Iranian Journal of Applied Animal Science

Interaction Effect of Dietary Fatty Acid (N-6 *vs.* N-3) and Rumen Undegradable Protein Source (Fish Meal *vs.* Corn Gluten Meal) on Plasma Metabolites, Hormones and Reproductive Performances of Lori Goats during the Breeding Season

Research Article

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Received on: 3 May 2024
Revised on: 20 Sep 2024
Accepted on: 15 Nov 2024
Online Published on: Dec 2024

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Online version is available on: www.ijas.ir
doi.org/10.71798/ijas.2024.1195020

ABSTRACT

This study aimed to investigate the effect of the interaction effect of fatty acid (FA) and rumen undegradable protein (RUP) source in diet around mating on body weight (BW), plasma metabolites, minerals, steroid hormones, and reproductive performances of Lori goats. Forty-eight Lori goats were divided into four groups (n=12) based on age (2-3 years), BW (39.85 kg), and body condition score (BCS) (2.5) and randomly assigned to one of four experimental diets. Goats were fed one of the experimental *flushing* diets supplemented with calcium (Ca) salt of n-6 FA (soybean oil) and corn gluten meal (CGM; n-6-CGM), Ca salt of n-6 FA and fish meal (FM; n-6-FM), Ca salt of n-3 FA (fish oil) and CGM (n-3-CGM) and Ca salt of n-3 FA and FM (n-3-FM). Results showed that dry matter intake (DMI), BW, BCS and plasma glucose, total cholesterol, urea nitrogen (UN), Ca, magnesium (Mg), sodium (Na) and potassium (K) concentrations did not influenced by experimental diets (P<0.05). However, fertility rate tended to be lower in goats fed n-6-CGM diet (P=0.07). Furthermore, numerical higher kidding rate, twinning rate and kid birth BW were observed in goats fed n-6-FM and n-3-FM diets compared to other groups. In conclusion, feeding a flushing diet containing Ca salt of n-6 or n-3 FA in combination with FM (n-6-FM and n-3-FM diets) rather than n-6 FA in combination with CGM around mating may improve the reproductive performances of Lori goats, however, further research is required to confirm these findings.

KEY WORDS flushing diet, goat, n-3 and n-6 fatty acid, reproductive performances, rumen undegradable protein source.

INTRODUCTION

Nutrition alters reproductive function through manipulation of gonadotropin secretion or through local effects on ovarian folliculogenesis and uterine environment. Effects appear to be mediated through metabolic hormones such as insulin and insulin like growth factor-1 (IGF-1) or through blood metabolites such as urea, glucose, and amino acids (Gong *et al.* 2002). Number of offspring born and consequently the reproduction efficiency in small ruminants have been influenced by the number of follicles that reach the ovulation stage. In ruminant animals, hormones and nutritional factors are used to increase the number, size and quality of ovarian follicles (Daniel *et al.* 2013). Follicle size and quality can affect embryo survival, pregnancy continuity, and fetal development and growth (Webb *et al.* 2004). Feeding a diet based on cereal grains in the time before the beginning of the breeding season (flushing) is one of the most significant management strategy for enhancing the number and quality of oocytes in small ruminants (Scaramuzzi *et al.*

2006). Flushing increases the plasma concentrations of metabolites and metabolic hormones and subsequently the chemical composition of the follicular fluid, causing an increase in the secretion of FSH and stimulating the growth of follicles (Greyling, 2010). In fact, these events influence by nutrients profile including AA fatty acid (FA), minerals and vitamins in flushing diet. Crude protein (CP) in ruminant diet is divided into rumen degradable protein (RDP) and rumen undegradable protein (RUP) fraction (NRC, 2007). High dietary CP and especially RDP content increases ruminal ammonia concentration and consequently plasma urea nitrogen (Calsamiglia *et al*. 2010). Reducing oocyte growth, high ammonia level in follicular fluid, high early embryo mortality, lower uterine pH and lower reproductive efficiency were reported in ruminant animals fed excess dietary CP and RDP (Rooke *et al*. 2004; Robinson *et al*. 2006). The RUP is the second-largest source of absorbable AA, especially essential AA (EAA), in the small intestine of ruminant animals (NRC, 2021). Geppert *et al.* (2016), reported higher follicle and corpus luteum (CL) growth and quality in beef cows fed diets contained more RUP. Fish meal (FM) and corn gluten meal (CGM) are among the most important sources of RUP in ruminant diets (Gargallo *et al*. 2020; NRC, 2021). These RUP sources differ in EAA profile. For example, FM contains more lysine and arginine than CGM (NRC, 2021). The EAA has different and important functions in the body and their deficiency compromise certain metabolic processes (Wu *et al.* 2014).

