Effects of different lipids and energy supplements on reproductive biological characteristics of 'Afshari' ewes in Iran

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ABSTRACT

Forty-eight 'Afshar' ewes, with an average body weight of 45±3 kg and an average age of 2.5 years were used in 4 groups (n = 12) in order to study the Effects of different lipids and energy supplements on reproductive characteristics of 'Afshari' ewes under heat stress. Ewes in each group received one of the following diets prepared based on the nutritional requirements of flushing period proposed by NRC tables: Group A) Flushing diet containing barley grains, Group B) flushing diet containing 5% of DMI (dry matter intake) CSFA (Calcium Salts of Fatty Acids) flaxseed oil (ω3), Group C) flushing diet containing 5% DMI CSFA sunflower oil (ω6) and Group D) control diet (basal diet only). Flushing diets had the same energy content with the energy sources. Treatments A, B and C improved both fertility and lambing rates. Group B with 16 lambs and A group with 9 lambs had the highest and the lowest number of offspring, respectively. Cholesterol and progesterone levels in group B were higher than the other groups. Results showed that the use of CSFA with different profiles increased the levels of blood metabolites and concentrations of hormones associated with reproduction. Therefore, the use of CSFA with source of flaxseed oil (ω3) during the flushing period can be very useful for sheep breeders under heat stress condition.

Keywords: CSFA; fertility; lambing; Afshar; sheep

Introduction

Animal feed includes more than 60 percent of animal raising costs. Thus, focusing on reduction of such costs together with increased reproductive performance may improve the efficiency of this part of production process. The optimization of ewe raising with the use of flushing diets for 2 weeks before and 3 weeks after the insemination, 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.* 2012). The feeding oil or oilseeds resulted in improved lambing performance in sheep (Daghigh Kia *et al.* 2012). Since the unsaturated fatty acids used in diets are being hydrogenated in rumen, it was therefore attempted to pass the unsaturated fatty acids through rumen to be used in intestines. The use of CSFA (Calcium Salts of Fatty acids) during the estrous period increased progesterone concentration in blood (Liel *et al.* 2010).

Considering the relatively limited information available about the effect of bypass unsaturated fatty acid supplements (ω3 and ω6) on reproductive performance in ewes during the flushing period, this study was performed to investigate the effects of feeding CSFA during the flushing period on reproductive performance. Heat stress is one of the limiting factors in dairy production in hot climates (Johnson et al., 1962) and is hard to account for by management in farming systems that practice semi extensive grazing.

Materials & methods

Animals, Feeding and Experimental Design

This study was conducted with 'Afshari' sheep in the Center of Ravand at Kashan, Iran with an average elevation of 975 m and the average annual temperature of 36°C. Forty eight 'Afshari' ewes, 2.5 years old with an average weight of 45±3 kg were used in for equal groups (n = 12). The experimental groups of ewes received the following diets respectively: Group A) Flushing diet containing barley, Group B) Flushing diet containing 5% CSFA with flaxseed oil (ω3) as an alternative to barley (5% of the diet dry matter), Group C) Flushing diet containing 5% CSFA with sunflower oil (ω6) as an alternative to barley and Group D) Control group (not receiving the flushing diet) (Table 1). All diets were prepared based on nutritional requirements for flushing (NRC, 1985). 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 2 weeks before insemination (ram introduction) and continued up to 3 weeks after insemination. 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.

Table 1. Ingredients and nutrient composition of experimental diets (dry matter basis)

	A	B	C	D
Ingredient (%)				
Wheat barn	10	10	10	10
Wheat straw	40	37	37	40
Alfalfa	25	41	41	34
Barley	25	7	7	16
CSFA (flaxseed oil)	0	5	0	0
CSFA (Sunflower oil)	0	0	5	0
Chemical components				
Digestible energy (Mcal/kg)	4.15	4.18	4.18	2.36
Total digestible nutrients (%)	86	85	85	54.3
Metabolism energy (Mcal/kg)	3.40	3.42	3.42	1.94
Crude protein (%)	15.02	15.02	15.02	9.52
Calcium (%)	4.32	4.3	4.3	4.2
Phosphorus (%)	2.85	2.83	2.83	2.78

A: Barley group; B: CSFA with source of flaxseed oil (ω3); C: CSFA with source of Sunflower oil (ω6);
D: Control group. Numbers or values within column with different superscripts are different ($p < 0.01$).

The estrus synchronization of ewes was accomplished using CIDR (controlled internal drug release)(EAZI_BREED; Pfizer NEW Zealand LTD, Auckland, NEW Zealand) for 14 days. The ewes then received

400 units of PMSG (pregnant mare's serum gonadotropin) hormone [Bioniche Animal Health (LA Asia) Pty Ltp/Australia (pregnecol injection)]. Natural insemination of ewes was done using 'Afshari' rams, 36 hours after the injection of PMSG.

