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Dietary polyunsaturated fatty acid supplementation of young post-pubertal dairy bulls alters the fatty acid composition of seminal plasma and spermatozoa but has no effect on semen volume or sperm quality

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ABSTRACT

The aim of this study was to examine the effects of dietary supplementation with rumen protected n-6 or n-3 polyunsaturated fatty acids (PUFA) on the quantity and quality of semen from young post-pubertal dairy bulls. Pubertal Holstein-Friesian (n = 43) and Jersey (n = 7) bulls with a mean ± s.e.m. age and bodyweight of 420.1 ± 5.86 days and 382 ± 8.94 kg, respectively, were blocked on breed, weight, age and semen quality (based on the outcomes of two pre-trial ejaculates) and randomly assigned to one of three treatments: (i) a non-supplemented control (CTL, n = 15), (ii) rumen-protected safflower (SO, n = 15), (iii) rumen-protected n-3 PUFA-enriched fish oil (FO, n = 20). Bulls were fed their respective diets, *ad libitum* for 12 weeks; individual intakes were recorded using an electronic feeding system for the initial 6 weeks of the feeding period. Semen was collected via electro-ejaculation at weeks −2, −1, 0, 7, 10, 11 and 12 relative to the beginning of the trial period (week 0). On collection, semen volume, sperm concentration and progressive linear motility (PLM) were assessed. On weeks −2, −1, 0, 10, 11, 12, semen was packaged into 0.25 mL straws and frozen using a programmable freezer. On weeks −1, 7 and 11; a sub-sample of semen was separated into sperm and seminal plasma, by centrifugation and stored at − 20 °C until analysis of lipid composition. Semen from 10 bulls per treatment were used for post-thaw analysis at weeks 10, 11 and 12 (3 straws per ejaculate). Sperm motility was analysed by computer assisted semen analysis (CASA). In addition, membrane fluidity, acrosome reaction and oxidative stress were assessed using flow cytometry. Sperm from bulls fed SO had a 1.2 fold higher total n-6 PUFA content at week 11 compared to week −1 ($P < 0.01$) while bulls fed FO had a 1.3 fold higher total n-3 PUFA content, in sperm by week 11 ($P < 0.01$). There was no effect of diet on semen volume, concentration or PLM of sperm when assessed either immediately following collection or post-thawing. Membrane fluidity and oxidative stress of sperm were also not affected by diet. The percentage of sperm with intact-acrosomes was lower in CTL bulls compared to those fed SO ($P < 0.01$). In conclusion, while the lipid composition of semen was altered following dietary supplementation with either n-6 or n-3 based PUFA, this did not lead to measurable improvements in the quantity or quality of semen produced by young post-pubertal dairy bulls.

1. Introduction

Polyunsaturated fatty acids (PUFA) are important components of cell membranes, and play an integral role in oocyte fertilization [1]. Fertile mammalian spermatozoa are characterized by a higher proportion of PUFA compared to saturated fatty acids (SFA) [2].

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Sperm utilise PUFA, in particular n-3 PUFA, to maintain membrane fluidity required for normal cell function [3]. Ruminants cannot synthesize n-6 or n-3 PUFA *de novo* as they lack the necessary fatty acid (FA) de-saturase enzymes. Thus, these animals must obtain PUFA, or their pre-cursors, from dietary sources [4]. Diet-derived PUFA are known to have positive effects on FA composition of spermatozoa in humans [5] as well as a variety of farm animals including pigs [6], sheep [7] and cattle [8]. In order to ensure that sufficient PUFA bypass the ruminal microbial mediated bio-hydrogenation process, they must be chemically protected [9].

Genomic selection has led to more accurate identification of elite sires, resulting in increased demand for their semen at a much younger age. This demand necessitates that bulls reach puberty as early as possible and produce an adequate volume of high quality semen, to meet this demand. Dietary supplementation with n-3 PUFA, derived from fish oil (FO) has been reported to improve certain semen parameters including sperm concentration in rams [7], as well as progressive motility and percentage of normal sperm in boars [10]. Other studies [6] however, found no improvement in semen quantity or quality in boars. Similarly, there are conflicting data from bulls in the literature regarding the effects of dietary n-3 PUFA supplementation.

The motility of fresh semen was improved in bulls supplemented with dietary DHA but there was no improvement detected in frozen-thawed semen in the same study [12]. Positive effects on progressive motility, morphology and viability in frozen-thawed sperm following FO supplementation of bulls [13], have also been reported. In contrast, supplementation of bulls with linolenic acid, a n-3 PUFA, using linseed oil, resulted in no improvement in fresh semen quality but did improve plasma membrane integrity post-thawing [14].

