Effects of omega-3 and omega-6 fatty acids on some reproductive parameters in ewes

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SUMMARY

n-6 and n-3 fatty acid families act as nutraceuticals to complement the sequential processes of follicle and embryo development. However, there is a lack of information on effect of dietary supplementation of n-6 and n-3 fatty acids on different reproductive events in the sheep. Accordingly, in this study, the effect of supplementation of n-6 PUFA rich SoyPreme (SP) or n-3 PUFA rich Flaxtech (FT) on plasma hormone concentrations and some ovarian activity in the sheep were studied. Following the first detected estrus, a total of 44 ewes were allocated into either bazal diet (C, n = 22) or SP (n = 22) treatments until next estrus (pre-mating). At the second estrus, the ewes were mated and again randomly allocated to either the C or FT allowance until day 15 (post-mating; mating = day 0). Hence, there were four nutrition treatments; CC (n = 11), SPC (n = 11), SPFT (n = 11) and CFT (n = 11). Blood samples were collected to monitor plasma hormon levels. Ewes were slaughtered on 16th day after mating, and the numbers and weights of corpora lutea (CL) and follicles were recorded. Plasma progesterone ($P<0.05$) and PGFM ($P<0.01$) concentrations including basal and peak PGFM in the SP ewes during pre-mating period were higher than those of the C ewes. The number of CL were higher in the SPFT ewes compared to the CC and SPC ewes ($P<0.05$). While the number of small follicules in the SPC, CFT and SPFT ewes were lower than those of CC, large follicles in the SPC and CFT ewes were lower than those of CC ($P<0.05$). It was concluded that short-term (15-17 days) changes in dietary n-6 and n-3 supplementation can have a beneficial effect on plazma hormon consentration and ovarian activity during pre-mating and post-maing, respectively, in ewes.

KEY WORDS

Sheep, nutrition, PUFA, ovarian activity, PGFM.

INTRODUCTION

Increasing the litter size by maximizing the ovulation rate and minimizing post-mating wastage are one of the most important approaches to increase the reproductive performance of the small ruminats[1]. Reproductive performance in domestic ruminants is influenced by dietary fat used to enhance reproductive status, as previously reviewed[2-3]. It is well accepted that dietary fat directly affects dairy ewes and fertility [3-5]. Therefore, nutrition influences ruminant fertility directly and indirectly. The direct effect relate to the supply of specific nutrients required for the processes of oocyte and spermatozoa development, ovulation, fertilization, embryo survival and the establishment of pregnancy, whereas the indirect influence is circulating concentrations of the hormones and other nutrient-sensitive metabolites that are required for the success of these processes[6]. Indeed, the positive effects of some nutritients such as fat and energy supplementation on improvements in reproduction of domestic ruminants are well documented[3]. A number of these documents have primarily focussed on the effects of total dietary fat and energy balance[3,5,6], rather than specific effects of n-3 or n-6, especially in the sheep.

Fatty acids of the n-6 and n-3 families act as nutraceuticals, altering innate immune responses and subsequent gene expression within the uterus to complement the sequential processes of follicle and embryo development and survival of the embryo and fetus. The n-3[7-8] and n-6 fatty acids[9-10] were reported to have effects on progesterone and prostaglandin release in cattle[6,8,11,12] , sheep[13,14] and goat[5]. The n-6 fatty acids increase the synthesis of prostaglandine series 2[7,14] while n-3 fatty acids increase the progesterone synthesis[15]. In recently, it has been reported that ALA affects prepubertal sheep embryo quality associated with alteration of releasing reproductive hormones[16] and supplementation of n-3 fatty acid increases the number of preovulatory follicles and ovulation rate, decreases the metabolites of serum prostaglandin F_2 and E_2 during the window of pregnancy recognition[5].

Ruminants obtain their unsaturated fatty acid needs from the green forages in pasture land[17]. Incorporation of fresh grasses into diets is vital to sustain the dietary ideal n-6/n-3 ratio[18]. However, in autumn season, i.e, mating season in most parts

