Letelier et al., 2008; Scaramuzzi et al., 2010; Ying et al., 2013). In the present study, increases in the number of ovulatory follicles could be due to the changes in blood concentrations of glucose and/or insulin. Cholesterol is the precursor of sex steroids such as progesterone, estradiol and testosterone in mammals (Rabiee and Lean, 2000; Kraemer et al., 2013; Kaminski et al., 2015). The effect of short-term and long-term nutritional supplements on blood cholesterol is similar. In agreement with the present results, Senosy et al. (2013) reported that short-term nutritional treatment increased serum cholesterol concentration and decreased serum urea nitrogen in ewes. Ying et al. (2013), however, reported that feeding supplementation during 6–12 days of the estrous cycle decreased cholesterol concentrations in Hu ewes. Circulating urea concentrations are reflected in follicular fluid and may affect the quality of both the oocyte and the granulosa cells (Leroy et al., 2004; Ying et al., 2013). In the present study, short-term feeding of diets (HE and HEe) had less serum urea nitrogen and greater numbers of large follicles compared with the control group.

3.4. Serum insulin and estradiol concentrations

The data of serum insulin and estradiol concentrations are depicted in Fig. 4. Results showed that 1 day after initiation of the short-term feeding program, serum insulin was affected by the treatments, so that feeding of the higher energy diets (HE and HEe) increased ($P < 0.05$) insulin concentration compared with the control group. No difference was found between the two short-term feeding groups (HE and HEe) throughout the sampling period with respect to serum insulin concentration. Feeding the high energy diet over a short time period increased serum glucose, therefore, the greater insulin concentration is a body response for uptake of glucose by the cells especially during follicular development (Scaramuzzi et al., 2006; Ying et al., 2011).

Insulin is influenced by dietary intake, therefore, overfeeding and underfeeding can drastically impact insulin concentrations in blood circulation (Diskin et al., 2003). Munoz-Gutierrez et al. (2002) reported that the

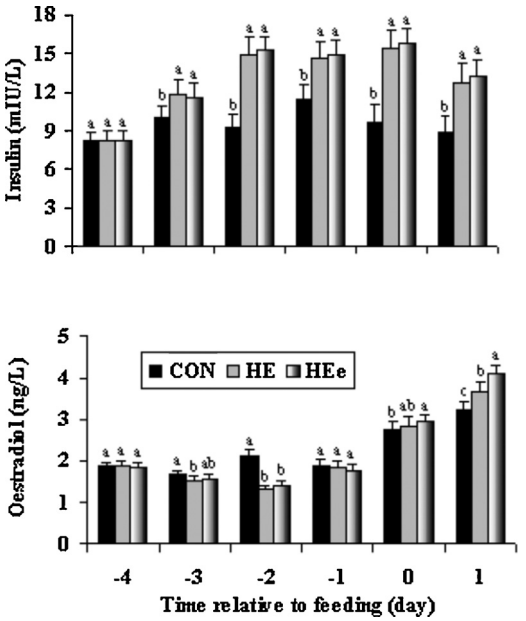

Fig. 4. Serum insulin and estradiol concentrations in Naeni ewes (CON = Control, HE = High energy short-term feeding, HEe = High energy short-term feeding + eCG injection).

nutritional supplements had a range of effects on circulating metabolites and hormones. In present study, serum glucose and insulin concentrations were increased in ewes fed the greater energy diets compared with the control diet similar to what was observed in previous studies with lupin grain and other supplements (Vinoles et al., 2010; Somchit et al., 2013; Kaminski et al., 2015).

After the start of a short-term feeding program the serum concentration of estradiol was reduced in HE and HEe groups compared to the control group. All treatment groups had basal serum estradiol concentrations during the days before sponge removal. One day after sponge removal, serum estradiol increased and ewes fed the greater energy diet with eCG injection had the greatest ($P<0.05$) concentration of estradiol. These observations are consistent with the present findings about the number of large follicles, ewes having two ovulations and twin births (Fig. 2 and Table 2).

Short-term feed supplementation effects on serum estradiol concentration are inconsistent (Somchit et al., 2007; Ying et al., 2011). Consistent with previous studies (Kosior-Korzecka and Bobowiec, 2003; Letelier et al., 2008; Somchit et al., 2013), the serum concentrations of estradiol decreased by a short-term supplementation before ovulation time in the present study. Results from the present study, however, are inconsistent with previous reports in ewes (Vinoles et al., 2005; Somchit et al., 2007; Ying et al., 2013) where it was found that short-term feed supplementation did not significantly influence the plasma estradiol concentrations. It is reported that glucose and metabolic hormones function directly on the ovary to regulate steroidogenesis (Munoz-Gutierrez et al., 2004; Vinoles et al., 2005). Downing et al. (1999) and Gallet et al. (2011) reported that infusion of glucose and glucose with insulin into the ovarian artery decreased estradiol secretion in granulosa cells. In the present study, before ovulation both serum glucose and insulin concentrations in the greater energy groups increased but estradiol concentrations decreased compared to the control group. These data suggest a specific inhibitory effect of glucose and insulin on the synthesis of estradiol by granulosa cells (Gallet et al., 2011). It is believed that this could be due to decreased availability of androstenedione substrate or decreased capacity of granulosa cells to convert androstenedione to estradiol (Somchit et al., 2007). Kosior-Korzecka and Bobowiec (2003) reported that a decrease in estradiol concentration causes the successive follicles to overcome the suppressive influence of estradiol and ovulation occurs from these follicles as a result of pre-ovulatory releases of LH. The greater concentration of glucose and insulin and lesser concentration of estradiol observed in the greater energy groups could, therefore, be responsible for more efficient large follicle development and ovulation rate in the ewes from these treatment groups in the present study. The estradiol concentrations observed after progestin-sponge removal in the present experiment were expected to reflect estradiol secretion by the ovarian follicles. These data are consistent with results from other reports demonstrating that the largest follicle is the principal source of ovarian venous estradiol (Munoz-Gutierrez et al., 2002).

4. Conclusion

It is concluded that the short-term feeding program of the present study with greater energy resulted in increased large follicle numbers near the time of ovulation. Before ovulation, feeding the greater energy diets increased serum glucose and insulin, but decreased serum estradiol concentrations compared with the control group. The ewes fed greater amounts of energy in the diets had greater serum estradiol 1 day after progestin sponge removal and these ewes needed a shorter time to express estrous behavior. During the breeding season and following progestin-based estrous synchronization using eCG had no beneficial effect for reproductive performance of Naeini ewes. Results provided a framework for future studies regarding the effect of transient feeding of high-energy diets on the reproductive performance of ewes reared in arid and semi-arid areas.

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