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Effects of calcium salts of fatty acids (CSFA) with different profiles (ω3 and ω6) during the flushing period on reproductive performance of 'Afshari' ewes

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ABSTRACT

The effects of supplementing the flushing diet of Afshari ewes with calcium salts of fatty acids (CSFA) with different profiles (ω3 and ω6) on their reproductive performance were studied using 48 sheep weighing 46 ± 3 kg and 2–3 years old. The ewes were assigned to four groups (n = 12 per group) and within a group received one of four diets for the period from the start of estrous synchronization to the end of mating (5 weeks). The diets all contained wheat bran (10%), wheat straw (37–40%), alfalfa (25–41%) and barley grain (7–25%). The control group received the basal (non-flushing) diet only. The remaining three groups received isoenergetic and isonitrogenous diets with higher energy contents formulated to meet the ewes' requirements during the flushing period. In the Barley group, the basal diet was enhanced using additional barley grain; in the CaFl group the flushing diet included 5% of DM as flaxseed oil (ω3 CSFA); and in CaSf group the diet was supplemented with 5% of DM as sunflower oil (ω6 CSFA). The estrous cycles of the ewes were synchronized by application of a CIDR for 14 d and administration of 400 units of PMSG at CIDR withdrawal. Afshari rams were introduced to the ewes 36 h later and the experimental diets continued to be fed for a further 3 weeks. The fertility and lambing rates were higher for Barley (91.7%; 125%), CaFl (100%; 150%) and CaSf (100%; 116.7%) than for the Control (83.3%; 83.3%). The number of lambs produced was significantly on the flushing treatments than the control. Serum cholesterol and progesterone levels were significantly higher on the diet supplemented with flaxseed oil (CaFl) than the other treatments. The results showed that supplementing the flushing diet with CSFA increased blood metabolites and hormones related to reproductive performance; and improved fertility, lambing rate and lamb birth weight. Ewes on the flaxseed oil supplemented flushing diet had the highest values for these variates. It was concluded that supplementing the flushing diet of ewes with flaxseed oil to provide a source of ω3 CSFA produces beneficial responses in their reproductive performance.

1. Introduction

Reaping the biggest benefits in sheep raising depends mainly on the improvement of reproductive performance through increasing the lambing rate and decreasing the mortality rate of lambs and, on the other hand, paying a special attention toward the subject of animal feed. Considering that animal feed includes more than 60% of animal raising costs, focusing on reduction of such costs together with increased reproductive performance may improve the efficiency of this part of production process. One of the issues related to the optimization of lamb raising is

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the use of flushing diets for 2 weeks before and 3 weeks after the natural mating that may increase the lambing rate. The use of nutritional supplements during the flushing period increases the ovulation and lambing rates in most races of ewes (Naqvi et al., 2011). So far, different sources of energy including both carbohydrate and fat sources have been used as supplements in flushing diets (Daghigh Kia et al., 2011). Adding supplemented fat to flushing diets in ewes may have a significant impact on animal's reproductive performance through an increase in plasma concentrations of cholesterol and progesterone, and also an increased supply of lipoproteins involved in the regulation of steroidogenesis system in ovaries (Williams, 1996). The use of sources of supplemented fat as oil or oilseeds resulted in an improved lambing performance in sheep (Daghigh Kia et al., 2011). In this respect, when cows were fed 0.75 kg of linseed rich in α-linolenic acid ($n-3$), or sunflower rich in linoleic acid ($n-6$), pregnancy rate tended to increase in cows of the first treatment (Ambrose et al., 2006). Since the saturated fatty acids used in diets are being hydrogenated in rumen, it was therefore attempted to pass the unsaturated fatty acids through rumen with minimal changes using the salt soaps in order for them to be used in intestines. The use of CSFA during the estrous period increased progesterone concentration in blood (Liel et al., 2010). Aranda-Ávila et al. (2010) observed a 12.8% increase in pregnancy rate when cows were supplemented with corn oil, relative to a control group (54.5 vs. 41.7% respectively) after 35 d supplementation. Unsaturated fatty acids, linoleic (ω6) and linolenic (ω3) play a major role in many physiological processes such as growth, reproduction, vision and brain development (Gurr et al., 2002). Due to the lack of relevant desaturase enzymes, animals are not able to synthesize ω3 and ω6 fatty acids through the De novo pathway. Studies have shown that linoleic acid is able to reduce the rate of $PGF_{2\alpha}$ synthesis by inhibiting the activity of delta-6-desaturase and cyclooxygenase (Staples et al., 1998) while the linolenic acid, which is a precursor for docosahexaenoic and icosapentaic acids, has an inhibitory effect on cyclooxygenase activity and subsequently stops $PGF_{2\alpha}$ synthesis. It is known that $PGF_{2\alpha}$ plays a direct role in the regression of corpus luteum (CL) and a subsequent decrease in blood progesterone and as a result, preventing the pregnancy. Results of a study showed that the highest number of grade 1 oocytes[1] were obtained from the ewes fed by a ω3 unsaturated fatty acid supplement (Zeron et al., 2002). Adding fat as CSFA in diet increased serum lipids and progesterone concentration in cows (Hightshoe et al., 1991). El-Shahat and Abo-El Maaty Amal (2009) reported that CSFA used with or without L-carnitine (LC) improved the ovarian response in ewes as an increase in medium and large follicles for ovulation and ultimately increased the ovulation rate and progesterone concentration in serum sample. Considering the relatively limited information available about the response of ewes to the bypass unsaturated fatty acid supplements (ω3 and ω6) in terms of reproductive performance during the flushing period, this study was performed in order to investigate the effects of feeding CSFA with different profiles on reproductive performance during the flushing period.