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of the World, pasture lands are poor in vegetation with respect to the specific nutrient such as fatty acids[19]. This situation can negatively affect reproduction in sheep. To avoid this, enriching the diets with special nutrients affect fertility positively. However, it is still not clear whether changing the fatty acid composition in diet before or during mating may affect reproduction parameters in sheep or not. In previous studies, the effect of n-6 and especially n-3 fatty acids, polyunsaturated fatty acids (PUFA) were found to be important for various reproductive processes, especially as steroid hormone and prostaglandin precursors[5,16]. However, there has been a lack of information related to the dietary n-3 and n-6 supplementations in sheep during follicular and luteal phases on ovarium activities, corpus luteum activities, embryo viability, pregnancy rate, concentration of reproduction hormones. Thus, it was hypothesized that short-term changes of n-6 and n-3 diet during pre- and post-mating period respectively could enhance not only the uterine environment and oocyte quality, but also ovarium activity, independent of a secondary effect on production or metabolism modifications. Therefore, the aim of this study was to determine the effect of supplementation of n-6 PUFA rich SoyPreme (SP) or n-3 PUFA rich Flaxtech (FT) on plasma hormones concentration and ovarium activity in the sheep during pre- and post-mating period.

MATERIALS AND METHODS

Animals and diets

The study was carried out during the breeding season at the experimental farm of the Gaziosmanpasa University, Tokat, Turkey (40°31' N, 36°53' E and 650 m above sea level). The ethical approval was received by Ondokuz Mayis University Ethical Commity. A total of 44, 4 year old multiparous Karayaka ewes with an average weight of 43.26 ± 3.97 kg were used. All ewes were fed on control diet composed of 65% forage and 35% concentrate feed at maintenance level[20] over an estrus cycle period during pre-mating and post-mating.

The n-6 was fed as SoyPreme (Boregaard UK, Warrington, UK), a heat-treated product of xylose and cracked soybean. The n-3 was fed as Flaxtech (Flaxtech, Virtus Nutrition, USA) a calcium salt of flaxeed and Ca salt containing 84% fat and 9% Ca. This process reduces the degradability of the protein and protects the PUFAs from biohydrogenation in the rumen[8].

Table 1 - Composition and nutrient content of basal diet (g/kg fed basis).

Fatty acid (%)	SoyPreme	Flaxtech
C 16:0	11	5.5
C 18:0	4	3.6
C 18:1	22	16.4
C 18:2 (n-6)	**53**	16.2
C 18:3 (n-3)	8	**55.3**
Others**	2	3.0

Provided from product cataloge
** Values below 1% were classified as "others".

Table 2 - Forages used in the study (% DM).

Forages	(%)
Grass hay	23.3
Corn silage	76.2
Beet Pulp	4.0

Synchronisation of ewes and allocation into experimental groups

The estrus cycles of ewes were synchronized using a 14-day treatment of progestagen impregnated vaginal sponges (40 mg Florogestone Acetate; Chronogest®, Intervet) combined with PGF_2 and GnRH injections. After synchronization, the ewes detected with referance heat by teaser ram were allocated to treatment groups without mating. The days detected reference estrus (day 16 pre-mating; -16) considered as starting experiment. Animals used in the experiment were waited until they show natural estrus. Then the animals were mated (mating; day 0) and allocated to feeding programme as planed in the experimental design. A 16-d (an estrus cycle period) feeding programme prior to mating and a 15-d feeding programme after mating were applied. During estrus cycle period, sheep were allocated to 2 groups fed basal diet (control) and n-6 diet, respectively. Following the first detected estrus, a total of 44 ewes were allocated into either bazal diet (C, n = 22) or SP (n = 22) treatment until next estrous (pre-mating). At the second estrus, the ewes were mated, and again randomly allocated to either the C or FT allowance until day 15 (post-mating; mating = day 0). Hence, there were four nutrition treatments; CC (n = 11), SPC (n = 11), SPFT (n = 11) and CFT (n = 11).

Blood sampling

Blood samples were taken 3-d intervals for plasma progesterone, daily for estradiol-17 analysis from the 15th day of estrus cycle to 3rd day after mating, and two hourly for prostaglandin (PGFM) from 13rd day to 16th day of natural estrus cycle. The blood samples in heparinized tubes were placed on ice and immediately centrifuged 15 min at 4000 rpm at 4 °C, frozen and stored at -20 °C until hormones assays. On day 16 post mating, ewes were slaughtered humanly to take ovary of each ewe to be transported to laboratory at 37 °C in PBS. The weights of corpus luteum and ovarium were recorded after isolation using scissors and forceps.

Hormone assays

Plasma progesteron and estradiol were measured by enzyme immunoassay using kit (DRG Instruments GmbH International, Marburg, Germany; progesteron: EIA-1561, estradiol: EIA-

Table 3 - Concentrates given to the control and treatment groups (%).

