Theriogenology 90 (2017) 78–87

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

The effect of dietary supplementation of algae rich in docosahexaenoic acid on boar fertility

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ARTICLE INFO

Article history:
Received 18 May 2016
Received in revised form
8 November 2016
Accepted 10 November 2016
Available online 28 November 2016

Keywords:
DHA
Sperm
Cryopreservation
PUFA
Farrowing rate

ABSTRACT

The objective of this study was to assess the effects of dietary supplementation of a commercial algal product rich in docosahexaenoic acid (DHA) on boar fertility as assessed *in vitro* and *in vivo*. Boars were fed one of three experimental diets for 19 weeks: (i) Control (Ctl) diet (n = 31), (ii) Ctl diet plus 75g All-G-Rich per day (n = 31) or (iii) Ctl diet plus 150g All-G-Rich per day (n = 30). Parameters assessed were (i) raw semen quality; volume, sperm concentration, total motility and morphology (ii) liquid semen quality; progressive motility, viability, hypotonic resistance and acrosomal integrity (iii) frozen-thawed semen quality; motility, thermal stress, viability, membrane fluidity and mitochondrial activity (iv) sperm and seminal plasma (SP) fatty acid composition (FAC) (v) total antioxidant capacity (TAC) of SP and (vi) farrowing rates and litter sizes of sows (n = 1158) inseminated with liquid semen. Boars consuming 75g All-G-Rich had a larger semen volume ($P < 0.05$) and a higher total sperm number ($P < 0.01$) than the Ctl treatment, however, there was no effect of treatment on any other semen quality parameter ($P > 0.05$). There was no effect of dietary treatment on the FAC and TAC of SP or on farrowing rate and litter size ($P > 0.05$). There was an effect of dietary treatment on the FAC of sperm, represented by an 1.72 and 1.60 fold increase in the DHA content for 75 and 150g treatments, respectively, compared to the Ctl treatment. In conclusion, a significant increase in semen volume and total sperm number in boars supplemented 75g All-G-Rich daily, resulted in an increase in production of 3 to 4 more doses per ejaculate, thus, indicating that the feeding regime described within this study has the potential for increasing the output of boar studs.

1. Introduction

Polyunsaturated fatty acids (PUFAs), especially omega-3 fatty acids, have been widely reported to have positive effects on human health, such as reducing the risk of both cardiovascular disease [1] and breast cancer [2] through their anti-inflammatory and chemo-preventative activities. They have been shown to have beneficial effects on female fertility through supporting fetal development [3] and, more recently, have been shown to positively influence male fertility in both human [4] and animal models, including; the boar [5,6], ram [7], bull [8], stallion [9] and chicken [10]. Dietary supplementation of PUFA's have been shown to alter the fatty acid composition (FAC) of the sperm and seminal plasma, increase libido, sperm concentration and, thus, total sperm numbers [6], decrease morphological abnormalities [11] and also increase sperm motility [12]. These benefits may be due to the influence of PUFA's on steroid production pathways, such as increasing testosterone concentration [13], the number of gonadotrophin receptors involved in the regulation of steroidogenesis and also as a result of modifying the phospholipid profile of the sperm membrane facilitating an increase in the fluidity and flexibility of the sperm membrane [14].

Docosahexaenoic acid (DHA) is a long chain omega-3 PUFA and is the most prevalent unsaturated fatty acid found in mammalian

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http://dx.doi.org/10.1016/j.theriogenology.2016.11.008
0093-691X/

sperm cells, comprising approximately, 38% of phospholipids and 20% to 30% of total omega-3 fatty acids in boar sperm [15]. The highest concentration of DHA in sperm is located within the tail region (99%) rather than the head (1%; [16]), giving the plasma membrane of the sperm tail a significant degree of flexibility and elasticity, facilitating the flagella movement required for motility [15]. The inclusion of DHA in the diet has been shown to contribute to sperm membrane fluidity and flexibility [17], enabling sperm to undergo membrane associated events such as capacitation and the acrosome reaction [15]. Two main approaches have been used to investigate the role of fatty acids in sperm cell function, namely; to define the lipid composition of both sperm and seminal plasma in normozoospermic and asthenozoospermic males [14,18]. The second approach is the addition of specific fatty acids to the male diet in an attempt to increase the rate of spermatogenesis and alter the fatty acid profile of sperm cell membranes and therefore, improve sperm quality [5,19].

Although most studies feeding PUFAs, from fish or vegetable oil sources, have reported a positive modification in the sperm lipid composition, in particular increasing the proportion of DHA and decreasing the level of saturated fatty acids [12,20], not all studies observed similar results in relation to sperm production, quality and function [11,20]. By far the most promising results are from studies which have supplemented DHA in the diet or through the addition of various oil types [5,8,20]. Rooke et al. [5] supplemented boars with 30g tuna oil diet for a period of 6 weeks and reported a decrease in the percentage of boar sperm with morphological abnormalities and an increase in progressive motility while Gholami et al. [8] reported an improvement in the viability and motility of fresh bovine semen. However, contradiction in the literature may be related to differences in breeds, boar age, sources of omega-3 PUFAs and the duration of supplementation. Most of these studies are focused on either the *in vitro* analysis of liquid or frozen-thawed semen and are not supported with field data. However, studies which have reported farrowing rate and litter size from boars, supplemented PUFAs in the diet, are confounded by small sample size [21].

