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Metabolic and reproductive parameters in prepubertal gilts after omega-3 supplementation in the diet

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ABSTRACT

Polyunsaturated fatty acids may benefit reproductive performance of female swine. This study evaluated metabolic and reproductive parameters of prepubertal finishing gilts fed with fish oil as a natural source of omega-3 fatty acids (6.88 g/d) (n = 12) over a period of 45 d. Gilts in the control group were fed soybean oil (n = 13). Body weight and backfat were determined at 15-d intervals. Serum levels of leptin, IGF-1, insulin, cholesterol and triglycerides were measured at the beginning (D0) and at the end of the period (D45). Immunolabeling intensity for leptin and its receptor (ObRb) was assessed in oocytes of preantral follicles. Gilts fed omega-3 presented slightly heavier uteri ($P = 0.09$) than control gilts, but there was no effect on body weight and backfat ($P > 0.05$). Cholesterol serum levels tended to be lower at D45 for omega-3 supplemented gilts than for controls ($P = 0.06$). Triglycerides and IGF-1 serum levels were lower at D45 than at D0 for control gilts ($P < 0.05$), but unaltered for supplemented gilts. Insulin levels were unaffected by supplementation ($P > 0.05$), but were greater at D45 than at D0 in both treatments ($P < 0.05$). Immunolabeling for leptin and ObRb in oocytes included in preantral follicles was more intense for supplemented gilts than for control gilts ($P < 0.05$). Omega-3 supplementation was associated with reduced serum cholesterol level and more intense staining for leptin in oocytes of prepubertal gilts, which suggests some involvement on triggering puberty.

1. Introduction

Polyunsaturated fatty acids (PUFA) are essential components of complex lipids required for embryo development and fetal growth in swine. When supplemented in gestation and lactation diets, PUFA can be incorporated into oocyte membranes, modulating the patterns of expression of enzymes involved in synthesis of prostaglandins, in metabolism of steroid hormones and in oxidative stress (Wathes et al., 2007; Jump, 2008). PUFA can also be incorporated in tissues of embryos and fetuses, leading to beneficial effects, such as accelerated embryo neural development (Wakefield et al., 2008; Brazle et al., 2009; Smits et al., 2011) and increased litter size (Smits et al., 2011). PUFA are classified based on the position of their first double bond relative to their methyl end. The n-6 (omega-6) and n-3 (omega-3) PUFA have linoleic (C18:2n-6; LIN) and α-linolenic (C18:3n-3; ALA) fatty acids as precursors, respectively. The most important PUFA in the omega-3 series are the eicosapentaenoic (C20:5n-3, EPA) and the docosahexaenoic fatty acids (C22:6n-3, DHA) (reviewed by Tanghe and De Smet, 2013).

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Diets containing high levels of n-3 from fish sources can reduce serum triglycerides (Harris and Bulchandani, 2006) and total cholesterol reviewed by Adkins and Kelley (2010), demonstrating that PUFA can affect energy metabolism. Other metabolic factors such as insulin and insulin-like growth factor type 1 (IGF-1) can also influence the reproductive axis in female swine (Prunier and Quesnel, 2000). Insulin is an important modulator of reproductive function, increasing during the reestablishment of follicular growth in anestrus sows after energetic supplementation (Flowers et al., 1989). Furthermore, insulin regulates leptin mRNA *in vitro* and *in vivo*, being positively correlated with plasma leptin levels (Houseknecht et al., 2000).

Diets with high inclusion of PUFA, particularly those of the n-3 series, may increase plasma concentrations of leptin (Cha and Jones, 1998). Leptin and its long form receptor (ObRb) were identified in the hypothalami of fetuses, pregnant sows, cyclic females and prepubertal gilts (Lin et al., 2001), acting as a permissive factor that signals the nutritional status to the hypothalamus-pituitary-gonads axis (Zieba et al., 2005). As gilts come close to puberty, leptin circulating levels increase (Qian et al., 1999) and immunostaining for ObRb in hypothalamus neurons becomes more intense (Moreira et al., 2014). Considering that backfat thickness is a reliable indicator of body condition in swine females and is positively associated with age at puberty (Zhuo et al., 2014), supplementation of gilts with diets including PUFA can increase their fat reserves and body weight (Amaral et al., 2010), which may help trigger puberty (Zieba et al., 2005). Nevertheless, studies associating reproductive performance with leptin levels in the ovarian tissue of gilts supplemented with sources of PUFA are scarce. The objectives of this study were to evaluate the effect of omega-3 PUFA supplementation on backfat thickness, body weight, serum levels of metabolic markers and immunolabeling intensity of leptin and ObRb in oocytes from prepubertal gilts.