Beneficial effects of EAA on many aspects of mammalian reproduction including blastocytes development, embryo growth and implantation, placentation, pregnancy recognition and maintenance of pregnancy has been reported previously (Bazer *et al*. 2015; Zhang *et al*. 2016; Halloran *et al*. 2021). Fat supplements are often used to enhance energy density, improve palatability, and decrease the diet's dryness (NRC, 2007; Jenkins *et al*. 2008). However, it is well recognized that fat supplements can improve ruminant fertility in several ways. Fat supplements improve reproductive performances through enhancing energy status, increasing plasma cholesterol concentration and steroid hormone synthesis, altering insulin secretion, stimulating follicle growth, and stimulating or inhibiting secretion of prostaglandin $F_{2\alpha}$ (Staples *et al*. 1998; Mattos *et al*. 2000). Results of many researches showed that FA profile in fat supplements is also very important in relation to the effect of fat supplements on reproduction (Gulliver *et al*. 2012). Polyunsaturated FA (PUFA) have more than one double bonds and are divided into 2 categories including n-6 FA and n-3 FA (NRC, 2021). Linoleic acid (18:2n-6) is the most important n-6 FA and linolenic acid (18:3n-3), eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid. (22:6n-3) are the most n-3 FA in ruminant diets (Gulliver *et al*. 2012; Moallem, 2018). In ruminants, feeding dietary n-6 or n-3 PUFA has led to several positive effects on female reproductive success (Robinson *et al*. 2002; Harvatine and Allen, 2005; Mirzaei Alamouti *et al*. 2018; Verma *et al*. 2018). For example, n-3 FA affects reproductive hormone synthesis, follicle growth, oocyte maturity, and quality, estrus and ovulation rate, embryo survival, pregnancy rate and duration, delivery, and offspring survival (Gulliver *et al*. 2012). To our knowledge, the interaction effect of dietary FA and RUP sources around mating on the reproductive performances of small ruminants has not previously been examined. Therefore, the objective of the present study was to determine the interaction effect of FA (n-6 *vs*. n-3) and RUP (FM *vs*. CGM) sources with different AA profiles in diets around mating on dry matter intake (DMI), body weight (BW), body condition score (BCS), plasma metabolites, minerals and hormones, plasma FA and AA profile and reproductive outcomes in Lori goats.

MATERIALS AND METHODS

The present study was conducted during the breeding season (May to June 2022) on a rural goat farm in Iran (Lorestan, Kuhdasht, Iran). All goats were reared and managed according to the guidelines of the Iranian Council of Animal care (ICAC, 1995).

Animals and experimental design

Forty-eight Lori goats were divided into four groups (n=12 per group) based on age (2-3 years), BW (39.85 kg) and BCS (2.5) and randomly allocated to one of four experimental diets with a 2 × 2 factorial arrangements. The factors were FA source (n-6 *vs*. n-3) and RUP source (CGM *vs*. FM). One month before the start of feeding flushing diets, all goats were vaccinated against foot and mouth disease (ARRIAH FMD Vaccine, Rooyan Daroo Pharmaceutical Co., Tehran, Iran), enterotoxaemia (Syva-Bax, Syva, Spain), and agalactia (Agalaksivac-Oil, Vetal, Turkey) and treated with anthelmintic drugs against external and internal parasites. Experimental flushing diets were: 1) diet containing n-6 FA and CGM (n-6-CGM), 2) diet containing n-6 FA and FM, 3) diet containing n-3 FA (fish oil) and CGM, and 4) diet containing n-3 FA (fish oil) and FM. Experimental diets were formulated according to the recommendations of the NRC (2007). Fat supplements including calcium (Ca) salts of soybean oil (Persia fat omega-6) and fish oil (Persia fat omega-3) were provided by Kimiya Danesh Alvand Co. (Tehran, Iran). The ingredients and chemical composition of experimental diets are shown in Table 1.