Blood Sampling

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

Analysis of Blood Metabolites

Analysis of serum metabolites was done using spectrophotometer (a STAT Faz-2100 state here city, country). Glucose (Pars Azmun Laboratory, Tehran, Iran; 019-510-1), total protein (Pars Azmun Laboratory, Tehran, Iran; 027-510-2), cholesterol (Pars Azmun Laboratory, Tehran, Iran; 011-510-3) and blood urea nitrogen (BUN) (Pars Azmun Laboratory, Tehran, Iran; 2-510-022) were analysed using commercial kits.

Hormonal Assay

Serum hormones concentrations were measured using an ELISA reader (STAT-FAX 3200, USA). Kits used to measure estrogen; progesterone and insulin were NO.ELA-2794, NO.ELA-1984 and NO.ELA-2025-250 (DRG), respectively.

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 analyzed using GLM and MIXED procedures. The statistical model used is as follows:

$Y_{ijkl} = \mu + Treat_i + Animal_j (Treat_i) + Time_k + (Treat*Time)_{ik} + B(X_{ijk} - X\ldots) + e_{ijkl}$

In which Y_{ijkl} = animal's performance, μ = mean of population, $Treat_i$ = treatment's effect, $Animal_j (Treat_i)$ = effect of the j^{th} animal on the i^{th} treatment, $Time_k$ = effect of the k^{th} time, $(Treat*Time)_{ik}$ = treatment * time interaction, $B(X_{ijk} - X\ldots)$ = effect of weight as a covariate and e_{ijkl} = residual effect or error.

Results & discussion

Groups A, B and C treatments improved reproductive traits and had a significant ($P < 0.05$) effect on lambing rate (Table 2). All the ewes in treatments B and C (CSFA treatments) were fertilized during the first stage of RI (Ram Introduction) (Table 2). Body condition score of the ewes during the RI was 3.

Table 2. Effects of CSFA on offspring frequency and fertility rate in ewes

Treatment	Birth weight (kg)	Twining rate (%)	Lambing rate (%)	Fertility rate (%)	Total offspring
A	4.35 [a]	33.33	125	91.7	13 [a]
B	4.9 [b]	50	150	100	16 [b]
C	4.75 [b]	8.33	116.66	100	12 [a]
D	4.12 [c]	0	83.33	83.3	9 [c]

A: Barley group; B: CSFA with source of flaxseed oil (ω3); C: CSFA with source of Sunflower oil (ω6);
D: Control group. Numbers or values within column with different superscripts are different ($p < 0.01$).

Blood Serum Metabolites and Hormones

Levels of blood proteins, urea, cholesterol, estrogen and progesterone were significantly different in flushing treatments compared with those in control treatment (group D). Such changes in levels of hormones and metabolites are related to reproductive performance improvement. The results of this study are similar to those obtained by other researchers in this regard (Chilliard *et al.* 1998; Scaramuzzi *et al.* 2006; Ricardo *et al.* 2000).

Figure 1 shows the significant difference between groups in this experiment for blood estrogen levels at various stages of sampling ($P < 0.01$). Treatment B had the highest concentration of estrogen during the estrous stage (third stage of blood sampling) with 123.4 pg/ml, which eventually resulted in the highest number of offspring (Table 2).

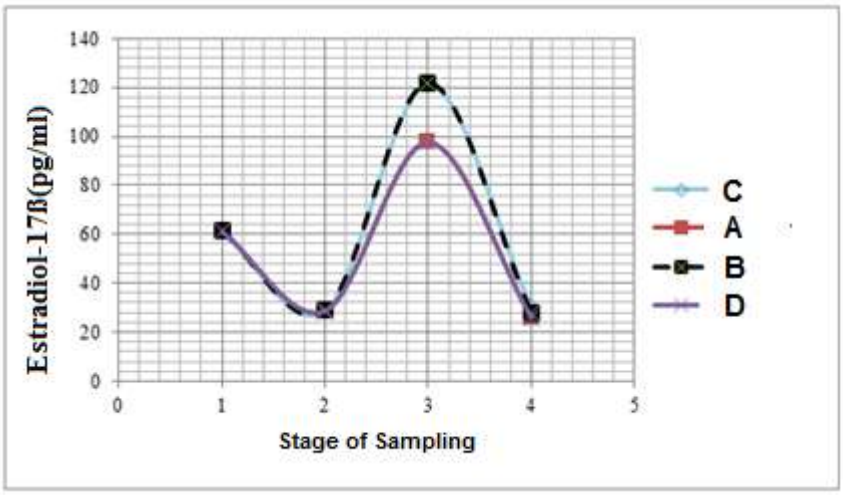

Fig. 1 Variations of blood serum estradiol-17β concentrations at four times.
A: Barley group; B: CSFA with source of flaxseed oil (ω3); C: CSFA with source of Sunflower oil (ω6); D: Control group. T1: Start of study; T2: 24 h before CIDR removal; T3: 24 h after CIDR removal; T4: 14 days after RI.

