Asian-Aust. J. Anim. Sci.
Vol. 21, No. 1 : 29 - 34
January 2008

www.ajas.info

Effects of Dietary Fish Oil on Semen Quality of Goats

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ABSTRACT : The aim of the study was to investigate the effect of dietary fish oil supplementation on the semen characteristics of the Markhoz buck. Sixteen bucks were randomly allocated into 4 groups and received four different diets: unsupplemented control diet, supplemented with fish oil at 2.50% dry matter (DM), supplemented with fish oil (2.50% DM) and vitamin E (0.30 g/kg DM), and supplemented with vitamin E (0.30 g/kg DM). All experimental diets were formulated according to AFRC (1998). Semen was collected at 14 d intervals from June 17, 2006 to September 2, 2006. Semen characteristics were evaluated. Significant effects ($p<0.05$) of the week (sampling time) were observed for all parameters except semen volume. Also a significant effect ($p<0.05$) of dietary treatment was observed for all parameters except for percent sperm with normal morphologies and semen volume. Fish oil supplementation with excess vitamin E had a significant effect ($p<0.05$) on total number and sperm density, motility and progressive motility, percentage viability and dead sperm. The interaction between fish oil feeding and sampling time was significant ($p<0.05$) for all of the parameters. The bucks that received fish oil in association with vitamin E, effect fish oil showed the greatest improvement in semen characteristics compared with the other groups ($p<0.05$). This study showed that fish oil supplementation with vitamin E may have a beneficial effect on the semen quality and fertility of Markhoz bucks. (**Key Words :** Goat, Semen, Nutrition, Fish Oil)

INTRODUCTION

Sperm membranes play an important role in fertilization capacity and in sperm-oocyte cross talk. In mammalian and non mammalian spermatozoa, there are natural fatty acids, cholesterol, phospholipids (mainly lecithin, cephalin and sphingomielin) and glycolipids. Phospholipids of mammalian sperm cell membranes characteristically contain very high proportions of long-chain (C_{22}) polyunsaturated fatty acids, particularly n-3 series. In most mammals, docosahexaenoic acid (DHA, 22: 6_{n-3}) is the dominant polyunsaturated fatty acid, although, in several species, docosapentaenoic acid (DPA, 22: 5_{n-3}) is also a major component of the semen cell membranes. Free cholesterol and phospholipids composed around 80% of sperm lipids. Analyses of the fatty acid pattern of membrane phospholipids and plasmalogen of human spermatozoa have demonstrated significant levels of polyunsaturated acids. PUFA are known to contribute to membrane fluidity, flexibility, acrosome responsiveness, and the packing of membrane-bound receptors. Furthermore, PUFA are the precursors of prostaglandins and leukoterienes important factors in both sperm motility and inflammatory processes. Prostaglandin E and 19-hydroxy-prostaglandin E are related to sperm motility. The higher contents of PUFA particular DHA in sperm has been shown significantly correlated with vital characteristics, increased both sperm total number and percentage sperm with normal morphologies, acrosome integration, and decreased percentage of sperm with abnormal morphologies. The reduction of sperm PUFA contents decreased the sperm number, motility, and sperm fertilizing ability in birds and mammals. The essential role of PUFA in membrane constitution and in the fertilization process has been confirmed by experimental data on rats fed with an essential fatty acid-deficient diet. Together with decrease concentrations of $PUFA_S$ in red blood cells and serum, these animals showed a degeneration of the somniferous tubules, a progressive decrease in germinal cells and an absence of spermatozoa in the lumen of somniferous tubules and epididymes. Moreover, $PUFA_S$ which are concentrated in the head and tail membrane regions of spermatozoa have been shown to play an important role in both sperm capacitating and the interaction between spermatozoa and uterine surface environment. The results of these studies demonstrated that n-3 PUFS had a beneficial effect on the characteristic of

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Received January 15, 2007; Accepted July 12, 2007

Table 1. The dietary ingredients, metabolizable energy and chemical composition of diets

Feed staffs	Diets			
	Control	Control+fish oil	Control+fish oil+vitamin E	Control+vitamin E
Alfalfa (% DM)	46.51	23.55	23.40	46.36
Concentrate* (% DM)	53.49	73.95	73.80	5.34
Fish oil (% DM)	-	2.50	2.50	-
Vitamin E (% DM)	-	-	0.30	0.30
ME (Mcal/kg DM)	2.15	2.15	2.15	2.15
CP (% in DM)	13.90	13.40	13.40	13.90
EE (% in DM)	2.27	4.57	4.57	2.27
NDF (% in DM)	40.20	44.40	44.40	40.20
Ca (% in DM)	0.71	0.77	0.77	0.71
P (% in DM)	0.50	0.34	0.34	0.50

* Concentrate had barley, wheat bran, cotton seed meal, sugar beet pulp, Dicalcium phosphate, mineral premix, Baggage, common salt and Zealot.