[1] Oocytes having a scattered polar body and wide perivitelline space.

2. Materials and methods

2.1. Animals, feeding and experimental design

This study was conducted in 'Afshari' sheep raising center of Kashan during the breeding season (summer) with an average elevation of 982 m and the average annual temperature of 35 °C (latitude 32°38″15″ N). In this study, 48 multiparous Afshari ewes, 2–3 years old with an average weight of 46 ± 3 kg (mean ± SD) were penned indoors in four equal groups ($n = 12$ per group). The ewes were group-fed their fixed ration twice per day and had water available ad libitum. Approximately 95% of the ration was consumed each day. The experimental groups of ewes received the following diets respectively; Group Barley: flushing diet containing barley, Group CaFl: flushing diet containing 5% CSFA with source of flaxseed oil (ω3) (CaFl) as an alternative to barley (5% of the diet dry matter), Group CaSf: flushing diet containing 5% CSFA with source of sunflower oil (ω6) (CaSf) as an alternative to barley and Control group (not receiving the flushing diet (Control group contained wheat bran (10%), wheat straw (40%), alfalfa (34%) and barley grain (16%))) (Table 1). All diets were prepared based on nutritional requirements table. The only difference between the flushing diets was their source of energy. All the flushing diets were both isoenergetic and isonitrogenous. The flushing period started on the day of CIDR insertion and continued for 3 weeks after ram introduction giving a total period of 5 weeks over which the experimental diets were fed. Ewes body condition score was approximately 2.5 at the start of this experiment. Ingredients and chemical analysis of diets are given in Table 1.

In order to conduct this study, the estrus synchronization of ewes was first accomplished using CIDR (EAZI_BREED; Pfizer NEW Zealand LTD, Auckland, NEW Zealand) for 14 d; then the ewes received 400 units of PMSG hormone [Bioniche Animal Health (LA Asia) Pty Ltp/Australia (pregnecol injection)]. Ewes were naturally mated using Afshari rams that were introduced 36 h after the administration of PSMG. A total of seven rams were used in rotation between the treatment groups of ewes.

2.2. Blood sampling

During the experiment, blood samples were collected from the jugular vein at four stages: start of the experiment, 24 h before CIDR removal, 24 h after CIDR removal and 14 d after ram introduction (RI) using a Venoject syringe. The blood samples serum was separated immediately after blood sampling using a centrifuge (3000 rpm for 15 min) and stored in microtubes inside a freezer at −20 °C until the time of analysis.