Table 1 Ingredients and chemical composition of experimental flushing diets

Ingredients, g/kg of DM	Experimental diet			
	n-6 FA		n-3 FA	
	CGM	FM	CGM	FM
Alfalfa hay, chopped	350.0	350.0	350.0	350.0
Wheat straw, chopped	150.0	150.0	150.0	150.0
Barley grain, ground	160.0	160.0	160.0	160.0
Corn grain, ground	160.0	160.0	160.0	160.0
Soybean meal	20.0	20.0	20.0	20.0
Corn gluten meal	50.0	0	50.0	0
Fish meal	0	50.0	0	50.0
Calcium salts of n-6 fatty acid[2]	50.0	50.0	0	0
Calcium salts of n-3 fatty acid[3]	0	0	50.0	50.0
Vitamin and mineral premix[4]	15	15	15	15
Chemical composition, g/kg of DM, unless stated otherwise				
Metabolizable energy, MJ/kg	10.45	10.45	10.45	10.45
Crude protein (CP)	164.0	167.0	164.0	167.0
Neutral detergent fiber (NDF)	334.0	328.0	334.0	328.0
Ether extract (EE)	65	64	65	64
Non-fiber carbohydrate (NFC)[5]	386.0	379.0	386.0	379.0
Rumen degradable protein (RDP)	103.0	110.0	103.0	110.0
Rumen undegradable protein (RUP)	61.0	57.0	61.0	57.0
Calcium (Ca)	10.0	12.0	10.0	12.0
Phosphorous (P)	4.0	5.0	4.0	5.0

[1] n-6-CGM: diet containing n-6 fatty acid with corn gluten meal; n-6-FM: diet containing n-6 FA with fish meal; n-3-CGM: diet containing n-3 FA with corn gluten meal and n-3-FM: diet containing n-3 FA with fish meal.
[2] Persia fat omega-6.
[3] persia fat omega-3.
[4] Containing per kg: vitamin A (IU): 500000; vitamin D (IU): 100000; vitamin E (IU): 100; Ca (g): 196; P (g): 96; Na (g): 46; Mg (g): 19; Fe (mg): 3000; Zn (mg): 3000; Cu (mg): 3000; I (mg): 100; Co (mg): 100; Mn (mg): 2000 and Se (mg): 1.
[5] Non-fibre carbohydrate was calculated as DM − (NDF + CP + EE + ash) (NRC, 2007).

All goats received their respective flushing diet twice daily (09:00 and 18:00) as a total mixed ration (TMR) from 28 days before to 21 days after mating. Goats confined individually in 1.2 m^2 (1.2×1.0 m) pens with a straw-covered floor, and had *ad libitum* access to experimental diets and water. All Goats were managed as a single group and kept in similar management and feeding conditions from d 22 after mating till kidding.

Estrous synchronization program and pregnancy diagnosis

Two weeks after the beginning of feeding the *flushing* diets, the estrous cycle of all Goats was synchronized using CIDRs (Eazi-Breed CIDR® Sheep and Goat Inseet, New Zealand) intra-vaginally for 14 days. All animals received an intramuscular PMSG (Gonaser 400 IU, Hipra, Spain) injection immediately after CIDR removal. After detecting estrus signs, all goats were mated with bucks (1 buck per 5 goats) for three consecutive days. To examine pregnancy status, blood samples of all goats were collected from jugular at d 30 after mating. Collected blood samples were centrifuged at 3,000 × g at 4 ^{o}C for 15 min and separated plasma was stored at – 20 °C until assayed for progesterone concentration using a commercial ELISA kit (Monobind Inc. USA, ELISA kit).

