

## **Reproductive performance and blood metabolites concentration in Iranian Afshari ewes fed calcium salts of fatty acids (CSFA) in flushing period**

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### **ABSTRACT**

This study investigated the effect of dietary supplementation with calcium salts of fatty acids using 48 Afshari ewes randomly allocated to four treatments. All groups used in flushing period and only the source of energy were different between rations. The ewes were divided into four groups: A-barely grain; group B-received 5% of CSFA with source of flaxseed oil (w3); group C-received 5% of CSFA with source of sun flower oil (w6) and group D-control (only received basal diet) . Treatments A, B and C improved fertility and lambing rates. Treatment C with 18 and control with 10 lambs represented the highest and the lowest number of progeny respectively. Our results indicate that using CSFA with different profiles increase metabolite levels related to reproductive. In conclusion, using CSFA supplementation in flushing period was effective on reproductive performance of Iranian Afshari ewes.

**Key words:** CSFA, Flushing, Reproductive performance, Afshari ewe

### **INTRODUCTION**

One of the most important advantages of sheep production is its high reproductive rate. Photoperiod, temperature and nutrition are three well-studied environmental cues that affect reproduction in sheep. Nutritional flushing is defined as the short-term provision of extra feed (with different sources) to raise the plane of nutrition immediately prior, during or after mating. Nutritional supplementation or feed flushing prior to mating has been reported to increase ovulation and lambing rates in many breeds of sheep (Naqvi et al., 2011). It is definite accepted in sheep production to provide ewe with additional energy supply (flushing) for 2-3 weeks prior and after mating, increasing the number of lamb produced. Nutritional flushing is defined as the short-term provision of extra feed (with different sources) to raise the plane of nutrition immediately prior, during or after mating. Calcium soaps of fatty acids supplementation increase

the number and size of ovarian follicles in estrous cyclic ewes and increase the circulating progesterone concentration (Liel et al., 2010). Higher numbers of grade 1 oocytes were collected from n-3 PUFA supplemented ewes (Zeron et al., 2002).

## MATERIAL AND METHODS

This study was conducted in Kashan-Iran advance livestock station at 982m altitude with a mean annual temperature of 35 C°. Forty-eight Iranian AFSHARI ewes aged between 2 and 3 years old weighting 46 average body weights were allocated into 4 equal treatments (n=12) and kept in different pens in separate groups according to the type of supplementation. Animals were fed a same basal diet and received one of the following treatments: Group A- Barley grain; Group B- received 5% CSFA (5% of dietary DM) with source of flaxseed oil ( $\omega$ 3); Group C- received 5% CSFA (5% of dietary DM) with source of sunflower oil ( $\omega$ 6) and Group D- control (basal diet). In the nutritional flushing treatments, only the source of energy was different between diets; however, they were isoenergetic and isonitrogenous. Flushing period started 2 weeks before mating and continued 3 weeks later. At the beginning of the experiment, all ewes had average BCS of 2/5.

**Table 1:** Ingredient and nutrient composition (dry matter basis) of experimental diets

	Treatment A	Treatment B	Treatment C	Treatment D
Wheat bran	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
<b>Chemical component</b>				
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

Table 1 shown ingredients and nutrient composition of experimental groups. Diets were formulated to meet the nutrient requirements of NRC (1985) for sheep. Estrous was synchronized using CIDR (EAZI\_BREED; Pfizer NEW zealand LTD, Auckland, NEW zealand), which insert in vagina for 14 days and injection of PMSG hormone[Bioniche Animal Health (LA Asia) Pty Ltp/Australia (pregnecol injection)] was done. Serum estrogen (E2), progesterone (P4) and Insulin levels were assayed ELIZA (Awareness model, WA, USA) using kit NO.ELA-2693 and kit NO.ELA-1561and kit NO.ELA-2425-300(DRG) respectively. This experiment was laid out in a completely randomized design. The pregnancy rate and other reproductive traits were analyzed applying FREQ, logistics procedures; oestrogen and

progesterone levels and blood metabolites were analyzed using GLM and MIXED procedures of SAS software (SAS, 2003). Statistical model included as following:

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

In which,  $Y_{ijkl}$  equals animals performance,  $\mu$  = population mean,  $\text{Treat}_i = i$  treatment effect,  $\text{Animal}_j(\text{Treat}_i)$  = effect of  $j$  animal in treatment,  $\text{Time}_k$  = effect of  $k$  time,  $(\text{Treat} * \text{Time})_{ik}$  = treatment by time interaction,  $B(X_{ijk} - \bar{X} \dots)$  = effects of weight and BCS as a covariate and  $e_{ijkl}$  = residual or error.

