Animal Reproduction Science 183 (2017) 132–142

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Dietary inclusion of fish oil changes the semen lipid composition but does not improve the post-thaw semen quality of ram spermatozoa

Rommy Díaz[b,d,*], Mariana A. Torres[c], Erwin Paz[a], John Quiñones[a,b], Silvana Bravo[a,b], Jorge G. Farías[b,d], Néstor Sepúlveda[a,b]

[a] Departamento de Producción Agropecuaria, Facultad de Ciencias Agropecuarias y Forestales, Universidad de La Frontera, Temuco, Chile
[b] Centro de Biotecnología de la Reproducción – Núcleo Científico y Tecnológico en Biorecursos (CEBIOR-BIOREN), Universidad de La Frontera, Temuco, Chile
[c] Departamento de Reprodução Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Pirassununga, São Paulo, Brazil
[d] Departamento de Ingeniería Química, Facultad de Ingeniería y Ciencias, Universidad de La Frontera, Temuco, Chile

ARTICLE INFO

Keywords:
Lipid composition
Omega-3 fatty acids
Cholesterol
Cryopreservation
Ram

ABSTRACT

The aim of this study was to investigate the effects of dietary fish oil (FO) time-response on the fatty acid profile, cholesterol levels and sperm cryosurvival in ram semen. Criollo Araucano rams were randomly assigned to two groups (n = 4) according to the type of supplementation: a control group without FO and a supplemented group fed a diet with 3% FO for 8 weeks. The semen lipid profile and post-thaw sperm quality were analyzed at weeks 0 (pre-supplementation), 4, 8, 12 and 16 (post-supplementation) to evaluate the effects of FO supplementation by time interaction. Post-thaw sperm quality was determined by CASA and flow cytometry. In spermatozoa, the supplemented group increased the linoleic acid (C18:2n6c) and docosahexaenoic acid (DHA; C22:6n3) with levels higher at week 16 ($P < 0.05$). The effect of FO on cholesterol concentration in sperm was significant at the end of the experiment (week 16). In seminal plasma, statistical differences of butyric acid (C4:0), palmitic acid (C16:0), stearic acid (C18:0), eicosatrienoic acid (C20:3n3) and DHA were observed at week 12. The cholesterol concentration was not affected by dietary treatments ($P > 0.05$). However, the post-thaw sperm quality of the FO treatment group decreased. Motility percentage decreased 50% and spermatozoa with permeable plasma membrane and reacted acrosome were higher (63%) at week 16 than the control group. These results showed that DHA was effectively incorporated into semen through dietary supplementation with FO, but evaluations of post-thaw sperm quality confirm alteration specificity related to the structure of the lipid bilayer.

1. Introduction

Semen lipid composition has been associated with membrane integrity and fluidity, sperm motility and cold sensitivity (White, 1993; Hossain et al., 2007; Mocé et al., 2010). In mammals, sperm membrane composition is characterized by a high proportion of omega-3 fatty acids (Parks and Lynch, 1992; Díaz et al., 2015). Omega-3 fatty acids including α-linolenic acid (ALA, 18:3n-3),

* Corresponding author at: Av. Francisco Salazar 01145, Universidad de La Frontera, Temuco, Chile. Tel.: +56-45-2325458; fax: +56-45-2325053.
E-mail addresses: rommy.diaz.pe@gmail.com (R. Díaz), torres.mandrade@gmail.com (M.A. Torres), paz.erwin45@gmail.com (E. Paz), johnbiotecnologia@gmail.com (J. Quiñones), silvana.bravo@ufrontera.cl (S. Bravo), jorge.farias@ufrontera.cl (J.G. Farías), nestor.sepulveda@ufrontera.cl (N. Sepúlveda).

http://dx.doi.org/10.1016/j.anireprosci.2017.05.002
Received 27 September 2016; Received in revised form 11 May 2017; Accepted 14 May 2017
Available online 18 May 2017

eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6n-3) are polyunsaturated fatty acids (PUFA) with a double bond at the third carbon atom from the end of the carbon chain, and they play an important role in the functionality of spermatozoa and cryoresistance (Towhidi and Parks, 2012; Fair et al., 2014).

Ram spermatozoa are more sensitive to cryopreservation than bull, rabbit or human spermatozoa (Brinsko et al., 2005). It is well known that spermatozoa are exposed to numerous damaging factors during the freezing-thawing process, and success depends largely on membrane resistance to these factors (Watson, 2000). These factors are associated with a loss and decrease of motility, viability, fertilizing capacity and the phenomenon commonly referred to as "cold shock" (Holt et al., 1992). Membrane lipid composition, especially the cholesterol/phospholipid ratio and free fatty acid composition, influences permeability to water and other molecules, membrane fluidity and lipid phase transitions in the membrane bilayer (Holt, 2000). For this reason, lipid composition is believed to be one of the primary determinants of semen quality and cryoresistance of spermatozoa (Samadian et al., 2010; Selvaraju et al., 2012).