2.3. Analysis of blood metabolites

Analysis of serum sample metabolites was done using a STAT Faz-2100 spectrophotometer. Kits of Glucose (Pars Azmun Laboratory, Tehran, Iran; 017-500-1), total protein (Pars Azmun Laboratory, Tehran, Iran; 028-500-1) and blood urea nitrogen (BUN) (Pars Azmun Laboratory, Tehran, Iran; 1-400-030) were used to measure blood metabolites. Blood cholesterol was also measured using a cholesterol kit (Pars Azmun Laboratory, Tehran, Iran; 010-500-1).

2.4. Hormonal assay

Serum sample hormones concentrations were measured using an ELISA reader (STAT-FAX 3200, USA). Kits used to measure estrogen, progesterone and insulin were NO.ELA-2693, NO.ELA-1561 and NO.ELA-2425-300 (DRG), respectively.

2.5. Statistical analysis

This study was conducted in a completely randomized design. SAS 2003 software was used for data analysis. FREQ procedure was utilized to analyze characteristics such as pregnancy rate and other reproductive traits. Blood hormones and metabolites concentrations were also analyzed using MIXED procedures and data are presented as mean ± SEM. The statistical model used is as follows:

$$Y_{ijkl} = \mu + \mathrm{Treat}_i + \mathrm{Animal}_j(\mathrm{Treat}_i) + \mathrm{Time}_k + (\mathrm{Treat} * \mathrm{Time})_{ik} + B(X_{ijk} - X\ldots) + e_{ijkl}$$

Table 1
Ingredients and nutrient composition of experimental diets (dry matter basis) fed during the flushing period to Afshari ewes.

	Control	CaFl	CaSf	Barley
Ingredient (%)				
Wheat bran	10	10	10	10
Wheat straw	40	37	37	40
Alfalfa	34	41	41	25
Barley	16	7	7	25
Flaxseed oil (CaFl)	0	0	5	0
Sunflower oil (CaSf)	0	5	0	0
Chemical components				
Digestible energy (Mcal/d)	2.55	6.68	6.68	6.64
Total digestible nutrients (%)	54.3	85	85	86
Metabolism energy (Mcal/d)	2.1	5.47	5.47	5.44
Crude protein (g/d)	95	150	150	150
Calcium (g/d)	4.12	4.33	4.33	4.33
Phosphorus (g/d)	2.8	2.9	2.9	2.9

CaFl: flaxseed oil diet supplied ω3 calcium salts of fatty acids (CSFA); CaSf: sunflower oil supplied ω6 CSFA.

where, Y_{ijkl} is animal's performance, μ is mean of population, Treat_i is treatment's effect, Animal_j (Treat_i) is effect of the jth animal on the ith treatment, Time_k is effect of the kth time, $(\text{Treat*Time})_{ik}$ is treatment × time interaction, $B(X_{ijk} - X\ldots)$ is effect of weight as a covariate and e_{ijkl} is residual effect.

3. Results

3.1. Reproductive performance

The body condition score of the ewes during the mating period was approximately 3. The control ewes (non-flushing diet) showed lower fertility (83.3%) than the Barley (91.7%) and CSFA treatments (100.0%). Control ewes also had a significantly lower ($P < 0.05$; Table 2) lambing rate (83.3%) than Barley (125.0%), CaFl (150.0%) and CaSf (116.7%). All the ewes in the CSFA treatments were fertilized soon after ram introduction. Lamb birth weights were significantly ($P < 0.01$) higher in treatments CaFl (4.90 kg) and CaSf (4.75 kg) than in the Barley (4.35 kg) and the Control (4.12 kg) treatments (Table 2).

3.2. Serum sample metabolites and hormones

Fig. 1 shows the variation of blood estrogen levels at the different stages of the experiment. The levels of estrogen were similar for all the treatments except at the third sampling 24 h after PRID removal when values for all the flushing treatments were higher than the control value. The values for the CaFl and CaSf treatments at this sampling were significantly higher ($P < 0.01$) than for Control.

The diets were Control (non-flushing) and flushing diets supplemented with Barley or sources of calcium salts of fatty acids (CSFA) in the form of flaxseed oil (CaFl; ω3) or sunflower oil (CaSf; ω6). T_1: start of study; T_2: 24 h before CIDR removal; T_3: 24 h after CIDR removal; T_4: 24 d after ram introduction.