Table 2. Fatty acids profile of fish oil and diets

Fatty acids (g/100 g FA)	Fish oil	Diet with fish oil	Diet without fish oil
14:0	8.2	2.3	0.62
16:0	16.6	25.3	27.48
16:1	9.6	2.23	1.13
18:0	3.7	3.38	3.62
18:1	13.0	13.05	20.72
18:2 (n-6)	1.4	21.3	34.44
18:3 (n-3)	2.9	5.34	5.82
20:0	0.5	0.85	0.68
20:5 (n-3)	11.5	1.83	0
22:6 (n-3)	10.3	1.47	0
Other FA	22.35	6.28	5.49

semen. In many cases, results of fish oil action depended on the fish oil quality which is easily Oxidized and products of it oxidation are toxic. However, there is no information regarding the effect of fish oil supplementation as n-3 fatty acid sources on the semen characteristics of goat. Since n-3 fatty acids are more escaped from rumen in ruminants, that it is used as n-3 fatty acids for improving of semen characteristics goat.

MATERIALS AND MATHODS

Animals and location

16 sexually mature bucks were used in this experiment. The experimental bucks (3 to 4 yr old) were kept at the animal husbandry station assistance affairs animal Sanandaj city, Iran. The bucks were randomly selected and allotted into the four groups (n = 4). They were maintained in individual pens.

Diets

The diets were formulated according to AFRC (1998) and fed to animals in two meals per day The Kilka (Clupeonnella engrauliformis) fish oil and vitamin E were used in four different diets respectively: 1- control (without fish oil and vitamin E), 2- supplemented with 2.50% in dry matter fish oil, 3- supplemented with 2.50% in dry matter fish oil and 0.3 g/kg DM vitamin E, and 4- supplemented with 0.3 g/kg DM vitamin E. The dietary ingredients, chemical composition and fatty acid profile of diets were showed in Table 1 and 2. Diets were fed to the bucks for 11 weeks.

Semen collection

16 bucks were trained for semen collection by an artificial vagina (AV). During a training period of two weeks, bucks succeeded in serving the AV, and ejaculating. Semen was collected from all bucks (n = 16) at 14 d intervals from June 17, 2006 to September 2, 2006, at week 0, 2, 4, 6, 8, 10, during 11 weeks of feeding the experimental diets (single sample per twice week). The semen was maintained at 37°C until evaluation on the farm and during transport to the laboratory for were evaluated the characteristics.

Semen evaluation

After the collected semen was diluted 1:200 with 3% NaCl solution, the concentration of spermatozoa was determined using a haemocytometer, and the volume of semen was measured with graduated tubes. The total number of spermatozoa per ejaculate was calculated by multiplication of the semen volume with sperm concentration. Sperm motility was also analyzed by placing a sample on a pre-warmed (37°C) microscopic slide covered with a cover slip, and examined under a high power microscope at a magnification ×200.

Morphological analysis of sperm

Stained semen smears were prepared by mixing diluted 10 μl semen with 40 μl nigrosin-eosin stains for 30 s to evaluate sperm morphology and viability. The mixed semen and stain were incubated for 2 to 5 min at 37°C before preparing smears on microscope slides and then leaving them to dry. The nigrosin-eosin-stained slides were evaluated by examining 100 spermatozoa per slide in duplicate slides. Viable spermatozoa were defined as those

Table 3. LSM (±SEM) of semen characteristics of Markhoz bucks in different experimental groups

Semen characteristics	Diets			
	Control	Fish oil	Fish oil-vitamin E	Vitamin E
Semen volume (ml)	0.839±0.036^{abc}	0.907±0.036^{a}	0.767±0.036^{c}	0.871±0.036^{ab}
Sperm concentration (10^8 cells ml^{-1})	386.57±13.657^{ab}	398.14±13.657^{a}	383.71±13.657^{ab}	301.79±13.657^{c}
Viable (%)	85.903±0.893^{ab}	87.029±0.893^{a}	86.992±0.893^{ab}	80.570±0.893^{c}
Motility sperm (%)	82.603±0.893^{ab}	83.729±0.893^{a}	83.682±0.893^{ab}	77.270±0.893^{c}
Normal sperm (%)	68.110±1.923^{ab}	72.369±1.923^{a}	68.986±1.923^{abc}	69.509±1.923^{abc}

Mean in a row without a common letter are different significantly ($p \leq 0.05$).

