Effects of diets enriched in different sources of fatty acids on reproductive performance of Zel sheep

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Summary

The present study evaluated the effects of diets enriched in saturated and polyunsaturated fatty acids (n-3 and n-6) on reproductive indices, metabolic hormones and metabolites prior to ram introduction in oestrus synchronized ewes. Zel ewes (n=188) were assigned to 4 groups. Ewes in the control group (CON) did not receive fat. Ewes in the 3 other groups received 3% oil/DM/day of palmolein oil (PLM), safflower seed (SAF), or flaxseed (FLX). Fat supplementation was carried out for 31 days (day 0 = initiation of fat supplementation). Oestrus was synchronized using CIDR for 14 days starting from day 16 of fat supplementation. Rams were introduced 24 h after CIDR removal. Blood samples were collected on days 0, 30, and 39. There was no difference in oestrus expression and mating parameters among groups. There was no difference in non-esterified fatty acids (NEFAs), insulin and insulin-like growth factor-1 (IGF-1) between day 0 and day 30 among groups. However, changes in cholesterol and LDL concentrations during the same occasions were greater in PLM, SAF, and FLX groups than in CON ($P<0.05$). There was no difference in reproductive indices, including: fertility rates, prolificacy and sex ratio of lambs among groups. In conclusion, diets enriched in n-6 and n-3 polyunsaturated fatty acid prior to mating did not affect reproductive performance, insulin, IGF-1 and progesterone in Zel sheep.

Key words: Fatty acids, Nutrition, Reproduction, Zel sheep

Introduction

Fat supplementation that increases energy concentration of diet (Santos *et al*., 2008) and milk quality (Fatahnia *et al*., 2010) could enhance reproductive performance of dairy cows (Santos *et al*., 2008). Polyunsaturated fatty acid (PUFA) has extra beneficial impacts on ruminant reproduction including greater follicular development (Santos *et al*., 2008; Zachut *et al*., 2010), better oocyte quality and chilling resistance (Zeron *et al*., 2002; Zachut *et al*., 2010), increased progesterone concentration (Thangavelu *et al*., 2007), more embryo development and greater number of high-quality embryos (Thangavelu *et al*., 2007; Cerri *et al*., 2009). The objective of this study was to evaluate the effects of diets, enriched in different sources of fatty acids, prior to ram introduction, on reproductive indices, sex ratio of lambs, and metabolic parameters in Zel ewes. Zel is a native meat-type and non-seasonal Iranian breed.

Materials and Methods

Experimental location and animals

This study was conducted during November-December, 2009 at the Zel Research Station, Golestan province, Iran. Multiparous (n=164; 81.5 ± 0.7 day postpartum and 1 week post weaning) and nulliparous (n=24) healthy Zel ewes (Age= 3.5 ± 0.08 year; body weight= 34.7 ± 0.4 kg) were selected for this study. To ease the managerial process, ewes in each dietary group were divided into two replicates.

Replicate 2 was initiated 8 days after replicate 1. The two replicates were treated exactly the same, except for blood sampling which was performed in replicate 1. Each experimental group consisted of 41 multiparous and 6 nulliparous ewes.

Experimental design and fat supplementation

Experimental ewes were assigned randomly into 4 dietary groups. Ewes in the control group did not receive fat supplementation (CON). Other ewes received palmolein oil (PAL, high in oleic and palmitic acids), whole safflower seed (SAF, high in linoleic acid), or whole flaxseed (FLX, high in α-linolenic acid) for 31 days (Fig. 1; day 0 = initiation of fat supplementation). Ewes in each experimental group were subjected to diet adaptation in which they received daily 10% increments in supplementary diet over 10 days (Fig. 1). After 10 day diet adaptation, ewes received complete experimental diets for 21 days.

Total mixed ration was formulated to meet the requirements of 35 kg ewes (NRC, 1985). The formulated diets were approximately isocaloric and isonitrogenic. Ingredients and diet composition are presented in Table 1. Rations formulated for PAL, SAF, and FLX groups contained 3% oil/DM/ewe/day. Palmolein oil contained 88.5% saturated and monounsaturated fatty acids, mainly oleic (C18:1; 41.5%) and palmitic (C16:0; 39.8%) acids. Safflower seed was high in linoleic acid (76.8%) whereas flaxseed was high in linolenic acid (54.4%). Ewes were housed in dry-lots and had free access to water and multi-mineral salt blocks. Level of feeding was set on 1.2 kg dry matter of experimental diet/ewe/day and ewes were fed twice daily at 07:00 and 17:00 h. The calculated daily intake of the main fatty acids from supplemental fat sources is shown in Table 2.