Concentrates	Control	Omega-3 (n-3)	Omega-6 (n-6)
Wheat	35.5	31.5	28.3
Canola meal	28.0	22.3	20.0
Megalac	12.2	-	5.0
Soypass	24.3	22.3	-
SoyPreme	-	-	46.7
Flaxtech	-	23.8	-

Table 4 - Nutrient contents of mixtures used in C and treatment groups (%).

Nutrient	Forage		Control		Omega-3 (n-3)		Omega-6 (n-6)	
	As-fed	DM	As-fed	DM	As-fed	DM	As-fed	DM
DM	41.00	100	90.60	100	93.65	100	92.12	100
OM	37.98	92.63	83.23	91.87	81.52	87.05	87.16	94.62
CP	2.33	5.70	22.54	24.88	23.80	25.41	24.02	26.07
EE	0.32	7.80	13.32	14.70	15.90	16.98	13.83	15.01
CF	16.25	39.63	5.84	6.45	3.61	3.85	6.27	6.80
NFE	19.08	46.53	41.53	45.84	38.21	40.81	43.04	46.74
Ash	3.02	7.37	7.37	8.13	12.13	12.95	4.96	5.38
ADF	20.70	50.39	13.75	15.18	8.79	9.40	11.81	12.82
NDF	30.20	73.56	22.17	24.47	15.07	16.09	21.12	22.93
ME, kcal/kg	1713		3630		3817		3632	

DM: Dry matter, OM: Organic matter, CP: Crude protein, EE: Ether extracts, CF: Crude fibre, NFE: Nitrogen free extracts, A: Ash, ADF: Acid detergent fibre, NDF: Neutral detergent fibre, ME: Metabolisable energy.

2693). Tests were adjusted according to the sheep experiment as the standards used in these test kits were prepared in human sera. For this purpose, sheep blood plasma treated with active coal (Activated-charcoal stripped) was prepared. Standard curves were formed by adding progesterone or estradiol (SIGMA) in different ratios in this plasma from which steroid hormones were removed by using charcoal. The intra- and inter-assay coefficents of variation were 8.8%, 18.1% and 0.1 ng/ml for progesteron, and 9.6%, 14.4% and 10.0 pg/ml for estradiol, respectively. The stable metabolite of $PGF_{2\alpha}$13,14 dihydryo-15 keto prostaglandin $F_{2\alpha}$ (PGFM) was measured by enzyme immunoassay using a kit (Cayman Chemical Company, USA). The intra- and inter-assay coefficents of variation for this metabolite were 9.6%, 15.4% and 7.8 pg/ml, respectively.

Table 5 - Fatty acid contents of forages and concentrates used in C, n-3 and n-6 groups (%).

C chain	Forage	Control	Omega-3 (n-3)	Omega-6 (n-6)
C14:0	1.49	-	0.57	0.64
C16:0	33.86	15.24	7.26	18.41
C16:1 (n-7)	1.08	0.00	0.95	0.37
C18:0	6.02	1.83	1.29	4.55
C18:1 (n-9)	18.18	63.48	24.33	30.27
C18:2 (n-6)	15.04	18.03	19.95	35.21
C18:3 (n-3)	12.01	1.42	40.50	8.32
C18:4 (n-3)	-	-	0.69	0.26
C20:0	1.78	-	-	0.42
C20:1	2.88	-	-	0.50
C20:2	1.47	-	0.29	-
C20:3 (n-6)	-	-	0.82	-
C20:5	1.34	-	1.16	0.53
C22:1 (n-9)	4.86	-	0.95	0.52
C22:6 (n-3)	-	-	1,24	0.64
n-6/n-3 ratio	1.25	12.69	0.49	3.81

Statistical analysis

GLM procedures of SPSS were used to evaluate the effects of treatment on parametric data with comparing them Duncan Multiple Range Test while non-parametric data regarding on degenerated and CL counts were tested by Khi Square (χ^2) in the same software (Windows version of SPSS, release 10.0). All other variables (P4, E2 and PGFM) were analyzed using a linear mixed model (MIXED procedure) for repeated measurements. Permutation test, a nonparametric method which was not affected by suppositions, was used to evaluate the effects of n-3 and n-6 fatty acids due to the fact that variance analyse results were not dependable as the curves related to the ovulation rate, small and large follicule counts, ovarium and CL weights did not show normal distribution[21] Advanced pairwise comparison permutation tests were used to investigate the source of variation among the averages. Data were presented as means ± standard error.