The serum progesterone concentrations were the same or similar for all the treatments at the first (1.74 ± 0.05 ng/dl), second (2.87 ± 0.05 ng/dl) and third (1.80 ± 0.05 ng/dl) samplings. At the final sampling, 14 d after the start of mating, progesterone levels for CaFl (5.50 ± 0.05 ng/dl) were significantly higher ($P < 0.01$) than for the other three treatments (overall mean 4.62 ± 0.05 ng/dl; Table 3).

The serum Glucose concentrations were similar for all the treatments at the first sampling (67.4 ± 1.04 mg/dl). At the second sampling Glucose concentrations for Barley (90.4 ± 1.04 mg/dl) were significantly higher ($P < 0.01$) than for the CaSf and control treatments and at the third for Barley (91.8 ± 1.04 mg/dl) were significantly higher ($P < 0.01$) than for the other three treatments. At the final sampling, 14 d after the start of mating, glucose levels for Barley (86.80 ± 1.04 mg/dl) were significantly higher ($P < 0.01$) than for the CaSf and control treatments (Table 4).

The serum protein concentrations were not significantly different between the treatments at the first, second and third samplings (overall means 8.54 ± 0.14, 8.60 ± 0.14, 7.95 ± 0.114 g/dl).

The serum insulin concentrations were not significantly different at the first sampling (overall mean 45.7 ± 0.42 ng/dl). At the second sampling, insulin concentrations were significantly ($P < 0.01$) lower for control (43.1 ± 0.42 ng/dl) than for the other three treatments whereas at the third sampling serum insulin levels were significantly lower ($P < 0.01$) for CaFl and CaSf than for Barley and Control. The same pattern of differences was

Table 2
Effects of calcium salts of fatty acids (CSFA) supplements in the flushing diets of Afshari ewes on their fertility and fecundity ($n = 12$ per treatment).

Birth weight (kg)	Twining (%)	Lambing rate (%)	Fertility (%)	Total offspring	Treatment
4.35 ± 0.22^a	33.33	125	91.7	15^a	Barley
4.9 ± 0.22^b	50.00	150	100	18^b	CaFl
4.75 ± 0.22^b	8.33	116.6	100	14^a	CaSf
4.12 ± 0.22^c	0	83.3	83.3	10^c	Control

CaFl: flaxseed oil diet supplied ω3 calcium salts of fatty acids (CSFA); CaSf: sunflower oil supplied ω6 CSFA. Means with different superscripts within a column are significantly different ($P < 0.01$).

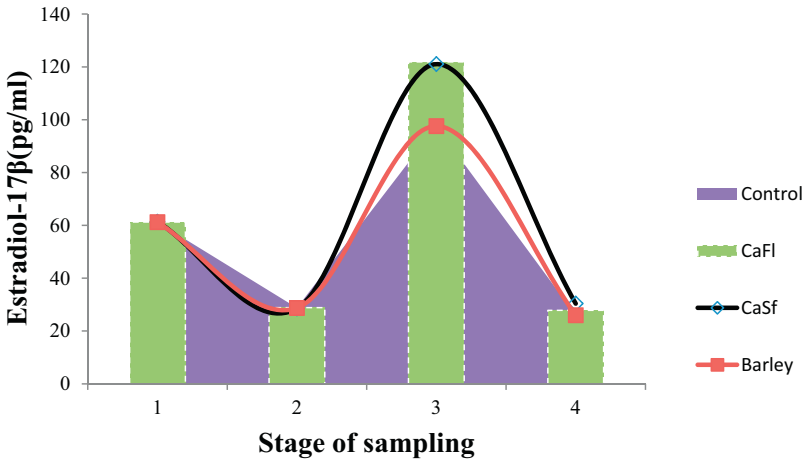

Fig. 1. The effect of four different diets during the flushing period on serum estradiol-17β concentrations of Afshari ewes.

Table 3
Effects of calcium salts of fatty acids (CSFA) supplements in the flushing diets of Afshari ewes on serum progesterone concentrations (ng/dl; mean ± SEM).