Data recording and sample collection

The BW and BCS of all goats were recorded one day before starting the flushing diet (day 0) and the day of CIDR removal. Experimental diets offered and refused was weighed daily to determine the DM intake for each doe throughout the study. Samples of experimental diets were collected twice a week and dried at 55 °C in a forced air oven for 72 h. At the end of the study, samples of each experimental diet were mixed thoroughly and ground through a 1-mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) before analysis. Standard methods as described in AOAC (2007), were used for the determination of crude protein (CP), and ether extract (EE). Neutral detergent fiber (NDF) was measured by the method described by Van Soest *et al.* (1991), without using a heat-stable α-amylase and sodium sulfite and not corrected for residual ash and N. The non-fiber carbohydrate (NFC) content of experimental diets was estimated as follows: NFC= DM - (CP+EE+Ash+NDF). The metabolizable energy (ME), RUP and RDP content of experimental diets were calculated using values reported for ingredients by (NRC, 2007). Blood samples were collected from the jugular vein of all goats into a 10-mL evacuated tube containing sodium heparin one day before feeding the experimental diets and at the day of CIDR removal approximately 3 h after the morning feeding meal. Blood samples were immediately placed on ice, centrifuged at 3000 x g at 4 ^{o}C for 15 min and separated plasma was kept at -20 ^{o}C until analysis. Plasma concentrations of glucose, total protein (TP), total cholesterol (TC), and UN was measured by a commercial analyzer (BT1500, Biotecnica, SRL, Italy) based on the manufacturer's procedure.

Plasma progesterone (P4) and estradiol (E2) concentrations were measured using an ELISA reader (STAT-FAX 3200, USA) and a commercial kit (Monobind Inc. USA, ELISA kit). A flame Atomic Absorption Spectrometer (Analytik Jena AG-novAA® 400p, Germany) was used for determination of sodium (Na), potassium (K), Ca and magnesium (Mg) contents in plasma samples.

Reproductive outcomes

All goats had ear tags and their progeny were identified at birth. Total number, sex and birth BW of kids were recorded. The reproductive performances in terms of estrous response (number of goats showing estrous/total treated goats in each group×100), the fertility rate (number of newborn kids /total number of goats×100), newborn kids rate (number of newborn kids /total number of goats in each group×100), and twining rate (number of newborn kids twin/total number of newborn kids in each group×100) were recorded (Mirzaei Alamouti *et al*. 2018).

Statistical analysis

Data for DMI, BW, BCS and plasma metabolites, minerals and hormones were analyzed using the MIXED procedure of SAS (2013). The mean comparison was performed by the least square mean (LSM) method. The differences were compared using Tukey test. Plasma concentrations of metabolites, minerals and hormones at the beginning of study were added as a covariate to analyze data at the day of CIDR removal. The reproductive parameters (%) were analyzed using the chi-square test. Data on birth weight of lambs were analyzed using the MIXED procedure. Effects of dam BW was added as covariate factors to the model. Significance and tendency differences between treatments were determined at P < 0.05 and P < 0.1 levels, respectively. Data were expressed as LSM ± SEM unless otherwise stated.

RESULTS AND DISCUSSION

DMI, BW and BCS

Effect of experimental diets on DMI, BW and BCS of goats during study are shown in Table 2. Dietary FA source (n-3 *vs*. n-6), RUP source (CGM *vs*. FM) and their interaction had no effect on DMI, BW and BCS of goats (P>0.05).

Plasma metabolites, minerals and hormones

Effect of experimental diets on plasma metabolites, minerals and hormones concentrations are shown in Table 2. Plasma glucose, UN, Ca, Mg and K concentrations did not influence by FA source (n-3 *vs*. n-6), RUP source (CGM *vs*. FM) and their interaction in flushing diet (P>0.05). The lowest plasma concentration of TP was observed in goats fed n-3-CGM (P<0.05). Whereas, dietary FA and RUP source had no effect on plasma TP concentration (P>0.05). Experimental diets had no effect on plasma concentration of TC (P>0.05). However, addition of n-3 FA and FM to flushing diets decreased plasma TC concentration compared to n-6 FA and CGM, respectively (P<0.05). Plasma Na concentration tended to be lower in goats fed n-3-CGM diet compared to other groups (P=0.06). Indeed, feeding flushing diets containing n-3 FA decreased plasma Na concentration than n-6 FA (P<0.05). Moreover, plasma Na concentration tended to be lower in goats fed diets containing CGM compared to FM (P=0.08). The highest plasma concentrations of P_4 and E_2 were observed in goats fed n-6-FM diets than other groups (P<0.05). Goats fed diets containing n-3 FA had lower plasma P_4 and E_2 concentrations compared to those fed n-6 FA (P<0.05). Dietary RUP sources (CGM *vs*. FM) had no effect on plasma P_4 and E_2 concentrations (P>0.05).