RESULTS

Ovarium activity

The effects of short-term variation of diet n-6 and n-3 contents during pre- and post-mating periods on CL numbers, ovulation rates and weights of ovarium and CL in ewes were presented in Table 6. CL numbers formed following ovulation and degenerated during slaughter were found higher in C+C and n-6+C groups compared to those in n-6+n-3 and C+n-3 ($P<0.05$), indicating that the groups which were not fed n-3 diet had degenerated CLs. Ovarian weights were found higher in sheep fed n-6+n-3 diet compared to those fed C+n-3 diet ($P<0.05$). Short-term changes in n-6 and n-3 contents of diets during pre- and post-mating periods did not cause any differences in terms of CL weights among the experimental groups. But, the the average weight of the CLs in n-6+n-3 group tend to be higher compared to the other groups ($P>0.05$).

Follicle numbers

The effects of short-term variation of diet n-6 and n-3 contents during pre- and post-mating periods on follicule counts and sizes in sheep are presented in Table 7. Small follicule counts

Table 6 - CL numbers, ovulation rates, ovarian weights and CL weights in sheep fed n-6 diets during pre-mating period and n-3 diets during post-mating periods (n=11).

Groups	CL number	Ovulation rate	Ovarian weights (g)	CL weights (g)
C+C	0.40^{b} (4/10)	0.91	1.80±0.25^{ab}	0.56±0.15
n-6+C	0.27^{b} (3/11)	1.00	1.93±0.08^{ab}	0.53±0.07
C+n-3	0.67^{a} (6/9)	0.82	1.47±0.07^{b}	0.56±0.06
n-6+n-3	0.73^{a} (8/11)	1.00	2.02±0.19^{a}	0.81±0.09

^{a,b}: Averages with different letters in the same column are statistically different (P<0.05).

Table 7 - Small, large and total follicle numbers in sheep fed C and n-6 diets during pre-mating period and C and n-3 diets during post-mating periods.

Treatment Groups	Small follicle numbers (1-3 mm)	Large follicle numbers (>3 mm)	Total follicle numbers
C+C	13.0±1.16^{a}	4.27±0.68^{a}	17.27±1.62^{a}
n-6+C	8.27±1.49^{b}	1.27±0.41^{b}	9.55±1.67^{b}
C+n-3	8.73±1.18^{b}	2.27±0.47^{b}	11.00±1.27^{b}
n-6+n-3	9.0±1.38^{b}	2.36±0.54^{ab}	11.36±1.21^{b}

^{a,b}: Averages with different letters in the same column are statistically different (P<0.05).

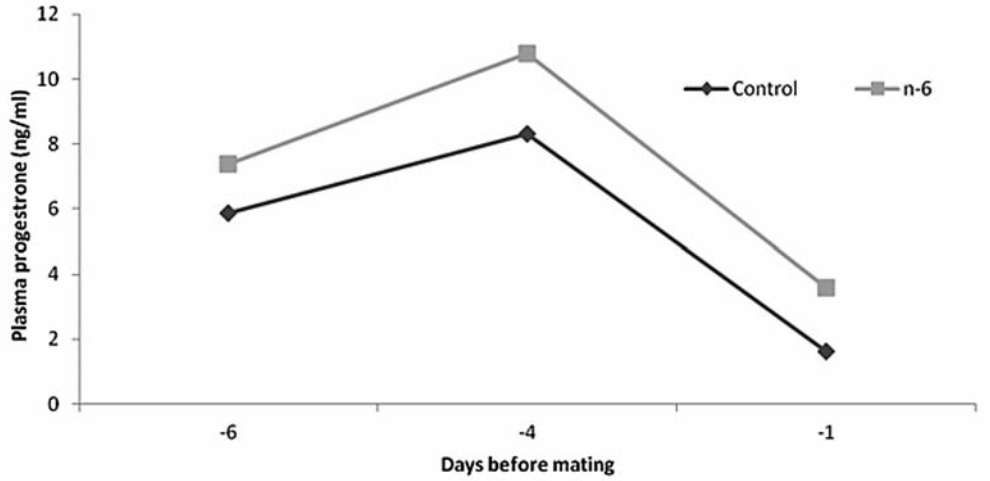

Figure 1 - Plasma progesterone concentrations in sheep fed basal diet (control) and n-6 diet (n-6) during pre-mating period (P<0.05).

were lower in ewes fed n-6+C, C+n-3 and n-6+n-3 diets compared to those in sheep fed C+C diet (P<0.05). Large follicle numbers were lower in ewes fed n-6+C and C+n-3 diets compared to those in sheep fed C+C diet (P<0.05). Total follicle numbers were found lower in sheep fed n-6+C, C+n-3 and n-6+n-3 diets compared to those in sheep fed C+C diet (P<0.05).

