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Relationship of Dietary Fat Sources with Semen Characteristics, Blood Plasma Metabolites and Scrotal Circumference in Mature Rams

Research Article

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ABSTRACT

The hypothesis of this experiment was that the various sources of dietary fat with different dietary ratios of polyunsaturated fatty acids (PUFAs) to saturated fatty acids (SFAs) would alter the reproduction parameters of mature rams. Twelve mature rams were randomly allotted to one of four dietary treatments in a completely randomized design. Dietary treatments were: 1) ration with 4% soybean oil (SOY-OIL), 2) ration with 8% full fat soybean (FULL-FAT), 3) ration with 4% calcium salts of soybean oil (Ca-SALT) and 4) ration with 4% tallow (TALL). All diets were kept isoenergetic and isonitrogenous and formulated to be similar in Ca and P contents, while meeting or exceeding the Cornell Net Carbohydrate and Protein System software requirements for the rams used in this study. Diets were supplied to the rams during four months (from August to late December). Semen characteristics, scrotal circumference, and concentrations of cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride and testosterone of plasma were measured at the end of the experimental period. Based on the results of this study, diets supplemented with Ca-SALT and TALL improved the health of plasma membrane, viability and sperm concentration ($P<0.05$). Rams supplemented with Ca-SALT had also greater volume of semen and total sperm count in ejaculate ($P<0.05$) which are two important factors determining the sperm quality. There was no beneficial effect for different treatments based on spermatozoa motility ($P>0.05$). Blood concentrations of cholesterol, LDL and HDL of rams supplemented with Ca-SALT were significantly higher than the other treatments. Concentrations of triglyceride, testosterone and scrotal circumference were not affected by the experimental treatments ($P>0.05$). As an overall conclusion, the results of this study have indicated the benefits for adding Ca-SALT to the diets of adult rams on their reproductive performance.

KEY WORDS dietary fat source, fertility, full fat soybean, ram, spermatozoid.

INTRODUCTION

The feeding of ruminants with fats differing in origin, type and degree of saturation has resulted in a variety of responses in ruminant production and reproduction. The feeding of supplemental fats rich in polyunsaturated fatty acids (PUFA) has been shown to increase the level of polyunsaturated fatty acids in meat (Felton and Kerley, 2004), spermatozoa and seminal plasma (Gliozzi *et al.* 2009; Safarinejad *et al.* 2010). Spermatozoa fatty acids composition has a very important role in male fertility. The different studies demonstrated that the composition of fatty acids in sperm are sensitive to the diet, since the relative enrichment of dietary lipids with either n-3 or n-6 PUFAS resulted in significant differences in the n-6:n-3 ratio in both spermatozoa and seminal plasma (Cerolini *et al.* 2003; Gliozzi *et al.* 2009; Safarinejad *et al.* 2010). In contrast, high fat levels in the ration of ruminant inhibit ruminal fermentation

and thus diminish the utilization of dietary fiber (Coppock and Willks, 1991; Vafa *et al*. 2009). Fatty acids that are released in the rumen disturb the function of microbial cell membranes (Calsamiglia *et al*. 2007). However, the degree of the toxic effect of fatty acids on ruminal bacteria depends on the amount and type of fat. Especially, oils with a high degree of unsaturation disturb ruminal fermentation (Hristov *et al*. 2009; Vafa *et al*. 2009). In other words, dietary lipids originated from common feedstuffs are readily and almost completely biohydrogenated in the rumen. One alternative to increase the supply of lipids to the small intestine is via supplementation with rumen-inert feeds such as calcium salts of fatty acids (Ca-SALT) (Lopez *et al*. 2009). Also, there is evidence that oil seeds are less toxic than purified oils which might relate to a protective effect of the pericarp that reduces exposure of the oil inside the seeds to ruminal bacteria (Felton and Kerley, 2004). There is little information on the effect of dietary fat sources on semen and scrotal circumference in mature rams. Therefore, this study was conducted in order to determine the effect of different sources of dietary fat on reproduction parameters of mature rams.

MATERIALS AND METHODS

Animals and management

Twelve mature rams were randomly allotted to one of four dietary treatments in a completely randomized design. Each ram was housed in individual pens, with three pens (replicates) per treatment. Before initiation of the study, all rams were adapted to the dietary treatments for 10 days. Diets were fed twice daily. Water was available *ad libitum*.

Diets

The rams were fed one of four dietary treatments: 1) ration with 4% soybean oil (SOY-OIL), 2) ration with 8% full fat soybean (FULL-FAT), 3) ration with 4% Ca-SALT and 4) ration with 4% tallow (TALL).