Fish oils are the major commercial source of omega-3 fatty acids, specifically DHA [22]. However, due to increases in global demand and the price of fish oils, the development of fish oil alternatives is imperative [24]. Microalgae mass culture is a renewable production technology at an industrial scale which enables the production of omega-3 fatty acids, particularly DHA, from algal sources [25] and is now providing an important source of DHA within the food industry [26]. However, there is no published study on the effect of the dietary supplementation of algal DHA on male fertility in any species. The objective of this study was to assess the effects of dietary supplementation of a commercial algal product rich in docosahexaenoic acid (DHA) on boar fertility as assessed *in vitro* and *in vivo*.

2. Materials and methods

2.1. Experimental design

Purebred maternal (Landrace; LR; n = 36, Largewhite; LW; n = 35) and terminal line boars (Maxgro; MG; n = 21; Hermitage Genetics 2014) of proven fertility, ranging between 10 and 18 months of age were used in this study. All boars were balanced across treatments according to age, breed and pre-experimental semen quality records from the boar stud (total motility, sperm concentration and morphology). Boars were fed one of three experimental diets for 19 weeks. These diets included (i) Control diet (Ctl; standard commercial grain based diet; n = 31 boars), (ii) Ctl diet plus 75g of All-G-Rich per day (75g; n = 31 boars) and (iii) Ctl diet plus 150g of All-G-Rich per day (150g; n = 30 boars). All-G-Rich is a DHA-rich algal commercial supplement (Hower 2014: Filer 2014) which contains the antioxidant ethoxyquin (Table 2: Alltech, Dunboyne, Co Meath, Ireland). Ethoxyquin, is a synthetic antioxidant, commonly used in animal feed to protect against lipid peroxidation but is also known for having a high antioxidant capacity [28]. This study was approved by the University of Limerick ethics committee (2013_12_1_ULAEC).

Semen was collected from boars once per week throughout the supplementation period and assessed on farm for volume, sperm concentration as well as motility and morphology (microscopy-based; Supplementary Material Fig. 1) from week one to 14. Laboratory technicians were blind to treatments. On three occasions (replicates; Supplementary Material Fig. 1), between week eight and 14, liquid semen from a subset of boars (n = 12 per treatment) was assessed on Days 1, three and six post collection for progressive motility using a phase-contrast microscope and viability, hypotonic resistance and acrosomal integrity *via* flow cytometry. Motility and viability were performed on liquid semen as these are standard parameters to assess semen quality while acrosomal integrity and hypotonic resistance were conducted to assess the versatility of sperm to increased duration of storage. Throughout the experimental period sows (n = 1158) were inseminated with liquid semen on commercial farms (n = 27) and farrowing rates and litter sizes were captured. On three occasions (replicates; Supplementary Material Fig. 1) between week 14 and 19, semen was collected and frozen from six boars per treatment. Frozen-thawed semen was assessed post-thaw for motility pre- and post−thermal stress using Computer Assisted Sperm Analysis (CASA; SCA Evolution, Microptics, Barcelona, Spain) as well as viability, membrane fluidity, mitochondrial activity and oxidative stress *via* flow cytometry. These tests were performed on frozen-thawed semen as again motility and viability are basic parameters to assess semen quality while thermal stress test, membrane fluidity and oxidative stress test were conducted to best assess if dietary supplementation reduced the damage sustained to sperm membrane due to the stress associated with the freeze-thaw process. On weeks one, eight and 18 ejaculates were collected from 12 boars per treatment, centrifuged and sperm and seminal plasma (SP) were harvested and frozen. The SP was later analyzed for total antioxidant capacity (TAC) while the sperm and SP samples were analyzed for their fatty acid profile (week eight only).

2.2. Composition of diets and feeding regime

All boars were located at one commercial boar stud in County Kilkenny, Ireland, individually housed and fed and maintained under similar management and feeding conditions. Boars were individually fed approximately 3 kg of Ctl diet daily as per routine procedure (depending on size and age), between 08.30 and 09.00 hours, and had *ad libitum* access to water. The All-G-Rich

Table 1
Chemical analysis (expressed as g/kg of dry matter (DM) unless otherwise stated) of the control diet (a standard commercial grain based diet) and the All-G-Rich supplement (a DHA-rich algal commercial supplement).

Ingredient	Control diet	All-G-Rich
DM (%)	87.4	97.3
Crude Protein (g/kg)	16.5	10.7
Crude Fiber (g/kg)	38.2	16.8
Ash (g/kg)	59.58	44.1
Acid detergent fiber (g/kg)	66.26	64.21
Neutral detergent fiber (g/kg)	104.47	81.04
Ether extract (g/kg)	2.3	33.2
Gross energy (MJ/kg DM)	17.0	31.8