Hormone levels

Dietary n-6 treatment increased (P< 0.05) plasma progesterone concentration level over the 9th, 12th and 15th days in ewes fed on n-6 diet during pre-mating period compared to control (7.31±0,64 vs 5.22±0.49). Plasma progesterone concentrations were 6.30 and 8.41 ng/ml in C+C and n-6+C groups at 12th day following mating and these values decreased to 5.03 and 7.11 ng/ml at 15th day (Figure 1). Plasma progesterone concentrations increased from 5.82 and 7.16 ng/ml at 12th day to 6.54 and 7.17 ng/ml at 15th day in C+n-3 and n-6+n-3 groups, respectively (Figure 2). Short-term variations in n-6 and n-3 contents

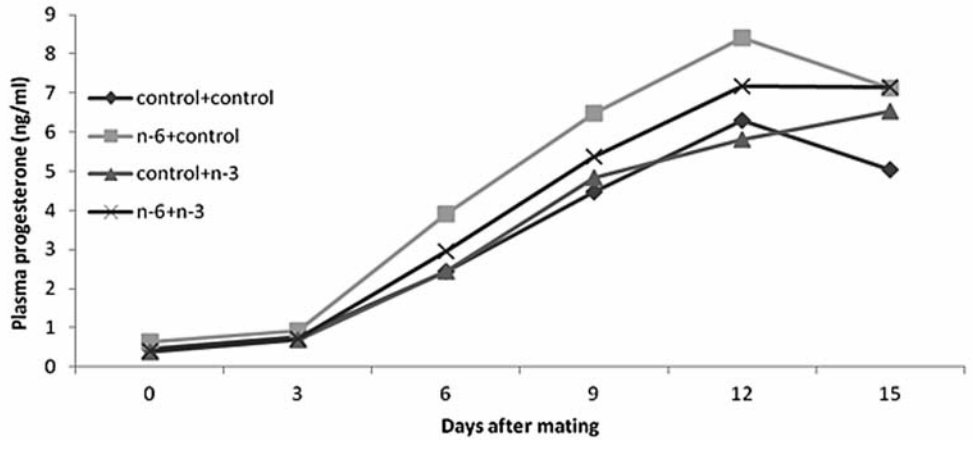

Figure 2 - Plasma progesterone concentrations during post-mating period in treatment groups.

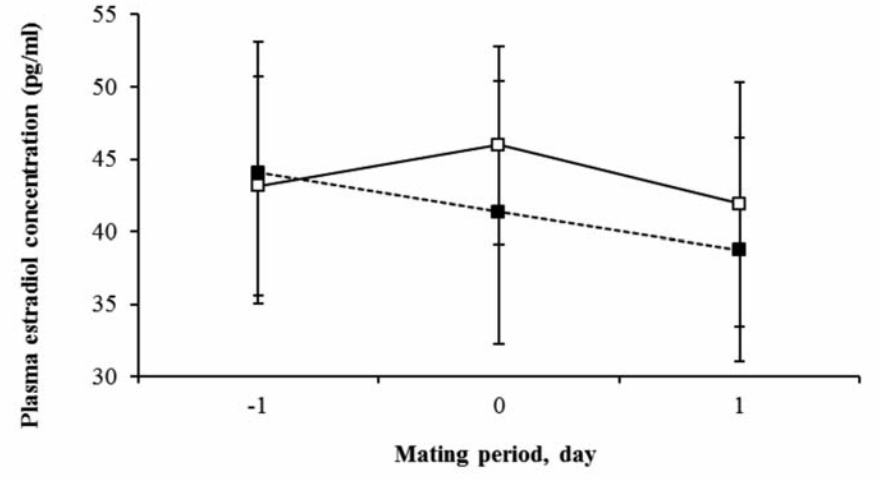

Figure 3 - Plasma estradiol concentrations of control goup C(—□—) and sheep fed n-6 during pre-mating and n-3 after mating period (---■--) (P> 0.05).