All diets were kept isoenergetic and isonitrogenous and formulated to be similar in Ca and P content (Table 1), while meeting or exceeding the Cornell Net Carbohydrate and Protein System software requirements for the rams used in this study. The dietary treatments were offered to the rams for a period of four months before semen and blood collection.

Semen and blood collection

Semen was collected by electro-ejaculator three times with 3-day intervals between sessions. In each collection session, one ejaculate per ram was collected (a total of thirty-six ejaculates). After collection, the volume of the ejaculates was recorded directly from the calibrated collection tube.

Then, the semen was diluted separately 1:1 (v:v) with Tris diluent (300 mMtris [hydroxymethyl] aminomethane, 95 m*M* citric acid monohydrate, 28 m*M* glucose, pH 7.0) and transported to the laboratory in an insulated Styrofoam box (30-33 ˚C) within 45 min of collection.

Table 1 Dry matter composition of treatment diets fed to mature rams

Item	Treatment[a]			
	SOY-OIL	FULL-FAT	CA-SALT	TALL
Ingredient, %				
Soybean oil	4	-	-	-
Full fat soybean	-	8	-	-
Ca-salts of soybean oil	-	-	4	-
Tallow	-	-	-	4
Alfalfa hay	20	20.22	20	20
Wheat-straw	40	40.44	40	40
Barley grain	20	20.22	20	20
Beet pulp	10	10.11	10	10
DCP	0.5	0.51	0.5	0.5
NaCl	0.5	0.51	0.5	0.5
Calculated				
ME, Mcal/day	2.05	2.074	2.051	2.055
MP, g/day	53	54	53	53
Ca, g/day	4	4	4	4
P, g/day	3	3	3	3
DMI, Kg/day	1.4	1.4	1.4	1.4
NDF, % DM	51.1	50.2	51.1	51.1
NFC, % DM	28.7	29.8	28.7	28.7
Ash, % DM	7.8	7.3	7.8	7.2

[a]SOY-OIL: diet contained 4% soybean oil; FULL-FAT: diet contained 8% full fat soybean; Ca-SALT: diet contained 4% calcium salts of soybean oil and TALL: diet contained 4% tallow.
DCP: dicalcium phosphate; ME: metabolizable energy; DM: dry matter; MP: metabolizable protein; DMI: dry matter intake; NDF: neutral detergent fiber and NFC: non fiber carbohydrates.

Sperm assessment

Immediately upon reaching the laboratory, the concentration of spermatozoa was determined by means of a Neubauer haemocytometer. The percentage of sperm motility was assessed by phase-contrast microscopy (400×magnification) on a warm stage at 37 ˚C. Samples were diluted with Tris-glucose 1:8 and then, a wet mount was made using a 5 µL drop of semen placed directly on a microscope slide and covered by a cover slip.

Sperm motility was estimated in 3-7 different microscopic fields for each semen sample. The subjective estimations were approximated to the nearest 5% by single technician. The mean of the successive estimations were recorded as the final motility score (Evans and Maxwell, 1987). The viability was assessed by means of a one-step eosin-nigrosin staining (Bjorndahl *et al*. 2003). Briefly, equal volumes of semen and stain solution (0.67 g eosin Y, 0.9 g sodium chloride and 10 g nigrosin in 100 mL distilled water) were incubated for 30 s at room temperature (22 ˚C). After that, one drop of mixture was put on a slide, instantly smeared and air dried.

A total of 200 sperms were evaluated under light microscope (1000×magnification, oil immersion). Sperm showing partial or complete pink or red color was considered dead and sperm showing strict exclusion of the stain was considered to be alive.

The hypo-osmotic swelling test (HOST) was used to evaluate the functional integrity of the sperm membrane. The procedure was described by Jeyendran *et al*. (1992) and adapted for ram semen by García-Artiga (1994). HOST was performed by incubating 5 µL of semen with 500 µL of a 100 mOsm hypo-osmotic solution (7.35 g sodium citrate dihydrate and 13.51 g fructose in 1 L distilled water) at 37 ˚C for 30 min.

One drop of the mixture was placed on a pre-warmed slide, covered with a cover slip and examined under a phase-contrast microscope (400×magnification). The sperm with swollen tails were considered intact. To assess the percentages of intact sperm, a total of 200 sperms were evaluated in at least five different microscopic fields. The total number of spermatozoa per ejaculate was calculated by multiplication of the semen volume with sperm concentration.

During semen collection, blood samples were obtained before the morning meal by jugular venipuncture into tubes without sodium heparin. The tubes were transported to the laboratory immediately and centrifuged at 1500 × *g* for 20 min. Blood serums were separated and stored in 2 mL vials at -20 ˚C until analyses.