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. 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 (High Density Lipids), because cholesterol is the main precursor for all the steroids (Carrol et al. 1990).

Figure 2 and table 3 show changes in blood serum progesterone concentrations during the reproductive period among experimental groups in this study. Progesterone concentrations in flushing treatments were not different at the second and third stages of blood sampling. At the last stage of blood sampling however, progesterone levels in group B were higher compared to other groups followed by a higher number of offspring (Figure 2, Tables 2 and 3) ($P < 0.01$).

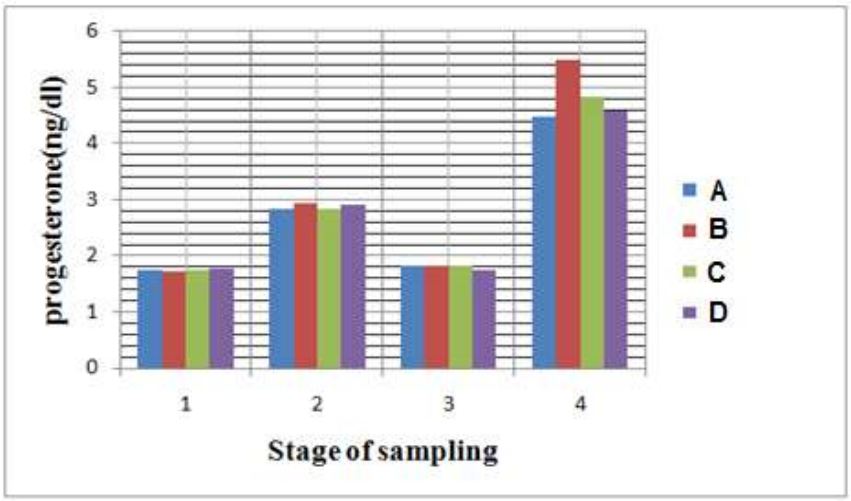

Fig. 2 Variations of blood serum progesterone concentrations at four times (stages of sampling).
A: Barley group; B: CSFA with source of flaxseed oil (ω3); C: CSFA with source of Sunflower oil (ω6); D: Control group. T1: Start of study; T2: 24 h before CIDR removal; T3: 24 h after CIDR removal; T4: 14 days after RI.

Table 3 Effects of CSFA on serum progesterone concentrations (ng/dl) at four stages of blood sampling in 'Afshari' ewes

Stages of sampling			
Treatment	T_1	T_2	T_3
A	2.82±0.05	1.80±0.05	$4.45±0.05^{a}$
B	2.93±0.05	1.81±0.05	$5.51±0.05^{b}$
C	2.82±0.05	1.81±0.05	$4.83±0.05^{c}$
D	2.88±0.05	1.73±0.05	$4.57±0.05^{ac}$
*BIC = -70			

A: Barley group; B: CSFA with source of flaxseed oil (ω3); C: CSFA with source of Sunflower oil (ω6); D: Control group. ; **T_1:** 24 h before CIDR removal**; T_2:** 24 h after CDIR removal**; T_3:** 14 days after RI. Numbers or values within columns with different superscripts are different ($p < 0.01$); **Bayesian Information Criterion*

Results obtained regarding the progesterone were in agreement with those of previous studies (Ishida *et al.* 1999; ZareShahneh *et al.* 2008). 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. Wonnacott *et al.* (2010) reported that progesterone concentrations in follicular fluid were higher in sheep that consumed a diet rich in omega-3 fatty acids compared to those who consumed omega-6 fatty acids in their diet. GnRH also increased the progesterone levels through an increase in blood LH levels (ZareShahneh *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 days after RI) were evident (Figure 2, $P < 0.01$) 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.* (2012). However, Titi and Awad (2007) did not observe a relationship between the use of fat and blood progesterone concentrations.

Glucose concentrations were different among treatments at different stages of blood sampling (Table 4, $P < 0.01$). Treatment A had the highest glucose concentration (8.91 mg/dl) at the third stage of blood sampling. It appears that the results observed in terms of glucose concentration are linked with the number of offspring obtained in treatment A. 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).

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.

Changes in blood serum protein levels during the reproductive period among the treatments in this experiment are reported in table 5. Serum protein levels in treatments were almost equal at early stages of reproductive cycle but significant differences were observed at later stages between flushing treatments and control group ($P < 0.01$). Barley group had the highest serum protein level among flushing groups, however the differences were not significant. 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/day and high levels of protein absorption increased FSH pulses and improved the fertility rate as a result.

