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Quality and lipid composition of spermatozoa in rabbits fed DHA and vitamin E rich diets

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Abstract

The effects of fish oil (FO) and vitamin E (vE) dietary supplementation on semen quality, sperm susceptibility to lipid peroxidation, tocopherols content and fatty acid profiles were studied in rabbits. Fifty-two rabbit bucks randomly divided in four groups received a control diet and enriched diets containing either FO (1.5%, w/w), vE (200 mg/kg) or both. Semen volume, concentration, motility and viability were analysed at various time-points and the lipid composition was assessed on sperm cells. The phospholipid fatty acid profile was determined: *n*-6 PUFA were the major fatty acids found, with a proportion of 42%, whereas the *n*-3 PUFA accounted for nearly 1%, mainly represented by C22:6*n*-3 (docosahexaenoic acid, DHA). FO supplementation produced a seven-fold increase in the content of DHA in sperm phospholipids and a comprehensive rearrangement of the phospholipid fatty acid composition, while an unexpected negative effect of feeding high level of vE on the proportion of total PUFA was found. Despite the remarkable changes observed in sperm lipid composition, semen quality parameters were not affected by the dietary treatments and the interaction between the two dietary supplements had a significant effect only on sperm concentration. An increase in semen production by ageing and a concomitant rise in sperm susceptibility to *in vitro* peroxidation was found. α- and δ-tocopherol, present in rabbit sperm in similar amount, were not affected by dietary treatment. δ-tocopherol content had a significant linear negative regression with age and showed a significant negative correlation with the susceptibility to peroxidation values.

Keywords: Rabbit; Sperm quality; Diet; *n*-3 fatty acids; Vitamin E

1. Introduction

The lipid composition of sperm has a recognized importance as structural and functional component. In particular, phospholipids (PL) of spermatic cells are characterized by very high proportions of long chain polyunsaturated fatty acids (PUFA), especially from the *n*-3 and *n*-6 series, with species-specific composition. The sperm of most mammalian species as bull, ram, monkey and man show very high levels of C22:6*n*-3 (docosahexaenoic acid, DHA) [1–7]; otherwise, rabbit and dog exhibit low or no DHA in sperm PL, and the major PUFA is C22:5*n*-6 (docosapentaenoic acid, DPA) [2,8]. Finally, boar sperm display an intermediate pattern [2,9]. As regards avian semen, *n*-3 series is essentially absent, with C22:4*n*-6 (docosatetraenoic acid) and C20:4*n*-6 (arachidonic acid) being the major PUFA present in the sperm PL [10,11].

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The high degree of unsaturation found in sperm is believed to contribute actively to regulation of cell movement and lipid metabolism; it also confers sufficient fluidity to the sperm plasma membrane for the fusion events that characterize fertilization [12]. The proportion of sperm PUFA has been directly related to semen quality in different species [6,13]. Particularly the DHA content has been related to sperm concentration and motility in men [6,7,14], boars and chickens [13]. A reduction in fertility during aging was associated with decreased proportions of specific PUFA in sperm PL both in bull (DHA) and chicken (C20-22 PUFA) spermatozoa [15,16]. Furthermore, in men lower proportions of DHA were observed in oligozoospermic and asthenozoospermic samples than in normozoospermic samples [7].

High levels of PUFA increase the susceptibility of the cells to free radical induced peroxidative damages, considered a significant cause of male infertility [17]. Within the antioxidant system of sperm vitamin E is the major natural lipid-soluble antioxidant present in cell membranes and plays a crucial role in breaking the chain reaction of peroxidation, initiated by reactive oxygen species (ROS). It also has a role in the direct stabilisation of membranes by interaction with PL and is involved in the regulation of several physiological functions [18,19]. ROS, at low and controlled concentrations, have a regulating role in specific sperm functions, such as capacitation and acrosomal reaction [20–22]. It is therefore fundamental to take into account the critical balance between lipids, ROS and the different components of the antioxidant system of the cell to ensure the efficient functionality of spermatozoa.

The lipid composition of the diet modify the acidic arrangement of spermatozoa and their fertilizing ability [23]: PUFA enriched diets have been successfully used to improve semen quality and fertility in mammalian and avian species [23–30] and the addition of antioxidants to the diets or during *in vitro* storage of spermatozoa was shown to reduce the sperm susceptibility to lipid peroxidation [31,32].

Limited reports are available regarding the administration of PUFA and antioxidant enriched diets to rabbit bucks [33–38]. Supplementation of ascorbic acid, vitamin E and their combination was found to have a protective effect by reducing the production of free radicals and improving rabbit semen quality [34–36]. In contrast, fertilizing ability was not modified by supranutritional level of dietary α-tocopheryl acetate and selenium [37]. Finally supplemental *n*-3 PUFA, vitamin E and C have been shown to affect the oxidative status and semen characteristics of rabbit bucks [38].

The aim of the present study was to investigate the effects of fish oil (FO) and vitamin E (vE) dietary supplementation on semen quality, sperm susceptibility to lipid peroxidation, tocopherols content and fatty acid profiles in rabbit bucks.

2. Materials and methods

All the chemicals used were of reagent grade. Unless otherwise indicated, reagents were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA).

2.1. Animals and diets

Fifty-two rabbit bucks of female line [39] were randomly divided in four groups and assigned to one of four diets. The bucks were housed in an experimental stable at the Institute for Agriculture and Fisheries Research, Merelbeke, Belgium, in controlled environment and fed ad libitum the experimental diets from the 20th week of age. The experimental diets were obtained by supplementation of the control diet as follows: CO diet = control diet, containing on average 16.5% CP, 3.2% EE, 19.5% ADF, 9.8 MJ DE/kg and 8 mg vE/kg; COvE diet = CO diet enriched with 200 mg vE/kg; FO diet = CO diet enriched with FO 1.5% (w/w); FOvE diet = CO diet enriched with 200 mg vE/kg and FO 1.5% (w/w).

2.2. Semen collection

Bucks were trained to semen collection from the age of 20 weeks and routinely ejaculated weekly thereafter. Semen production was controlled weekly from 26 to 28 and from 37 to 39 weeks of age. Semen was collected via an artificial vagina (IMV Technologies®) and two ejaculates were obtained from each male on the same day. The volume of each ejaculate was recorded by graduated tubes before to dilute semen 1:2 with Tris extender (Tris–glucose–citrate, 300 mOsm/g, pH 7.1). Diluted ejaculates were pooled per treatment, immediately stored in a water-bath at 37 °C and pooled again after 30 min with the samples from the second collection before analyses.

2.3. Sperm quality parameters

Sperm motility of the 1st and 2nd pooled ejaculates was assessed subjectively, using a microscope with a warmed stage, at ×400 magnification; a five (1–5) category classification, corresponding to a motility of 0, 0–25, 25–50, 50–75, or >75%, was used [40]. Sperm