Oestrus synchronization, mating, expression of oestrus, and reproductive performance

Oestrus was synchronized using CIDR (Pfizer Animal Health, Australia) for 14 days (Fig. 1). Rams (n=12) were introduced 24 h after CIDR removal and maintained with the ewes for 48 h (1 ram per 8 ewes). Mating was observed continuously from ram introduction. Oestrus length (h; for ewes with more than 1 mating) was determined as the difference between the first and the last mating. Fertility rate was calculated as the percentage of ewes lambed per ewes exposed. Prolificacy was calculated as the number of lambs born per number of ewes lambing. Sex ratio of lambs was calculated as ratio of ram lambs per number of ewe lambs.

Blood sampling and measurements

In replicate 1, blood samples were collected from 6 nulliparous and 12 multiparous ewes. Samples were collected through the jugular vein using heparinized venipuncture tubes on day 0 (prior to the commencement of feeding with experimental diets), and day 30 to determine the concentrations of NEFAs, cholesterol, LDL, insulin, and IGF-1, and on day 39 (8 days after introducing ram) to determine

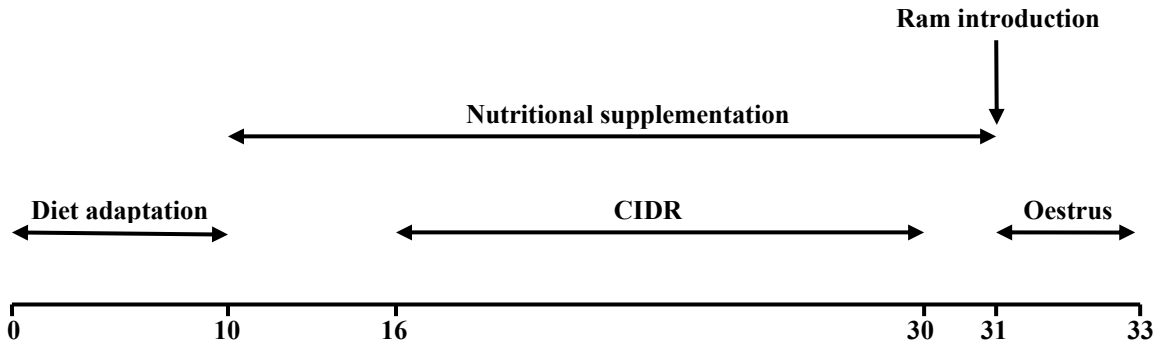

Fig. 1: Experimental design. Nutritional supplementation consists of no fat source (control: CON), palmolein oil (high in oleic and palmitic acids; PLM), whole safflower seed (high in linoleic acid; SAF), whole flaxseed (high in α-linolenic acid; FLX) in Zel sheep

Table 1: Ingredients and chemical composition of the experimental diets containing no fat source (CON), palmolein oil (PLM), whole safflower seed (SAF), or whole flaxseed (FLX)

Item	CON	PLM	SAF	FLX
Ingredients (% of dry matter)				
Corn silage	40	40	40	40
Barley straw	20	20	20	20
Wheat bran	6	34.4	12.1	7.6
Barley	29.5	0	15.5	19.8
Rape seed meal	4	2	0.5	0
Palmolein oil	0	3	0	0
Safflower seed	0	0	11.4	0
Flaxseed	0	0	0	12.1
DCP	0.3	0	0.2	0.3
Limestone	0.2	0.6	0.3	0.2
Nutrient composition				
ME (Mcal/kg)	2.3	2.3	2.3	2.3
DM (%)	63.5	63.3	64.1	64.2
CP (%)	10.2	10.3	10.2	10.4
ADF (%)	23.3	22.4	25.6	23.3
Lipid (%)	2.2	5.5	5.2	5.2

Table 2: Calculated daily intake of fatty acids (g/d/ewe) from supplied fat sources in ewes' rations

Main fatty acids	CON		PLM		SAF		FLX	
	%	g/d	%	g/d	%	g/d	%	g/d
Myristic (C14:0)	0.00	0.00	1.16	0.42	0.11	0.04	0.05	0.02
Palmitic (C16:0)	0.00	0.00	39.84	14.34	6.75	2.43	5.83	2.10
Stearic (C18:0)	0.00	0.00	4.39	1.58	2.4	0.86	4.16	1.50
Oleic (C18:1)	0.00	0.00	41.50	14.94	12.53	4.51	22.19	7.99
Linoleic (C18:2)	0.00	0.00	11.24	4.05	76.76	27.63	12.34	4.44
Linolenic (C18:3)	0.00	0.00	0.27	0.10	0.34	0.12	54.44	19.60

progesterone concentration. Blood samples were centrifuged for 15 min at 3000 rpm. Plasma was refrigerated at -20°C until hormone and metabolite assays.