Reproductive outcomes

Reproductive outcomes including estrus response, kidding rate, twinning rate, and kid birth BW and sex were not influence by experimental diets (P>0.05), Table 3. Whereas, the fertility rate tended to be lower in goats fed n-6-CGM diet compared to other groups (P=0.07). Dietary FA (n-3 *vs*. n-6) and RUP (CGM *vs*. FM) sources did not affect reproductive outcomes (P>0.05). Although, numerically but not significantly higher kidding rate (P=0.13) and lower twinning rate (P=0.16) were observed in goats fed n-6-FM and n-6-CGM, respectively.

The lack of effect of dietary FA source on DMI of goats in the present study was similar to the results of Mirzaei-alamouti *et al*. (2018), who reported no difference in DMI of Afshari ewes fed flushing diets contained n-6 FA, n-3 FA or their combination pre-mating. Control diets in their studies contained no RUP source, which may be a reason to Supplemental FM (5% of DM) increased DMI of lactating dairy cows compared to the control group fed a diet without FM. Indeed, a feeding diet containing CGM compared to soybean meal decreased the DMI of lactating dairy cows. Researchers studied the impact of providing Afshari sheep with pre-mating meals high in sunflower oil, fish oil, or palm oil on their reproductive function and blood metabolites.

Table 2 Least square means for dry matter intake (DMI), body weight (BW), body condition score (BCS) and plasma metabolites, minerals and hormones in Lori goats fed flushing diets containing different fatty acid (n-6 *vs.* n-3) and rumen undegradable protein (corn gluten meal *vs.* fish meal) sources during breeding season (n=12 goats per experimental diet)

Item	Experimental diet				SEM	P-value		
	n-6 FA		n-3 FA					
	CGM	FM	CGM	FM		RUP	FA	RUP×FA
DMI, kg/d	1.35	1.29	1.38	1.25	0.08	0.92	0.90	0.85
BW, kg	43.03	43.22	42.93	42.58	0.78	0.17	0.25	0.87
BCS	2.74	2.74	2.77	2.78	0.05	0.85	0.30	0.99
Glucose, mg/dL	60.67	59.33	60.17	57.83	1.22	0.15	0.42	0.67
Total protein, g/dL	6.35[a]	5.82[a]	5.53[b]	5.93[a]	0.21	0.76	0.12	0.04
Urea nitrogen, mg/dL	12.67	13.17	12.00	11.83	1.13	0.88	0.39	0.77
Total cholesterol, mg/dL	67.17	65.17	61.83	53.13	2.43	0.04	0.01	0.19
Calcium, mg/dL	11.93	12.97	12.11	12.20	0.64	0.31	0.38	0.75
Magnesium, mg/dL	3.52	3.06	3.18	2.89	0.23	0.15	0.21	0.92
Sodium, mEq/L	135.09	134.79	128.32	133.91	1.45	0.08	0.01	0.06
Potassium, mEq/L	4.49	4.14	4.22	4.30	0.28	0.61	0.85	0.41
Progesterone, ng/mL	0.49[a]	0.51[a]	0.38[b]	0.32[b]	0.03	0.57	0.01	0.05
Estradiol 17-β, pg/mL	119.57[b]	137.16[a]	103.56[c]	95.97[c]	3.21	0.14	0.01	0.02

n-6-CGM: diet containing n-6 fatty acid with corn gluten meal; n-6-FM: diet containing n-6 FA with fish meal; n-3-CGM: diet containing n-3 FA with corn gluten meal and n-3-FM: diet containing n-3 FA with fish meal.
RUP: rumen undegradable protein source (corn gluten meal *vs.* fish meal); FA: n-6 fatty acid *vs.* n-3 fatty acid source; RUP×FA: interaction of RUP source and FA source.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Least square means for reproductive outcomes in Lori goats fed flushing diets containing different fatty acid (n-6 *vs.* n-3) and rumen undegradable protein (corn gluten meal *vs.* fish meal) sources during breeding season (n=12 goats per experimental diet)