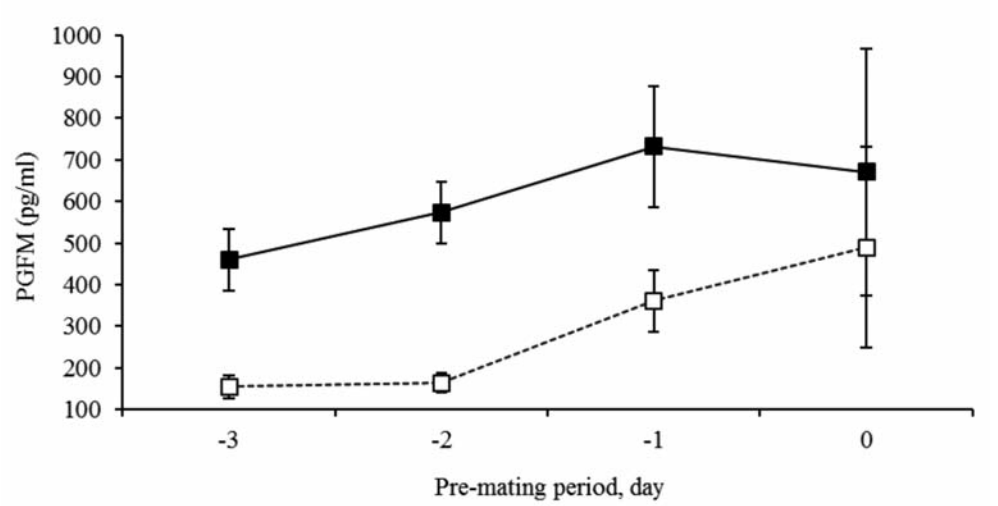

Figure 4 - Plasma PGFM concentrations in sheep fed C(□) and n-6 (■) diets during pre-mating period (P<0.01).

of diets during pre- and post-mating periods did not cause any differences in terms of plasma progesterone concentrations among the experimental groups.

The effects of short-term variation of diet n-6 contents during pre- mating period on plasma estradiol content are presented in Figure 3. Plasma estradiol concentrations during mating period (-1, 0, +1) were found as 41.30±4.87 pg/ml and 43.72 pg/ml in ewes fed diets rich in n-6 and those fed C diet, respectively, during pre-mating period (Figure 3). Plasma estradiol concentrations in -1., 0. and +1 days were found as 43.16±7.59, 45.99±6.85 and 41.89±8.45 pg/ml in sheep fed C diet at pre-mating period and 44.07±9.04, 41.35±9.08 vs 38.76±7.72 pg/ml in sheep fed n-6 diet, respectively (Figure 3). Short-term variations in n-6 contents of diets during pre-mating period did not cause any differences in terms of plasma estradiole concentrations among experimental groups.

Plasma PGFM concentrations in sheep fed n-6 diets during pre-mating period were given in Figure 4. The plasma PGFM concentration in sheep fed n-6 diet during pre-mating period (656.854±73.44 pg/ml) was found higher compared to that in sheep fed C diet (252.15±35.91 pg/ml) (P<0.01) (Figure 4). Basal and peak plasma PGFM concentrations were found as 176.71 and 523.47 pg/ml (P<0.01) and 679.58 vs 1487.71 (P<0.01) for sheep fed C diet and for those fed n-6 diet during pre-mating period (Figure 5).

DISCUSSION

The results of the present study indicate that short-term (15-17 days) changes in dietary n-6 and n-3 supplementation can have a beneficial effect on plazma hormon consentration and ovarian activity during pre-mating and post-maing, respectively, in ewes. These results support the idea that fats in diet can influence reproduction positively by altering both ovarian follicle and corpus luteum function via improved energy status and by increasing precursors for the synthesis of reproductive hormones such as steroids and prostaglandins[2,3,8,16]. In the present study, the SoyPreme and Flaxtech as a source of protected n-6 and n-3 FA was chosen in order to maximize the proportion of n-3 PUFA and n-6 PUFA in plasma. It has been reported that the rumen-protected form as in the present study is sufficient to enable a significant increase in plasma between 7-30 days after feeding, even with the moderate amounts in the diet[8]. It is known that some scientific articles on use of the protected oil as a PUFA source in ruminant animal diets to enhance