Ewes were weighed at the beginning of the experiment and on day 29. Concentration of NEFAs (Anzan Chimy Mandegar, Iran), cholesterol (Pars Azmoon, Iran), and LDL (Pars Azmoon, Iran) were measured using biochemical kits. Insulin (DRG Diagnostics GmbH, Germany) and IGF-1 (DIA source Immunoassays S.A., Norway) were measured by ELISA. Progesterone concentration was determined using RIA method.

Statistical analysis

Changes in the concentrations of cholesterol, NEFAs, insulin, LDL and IGF-1 over time were analysed using GLM procedure including repeated measures in the model in SAS (2001). Treatment by day interactions were analysed using LS means. Single-point measurements for cholesterol, NEFAs, insulin, LDL and IGF-1 and progesterone were analysed using GLM procedure followed by Tukey's Honestly significant difference. In cases when the assumptions of parametric tests were not achieved, the non-parametric ANOVA for a single factor (Kruskal-Wallis One way ANOVA) in SAS (2001) was used. The response variables with a discrete nature that had binomial distribution were subjected to Genmod procedure in SAS (2001), including logit statement in the model. Frequency data such as oestrus expression were subjected to Chi-square analysis. Data were presented as mean ± standard error of the mean.

Results

Three ewes (one from SAF and two from control group) had lost their CIDR and were excluded from the experiment. There was no difference between multiparous and

nulliparous ewes in any parameters measured in this study. Therefore, the data for multiparous and nulliparous ewes in each experimental group were pooled and analysed.

Feed composition and body weight

Ewes in all groups showed an increase in body weight from day 0 to day 29 of the experiment (P<0.0001; CON: 34.9 ± 0.88 versus 37.3 ± 0.87 kg; PLM: 34.7 ± 0.86 versus 37.3 ± 0.83 kg; SAF: 34.7 ± 0.86 versus 37.7 ± 0.84 kg; FLX: 34.8 ± 0.87 versus 37.8 ± 0.87 kg). However, the amount of weight gain did not differ among experimental groups (P>0.05).

Expression of oestrus

There was no difference in the commencement of oestrus among experimental groups (P>0.05). There was a delay in oestrus expression in replicate 1 compared to replicate 2 in each experimental group (P<0.05; Table 3). Length of oestrus did not differ among experimental groups (P>0.05; Table 3). In all experimental groups, replicates 1 had less cumulative percentage of mated ewes at 18 h following ram introduction compared to replicates 2 (P<0.05); but there was no significant difference between the two replicates at 48 h following ram introduction (P>0.05; Fig. 2).

Metabolic hormones, metabolites, and plasma progesterone

The change in NEFAs, insulin, and IGF-1 concentrations between day 0 and 30, and among different groups were not different (P>0.05; Table 4). Cholesterol concentrations remained unchanged between day 0 and 30 in the CON group (P>0.05), but increased in the PLM, SAF, and FLX groups (P<0.001). There was no difference in the change of cholesterol concentration among the PLM, SAF, and FLX groups (P>0.05). LDL concentrations increased after feeding with experimental diets in CON, PLM, SAF, and FLX groups (P<0.01). There was no significant difference in LDL concentrations among diets on day 0 (P>0.05); however, on day 30, LDL concentrations were higher in the PLM, SAF, and FLX groups than the CON group (P<0.05) and were also higher in PLM group compared to SAF group (P<0.05; Table 4). On day 39, progesterone concentrations of ewes belonging to CON, PLM, SAF, and FLX groups were 5.2 ± 0.65, 5.5 ± 0.47, 5.8 ± 0.70, and 5.2 ± 0.61 ng/mL, respectively (P>0.05).

