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Effects of dietary $n-3$ fatty acids and vitamin C on semen characteristics, lipid composition of sperm and blood metabolites in fat-tailed Moghani rams

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ABSTRACT

Sixteen fertile rams were randomly allotted to four groups and fed either of the four diets for 14 weeks: (1) control diet (COD) without fish oil (FO) and vitamin C (VC), (2) diet containing 2.5% FO (FOD), (3) diet containing 300 mg/kg DM VC (VCD), and (4) diet containing 2.5% FO and 300 mg/kg DM VC (FCD). Semen was collected at 14-d intervals from 1 April to 10 July (out of the physiologic breeding season in Iran). Semen volume and percentages of motile and progressively motile sperm were increased by FO and VC feeding. A significant interaction was also found between FOD and VCD on motility and progressive motility percentage ($P<0.05$). HOS-test and percentage of sperm with normal acrosome improved significantly by FO and VC. Rams fed FCD had better HOS-test and higher proportion of sperm with normal acrosome than rams in other groups (82.4 and 93.6%, respectively). Diets containing FO and FO and VC increased the proportion of docosahexaenoic acid in sperm ($P<0.05$). The activity of lactate dehydrogenase in the seminal fluid was significantly affected by VC and the interaction between FO and VC ($P<0.05$). Blood metabolites, except glucose, were affected positively by FO. The results showed that dietary supplementation with FO and VC improved seminal quality and may have beneficial effects on fertility in Moghani rams.

1. Introduction

Lipids, present in both the sperm and seminal plasma, are involved not only in sperm energy metabolism, but also in many functions and events that lead to fertilization (Zaniboni et al., 2006). Semen from all domestic species contains high levels of polyunsaturated fatty acids (PUFAs) (Brinsko et al., 2005), but the nature of lipids depends on animal species. Whereas, fatty acids of the $n-6$ series predominate in birds semen (Kelso et al., 1997), in most

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mammals, the fatty acid composition of sperm is characterized by very high proportions of omega-3 polyunsaturated fatty acids ($n-3$), particularly docosahexaenoic acid (22:6 $n-3$) (Kelso et al., 1997; Samadian et al., 2010). The ratio of omega-6 ($n-6$) to $n-3$ polyunsaturated fatty acids may play an important role in several aspects of animal health, production and reproduction (Abayasekara and Wathes, 1999). Furthermore, there is considerable evidence that lipid composition of the sperm membrane is a major determinant of motility, cold sensitivity and overall viability (Hammerstedt, 1993), lipid metabolism, and fusion capacity of sperm (Stubbs and Smith, 1984). These unsaturated fatty acids give the sperm plasma membrane the fluidity that it needs to participate in the membrane fusion occurrences associated with fertilization (Conquer et al., 2000).

Long chain $n-3$ PUFAs, including eicosapentaenoic (EPA, 20:5 $n-3$) and docosahexaenoic (DHA, 22:6 $n-3$) acid are synthesized in the body from the short chain $n-3$ α-linolenic acid (ALA, 18:3 $n-3$) through a number of steps involving desaturation and elongation (Gulliver et al., 2012), but The ALA cannot be synthesized by animals (Lands, 1992). There are several sources of short chain $n-3$ ALA in ruminant diets, including forages and linseed. Palmquist et al. (2005) stated that dietary PUFAs are extensively metabolized in the rumen; however, long chain $n-3$ fatty acids (20 carbons or more), purified from sources such as fish oil and fishmeal, are usually protected against rumen biohydrogenation (Ashes et al., 1992). Now, it is well recognized that fish oil is one of the best sources of DHA and EPA in the human and animal diets (Conquer et al., 2000; Gulliver et al., 2012).