Table 4. Analysis of dietary treatment, time and treatment×time effect on semen quality Markhoz bucks

Semen characteristics	Treatment effect	Time effect	Treatment×time effect
Semen volume (ml)	*	NS	**
Sperm concentration (10^8 cells ml^{-1})	**	**	**
Viable (%)	**	**	**
Motility sperm (%)	**	**	**
Normal sperm (%)	NS	**	**

NS: $p>0.05$, * $p<0.05$, ** $p<0.01$.

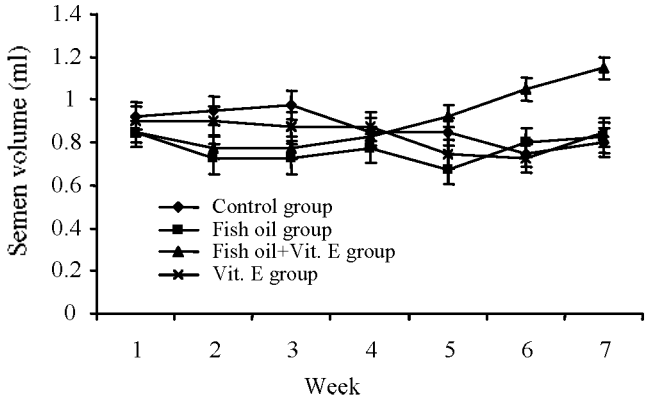

Figure 1. Means±SE of semen volume in during of period experiment.

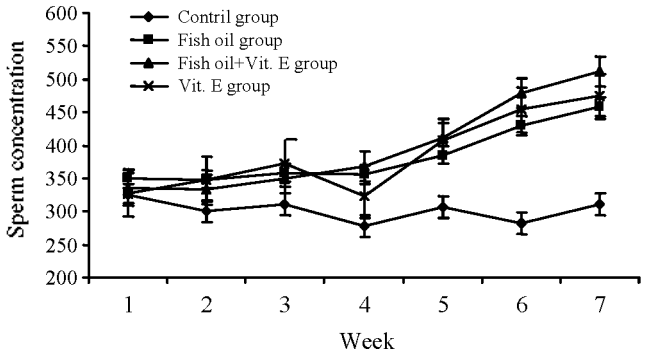

Figure 2. Means±SE of sperm concentration in during of period experiment.

that did not take up stain. Spermatozoa were examined for the following abnormal morphologies: detached head, abaxial head, malformed head, and damage to acrosome cap, bent tail, coiled tail and presence of cytoplasmic droplets (Evans et al., 1989).

Statistical analysis

Data were analyzed by using the PROC MIXED of the SAS program for repeatedly measured data. The data in range 30%≤ and ≥70% were normalized before analysis. All results are presented as means±standard error of means (SEM). Calculation was carried out using the STATISTICA computer package (STATISTICA for Windows, Stat soft Incorporation, Tulsa, OK., USA). Difference between means was calculated for statistical significance ($p<0.05$).

RESULTS

The effect of dietary fish oil supplementation as n-3 fatty acid sources on the semen characteristics of the Markhoz bucks is shown in Table 3 and 4. The significant increase observed on total number and sperm density, motility and progressive motility, percentage of viability and significant decrease on percentage dead sperm in week 11 of experiment. Changes in semen volume in response to dietary supplements are shown in Figure 1. There was no effect of time, but there was an effect of treatment and treatment×time for semen volume in fish oil+vitamin E group than other three groups, and also there was no effect of time, but there was an effect of treatment and treatment×time for semen volume in Fish oil and vitamin E groups than control group. Higher semen volume was concurrent with an increased number of spermatozoa per ejaculate during experimental period of the dietary supplementation. Changes in sperm concentration in response to dietary supplements are shown in Figure 2. There was an effect of treatment, time, or treatment×time for sperm concentration in fish oil+vitamin E group than other three groups, and also there was an effect of treatment, time, or treatment×time for sperm concentration in vitamin

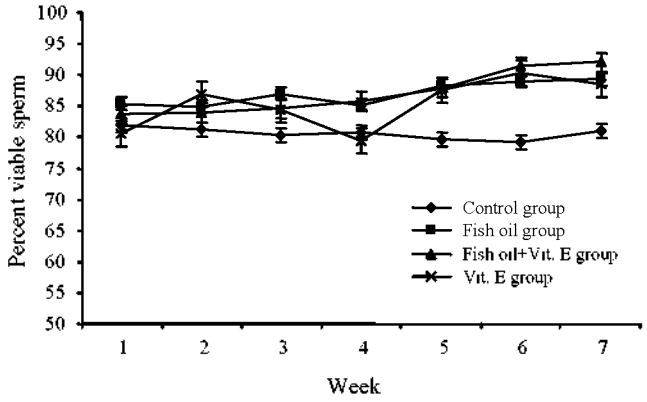

Figure 3. Means±SE of percent viable sperm in during of period experiment.