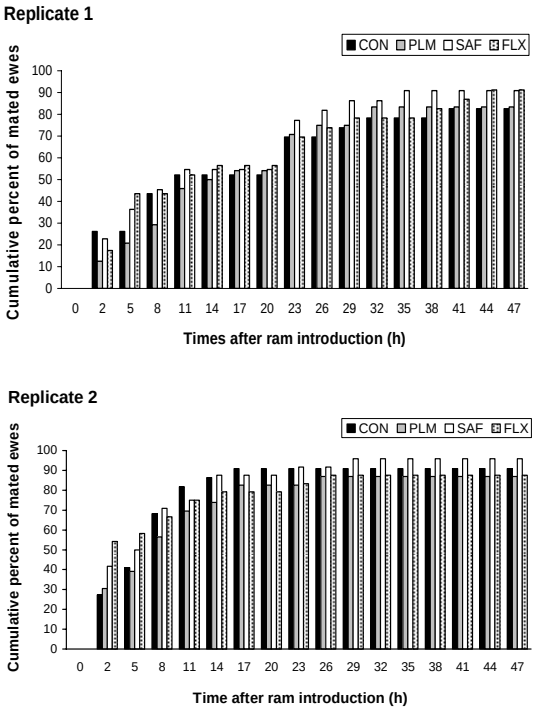

Fig. 2: Cumulative percentage of mated ewes in two replicates. Ewes were fed (control: CON), palmolein oil (high in oleic and palmitic acids; PLM), whole safflower seed (high in linoleic acid; SAF), whole flaxseed (high in α-linolenic acid; FLX)

Table 3: Mean (±SEM) parameters of oestrus expression in ewes fed no fat source (CON), palmolein oil (PLM), safflower seed (SAF), and flaxseed (FLX)

Parameter	CON	PLM	SAF	FLX
Oestrus commencement (replicate 1)	13.69 ± 2.86[a]	15.09 ± 0.99[a]	12.98 ± 2.41[a]	14.96 ± 3.08[a]
Oestrus commencement (replicate 2)	6.02 ± 1.05[b]	7.63 ± 1.44[b]	6.56 ± 1.56[b]	4.74 ± 1.30[b]
Length of oestrus (h)[†, ‡]	14.97 ± 1.51	14.75 ± 1.17	10.70 ± 1.30	13.13 ± 1.20

[†] Data were pooled because of having no difference between two replicates, [‡] Length of oestrus was calculated for ewes with more than 1 mating event, and [a, b] Values with different superscripts between replicates differ (P<0.05)

Table 4: Mean (±SEM) concentrations of metabolic hormones and metabolites in ewes (n=18 in each group) fed no fat source (CON), palmolein oil (PLM), safflower seed (SAF), and flaxseed (FLX)

Parameter	Day	CON	PLM	SAF	FLX
Age (year)		2.92 ± 0.27	3.00 ± 0.29	3.19 ± 0.25	2.92 ± 0.25
Weight (kg)	0	32.88 ± 1.03	32.94 ± 0.94	33.98 ± 0.86	32.99 ± 1.05
NEFA (mmol/L)	0	17.64 ± 1.33	14.06 ± 0.80	14.56 ± 1.16	15.29 ± 1.26
NEFA (mmol/L)	30	18.38 ± 1.89	14.04 ± 1.11	16.02 ± 1.10	16.91 ± 1.39
Cholesterol (mg/dL)	0	86.35 ± 5.87	82.17 ± 3.98	72.00 ± 3.96	82.11 ± 4.61
Cholesterol (mg/dL)	30	85.06 ± 4.40^{A}	119.28 ± 6.02^{B}	106.00 ± 7.61^{B}	108.53 ± 4.85^{B}
LDL (mg/L)	0	16.82 ± 1.64^{a}	15.83 ± 1.09^{a}	17.69 ± 1.83^{a}	19.16 ± 1.71^{a}
LDL (mg/L)	30	23.83 ± 1.62^{Ab}	35.83 ± 2.94^{Bb}	29.83 ± 2.64^{Cb}	$34.00 \pm 2.00^{B, Cb}$
Insulin (µIU/mL)	0	9.67 ± 0.88	8.66 ± 0.85	10.79 ± 1.13	10.22 ± 1.00
Insulin (µIU/mL)	30	9.03 ± 1.00	8.37 ± 0.68	9.77 ± 1.12	9.73 ± 1.01
IGF-1 (ng/mL)	0	50.01 ± 6.96	41.70 ± 6.64	36.98 ± 4.46	51.57 ± 8.43
IGF-1 (ng/mL)	30	54.72 ± 5.66	44.74 ± 6.25	48.64 ± 7.39	55.83 ± 5.44

$^{A, B, C}$ Values with different superscripts within rows differ (P<0.05), and $^{a, b}$ Values with different superscripts between LDL (day 0 and 30) differ (P<0.05)

Reproductive performance

There was no significant difference in reproductive indices (fertility rates, prolificacy and sex ratio of lambs) among groups (P>0.05; Table 5). As a result, data for all groups of each replicate were pooled and analysed. There was no difference between the two replicates in terms of fertility rates (replicate 1=70.21% versus replicate 2=57.45%; P>0.05) and prolificacy (replicate 1=1.08 versus replicate 2=1.09; P>0.05). However, sex ratio of offspring was higher in replicate 1 than in replicate 2 (replicate 1=1.73 versus replicate 2=1.19; P<0.05).