One difference in the ability of spermatozoa from various species to resist cold shock appears to be related to the membrane lipid composition (White, 1993). Therefore, there is great interest in modifying the membrane lipid composition and improving freezability of spermatozoa (Castellano et al., 2011; Towhidi and Parks, 2012; Fair et al., 2014). Different feeding strategies have been developed to improve post-thaw sperm quality (Samadian et al., 2010; Jafaroghli et al., 2014). In this context, dietary supplementation of PUFA, especially diets rich in omega-3 or omega-6 fatty acids, affects the composition of phospholipids, helps maintain membrane fluidity, motility and normal morphology, influences the assembly of enzymes involved in sperm-egg interaction as well as resistance to physicochemical stress and the fertilizing capacity of spermatozoa (Rooke et al., 2001; Safarinejad et al., 2010).

Fish oil (FO) is a source of dietary PUFA omega-3 because of its high EPA and DHA content (Ashes et al., 1992; Gama et al., 2008). In addition, several studies have shown that the FO fatty acids can be incorporated into ram semen despite the biohydrogenation of dietary fatty acids in the rumen (Samadian et al., 2010; Esmaeili et al., 2014; Fair et al., 2014; Jafaroghli et al., 2014). However, there is no information on the duration of the effect of FO supplementation on the ram semen lipid profile.

Accordingly, we believe that direct FO intake changes the fatty acid profile of ram semen, improves freezability during the supplementation period and that its effect persists after supplementation. Therefore, the aim of this study was to assess the effects of dietary FO supplementation on fatty acid profile and cholesterol levels of spermatozoa and seminal plasma to determine the persistence of FO fatty acids and the effects on cryoresistance of ram spermatozoa.

2. Materials and methods

2.1. Animals and experimental design

Data were collected from March 1 to June 30, 2016 (physiological breeding season of Criollo Araucano rams in Chile). This study received the approval of the ethics committee of the Universidad de La Frontera. The rams were located at the Maquehue Experimental Farm of Universidad de La Frontera, Temuco, Chile (38°44′S, 72°35″W). Eight Criollo Araucano rams sexually mature at 3–5 years of age and with a mean live weight of 81 ± 5 kg were chosen at random for use in this study. The rams were housed separately from the ewes. Before the commencement of the trial and following an acclimation period of 4 weeks allowed for adaptation to the diets, rams were assigned to 2 groups (n = 4) and placed in a different pen according to the type of supplementation. The rams were kept under natural photoperiod conditions and water and mineral blocks were available *ad libitum* throughout the experimental period. The period of supplementation was during 8 weeks, since the duration of the spermatogenesis in the ram is 49 days and the epididymal transport 9 days. Therefore, it is recommended that dietary supplementation of rams should be for a similar period to ensure the incorporation of fatty acids into spermatozoa (Samadian et al., 2010). After the trial period all groups were offered basal diet without any supplementary fat sources to evaluate the effect treatment-by-time response for 8 weeks more. Semen was collected from each ram twice per week for 16 weeks (in each group the total number of ejaculates per week = 8). Rams were routinely ejaculated at the same interval throughout the experiment. Ejaculates at weeks 0, 4, 8, 12 and 16 were analyzed individually to determine the fatty acid profile and cholesterol concentration and processed to semen freezing.

2.2. Diets

Basal diet (1.48 kg DM/day/ram) for both groups consisted of alfalfa hay (0.74 kg DM), oat grain (0.37 kg DM), wheat straw (0.30 kg DM) and sugar beet molasses (0.07 kg DM). Rams were randomly allocated to receive one of the following two diets: 1) control group (CON) without FO and 2) treatment group (SUP) fed a diet with 25 ml/day of FO equivalent to 0.3 ml/kg live weight/day. Rams were drenched orally with a dose of FO (*Fish oil from Menhaden fish*; Sigma Aldrich Inc., St. Louis, USA, No. F8020). The FO fatty acid composition is presented in Table 1. The daily dose was divided into two equal quantities and administered at 8:30 a.m. and 3:30 p.m.

2.3. Semen collection and evaluation

Rams were routinely used as semen donors and had been trained for semen collection before the study outset. Semen samples were collected by artificial vagina using a female as the stimulus. To eliminate any potential differences in spermatozoa quality due to serial ejaculates, the samples were obtained only once a day. Semen was collected in the morning and transported to the laboratory (at 37 °C). The parameters sperm concentration, sperm mass movement and morphology were assessed at the farm (Santiani et al.,