Stages of sampling				
Treatment	T_1	T_2	T_3	T_4
Barley	1.75 ± 0.05	2.83 ± 0.05	1.81 ± 0.05	4.46 ± 0.05[a]
CaFl	1.73 ± 0.05	2.94 ± 0.05	1.82 ± 0.05	5.50 ± 0.05[b]
CaSf	1.74 ± 0.05	2.83 ± 0.05	1.82 ± 0.05	4.82 ± 0.05[c]
Control	1.76 ± 0.05	2.89 ± 0.05	1.74 ± 0.05	4.58 ± 0.05[ac]
[A]BIC = −70				

CaFl: flaxseed oil diet supplied ω3 calcium salts of fatty acids (CSFA); CaSf: sunflower oil supplied ω6 CSFA. T_1: start of study; T_2: 24 h before CIDR removal; T_3: 24 h after CIDR removal; T_4: 24 d after ram introduction.
Means with different superscripts within a column are significantly different ($P < 0.01$).
[A] Bayesian Information Criterion.

Table 4
Effects of calcium salts of fatty acids (CSFA) supplements in the flushing diets of Afshari ewes on serum glucose concentrations (mg/dl; mean ± SEM).

Stages of sampling				
Treatment	T_1	T_2	T_3	T_4
Barley	65 ± 1.04	90.4 ± 1.04[a]	91.8 ± 1.04[a]	86.8 ± 1.04[a]
CaFl	67.8 ± 1.04	86.4 ± 1.04[ab]	79.2 ± 1.04[b]	85.8 ± 1.04[a]
CaSf	70.8 ± 1.04	83.2 ± 1.04[b]	86 ± 1.04[c]	79.6 ± 1.04[b]
Control	66 ± 1.04	63.4 ± 1.04[c]	56.6 ± 1.04[d]	63.8 ± 1.04[c]
[A]BIC = 319.1				

CaFl: flaxseed oil diet supplied ω3 calcium salts of fatty acids (CSFA); CaSf: sunflower oil supplied ω6 CSFA. T_1: start of study; T_2: 24 h before CIDR removal; T_3: 24 h after CIDR removal; T_4: 24 d after ram introduction.
Means with different superscripts within a column are significantly different ($P < 0.01$).
[A] Bayesian Information Criterion.

Table 5
Effects of calcium salts of fatty acids (CSFA) supplements in the flushing diets of Afshari ewes on serum protein concentrations (g/dl; mean ± SEM).

Stages of sampling				
Treatment	T_1	T_2	T_3	T_4
Barley	8.21 ± 0.14	8.44 ± 0.14	8.12 ± 0.14	9.38 ± 0.14[a]
CaFl	8.66 ± 0.14	8.72 ± 0.14	7.89 ± 0.14	9.21 ± 0.14[a]
CaSf	8.78 ± 0.14	8.91 ± 0.14	7.66 ± 0.14	9.00 ± 0.14[a]
Control	8.53 ± 0.14	8.31 ± 0.14	8.14 ± 0.14	8.26 ± 0.14[b]
[A]BIC = 65.8				

CaFl: flaxseed oil diet supplied ω3 calcium salts of fatty acids (CSFA); CaSf: sunflower oil supplied ω6 CSFA. T_1: start of study; T_2: 24 h before CIDR removal; T_3: 24 h after CIDR removal; T_4: 24 d after ram introduction.
Means with different superscripts within a column are significantly different ($P < 0.01$).
[A] Bayesian Information Criterion.

Table 6
Effects of calcium salts of fatty acids (CSFA) supplements in the flushing diets of Afshari ewes on serum insulin concentrations (ng/dl; mean ± SEM).

Stages of sampling				
Treatment	T_1	T_2	T_3	T_4
Barley	45.3 ± 0.42[a]	46.4 ± 0.42[a]	46.9 ± 0.42[a]	45.7 ± 0.42[a]
CaFl	46.3 ± 0.42[a]	47.3 ± 0.42[a]	37.1 ± 0.42[b]	37.1 ± 0.42[b]
CaSf	45.8 ± 0.42[a]	48.7 ± 0.42[a]	36.8 ± 0.42[b]	37.2 ± 0.42[b]
Control	45.4 ± 0.42[a]	43.1 ± 0.42[b]	45.1 ± 0.42[a]	43.4 ± 0.42[c]
[A]BIC = 201.9				

CaFl: flaxseed oil diet supplied ω3 calcium salts of fatty acids (CSFA); CaSf: sunflower oil supplied ω6 CSFA. T_1: start of study; T_2: 24 h before CIDR removal; T_3: 24 h after CIDR removal; T_4: 24 d after ram introduction.
Means with different superscripts within a column are significantly different ($P < 0.01$).
[A] Bayesian Information Criterion.