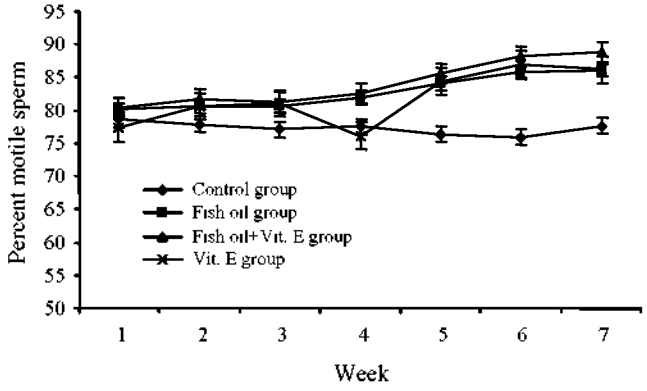

Figure 4. Means±SE of percent motile sperm in during of period experiment.

E and fish oil groups than control group. Changes in percent of viable sperm in response to dietary supplements are shown in Figure 3. There was an effect of treatment, time, or treatment×time for percent of viable sperm in fish oil+ vitamin E group than other three groups, and also there was an effect of treatment, time, or treatment×time for percent of viable sperm in fish oil and vitamin E groups than control group. Changes in percent of motile sperm in response to dietary supplements are shown in Figure 4. There was an effect of treatment, time, or treatment×time for percent of motile sperm in fish oil+vitamin E group than other three groups, and also there was an effect of treatment, time, or treatment×time for percent of motile sperm in vitamin E and fish oil groups than control group. Changes in percent of normal sperm in response to dietary supplements are shown in Figure 5. There was no effect of treatment, but there was an effect of time, treatment×time for percent of normal sperm in fish oil+vitamin E group than other three groups, and also there was no effect of treatment, but there was an effect of time, treatment×time for percent of normal sperm in fish oil and vitamin E groups than control group. At bucks that received fish oil to association with vitamin E, effect fish oil on improvement

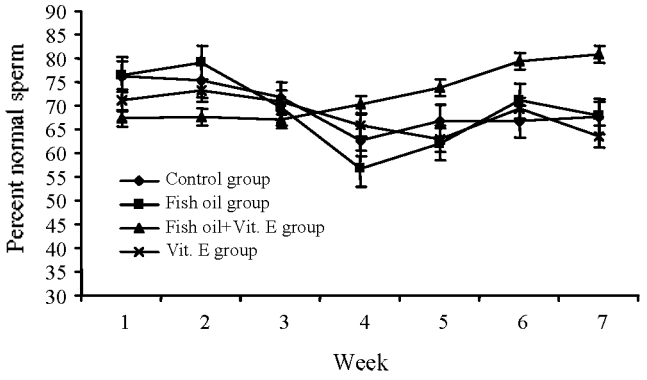

Figure 5. Means±SE of percent normal sperm in during of period experiment.

semen characteristics was clearer than control, fish oil, and vitamin E groups.

DISCUSSION

The objective of this study was to evaluate whether feeding fish oil supplementation changes semen quality in the Markhoz bucks. To date, there were not studies in relation to effects of dietary fish oil supplementation on semen characteristics of ruminant. Previous studies were investigated the effect of dietary fatty acids on meat quality (Choct et al., 2005; Huang et al., 2006) or fertility of female mammals (Hightshoe et al., 1991; Carroll et al., 1992; Thomas et al., 1997; Saples and Mattos, 2000; Petit, 2002; Ambrose and Divakar, 2003; Chelikani et al., 2004). Whereas, few studies investigated the effect of feeding fish oil supplementation on semen characteristics in mammals (Brian, 1981; Surai et al., 2000; Culver and Rooke, 2001; Strzezek et al., 2004). Since $PUFA_S$ cannot be synthesized in vertebrates, they must be provided by the diet. Indeed, fatty acid composition of any tissue is the result of 1) de novo fatty acid synthesis, which takes place in the liver and provides mostly palmitic (16:0) and stearic (18:0) acids, together with their monounsaturated derivatives, palmitoleic (16:1n-7) and oleic (18:1n-9) acids, and 2) the deposition of more or less dietary fatty acids. The previous studies demonstrated that the composition of long-chain $PUFA_S$ in sperm is sensitive to the diet, since the relative enrichment of dietary lipids with either n-3 or n-6 $PUFA_S$ resulted in significant differences in the n-6/n-3 ration in both spermatozoa and seminal plasma. The difference in phospholipids fatty acids of spermatozoa induced by the diet may modify the membrane structures, fluidity, and/or susceptibility to peroxidative damage. These modifications may affect the viability of the spermatozoa in the female reproductive tract and/or their fusion capacity, inducing modifications of fertility rates but not of embryonic mortality (Blesbois et al., 1997). For example, Strzezek et al. (2000) indicated that unsaturated fatty acid supplementation