## RESULTS AND DISCUSSION

Number of progeny, fertility, lambing rate, are shown in Table 2. Treatments A, B and C improved reproductive traits and had significant effects on the lambing rate ( $x^2 = 10.50$ ,  $p < 0.05$ ). All of ewes became pregnant with the first service in both CSFA<sup>1</sup> treatments (Table 2). The BCS of ewes was 3 during mating operation.

**Table 2:** Effects of calcium salt of fatty acids on offspring frequency and fertility rate in ewes

Treatment	Total offspring	Fertility (%)	Lambing rate (%)	Twining (%)	Birth weight(kg)
A	15 <sup>a</sup>	91.7	125	33.33	4.35 <sup>a</sup>
B	18 <sup>b</sup>	100	150	50	4.9 <sup>b</sup>
C	14 <sup>a</sup>	100	116.66	8.33	4.75 <sup>b</sup>
D	10 <sup>c</sup>	83.3	83.33	0	4.12 <sup>c</sup>

A, barley grain; B, 5% of CSFA with source of flaxseed oil (w3); C 5% of CSFA with source of sun flower oil (w6); D, control group. Numbers or values within column with different superscripts are different ( $p < 0.01$ ). Glucose concentration was differences at four times among treatments ( $p < 0.01$ ; Table 3). Treatment A with 91.80mg/dl had the highest glucose level at 24 hours after withdrawal CIDR. It seems that this result has relation to number of offspring in Treatment A. Glucose is a one of the most important metabolic substrate for adequate reproductive performance (Hess et al. 2005) and influence on the hypothalamus-hypophysis axis (Downing et al. 1995). Glucose is a important source of energy for ovaries (Rabiee and Lean, 2000 and Rabiee et al. 1999) and as a primary metabolic fuel that useful by central nervous system.

<sup>1</sup> Calcium salt of fatty acids

**Table 3:** Effects of CSFA on serum glucose concentrations (mg/dl) at four times in ewes

Treatment	Stages of sampling			
	A <sub>1</sub>	B <sub>1</sub>	C <sub>1</sub>	D <sub>1</sub>
A	65± 1.04 <sup>a</sup>	90.40± 1.04 <sup>a</sup>	91.80± 1.04 <sup>a</sup>	86.80± 1.04 <sup>a</sup>
B	67.80± 1.04 <sup>a</sup>	86.40± 1.04 <sup>ab</sup>	79.20± 1.04 <sup>b</sup>	85.80± 1.04 <sup>a</sup>
C	70.80± 1.04 <sup>a</sup>	83.20± 1.04 <sup>b</sup>	86± 1.04 <sup>c</sup>	79.60± 1.04 <sup>b</sup>
D	66± 1.04 <sup>a</sup>	63.40± 1.04 <sup>c</sup>	56.64± 1.04 <sup>d</sup>	63.80± 1.04 <sup>c</sup>
BIC* = 319.1				

Serum proteins level changes in reproductive period between treatments are reported in Table 4. Treatment A represented the highest level of serum protein ( $p < 0.01$ ). Flushing groups showed higher levels of serum protein, probably owing to higher protein intake. A positive correlation between protein and urea concentrations was observed ( $r = + 0.25$ ;  $p < 0.05$ ) and consequently the number of offspring (Tables 2 and 4).

**Table 4 :** Effects of CSFA on serum protein concentration (mg/dl) at the four times in ewes

Treatment	Stages of sampling			
	A <sub>1</sub>	B <sub>1</sub>	C <sub>1</sub>	D <sub>1</sub>
A	8.21±0.14 <sup>a</sup>	8.44±0.14 <sup>a</sup>	8.12±0.14 <sup>a</sup>	9.38±0.14 <sup>a</sup>
B	8.66±0.14 <sup>a</sup>	8.72±0.14 <sup>a</sup>	7.89±0.14 <sup>a</sup>	9.21±0.14 <sup>a</sup>
C	8.78±0.14 <sup>a</sup>	8.91±0.14 <sup>a</sup>	7.66±0.14 <sup>a</sup>	9.00±0.14 <sup>a</sup>
D	8.53±0.14 <sup>a</sup>	8.31±0.14 <sup>a</sup>	8.14±0.14 <sup>a</sup>	8.26±0.14 <sup>b</sup>
BIC* = 65.8				

A, barley grain; B, 5% of CSFA with source of flaxseed oil (w3); C 5% of CSFA with source of sun flower oil (w6); D, control group; **A<sub>1</sub>**, start testing; **B<sub>1</sub>**, 24 h before removal CIDR; **C<sub>1</sub>**, 24 h after removal CIDR; **D<sub>1</sub>**, 14 days after mating. Numbers or values within column with different superscripts are different ( $p < 0.01$ ); \**Bayesian Information Criterion*.