Table 7
Effects of calcium salts of fatty acids (CSFA) supplements in the flushing diets of Afshari ewes on serum BUN concentrations (mg/dl; mean ± SEM).

Stages of sampling				
Treatment	T_1	T_2	T_3	T_4
Barley	35.2 ± 0.60	39.8 ± 0.60[a]	38.5 ± 0.60[a]	41.6 ± 0.60[a]
CaFl	36.5 ± 0.60	39.6 ± 0.60[a]	40.3 ± 0.60[a]	40.4 ± 0.60[a]
CaSf	36.4 ± 0.60	41.6 ± 0.60[a]	41.0 ± 0.60[a]	41.1 ± 0.60[a]
Control	35.5 ± 0.60	32.1 ± 0.60[b]	33.8 ± 0.60[b]	33.3 ± 0.60[b]
[A]BIC = 249.7				

CaFl: flaxseed oil diet supplied ω3 calcium salts of fatty acids (CSFA); CaSf: sunflower oil supplied ω6 CSFA. T_1: start of study; T_2: 24 h before CIDR removal; T_3: 24 h after CIDR removal; T_4: 24 d after ram introduction.
Means with different superscripts within a column are significantly different ($P < 0.01$).
[A] Bayesian Information Criterion.

Table 8
Effects of calcium salts of fatty acids (CSFA) supplements in the flushing diets of Afshari ewes on serum cholesterol concentrations (mg/dl; mean ± SEM).

Stages of sampling				
Treatment	T_1	T_2	T_3	T_4
Barley	67 ± 0.92[a]	73 ± 0.92[a]	73.4 ± 0.92[a]	80.2 ± 0.92[a]
CaFl	68.4 ± 0.92[a]	94.4 ± 0.92 [b]	95.4 ± 0.92[b]	90.2 ± 0.92[b]
CaSf	68.5 ± 0.92[a]	92.8 ± 0.92[b]	93 ± 0.92[b]	89.4 ± 0.92[b]
Control	66.4 ± 0.92[a]	67 ± 0.92[c]	66.4 ± 0.92[c]	70 ± 0.92[c]
[A]BIC = 303.3				

CaFl: flaxseed oil diet supplied ω3 calcium salts of fatty acids (CSFA); CaSf: sunflower oil supplied ω6 CSFA. T_1: start of study; T_2: 24 h before CIDR removal; T3: 24 h after CIDR removal; T_4: 24 d after ram introduction.
Means with different superscripts within a column are significantly different ($P < 0.01$).
[A] Bayesian Information Criterion.

observed at the final sampling 14 d after the start of mating, but at this sampling the difference between Barley (45.8 ± 0.42 ng/dl) and Control (43.5 ± 0.42 ng/dl) was significantly different (Table 6).

The serum BUN concentrations were the similar for all the treatments at the first (overall mean 35.9 ± 0.60 mg/dl). At the second, third and final sampling, BUN concentrations for control (32.1 ± 0.60 mg/dl, 33.8 ± 0.60 mg/dl and 33.3 ± 0.60 mg/dl, respectively) were significantly lower ($P < 0.01$) than the other three treatments (Table 7).

The serum cholesterol concentrations were not significantly different between the treatments at the first sampling (overall mean 65.6 ± 0.92 mg/dl). At the other three samplings the levels of serum cholesterol were substantially higher ($P < 0.01$) for the Ca treatments (CaFl and CaSf) than for Barley and Control. Moreover, at these samplings the level of serum cholesterol for the Barley treatment was significantly higher ($P < 0.01$) than for the Control.