2. Material and methods

2.1. Animals and sampling

All experimental procedures were approved by the UFPel's Ethics for Animal Experimentation Committee (protocol number 9648). The study was conducted in a commercial swine farm located in Southern Brazil (latitude 31°38′47″S and longitude 52°21′03″W). As supplementation and slaughter of replacement gilts were not allowed on that farm, 25 prepubertal crossbred F2 gilts destined for slaughter were used as experimental models. They were housed in naturally ventilated split-sex finishing pens and fed 2.5 kg daily of a diet containing 14.7% (w/w) crude protein and 3205 kcal/kg metabolizable energy (NRC, 1998). At the beginning of the experiment, gilts were 120 d-old and weighed 50.6 kg, on average.

Gilts were randomly allocated to two treatments: a control group (n = 13) that received commercially available soybean oil; and a second group (n = 12) that received fish oil (Idealfarma, São Paulo, SP, Brazil) as an omega-3 supplemented source. Each group was housed in separate pens. Gilts from both groups were orally supplemented with 9 mL of each oil with a disposable syringe daily during 45 days. The energy value in 1.0 g [1.0 g = 1 mL (w/v)] of soybean oil was 8.3 kcal (35.0 kj), containing 0.9 g of total fat, including 0.55 g of unspecified PUFA, but no omega-3. The energy value in 1.0 g [1.0 g = 1 mL (w/v)] of the fish oil was 9.5 kcal (40.0 kj), containing 0.9 g of total PUFA, which included 0.35 g DHA and 0.5 g EPA. Thus, gilts were supplemented with a total of 8.1 g of PUFA, which corresponded to 6.88 g of omega-3.

At four 15-d periods (at 0, 15, 30 and 45 d), gilts were weighed and their backfat thickness was measured at the P2 position, 65 mm down the left side from the midline, at the level of the head of the last rib, by ultrasound (Pie Medical, Aquila Vet) with a convex 5 MHz probe. The last weighing and backfat measurement occurred 15 d prior to the scheduled date of slaughter. At an abattoir, the ovaries and the uterus were collected. Uteri was weighed and the presence of follicles and corpora lutea in the ovaries was recorded.

2.2. Metabolic markers

Blood samples were collected from the jugular vein prior to feeding at the beginning (D0) and on the last day (D45) of the experiment, Serum leptin levels were determined by ELISA (CAN-L-4260, Diagnostic Biochem Canada Inc, Dorchester, ON, Canada) (sensitivity: 0.42 ng/mL). Levels of IGF-1 were determined using chemiluminescence (Siemens Immulite IGF-1 L2KGF2: Siemens Healthcare Ltda, São Paulo, SP, Brazil) (sensitivity: 20 ng/mL). Insulin levels were quantified using eletrochemiluminescence (Roche, São Paulo, SP, Brazil), using commercial kits and following the manufacturer's protocol (sensitivity 0.2 μU/mL). Standard enzymatic procedures were used to assay total cholesterol (11539, BioSystems, Curitiba, PR, Brazil) (sensitivity 0.3 mg/dl) and triglycerides (87.1/250, for human serum, Labtest, Lagoa Santa, MG, Brazil) (sensitivity 3.0 mg/dl). All kits were developed for human serum, but were validated for multi species use. According to the laboratory that conducted the analyses, all kits present intra and inter assays CV below to 10%. All samples were analyzed in duplicate

2.3. Classification of structures

Considering known sites of action for leptin and ObRb (Lin et al., 2001; Smolinska et al., 2007), the cytoplasm of oocytes was evaluated in slides. Oocytes were classified as: included in primordial/primary follicles (OIPF), when surrounded by one layer of flat to cuboidal granulosa cells; included in secondary follicles (OISF), when surrounded by two or more layers of cuboidal granulosa cells; or included in tertiary follicles (OITF), when surrounded by various granulosa cell layers with antrum formation (Silva et al., 2011).

2.4. Immunohistochemistry (IHQ)

The analyses of the leptin antibody considered 79 OIPF, 35 OISF and 28 OITF, whereas the analyses of the ObRb antibody considered 44 OIPF, 34 OISF and 21 OITF, using ovaries