Gulliver et al. (2012) reviewed the role of omega-3 PUFAs on reproduction in sheep and cattle and stated no published studies have specifically examined the effects of $n-3$ supplementation on male fertility and semen quality. They suggested that direct feeding studies in ruminants examining the effects of $n-3$ on male fertility are required. Recently, the effects of intact fish oil (Samadian et al., 2010; Esmaeili et al., 2014) and rumen protected fish oil (Fair et al., 2014) on the semen characteristics of rams were studied and their positive effect were reported. But, these studies were done in the physiologic breeding season and there are no published study concerning the effects of $n-3$ supplementation on semen quality of sheep out of breeding season. However, PUFAs are also associated with increased oxidative stress, which can reduce semen quality (Wathes et al., 2007); therefore, several mechanisms need to be considered when examining the overall effects of $n-3$ PUFAs (Gulliver et al., 2012).

PUFAs are prone to oxidation, and their oxidation products are absorbed by the intestine with deleterious effects (Cohn, 2002). Dietary lipids, if not stabilized, can be significant contributors to the load of free radicals in the animal (Andrews et al., 2006). Inclusion of dietary antioxidants ameliorates these negative effects by scavenging peroxides and reducing peroxidation of fatty acids (Frankel, 2005). Vitamin C is a water-soluble reactive oxygen scavenger with high potency (Rolf et al., 1999). Vitamin C can regenerate other small antioxidant molecules, such as α-tocopherol, glutathione (GSH), and β-carotene, from their respective radical species (Halliwell, 1996). Knight et al. (1940) stated that adult ruminants are incapable of using orally administrated ascorbic acid to increase serum concentration of ascorbic acid because of its degradation in the rumen. However, increased plasma ascorbic acid concentration after oral administration of finely powdered vitamin C has been reported in sheep (Hidiroglou et al., 1997) and cattle (Hidiroglou, 1999). These observations supported the notion that unprotected vitamin C is partially destroyed in the rumen. Therefore, the objective of this study was to determine the effect of dietary fish oil and Vitamin C supplementation on semen characteristics, sperm lipid composition and blood metabolites in Moghani rams.

2. Materials and methods

2.1. Animals and location

An experiment was performed at the Moghani Sheep Breeding Station, located in Jafar-Abad, Iran (39°, 26′, 20.6″N latitude and 48°, 5′, 26.4″E longitude). Sixteen fertile rams at 3–4 years of age and with a mean live weight of 63 ± 2 kg, were randomly allotted to four dietary groups and housed in individual pens. Rams were cared according to the guidelines of the Iranian Council of Animal Care (1995). The animals were maintained under natural photoperiod and during the trial, the rams were housed separately from the ewes. Data collection was performed for 14 weeks, from 1 April to 10 July (out of the physiologic breeding season in Iran), with the first two weeks allowed for adaptation to the diets.

2.2. Diets

Diets were formulated to support the maintenance requirements (NRC, 1985). The experimental diets consisted of 1) control diet (COD) without fish oil (FO) and vitamin C (VC), 2) diet containing 2.5% FO (Anchovy fish oil, Khazar Fish Powder Co., Kiyashahr, Iran) (FOD), 3) diet containing 300 mg/kg DM VC (Rovimix C, Hoffmann-La Roche Ltd.) (VCD), and 4) diet containing 2.5% FO and 300 mg/kg DM VC (FCD). Vitamin C was added to diets as an antioxidant to protect fish oil PUFAs from oxidation. All diets were iso-energetic (2.25 Mcal/kg DM metabolizable energy) and iso-nitrogenous (12% CP in DM). Water and mineral blocks were freely available. For detecting any effects of fish oil on metabolic and semen parameters, the iso-energetic status in the diets without fish oil was obtained by changing forage to concentrate ratio and no fat source was used in their composition. The experimental diets were mixed completely and fed as a total mixed ration (TMR) twice daily at 0800 and 1400 h. Table 1 presents ingredients and chemical composition of the experimental diets and fatty acid profile of the diets and fish oil were shown in Table 2.

2.3. Semen collection and evaluation

Semen was collected by artificial vagina, at 14-d intervals, on seven occasions. Prior to collection, the prepuce was wiped clean to prevent contamination of the semen sample. Semen was collected in the morning, and