4. Discussion

The use of oil supplements in diet, stimulates the growth and development of follicles and as a result increases the levels of estrogen and ovulation rate. Number of offspring has a high correlation with concentration of estrogen at proestrus and estrus stages, because the high concentrations of estradiol during the follicular phase with positive feedback, causes a surge in gonadotropins secretion. This mechanism causes the release of LH and FSH through both increasing the secretion frequency of GnRH and the sensitivity of anterior pituitary to it. In current study, the use of flaxseed oil increased the estrogen concentration in plasma and consequently the number of offspring. Granulosa cells collected from follicles in cows fed unsaturated fatty acids (ω6) supplement, increased both the steroid secretions and steroidogenesis performance and estrogen concentration during the follicular phase was also high in cows fed an α-linolenic acid supplement (Robinson et al., 2002). An increase in GnRH/FSH pulses also increases blood estrogen

levels, ovulation rate, fertility and delivery rate in goats (Medan et al., 2004; Sasaki et al., 2006; Rahman et al., 2008). Another factor that may justify blood estrogen concentration increase is high levels of plasma cholesterol and HDL, because cholesterol is the main precursor for all the steroids (Carrol et al., 1990).

The increase in levels of blood progesterone concentration may be due to an increase in levels of cholesterol available which is a precursor for progesterone biosynthesis by corpus luteum. Ghoreishi et al. (2007) demonstrated that feeding the animals with bypass fat sources directly affected the ovulation, formation of corpus luteum and also the continuation of pregnancy especially in its early stages. In addition, increased concentrations of serum sample lipids was completely associated with an increase in intercellular lipids in both small and large steroidogenic cells which were responsible for the formation of corpus luteum followed by an increase in progesterone synthesis precursors. Blood progesterone levels were associated with the percentage of lipids in large luteal cells, the synthesis rate of steroidogenic products derived from lipids and, cholesterol and HDL concentrations (Hawkins et al., 1995). High absorption of lipids from the diet content increased the serum progesterone concentrations in dairy cattle (Spicer et al., 1993). Wonnacott et al. (2010) reported that progesterone concentrations in follicular fluid were higher in sheep that consumed a diet rich in ω3 fatty acids compared to those who consumed ω6 fatty acids in their diet. GnRH also increased the progesterone levels through an increase in blood LH levels (Zare Shahneh et al., 2008) and this was directly related to many reproductive factors such as ovulation and embryo replacement at early stages of pregnancy (Akifcam and Kuran, 2004).

Progesterone concentrations in flushing treatments were lower at early reproductive stage (the time of CIDR removal). However, an increase in progesterone levels after the ovulation period (14 d after RI) were evident (Table 3, $P < 0.01$) which seems is due to changes in progesterone levels as a result of an increase in concentrations of its precursors i.e. cholesterol and acetate or the use of bypass fats have caused such changes by increasing the levels of precursors such as cholesterol. Results of this study were similar to those obtained by Daghigh Kia et al. (2011). However, Titi and Awad (2007) did not observe a relationship between the use of fat and blood progesterone concentrations.

It appears that the results observed in terms of glucose concentration are linked with the number of offspring obtained in treatment Barley. Glucose is one of the most important ingredients for a suitable reproductive performance (Hess et al., 2005) and affects the hypothalamus–pituitary axis (HPA) (Downing et al., 1995). Vegetable oils rich in oleic acid (18:1) and linoleic acid (18:2) increase gluconeogenesis process by producing propionate in rumen (Chalupa et al., 1986) and therefore cause an increase in the rate of glucose synthesis and consequently the insulin concentration (Ryan et al., 1995). Glucose absorption and utilization rates were not measured in this study but the positive impact of glucose on folliculogenesis and increasing the rate of ovulation has been confirmed based on the findings by Scaramuzzi et al. (2006). This increase in blood glucose concentration may be due to a diet rich in those unsaturated fatty acids that pass through the rumen because of their calcified nature and increase blood sugar after breaking at the beginning of the intestines to form glycerol and then glucose.

It seems that the reason behind higher serum protein levels (although non-significant) in this group compared to other groups in this experiment especially during the middle or late stages of flushing, is the adaptation of rumen microbial population to the feed and increased production of microbial biomass or microbial protein as a result. Considering the higher fermentability of barley compared to other experimental diets, increased production of microbial protein followed by an increase in serum protein levels seems quite logical. There was also a positive correlation between protein and urea ($r = +0.25$, $P < 0.05$) which also affected the number of offspring (Tables 2 and 5). Hoon et al. (2000) reported that diets containing protein supplements affected the fertility rate at 400 g/d and high levels of protein absorption increased FSH pulses and improved the fertility rate as a result.