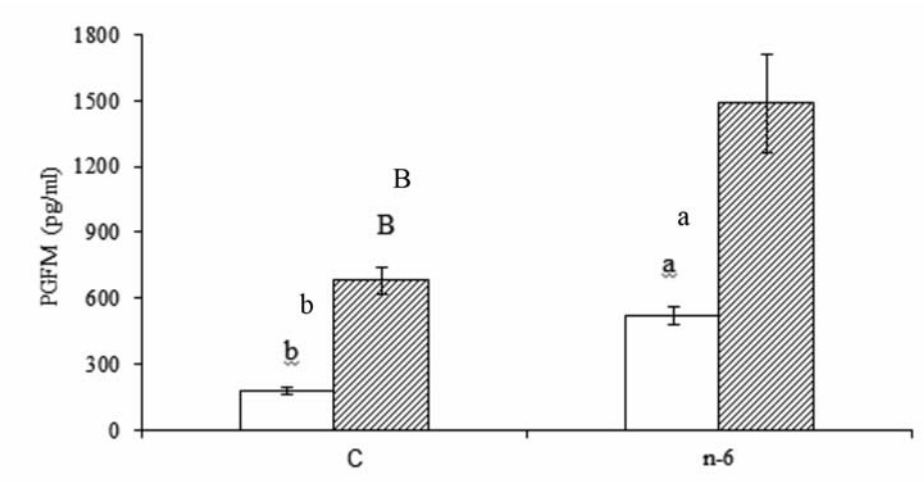

Figure 5 - Basal (□) and peak (▨) plasma PGFM concentrations in sheep fed C and n-6 diets during pre-mating period (A, B; a, b:P<0.01).

reproductive status contain also some data such as: blood fatty acid concentrations before and after insemination, non-fertilization or early embryo mortality on 17 d after insemination and body weight change during supplementation periods. Unfortunately, these physiological traits were not investigated in the present study. The aim of the present study was to determine dietary supplementation of n-6 and n-3 fatty acids whether enhance plasma hormone concentrations and ovarian activity rather than modify blood fatty acid concentrations of ewes. To ensure a specific effect of n-3 FA and n-6 FA, a basal diet without oil, especially PUFA was chosen as the control, because among the PUFA, we have focused on the effect of short-term (15-17 days) changes in n-6 and n-3 FA supplementation.

Our findings did not support the idea that n-3 supplementation during post-mating period inhibit prostaglandin secretion and increase the progesterone secretion by stimulating the luteal and steoroidogenic activity. Kuran et al. (1999) reported that luteal cells produced higher amounts of progesterone in *in vitro* conditions in ewes fed diets supplemented with palmitic acid protected with Ca soaps[22]. Thatcher et al. (1995) reported that high linoleic acid diets stimulated progesterone synthesis due to their effects on the luteal cells in corpus luteum[23]. Cholesterol side chain cleavage, the first step in progesterone synthesis, was unchanged by n-3 supplementation[24]. Thus, the lacking effect of n-3 fatty acids on progesterone synthesis can be explained by this mechanism.

The differences among the studies might be caused by the experimental design, animal species, fatty acid amount and source (fish oil vs linseed oil), n-3:n-6 ratio or n-3/other fatty acids (n-7, n-9 etc.) ratio, the protection types of fatty acids from biohydrogenation in the rumen (formaldehit treatment vs Ca-soap treatment). Indeed, progesterone synthesis decreased when the luteal cells incubated with n-3 fatty acids in cows[25] and plasma progesterone synthesis decreased in cows fed diets rich in n-3 fatty acids[6,12,26,27]. Moussavi et al. (2007) reported that protected fish oil, a source of n-3, led to an increase in n-3 fatty acid amount in uterus endometrium and consequently a decrease in n-6/n-3 ratio[28]. Moreover, there are some studies indicating that progesterone concentrations were not affected following the incubation of cow endometrium cells with different n-3 sources[29]. Therefore, results of our study are in accordance with previous works.

The results of the present study on the plasma $PGF_{2\alpha}$ concentration of ewes are conflicting literature related to the effects of n-6 fatty acid supplementation on blood PGFM concentrations[11,30]. Indeed, Chassagne and Bornouin (1992) reported that linoleic acid/linolenic acid ratio in diet had significant effect on reproduction functions and that decrease in this ratio lowered the prostaglandine synthesis and activity[31]. Linoleic and arachidonic acids are considered as limiting precursors in the $PGF_{2\alpha}$ synthesis[14,32]. In the same study, an increase in PGF_2 synthesis was found in placental tissues and uterus endometrium in ewes fed high linoleic acid ratio. Higher PGF_2 concentrations in ewes fed high linoleic acid compared to the control group might be attributed to the fact that n-6 fatty acids increased arachidonic acid concentrations in blood circulation. Mattos et al. (2000) and Robinson et al. (2002) found similar results in their studies in which SoyPreme was used in cattle[6,33]. The results on plasma estradiol concentration are agreed with suggestions of Lammoglia et al. (1997) in cows and of Elmes et al. (2005) in ewes[14,34]. Conversely, Robinson et al. (2002) reported that n-6 supplementation in cows had no effect on plama estradiol concentration[6]. Because of the conflicts between studies there is no precise judgment related to the mechanism of the effects of n-6 supplementation on plasma estradiol concentration.