Item	Experimental diet[1]				SEM	*P-value*[2]		
	n-6 FA		n-3 FA					
	CGM	FM	CGM	FM		RUP	FA	RUP×FA
Estrus response, %	83.30	91.70	100.00	91.70	-	1.00	0.29	0.40
Fertility rate, %	66.70	91.70	100.00	83.30	-	0.68	0.21	0.07
Kidding rate, %	150.00	181.80	141.70	170.00	-	0.13	0.51	0.49
Twinning rate, %	37.50	63.60	41.70	60.00	-	0.16	0.87	0.56
Kid birth BW, kg	5.27	6.02	5.25	5.82	0.50	0.27	0.74	0.74
Female kid, %	33.30	25.00	41.20	29.4	-	0.53	0.35	0.76
Male kid, %	66.70	75.00	59.80	60.6	-	0.53	0.35	0.76

n-6-CGM: diet containing n-6 fatty acid with corn gluten meal; n-6-FM: diet containing n-6 FA with fish meal; n-3-CGM: diet containing n-3 FA with corn gluten meal and n-3-FM: diet containing n-3 FA with fish meal.
RUP = rumen undegradable protein source (corn gluten meal *vs.* fish meal); FA; n-6 fatty acid *vs.* n-3 fatty acid source; RUP×FA= interaction of RUP source and FA source.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The results showed that dry matter intake and body weight were affected by omega-3 and omega-6 fatty acids. The amino acid arginine increases the concentration of somatotropin and insulin-like growth factor, so it is expected to improve growth performances. Results of experiments to determine the growth responses of beef cattle and other ruminants have been inconsistent. Therefore, it can be concluded that amino acids have other metabolic roles besides the synthesis of proteins, and it is possible that some amino acids are limiting for protein synthesis (Guoyao, 2021).

Corn gluten is deficient in two limiting amino acids lysine and arginine (Amerio *et al.* 1998). According to the above information, it was expected that the score of body condition and weight of goats fed with fish meal would be higher, but according to our results, the score of body condition and body weight were not affected by non-degradable protein sources. Displays the impact on goat plasma hormone concentrations of dietary fatty acid source (omega-6 *vs.* omega-3) and rumen non-degradable protein (corn gluten meal *vs.* fish meal) around the time of mating. The concentration of plasma progesterone on the day of estrus was not affected by the experimental diets, nor were the concentrations of testosterone or the overall ratio of estradiol and testosterone to progesterone on the day of CIDR removal or the day of estrus (P>0.05). Goats fed a diet high in omega-6 fatty acids and either corn gluten meal or fish meal had higher plasma concentrations of progesterone on the day of CIDR removal and estradiol on both the day of CIDR removal and the day of estrus than goats fed a diet high in omega-6 fatty acids and any other treatment. Investigate showed that the level of plasma progesterone in ewes fed with omega-3 fatty acids was higher than in ewes fed with omega-6 fatty acids. The relationship between dietary protein concentration and progesterone is complex and conflicting results have been reported. Increasing the crude protein content of the diet from 15 to 25%, with soy and urea supplementation, did not have a significant effect on plasma progesterone concentration (Blauweikel and Kincaid, 1986).

According to Childs *et al.* (2008), plasma cholesterol is the most important precursor for the production of progesterone. In the current study, the consumption of omega-3 fatty acids resulted in a reduction in total cholesterol on the day of CIDR removal and the day of estrus. Additionally, this reduction in total cholesterol resulted in a reduction in the synthesis of progesterone on both of these days. It is important to feed both rumen-protected protein and rumen-degradable protein to ruminants, although the ratio between the two should vary with reproductive status, lactation status, and body size (NRC, 2007).