Diets containing high amounts of starch are responsible for the increase in blood glucose in livestock by producing more propionate which is considered as the major glucose production pathway in ruminants (Evans et al., 1975). The level of glucose dropped in blood was directly related to insulin level in plasma (Bassett, 1971). The ratio of forage in a diet may have a significant impact on levels of both glycogenic substrates and insulin (Trenkle, 1970). In addition to the ratio of forage in a diet, other factors that could modify the ratio of volatile fatty acids in rumen, especially acetate and propionate, through affecting the microbial fermentation, could have a significant impact on blood glucose level. Lower levels of serum glucose in flushing treatments containing CSFA are probably the main reason behind lower levels of insulin too. The differences observed in average serum insulin concentrations in current study could be attributed to the differences in the rate of propionate production among the treatments. Insulin is a known factor for follicular function in several species of livestock is responsible for tasks such as: increased steroidogenic activity in granulosa cells, cell mitosis, morphological changes and differentiation of follicles and increased progesterone concentration in follicular fluid (Spicer et al., 1993a; Savion et al., 1981). Thus, it seems that increased insulin concentrations stimulate the further growth and development of follicles, therefore increase the rate of ovulation and ultimately lead to the birth of a greater number of offspring.

Naqvi et al. (2012) reported that a significant increase in levels of blood urea was related to the increased rate of protein catabolism in the body and urea was actually a metabolic product of protein catabolism. High levels of protein catabolism helps the process of gluconeogenesis, thus controls the balance of energy in body and as a result of this, reproductive activities would be followed normally.

There was a positive correlation between blood cholesterol and progesterone concentrations ($r = +0.3$, $P < 0.01$), and consequently the number of offspring (Tables 2 and 8). CaFl treatment (CSFA with source of flaxseed oil) had the highest level of serum sample cholesterol ($P < 0.01$). Thomas and Williams (1996) found that the use of fat

supplements in diet increased serum concentrations of cholesterol and HDL. Previous studies also showed that fat supplements rich in unsaturated fatty acids such as raw cottonseed, soybean oil and wheat bran increase the number of medium-sized follicles (Wehrman et al., 1991; Ryan et al., 1992). The use of nutritional supplements with rumen bypass fat could also be used as a source of energy during the RI (ram introduction) to improve metabolism, pregnancy and lambing rate in ewes (Hashem and El-Zarkouny, 2013). Increased triglyceride levels in ewes fed Calcium Slats of fatty Acids (CSFA) has been reported by Ghoreishi et al. (2007) and Liel et al. (2010). This increase may be due to the reduced activity of lipogenic enzyme in liver and fatty tissues (Starry, 1981) or the production of lipoproteins of fat inside the intestines (Grummer and Carroll, 1991).

5. Birth weight

Birth weight in treatments CaFl and CaSf (with sources of ω3 and ω6, respectively) was higher than treatment Barley and control treatment (Table 2, $P < 0.01$). Higher birth weights and twining rates in CSFA treatments were probably due to the use of fat supplements in diet which resulted in an increased base concentration of LH and follicles' diameter (Hightshoe et al., 1991; Lucy et al., 1992; De Fries et al., 1998). Sabra and Hassan (2008) demonstrated that performing the flushing process for a month before fertilization significantly improved the reproductive performance in 'Barki' ewes due to increased estrus, reducing the duration of estrus periods, increased lambing percentage and birth weight. Flushing process also significantly affected the initial birth weight and weaning weight of lambs (Sormunen-Cristian and Jauhiainen, 2002).

6. Conclusion

It is concluded that the inclusion of ingredients that supply calcium salts of fatty acids (CSFA) in the flushing diets of Afshari ewes beneficially increases metabolites and hormones related to reproductive performance especially cholesterol, glucose, progesterone and estrogen with a consequent improvement in their fertility and fecundity.

Conflict of interest

No conflict of interest with any financial organization regarding the material discussed in the manuscript.

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