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Dietary n-3 PUFAs improve fresh and post-thaw semen quality in Holstein bulls via alteration of sperm fatty acid composition

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ABSTRACT

The goal of this study was to investigate the effect of fish oil–supplemented diet on fresh and post-thaw semen quality and sperm lipid composition in bulls. Bulls were randomly assigned to two groups (n = 6). Six bulls were used as the control group and six received the fish oil (1.2% dry matter of total diet) for 11 weeks. Semen was individually collected from each bull and frozen biweekly. Semen volume, sperm concentration, viability, progressive motility, and fatty acid profile of sperm were measured in 1st, 3rd, 5th, 7th, 9th, and 11th week of experiment. Viability, progressive motility, and fatty acid profile of post-thaw sperm were also measured in 3rd, 5th, 9th, and 11th week of experiment. Data were analyzed with using Proc GLM or MIXED (for repeated measurement data) in SAS program. The fish oil–supplemented diet increased the semen volume and sperm concentration. The fish oil–supplemented diet also altered the viability, progressive motility, and fatty acid profile of fresh and post-thaw sperm. In conclusion, feeding a fish oil–enriched diet via alteration of fatty acid profile of sperm lipid could improve *in vitro* quality of fresh and post-thaw sperm in Holstein bulls.

1. Introduction

The plasma membrane of sperm has a critical role in the fertilization capacity and sperm–oocyte interaction [1]. Current membrane fusion theories have been suggested that membrane fluidity has a pivotal role in normal cell function and that the cell membrane fluidity and flexibility are mainly depended on their lipid composition [2]. Difference in sperm fatty acid composition among various species is an important factor in the freezability of sperm [3]. Docosahexaenoic acid (DHA; C22:6n-3) is present at high concentrations in plasma membrane of bull sperm and plays a major role in membrane fluidity [3]. Docosahexaenoic acid is an important fatty acid for membrane integrity [4,5], motility, viability, and morphology of sperm [6,7]. It has been also known that there is a positive correlation between DHA content in plasma membrane of sperm and the sperm motility in the studied species [6,8]. Freezing–thawing process damages sperm's membrane integrity [9,10]. The specific physical change in membranes, through insertion of polyunsaturated fatty acids (PUFAs) into plasma membrane, may result in more defiance to arising damage from the ice crystal formation. The deficiency in the content of PUFAs of the sperm plasma membrane leads to reduction in their survival and fertilizing ability. [11]. Also, there is a significant decrease in the proportion of PUFAs especially DHA after freezing–thawing process [12,13]. The lipid composition of the diet can modify the

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fatty acid composition of the semen, plasma membrane integrity, and fertilizing ability of sperm [4,5,12]. Recently, we have indicated that feeding fish oil–supplemented diet to Holstein bulls (%0.5 dry matter [DM] of total diet) improved *in vitro* quality of fresh semen but not post-thaw parameters. [14]. Fatty acid composition of sperm lipid was not also detected in that experiment. Therefore, the aim of this study was to investigate the effect of higher level of dietary fish oil (1.2% DM of total diet) as an n-3 PUFA source on fresh and post-thaw semen quality and sperm lipid composition in Holstein bulls.

2. Materials and methods

2.1. Animals and location

Twelve mature Holstein bulls (874 ± 45.38 Kg) were used in this experiment. The bulls were kept at individual pens throughout the experiment in Center of Animal Breeding in North-Eastern (Abbas Abad) of Iran, Jahad-Keshavarzi Khorasan Razavi, Mashhad, Iran (60°27′N, 36°27′E) during summer 2008. The animals were randomly assigned to two groups (n = 6 per group).

2.2. Diets

The diets were formulated according to NRC (2001) and fed at maintenance level to two following groups: (1) control (C) group fed a diet without fish oil and (2) fish oil group fed a diet with 3.5% of fish oil mixed in concentrate (1.2% DM of total diet; Kilka fish oil, fish powder and oil Co., Babolsar, Iran) per day in dry matter intake. Both diets were isocaloric and isonitrogenic. Animals had free access to water. Ingredients and chemical composition of diets are shown in Table 1. Fatty acid profiles of diets and fish oil are indicated in Table 2. Diets were fed to the bulls for 13 weeks (2 weeks as adaptation and 11 weeks as treatment).

2.3. Semen collection and processing

Semen was individually collected from each bull using an artificial vagina and frozen biweekly for the 11 weeks of feeding the experimental diets during summer to early autumn (July 5 to October 26). Semen was immediately transferred to the laboratory and held at 35 °C in a water bath before evaluation. Semen volume was measured using graduated tubes, and sperm concentration was determined by means of an Accueil photometer (IMV, L'Aigle, France) after dilution with 9% (wt/vol) NaCl solution (1:100). A commercial diluent (Bioxcell; IMV) was used as a freezing extender. Semen samples were diluted to a final concentration of 40 × 10^6 sperm/mL, allowing 5 minutes for the extender and semen to interact. After that, diluted semen was packaged into 0.5-mL straws (Minitube, Germany) and maintained for 6 hours in 4 °C before freezing by computer-controlled freezing system (Minitube). After the freezing process, the straws were plunged into liquid nitrogen.

Table 1
Ingredients and chemical composition of the experimental diets.

Item	Fish oil diet	Control diet
Ingredient, % of DM		
Cottonseed meal	8.5	8.4
Barley	67	70
Wheat bran	19.4	20
Fish oil	3.5	—
NaCl	0.6	0.6
Vitamin and mineral premix	1	1
Chemical composition		
ME (Mcal.kg DM)	2.20	2.20
CP (% in DM)	14.5	14.5
Ether extract (% in DM)	2.84	2.3
NDF (% in DM)	39	38.5
Ca (% in DM)	0.8	0.8
P (% in DM)	0.41	0.41

Abbreviations: CP, crude protein; ME, metabolisabe energy; NDF, neutral detergent fiber.

Table 2
Fatty acid profile of fish oil and diets (control diet and fish oil diet).

Fatty acids (g/100 g FA)	Fish oil	Control diet	Fish oil diet
14:0	3.59	4.65	2.86
16:0	22.86	25.74	31.41
16:1	6.2	3.13	2.77
17:0	1.20	0.00	0.67
17:1	0.28	0.21	0.00
18:0	5.22	11.89	4.20
18:1	31.37	34.81	16.20
18:2	4.62	14.03	26.55
18:3	1.56	1.22	6.63
20:0	0.32	0.64	3.52
20:1	2.28	0.05	0.00
20:2	0.52	0.36	0.81
20:4 (n-6)	0.65	0.75	0.38
20:5 (n-3)	5.49	0.31	2.28
22:2	0.70	0.55	0.22
22:5 (n-6)	0.57	0.00	0.35
24:0	0.39	0.06	0.00
22:6 (n-3)	10.3	—	2.94

2.4. Sampling and thawing

Fresh and associated frozen samples were collected after 1 (only for fresh sample), 3, 5, 7 (only for fresh sample), 9, and 11 weeks of feeding in two groups and analyzed for the following sperm characteristics. For frozen samples, straws were thawed for 1 minute at 37 °C in a water bath.

2.5. Fresh and post-thaw semen evaluation

During the semen analysis, there were two expert technicians helping in motility, progressive motility, abnormality, and viability estimation. These technicians had 17 years of experience in semen analysis working for Center of Animal Breeding in North-Eastern (Abbas Abad) Iran. Although there were a team working for semen analysis, one decision for each sample was stated by them. Therefore, there could not be any potential technician differences.

2.5.1. Progressive motility

A drop (6 μL) of fresh or frozen–thawed semen was placed on a prewarmed slide and covered with a cover slip.