has positive effect on the sperm number and semen volume, but did not affect the both of sperm motility and percentage sperm with abnormal morphology. So as Rooke et al. (2001) have reported that diet supplement with tuna oil with excess antioxidants in boar sperm characteristics for example increased viability, and the proportions of spermatozoa with progressive motility and normal morphology. This finding is in contrast to that of our results (increased total number and sperm density, motility and progressive motility, percentage of viability and decreased dead sperm), who found no improvements in sperm with normal morphology. Indeed feeding tuna oil to working boars specifically changed sperm phospholipids fatty acid proportions and improved sperm quality *in vitro*. Since, in humans, Zalata et al. (1998) have reported significant positive correlations between phospholipids 22:6 (n-3) status with motile spermatozoa and normal and abnormal sperm. Nutritional supplements of fish oil may also improve the quality of human spermatozoa. Also, a previous study showed that a reduction in the output, quality, fertilizing ability, motility and the number of spermatozoa in ejaculates from ageing bulls was accompanied by a decrease in DHA proportion in the sperm phospholipids (Rooke et al., 2001).

Furthermore, an experiment on broiler chickens has demonstrated that feeding of long-chain fatty acids increased both sperm number and motility (Surai et al., 2004). One hypothesis explains that an increase in sperm output as a result of dietary fish oil is due to correction of the deficiency of 22:4n-6 decreasing the production of viable spermatozoa. In addition, the enzymatic ability to synthesize or incorporate this fatty acid may decrease during ageing. Secondary hypothesis of favorable effect of feeding fish oil on semen quality is that it increases concentrations of eicosanoids. Those compositions of eicosanoids may be relevant to the changes in sperm output. Other possible mechanisms whereby dietary fatty acid could promote spermatogenesis, with may be regulation of gene expression. An important consideration is the potential interaction of polyunsaturated fatty acids or their derived eicosanoids with the hypothalamic-pituitary axis and the hormonal control of spermatogenesis. Thus, the effects of dietary polyunsaturated fatty acids on the secretion of GnRH, LH, FSH, and on the responsiveness of the relevant types of cells to these hormones, may be worthy of investigation (Surai et al., 2000). Speculation about the function of PUFAS, particular 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 (Lenzi et al., 1996; Blesbois et al., 1997). In conclusion; whereas dietary fat with increased stroidogenesis, changing metabolic hormones concentration and stimulated or inhibitory production or releasing prostaglandins cause reproduction performance improvement and increased fertility (Surai et al., 2000; Funston, 2004). The purpose of vitamin E supplementation was to prevent any peroxidation of the polyunsaturated fatty acid, which could potentially reduce the level of the DHA in the sperm phospholipids, and decreased also production of free radical which it was damaged to animal tissues (Strzezek et al., 2004). The lower level of vitamin E in diet markedly depleted vitamin E from tissues and decreased the concentration of vitamin E in the semen; these effects were largely prevented by the higher level of vitamin E in the diet (Surai et al., 2000; Moreno et al., 2004).

Therefore, the dietary fat increased sperm number total, motility, and percentage sperm with normal morphologies (Zalata et al., 1998; Strzezek et al., 2004; Skuladottir et al., 2006), and decreased both dies sperm and sperm with abnormal morphologies (Bottger et al., 2002), which that cause sperm characteristics improvement and increased male animal fertility.

CONCLUSION

Irrespective of the underlying mechanism, the present study indicates that dietary of fish oil supplementation can improve the quality and quantity of goat semen through dietary supplementation of fish oil, and also emphasizes the importance of adequate dietary vitamin E in preventing peroxidative reactions. This study showed that fish oil supplementation with excess vitamin E may have a beneficial effect on the semen quality and fertility in the Markhoz bucks.

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