Testis measurement

Scrotal circumference was measured with a measuring tape around the testes of each ram, in certain times of the morning.

Statistical analyses

Semen characteristics, blood plasma parameters and scrotal circumference data from this study were analyzed by PROC MIXED of the SAS program for repeatedly measured data SAS (1996). The statistical design for dietary treatments was a completely randomized design. Results are reported as least-squares means (LSM±SE). Differences were considered to be statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Feedlot performance

Feeding different source of fat did not alter final body weight (64.8 kg), total weight gain (250 g), or average daily gain (2.08 g/d). Some researchers have also reported little effect for fat source on gain, while improving feed efficiency through decreasing feed intake (Andrae *et al*. 2000; Ramirez and Zinn, 2000).

Spermatozoa and semen

The effects of dietary fats on semen characteristics of mature rams are represented in Table 2. As can be seen from Table 2, the viability and functional membrane integrity of spermatozoa (Host) were higher in Ca-SALT and TALL groups compared to SOY-OIL or FULL-FAT ($P<0.05$). There were also significant differences between dietary treatments for sperm concentration. The lowest and the highest sperm concentration were found with TALL and SOY-OIL, respectively. No significant differences were indicated for sperm concentration in rams fed Ca-SALT and FULL-FAT diets. The semen volume and total sperm count were higher in rams treated with Ca-SALT than the other groups ($P<0.05$). These results are consistent with several previous studies in which adding PUFA sources to the diet resulted in a concomitant increase in semen quality in boars (Rooke *et al*. 2001; Mitre *et al*. 2004) cockerels and stallions (Brinsko *et al*. 2005). The dietary fat sources (Table 2) did not show any significant effect on motility percentage ($P>0.05$) which is in agreement with the results obtained by Strzezek *et al*. (2004) and Safarinejad *et al*. (2010). In the present study, the effect of sampling time and the interaction of time and treatment were also not significant for spermatozoa and semen characteristics ($P>0.05$).

The membrane structure of spermatozoa plays a crucial role in fertilization. The lipids of the spermatozoa have been suggested to be important for the viability, maturity and functions of spermatozoa. It has also been suggested that the proportion of unsaturated fatty acids may have an influence over the physical properties of the sperm membrane including membrane fluidity (Miller *et al*. 2004; Aksoy *et al*. 2006). Furthermore, PUFAs are the precursors of prostaglandins, important factor in sperm motility. The negative effects of low levels of seminal prostaglandins on sperm concentration and motility might be correlated respectively with decreased adenylcyclase and testicular androgen activity. There is an important consideration in the potential interaction of PUFAs or their derived eicosanoids with the hypothalamic-pituitary axis and the hormonal (GnRH, LH and FSH) control of spermatogenesis (Surai *et al*. 2000). Speculation about the function of PUFAs such as DHA in testis has been related to their possible effect on the fluidity of the sperm plasma membrane, the packing of membrane-bound receptors and activity membrane-banding enzymes as enzymes associated in spermatozoon-oocyte cross-talk, secondary messenger systems and membrane resistance in physical and chemistry stress (Blesbois *et al*. 1997). The previous studies also showed a reduction in the output, quality, fertilizing ability, motility and the number of spermatozoa in ejaculates from ageing bulls by a decrease in DHA proportion in the sperm phospholipids (Rooke *et al*. 2001).

Table 2 Least square means (±SE) of semen characteristics of mature rams in different experimental groups

Semen characteristics	Diets[a]			
	SOY-OIL	FULL-FAT	Ca-SALT	TALL
Motility (%)	72.2±3.6	67.7±3.6	73.3±3.6	70.0±3.6
Viability[b] (%)	75.4±1.8[b]	77.6±1.8[b]	83.4±1.8[a]	83.3±1.8[a]
Host[c] (%)	79.3±1.6[b]	79.5±1.6[b]	85.7 ±1.6[a]	84.7±1.6[a]
Sperm concentration (10^6 cells/mL)	910±.233[c]	1387±233[bc]	1936±233[ab]	2263±233[a]
Semen volume (mL)	0.80±0.1[ab]	0.83±0.1[ab]	1.07±0.1[a]	0.46±0.1[b]
Total sperm count ($\times 10^6$)	806±327[b]	1258±327[ab]	2119±327[a]	1046±327[b]

[a] SOY-OIL: diet contained 4% soybean oil; FULL-FAT: diet contained 8% full fat soybean; Ca-SALT: diet contained 4% calcium salts of soybean oil and TALL: diet contained 4% tallow.
[b] Eosin-nigrosin staining.
[c] The hypo-osmotic swelling test.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Dietary fat with increased stroidogenesis, through changing metabolic hormones concentration and stimulated or inhibitory production or releasing prostaglandins, caused reproduction performance improvement and increased fertility (Surai *et al*. 2000). PUFAs which are concentrated in the head and tail membrane regions of spermatozoa have been shown to play an important role in both sperm capacitation and the interaction between spermatozoa and uterine surface environment.