Lysine and methionine are two amino acids that have been the subject of much research. This is because lysine and methionine are the two most often growth-limiting amino acids in ruminants, as shown by investigations of colostrum or duodenal infusion (Merchen and Titgemeyer, 1992). Depending on the animal's growth stage and food, arginine and histidine have been suggested as potential limiting variables for weight increase. Ovulation and progesterone release can be suppressed by taking arginine supplements. The ovulation rate and embryo development of sows that were given arginine supplements during early estrus were negatively affected (Bazer *et al*. 2014). Progesterone production was shown to be unaffected by the presence or absence of non-degradable protein sources in the rumen in the current investigation. The effect of the source of fatty acid (omega-6 *vs*. omega-3) and undegradable protein in the rumen (corn gluten meal *vs*. fish meal) in flushing diet around mating on the concentration of parameters in the plasma of goats is given in Table 2. On the day of CIDR removal and the day of estrus, goats fed experimental diets showed no significant difference in the levels of urea nitrogen and plasma glucose. Goats fed a diet rich in omega-3 fatty acids and corn gluten meal had the lowest concentration of total plasma protein on the day of CIDR removal, and goats fed a diet rich in omega-3 fatty acids and fish meal had the lowest concentration of total cholesterol on the day of estrus ($P<0.05$). The concentration of glucose and cholesterol at the time of flushing, the day of CIDR removal, and the day of estrus are not influenced by the source of omega-3 and omega-6 fatty acids, according to the findings of an investigation into the effect of short-term feeding of different sources of fatty acids in pre-mating rations on reproductive performances and blood metabolites of Afshari ewes.

According to Mirzaei-alamouti *et al*. (2018), short-term nutrition and physiological condition might be the reason why varied outcomes are obtained when different sources of fat are employed. Akbarinejad *et al*. (2012) investigated the effect of diets enriched with different sources of fatty acids on the reproductive performances of sheep and showed that cholesterol concentration was not affected by omega-3 and omega-6 fatty acids. Compared to other vegetable protein sources, corn gluten has a more favorable condition in terms of amino acid and fatty acids profile and is considered a rich source of methionine (NRC, 2007) and unsaturated fatty acids (linoleic acid) (Parkhurst and Mounteney, 1987). Corn gluten is deficient in two limiting amino acids (lysine and arginine) (Amerio *et al*. 1998). Higher concentrations of cholesterol have been reported in lactating cows fed rumen-protected conjugated linoleic acid (Rezaei Roodbari *et al*. 2016). According to the above information, the increase in cholesterol concentration in goats fed with corn gluten can be related to the high amount of fatty acids in it. Increasing the amount of linoleic acid in omega-6 fatty acids can also be a reason for increasing the amount of cholesterol. Increasing or decreasing mineral concentration in oviduct and uterine secretions of ruminant animals has an important effect on oocyte and sperm maturation, fertilization and early embryo development (Grippo *et al*. 1992).

A positive correlation between blood calcium and magnesium concentration with their concentration in oviduct secretions on the day of cow estrus has been reported (Hugentobler *et al*. 2007). The ratio of the total sodium and potassium to the total calcium and magnesium in the plasma of mammals is used as an effective indicator of the sex of the offspring at the time of birth, and in most of the researches, an increase in this ratio is usually associated with an increase in the number of male offspring (Navara, 2018). Considering that in the present study, the experimental treatments (fatty acid source (omega-6 *vs*. omega-3) and non-degradable protein in the rumen (corn gluten meal *vs*. fish meal) did not affect the ratio of total sodium and potassium to total calcium and magnesium. The number of male and female offspring were not affected. The effect of fatty acid source (omega-6 *vs*. omega-3) and non-degradable protein in the rumen (corn gluten meal *vs*. fish meal) in flushing diet around mating on reproductive traits of goats

The type of fatty acid, non-degradable protein in rumen and their interaction had no effect on estrus rate, twinning rate, kidding rate and birth weight and sex of kids. In mammals, it has been shown that maternal diet may significantly influence sex in some animal species (Cameron and Linklater, 2007).