The BUN concentrate was different between groups (Table 5,  $p < 0.01$ ). Many studies reported earlier are in agreement to our result that supplemental feeding influenced the plasma glucose level, serum total protein, albumin, and urea in the ewes (Abdelatif et al. 2009; Naqvi et al. 2011). Naqvi et al. (2012) reported that the significant ( $p < 0.01$ ) increase in plasma urea

suggests the increased rate of protein catabolism and urea is the metabolic end product of protein catabolism.

**Table 5:** Effects of CSFA on serum BUN concentrations (mg/dl) at four times in ewes

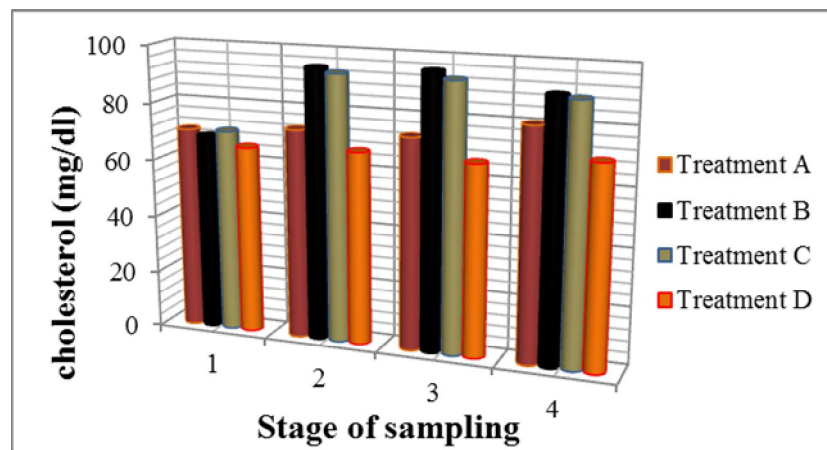
Treatment	Stages of sampling			
	A <sub>1</sub>	B <sub>1</sub>	C <sub>1</sub>	D <sub>1</sub>
A	35.28± 0.60 <sup>a</sup>	39.88± 0.60 <sup>a</sup>	38.58± 0.60 <sup>a</sup>	41.62± 0.60 <sup>a</sup>
B	36.52± 0.60 <sup>a</sup>	39.68± 0.60 <sup>a</sup>	40.30± 0.60 <sup>a</sup>	40.48± 0.60 <sup>a</sup>
C	36.46± 0.60 <sup>a</sup>	41.60± 0.60 <sup>a</sup>	41.06± 0.60 <sup>a</sup>	41.18± 0.60 <sup>a</sup>
D	35.52± 0.60 <sup>a</sup>	32.10± 0.60 <sup>b</sup>	33.84± 0.60 <sup>b</sup>	33.36± 0.60 <sup>b</sup>

BIC<sup>\*</sup>=249.7

A, barley grain; B, 5% of CSFA with source of flaxseed oil (w3); C 5% of CSFA with source of sun flower oil (w6); D, control group; **A<sub>1</sub>**, start testing; **B<sub>1</sub>**, 24 h before removal CIDR; **C<sub>1</sub>**, 24 h after removal CIDR; **D<sub>1</sub>**, 14 days after mating. Numbers or values within column with different superscripts are different (p < 0.01); \**Bayesian Information Criterion*.

The cholesterol had a significant difference between treatments among different reproductive cycles (p < 0.01). Figure 1 shows differences in cholesterol levels of experimental groups during reproductive cycles. Treatment B represented the highest level of serum cholesterol (p < 0.01). Tomas et al. (1996) founded that use of fat supplement in diet cause increase in cholesterol serum concentration and HDL.