Blood parameters

The effects of dietary fat source on blood metabolites and testosterone concentration of rams are presented in Table 3. In general, the concentration of cholesterol, HDL and LDL was higher in Ca-SALT group than the other groups (P<0.05).

The concentration of blood plasma testosterone was also higher in Ca-SALT group (11.03 ng/dL), but the effect was not significant (P>0.05). There were no significant differences between the dietary treatments in triglycerides concentration (P>0.05). The effect of time and interaction between treatments and sampling time were not significant for measured blood metabolites (P>0.05). The cholesterol content of the sperm membrane and the ratio of cholesterol to phospholipids are species specific.

This may be the reason for the differences in the sperm tolerance to cold shock (Darin-Bennett and White, 1977). In comparison with bull and human spermatozoa, ram spermatozoa have lower molar rate of cholesterol / phospholipids (Darin-Bennett and White, 1977) and are more sensitive to cold shock (Mui~no-Blanco *et al*. 2008). The detrimental effect of the osmotic stress and cold shock can be diminished by increasing cholesterol content of the membrane (Glazar *et al*. 2009; Moce *et al*. 2010). It has been shown that cholesterol plays an important role in regulating fluidity and stability and thereby increasing the integrity of membrane and decreasing the osmotic stress (Walters *et al*. 2008). Amidi *et al*. (2010) reported that increase in cholesterol in the membrane of spermatozoa improved the vitality and acrosome integrity significantly and prevented the sperm from premature capacitation after freezing.

Scrotal circumference

Scrotal circumference (width of the testicles at the widest point) should be measured as it gives a good indication of a ram's breeding ability. In this study, despite the non-significant effect of dietary fat sources on scrotal circumference, there was a close relationship between scrotal circumference and sperm production (Tables 2 and 4).

Table 3 Least square means (±SE) of blood parameters of mature rams in different experimental groups

Blood parameters	Diets[a]			
	SOY-OIL	FULL-FAT	Ca-SALT	TALL
Cholesterol (mg/dL)	54.80±5.0[b]	53.08±5.0[b]	74.33±5.0[a]	59.16±5.0[b]
Triglyceride (TG) (mg/dL)	18.10±2.6	16.10±2.6	15.90±2.6	17.70±2.6
Low-density lipoprotein (LDL) (mg/dL)	22.50±3.1[b]	20.70±3.1[b]	33.20±3.1[a]	21.80±3.1[b]
High-density lipoprotein (HDL) (mg/dL)	28.60±2.3[b]	29.00±2.3[b]	38.00±2.3[a]	33.60±2.3[ab]
Testosterone (ng/dL)	10.98±0.1	10.84±0.1	11.03±0.1	11.02±0.1

[a] SOY-OIL: diet contained 4% soybean oil; FULL-FAT: diet contained 8% full fat soybean; Ca-SALT: diet contained 4% calcium salts of soybean oil and TALL: diet contained 4% tallow.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 4 Least square means (±SE) of appearance of testicular of mature rams in different experimental groups

	Diets[a]			
	SOY-OIL	FULL-FAT	Ca-SALT	TALL
Scrotal circumference (cm)	31.50±1.06	33.30±1.06	33.77±1.06	31.94±1.06

[a] SOY-OIL: diet contained 4% soybean oil; FULL-FAT: diet contained 8% full fat soybean; Ca-SALT: diet contained 4% calcium salts of soybean oil and TALL: diet contained 4% tallow.

The lack of statistical significance for scrotal circumference could simply be the consequence of small sample size used with this study.

CONCLUSION

The lipid composition of semen is unique in its content of long chain polyunsaturated fatty acids which are essential components of sperm membrane and also give rise to many bioactive molecules. As a basic inception in ruminant, biohydrogenation of PUFA is a part of lipid digestion in the rumen and lipids are extensively altered in the rumen, resulting in marked differences between the fatty acid profile of lipids in the diet (mostly UFA) and lipids leaving the rumen (mostly saturated fatty acids). One alternative to increase the supply of PUFA to ruminants is via supplementation with rumen-inert feeds (Lopez *et al*. 2009) which was confirmed by the results achieved with the current study for the beneficial effects of dietary supplementation with calcium salts on semen production and its quality in mature rams.

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