Our results on small and large follicle numbers and follicle sizes show that treatments aimed at increasing the amount and qualities of animal products by changing the n-6 and n-3 fatty acid amounts and ratios might negatively affect follicle growth. Lower large follicle numbers in ewes fed n-3 diet compared to those in ewes fed control diet might be attributed to the fact that n-3 fatty acids suppress follicle growth by stimulating the negative feedback mechanism between GnRH and FSH due to their (n-3 fatty acids) increasing effects on luteal activity of corpus luteum. This stuation may be explained the results with regard to plasma progesterone level and active corpus luteum numbers of the present study.

Negative effects of both fatty acids on small and large follicle numbers and sizes might be caused by the amount of fatty acids and ratios between these fatty acids (n-3:n-6). Stanko et al. (1997) reported that vegetable oil supplementation below 4% of diet dry matter led to maximum follicular growth[35]. Chassagne and Bornouin (1992) reported that linoleic acid/linolenic

acid (n-6:n-3) had significant effect on reproduction functions[31]. In this study, total fat amount is at the level of 5.75% of diet DM. The findings of the present study on follicler numbers and sizes and ovulation rate are inconsistent with those obtained in many studies[6,36,37]. This inconsisteny might be caused by the differences in experimental designs, physiological phase, fatty acid amounts and sources and/or diet's linoleic acid/linolenic acid contents.

The results on CL numbers may be related to the contributing effect of n-3 supplementation during post-mating period on the viability of the corpus luteum. n-3 fatty acids achieve this contribution by inhibiting the $PGF_{2\alpha}$ synthesis in the uterus endometrium. n-3 supplementation decreased or suppressed $PGF_{2\alpha}$ secretion by decreasing COX-2 proteins[38], by changing n-3/n-6 ratio in uretus endometrium[28] and/or by decreasing the arachidonic acid synthesis[30]. Mattos et al. (2000) reported that n-3 fatty acids inhibited $PGF_{2\alpha}$ synthesis in uterus endometrium cells[33]. n-3 fatty acids might have stimulated luteal cells in the corpus luteum to secrete higher amounts of progesterone. Consequently, the presence of a strong feedback mechanism between progesterone and $PGF_{2\alpha}$ might have affected CL activity and viability positively.

Early embryonic deaths are among the most significant factors which limit the optimum reproduction performance in livestock. The incidence of embrionic losses were 30-40% in the first 3 weeks of pregnancy in ewes and 70-80% of these are between 8th and 16th days. Moreover, it was suggested that most of the early embrionic losses occurred due to the luteal cells' insufficient functions and fertility can be increased 20% by stimulating the luteal activity[39]. Cam and Kuran (2004) and Cam et al. (2004) claimed that progesterone levels can be increased by enhancing luteal activity via hormonal applications and consequently embrionic deaths can be diminished[39,40]. Degenerated CL counts during post-mating period is one of the most important indicators of embrionic deaths. Thus, lower degenerated CL counts in groups fed n-3 diets during post-mating period supports the findings that blood progesterone synthesis increases[15,41-43] and consequently incidence of early embrionic deaths decreases[39]. In present study, while plasma progesterone concentrations began to diminish from the 12th day after mating in ewes fed control diet, it progressively increased in ewes fed n-3 diet. Our results thus indicate that n-3 fatty acid supplementation during post-mating period may affect the viability of CL and so may diminish the incidence of early embrionic losses.

Our results on the plasma progesterone, PGFM and estradiol concentrations showed that short-term variations of fatty acid composition during pre-mating and post-mating period decreased both small and large follicle numbers, increased the CL counts and did not affect the ovulation rate. It can be said that n-3 supplementation during post-mating period might decrease the incidence of embrionic losses due to its positive influence on CL numbers. These results indicate that fatty acid contents of diet at mating are important especially in terms of enhancing the pregnancy rate.

In conclusion, short-term (15-17 days) changes in dietary n-6 and n-3 supplementation can have a beneficial effect on plasma hormon concentration and ovarian activity during pre-mating and post-maing, respectively, in ewes.

Conflict of Interest

None of the authors have any conflict of interest to declare.

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