Ambrose *et al.* (2006) investigated the reduction of pregnancy losses in lactating cows fed diets rich in alpha-linolenic acid and showed that feeding diets rich in linolenic acid increased the size of follicles before ovulation and also increased the fertility rate in became milk cows. Several studies have shown that high-protein diets lead to increased blood urea nitrogen (BUN), which is associated with reduced fertility in cows and ewes (Ferguson *et al.* 1993). Considering that in the present study, the amount of urea nitrogen was not affected by the non-degradable protein source in the rumen, it was expected that the fertility rate would not be affected by the non-degradable protein source. Hoon *et al.* (2000) by investigating the effect of protein supplement on reproductive performances of Merino sheep showed that sheep fed with urea and protein did not differ from sheep fed with control diet in terms of fertility percentage, they also showed that the birth weight of lambs there was more births from urea-fed sheep than from control ration-fed sheep. Diets rich in crude protein and rumen-degradable protein, which increase blood urea nitrogen levels, often have a negative effect on fertility. The negative effects of a deficit energy balance on fertility are most likely due to energy deficiency and imbalance between lipogenic, glucogenic and aminogenic nutrients in early lactation. Ammonia and urea, the end products of aminogenic excess energy breakdown, may also directly interfere with fertility both during the preovulatory stage and during the early stages of embryo development. High non-degradable protein diets may exacerbate a deficit energy balance, the mechanism of which is still unclear (Tamminga, 2006). It has been reported that arginine supplementation in pregnant pigs increased placental weight and offspring birth weight (Chacher *et al.* 2013; Bazer *et al.* 2014). Intravenous administration of arginine increased embryo survival and growth in sheep and milk production in dairy cows (Chacher *et al.* 2013). Therefore, it is not surprising that in pregnant ewes, arginine supplementation has beneficial effects from the first trimester until the birth of the fetus. At early stages, rumen-protected arginine supplementation rescues weaker embryos from death. Arginine supplementation between days 100 and 121 of pregnancy increased the weight of quadruplet lambs (Lassala *et al.* 2011). According to the results of the present experiment, the use of corn gluten (which has a limited amount of arginine) although it did not have a significant effect on birth weight, numerically, goats fed with corn gluten compared to goats fed with fish meal, goats with they were born with lower birth weight, which lack of amino acid arginine in corn gluten could not be without effect.

CONCLUSION

The results of the present experiment showed that the consumption of dry matter, body weight and body condition score of the goats on the day of CIDR removal were not affected by the experimental rations. The highest concentration of plasma progesterone was observed on the day of CIDR removal in goats fed diets containing omega-6 fatty acids and corn gluten meal or fish meal. The highest concentration of plasma estradiol was observed in goats fed with diets containing omega-6 fatty acids and fish meal and diets containing omega-6 fatty acids and corn gluten meal on the day of CIDR removal and the day of estrus, respectively. The addition of omega-3 fatty acids compared to omega-6 to the flushing diet of goats caused a decrease in plasma progesterone and estradiol concentrations on the day of CIDR removal and on the day of estrus. The lowest plasma total protein concentration on the day of CIDR removal was found in goats fed with diets containing fatty acids. Omega-3 and corn gluten meal were observed. The lowest plasma cholesterol concentration on the day of estrus was observed in goats fed with diets containing omega-3 fatty acids and fish meal. The addition of omega-3 fatty acids compared to omega-6 to the flushing diet of goats decreased the plasma cholesterol concentration on the day of CIDR removal and the day of estrus. The lowest plasma sodium concentration on the day of estrus was observed in goats fed with diets containing omega-3 fatty acids and corn gluten meal. Fertility rates tended to decrease in goats fed diets containing omega-6 fatty acids and corn gluten meal compared to other diets. According to these results, the simultaneous addition of omega-6 fatty acids and corn gluten meal to the flushing diet of goats was associated with a decrease in fertility rate.

ACKNOWLEDGEMENT

The manuscript was extracted from the first author MSc. thesis (Grant No. 9815010004) that was financially supported by Ilam University, Iran. The hands of staff for calf rearing stalls are appreciated during experimental period.

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