Although some positive effects of PUFA supplementation on semen quality have been detected, increasing dietary PUFA intake can also cause vulnerability of spermatozoa to reactive oxygen species (ROS) damage, leading to an increase in lipid peroxidation [15]. In humans, increased levels of lipid peroxidation have been associated with loss of sperm motility [16] and thus is likely to have a negative impact on fertility. Increases in oxidative stress are also associated with DNA damage [17] and damage to DNA of spermatozoa can reduce fertilizing ability as well as leading to an increase pre-implantation early embryo loss [18]. In addition, a significant reduction in sperm PUFA concentration, particularly in docosahexaenoic acid (DHA; C22:6n-3), has been reported with increasing age In bulls [11]. This has stimulated commercial interest in the use of dietary supplementation to alter the PUFA content of sperm, and increase reproductive potential.

Given the conflicting nature of in the published literature on the consequences of dietary PUFA supplementation on semen characteristics of cattle, the aim of this study was to examine the effects of dietary rumen-protected n-6 and n-3 PUFA on semen quantity and quality in young post-pubertal dairy bulls.

2. Material and methods

All animal procedures performed in this study were conducted under experimental licence from the Irish Department of Health and Children (licence number B100/2869). Protocols were in accordance with the Cruelty to Animals Act (Ireland 1876, as amended by European Communities regulations 2002 and 2005) and the European Community Directive 86/609/EC.

2.1. Animal management

Holstein-Friesian (n = 43) and Jersey (n = 7) bulls with a mean ± s.e.m. age and bodyweight of 420.1 ± 5.86 days and 382.0 ± 8.94 kg, respectively, were blocked on breed, weight, age and semen quality (based on the outcomes of two pre-trial ejaculates) and randomly assigned to one of three concentrate-based dietary treatments (Table 1), namely: (i) a non-supplemented control (CTL, n = 15), (ii) rumen-protected safflower (Safflower; SO, n = 15), or (iii) rumen-protected n-3 PUFA-enriched FO (Incromega; FO, n = 20). Both fat supplements were supplied by Trouw Nutrition; Belfast, Ireland. All diets were isonitrogenous and isocaloric (Table 2). Animals were housed in a concrete slatted floor shed and individually fed using an electronic feeding system (Calan Inc., Northwood, NH, USA) for the initial six weeks of the feeding period, followed by group feeding (5 bulls per treatment/pen), for the remaining six weeks. Animals were allowed two weeks to acclimatise to the individual feeding facility followed by ten days acclimatisation to their respective diets and were then offered diets *ad libitum* for 12 weeks. All animals received 5 kg (fresh weight) of grass silage daily.

2.2. Semen collection

Semen collections were carried out in the summer, between June and August. Semen was collected using the trans-rectal electro-ejaculation (Pulsator, Lanes, CO, USA) technique [19] at weeks −2, −1, 0, 7, 10, 11 and 12 relative to the beginning of the trial period (week 0.). Following collection, semen volume was recorded and progressive linear motility (PLM) was assessed subjectively using a phase contrast microscope incorporating a heated stage at 37 °C (100 sperm per assessment). Spermatozoa concentration was assessed using a photometer (Minitub, Tiefenbach, Germany). On weeks −2, −1, 0, 10, 11, and 12, semen was diluted to 80×10^6 sperm per mL in Bioxcell (IMV, L'Aigle, France) and loaded into 0.25 mL straws (IMV). Straws were cooled gradually from room temperature to 4 °C over a period of 90 min and allowed to equilibrate at 4 °C for 3 h. They were then frozen to −140 °C over a 9 min period (−15.5 °C/min) in a programmable freezer (Planar, Birmingham, UK) followed by immersion and storage in liquid nitrogen, pending

Table 1
Composition of ration offered to young post-pubertal dairy bulls for 12 weeks.

Ingredient	%
Rolled barley	25
Maize	20
Soya bean	15
Beet pulp	17
Soya hulls	12
Oil	4
Minerals/Vitamins	2
Molasses	5

Table 2
Chemical composition of diets offered to young post-pubertal dairy bulls for 12 weeks (mean as g/kg, unless otherwise stated).

	Ration				Silage	
	CTL	SO	FO	s.e.m.		s.e.m.
DM	829	837	838	177.8	230	0.8
Crude protein	17.9	15.7	19.2	0.65	10.9	0.63
ADF	107.6	101.0	78.0	5.35	368.3	4.09
NDF	211.2	199.5	161.2	11.6	580.6	8.33
Ash	78.9	110.1	112.0	9.73	88.9	3.84
Ether extract	1.30	2.65	2.56	0.261	3.04	0.26
Gross energy (MJ/kg DM)	16.46	15.89	15.73	0.178	16.8	0.05

DM: dry matter, ADF: acid detergent fibre, NDF: neutral detergent fibre, CTL: control diet, SO: safflower oil diet, FO: fish oil diet.