Changes in levels of insulin among treatments during different reproductive periods are reported in table 6. Treatment A (barley flushing) had the highest blood insulin level ($P < 0.01$). 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.

Table 4. Effects of CSFA on serum glucose concentrations (mg/dl) at four stages of blood sampling in 'Afshari' ewes

Stages of sampling			
Treatment	T_1	T_2	T_3
A	90.40±1.04^{a}	91.80±1.04^{a}	86.80±1.04^{a}
B	86.40±1.04^{ab}	79.20±1.04^{b}	85.80±1.04^{a}
C	83.20±1.04^{b}	86.00±1.04^{c}	79.60±1.04^{b}
D	63.40±1.04^{c}	56.64±1.04^{d}	63.80±1.04^{c}
*BIC = 319.1			

Table 5 Effects of CSFA on serum protein concentrations (mg/dl) at four stages of blood sampling in 'Afshari' ewes

Stages of sampling			
Treatment	T_1	T_2	T_3
A	8.44±0.14	8.12±0.14	9.38±0.14 ^{a}
B	8.72±0.14	7.89±0.14	9.21±0.14^{a}
C	8.91±0.14	7.66±0.14	9.00±0.14^{a}
D	8.31±0.14	8.14±0.14	8.26±0.14^{b}
*BIC = 65.8			

Table 6 Effects of CSFA on serum insulin concentrations (ng/dl) at four stages of blood sampling in 'Afshari' ewes

Stages of sampling			
Treatment	T_1	T_2	T_3
A	46.40±0.42^{a}	46.96±0.42^{a}	45.76±0.42^{a}
B	47.30±0.42^{a}	37.10±0.42^{b}	37.12±0.42^{b}
C	48.72±0.42^{a}	36.86±0.42^{b}	37.22±0.42^{b}
D	43.10±0.42^{b}	45.16±0.42^{a}	43.48±0.42^{c}
*BIC = 201.9			

Table 7. Effects of CSFA on serum BUN concentrations (mg/dl) at four stages of blood sampling in 'Afshari' ewes

Stages of sampling			
Treatment	T_1	T_2	T_3
A	39.88± 0.60 ^{a}	38.58±0.60 ^{a}	41.62±0.60 ^{a}
B	39.68± 0.60 ^{a}	40.30±0.60 ^{a}	40.48±0.60 ^{a}
C	41.60± 0.60 ^{a}	41.06±0.60 ^{a}	41.18±0.60 ^{a}
D	32.10± 0.60 ^{b}	33.84±0.60 ^{b}	33.36±0.60 ^{b}
*BIC = 249.7			

Table 8 Effects of CSFA on serum cholesterol concentrations (mg/dl) at four stages of blood sampling in 'Afshari' ewes

Stages of sampling			
Treatment	T_1	T_2	T_3
A	73.00±0.92^{a}	73.40±0.92^{a}	80.20±0.92^{a}
B	94.40±0.92 ^{b}	95.40±0.92^{b}	90.20±0.92^{b}
C	92.80±0.92^{b}	93.00 ±0.92^{b}	89.40±0.92^{b}
D	67.00±0.92^{c}	66.40±0.92^{c}	70.00±0.92^{c}
*BIC = 303.3			

A: Barley group; B: CSFA with source of flaxseed oil (ω3); C: CSFA with source of Sunflower oil (ω6); D: Control group. ; **T_1:** 24 h before CIDR removal**; T_2:** 24 h after CDIR removal**; T_3:** 14 days after RI. Numbers or values within columns with different superscripts are different ($p < 0.01$); **Bayesian Information Criterion*

Blood serum urea concentrations during the reproductive period were significantly different among different groups in this experiment (Table 7, $P < 0.01$). Many recent studies reported results similar to those obtained in the current study that shows the effect of nutritional supplements on blood glucose, total protein, albumin and urea in ewes (Abdelatif *et al.* 2009; Naqvi *et al.* 2011). 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 level 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.

Levels of blood serum cholesterol were significantly different during various reproductive periods among different groups in this experiment (Table8, $P < 0.01$). 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). Treatment B (CSFA with source of flaxseed oil) had the highest level of blood serum cholesterol ($P < 0.01$). 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 2014). 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).

Birth Weight

Birth weight in treatments B and was higher than treatment A 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 (Lucy *et al.* 1992). Sabra and Hassan (2008) demonstrated that performing the flushing process for a month before fertilization significantly improved birth weight. Flushing process also significantly affected the initial birth weight and weaning weight of lambs (Sormunen-Cristian and Jauhiainen 2002).

Conclusion

Results of this study showed that the use of Calcium Salts of Fatty Acids (CSFA) with different sources of flaxseed oil (ω3) during the flushing period improved the reproductive performance and in particular the fertility rate and lambing percentage in 'Afshari' ewes under heat stress condition, significantly.

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