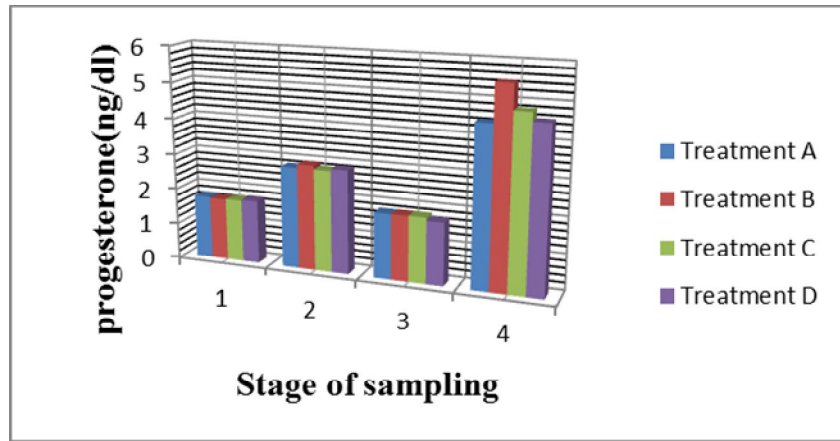
**Fig. 1:** Variations of blood serum cholesterol levels at four times (stages of sampling)



A, barley grain; B, 5% of CSFA with source of flaxseed oil (w3); C 5% of CSFA with source of sun flower oil (w6); D, control group. Stage1, start testing; Stage 2, 24 h before removal CIDR; Stage 3, 24 h after removal CIDR; Stage 4, 14 days after mating.

In conclusion, Result reveled that feeding flushing ration before mating and consequent increase in BCS, improved fertility and reproductive traits. Therefore, the source of supplemental CSFA during flushing is effective in improving fertility and lambing rate. Negative correlation between progesterone and oestrogen concentrations was observed ( $r = -0.67$ ;  $p < 0.05$ ) and consequently the number of offspring (Fig. 2; Tables 2).

**Fig. 2:** Variations of blood serum progesterone concentrations at four times (stages of sampling)



A, barley grain; B, 5% of CSFA with source of flaxseed oil (w3); C 5% of CSFA with source of sun flower oil (w6); D, control group. Stage1, start testing; Stage 2, 24 h before removal CIDR; Stage 3, 24 h after removal CIDR; Stage 4, 14 days after mating. Wonnacott et al.(2010) (15) reported that concentration of progesterone in the follicular fluid was greater in sheep, which consumed the ration enriched with n-3 fatty acids, than those, which were fed with a greater amount of n-6 fatty acids. Serum progesterone level changes in reproductive period between treatments are reported in Table 6.

**Table 6:** Effects of CSFA on serum progesterone concentrations (ng/dl) at four times in ewes

Treatment	Stages of sampling			
	A <sub>1</sub>	B <sub>1</sub>	C <sub>1</sub>	D <sub>1</sub>
A	1.75± 0.05 <sup>a</sup>	2.83± 0.05 <sup>a</sup>	1.81± 0.05 <sup>a</sup>	4.46± ± 0.05 <sup>a</sup>
B	1.73± 0.05 <sup>a</sup>	2.94± 0.05 <sup>a</sup>	1.82± 0.05 <sup>a</sup>	5.50± 0.05 <sup>b</sup>
C	1.74± 0.05 <sup>a</sup>	2.83± 0.05 <sup>a</sup>	1.82± 0.05 <sup>a</sup>	4.82± 0.05 <sup>c</sup>
D	1.76± 0.05 <sup>a</sup>	2.89± 0.05 <sup>a</sup>	1.74± 0.05 <sup>a</sup>	4.58± 0.05 <sup>ac</sup>

BIC\* =70.3

A, barley grain; B, 5% of CSFA with source of flaxseed oil (w3); C 5% of CSFA with source of sun flower oil (w6); D, control group;; **A<sub>1</sub>**, start testing; **B<sub>1</sub>**, 24 h before removal CIDR; **C<sub>1</sub>**, 24 h after removal CIDR; **D<sub>1</sub>**, 14 days after mating. Numbers or values within column with different superscripts are different ( $p < 0.01$ ); \**Bayesian Information Criterion*.

These results are similar with some previous reports (Ishida et al., 1999; Zare Shahneh et al., 2008)(100). P4 was related to the percentage of lipid in large luteal cells, percentage of total steroidogenic area occupied by lipids, and serum concentrations of cholesterol and HDL (Hawkins et al., 1995) (1).

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