Discussion

In the present study, diets containing different sources of fatty acids, proved to be isonitrogenic and isocaloric by similar weight gain and NEFAs among groups, were used to evaluate the effects of different fatty acids on reproductive performance of Zel ewes. Although several studies have reported that feeding with fatty acids and PUFAs enhanced different aspects of reproductive performance in cattle (Santos *et al*., 2008), there was no difference in fertility rates, prolificacy and sex ratio of the lambs among experimental groups in this study.

Insulin and IGF-1 are important growth factors in ruminant reproduction (Velazquez *et al*., 2008). In the current study, neither insulin nor IGF-1 was influenced by different experimental diets. This finding agrees with the previous study of Childs *et al*. (2008) in cattle.

Diets containing fat sources increased cholesterol values with no difference among groups. This is in agreement with previous studies in cattle, in which plasma cholesterol and cholesterol content in follicular fluid increased following fat supplementation (Staples *et al*., 1998). Cholesterol serves as a precursor for steroidogenesis by ovarian cells and LDL delivers cholesterol to ovarian tissue (Santos *et al*., 2008). In this study, the increase in LDL concentrations was greater in fat supplemented groups compared to CON group. LDL concentrations were not different in ewes fed FLX and SAF, though PLM increased LDL more than SAF.

Replicate 1, in which blood sampling as a stress factor was implemented, had shown

Table 5: Effect of feeding with no fat source (CON), palmolein oil (PLM), safflower seed (SAF), and flaxseed (FLX) on fertility rates (ewes lambing/ewes exposed; %), prolificacy (lambs born/ewes lambing) and sex ratio of lambs (males/females; ratio)

Parameter	CON	PLM	SAF	FLX
Fertility rates	30/45 (66.67)	28/47 (59.57)	34/46 (73.91)	28/47 (59.57)
Prolificacy	32/30 (1.07)	29/28 (1.03)	38/34 (1.12)	31/28 (1.11)
Sex ratio of lambs	19/13 (1.46)	18/11 (1.67)	23/15 (1.53)	17/14 (1.21)

delayed oestrus commencement and cumulative oestrus expression compared to replicate 2. Stresses resulted from blood sampling in ewes without habituation to sampling procedures alters LH and FSH pulses (Adams *et al*., 1993). Regardless, cumulative percent of oestrus expression at 48 h after ram introduction and oestrus length of the two replicates were not different in the present study. Therefore, in the present study, stress of blood sampling simply altered the time of oestrus commencement.

The number of male and female lambs was not significantly different among experimental groups. The influence of maternal diet and body condition on sex ratio of offspring has been demonstrated in many mammalian species (Trivers and Willard, 1973; Rosenfeld and Roberts, 2004; Cameron and Linklater, 2007). Mice that received a diet fortified with fat delivered more male offspring compared to those that received a diet low in fat and high in carbohydrate (Rosenfeld *et al*., 2003). This effect was more pronounced by increasing the consumption of saturated fatty acids (Alexenko *et al*., 2007). However, effect of feeding with PUFAs on sex ratio of offspring is debatable. In one study n-6 PUFA resulted in more female offspring in mice (Fountain *et al*., 2008). In another study, feeding rumen-protected PUFA around conception shifted the sex ratio towards male embryos (Green *et al*., 2008).

The number of male offspring was significantly higher in replicate 1 (ewes under stress of blood sampling) compared to replicate 2 (unstressed ewes). It could be hypothesized that stress of sampling might affect the sex ratio in the present study. This result is similar to those reported in mice (Krackow and Hoeck, 1989) and in Lemur (a prosimian primate; Perret, 1990). Helle *et al*. (2008) have recently indicated that there was no association between preconceptional maternal corticosterone concentration and sex ratio in vole; however, females with high glucose delivered more male offspring. Male and female embryos respond differently to the presence of glucose in the media (Gutiérrez-Adán *et al*., 2001; Larson *et al*., 2001). Extra glucose in the culture media may enhance the development of the male embryos, but may inhibit female embryonic growth and development (Larson *et al*., 2001). As stress could increase glucose concentrations (Apple *et al*., 1995), the change in sex ratio of lambs in replicate 1, might be due to the stress of blood sampling leading to an increase in glucose concentrations.

In conclusion, fat supplementation and diets enriched in n-6 and n-3 PUFA prior to mating increased cholesterol, a precursor for steroidogenesis, and LDL, a carrier for delivering cholesterol to ovarian tissues, but did not enhance reproductive indices including fertility rates, prolificacy and sex ratio of lambs nor affect insulin, IGF-1 and progesterone in Zel sheep.

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