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Composition of α-tocopherol and fatty acids in porcine tissues after dietary supplementation with vitamin E and different fat sources

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ABSTRACT

The present study evaluated the effect of increasing supplementation of all-rac-α-tocopheryl acetate and dietary fatty acid composition during a four week period after weaning on porcine tissue composition of α-tocopherol stereoisomers and fatty acids, and on hepatic expression of genes involved in transfer of α-tocopherol, and oxidation and metabolism of fatty acids. From day 28 to 56 of age, pigs were provided 5% of tallow, fish oil or sunflower oil and 85, 150, or 300 mg/kg of all-rac-α-tocopheryl acetate. Samples of liver, heart, and adipose tissue were obtained from littermates at day 56. Tissue fatty acid composition was highly influenced by dietary fat sources. Dietary fatty acid composition ($P<0.001$) and vitamin E supplementation ($P<0.001$) influenced the α-tocopherol stereoisomer composition in liver, i.e. less proportion of the RRR-α-tocopherol was observed in pigs provided fish oil and the highest dose of vitamin E in comparison with other dietary treatments. In addition, the stereoisomer composition of α-tocopherol in heart, and adipose tissue was influenced by dietary treatments. Expression of genes in liver involved in the regulation of FA conversion, SCD ($P=0.002$) and D6D ($P=0.04$) were lower in pigs fed fish oil compared to other treatments, whereas the fatty acid oxidation, as indicated by the expression of PPAR-α, was higher when sunflower and fish oil was provided ($P=0.03$). Expression of α-TTP in liver was higher in pigs fed fish oil ($P=0.01$). Vitamin E supplementation did not influence significantly the hepatic gene expression.

1. Introduction

Vitamin E (α-tocopherol) and essential n-3 and n-6 fatty acids are important nutrients for growth, development and health of animals. The specific vitamin function of α-tocopherol is to protect the long-chain PUFA and thus maintain their concentrations for important events, such as membrane fluidity and eicosanoid signaling. α-Tocopherol is most concentrated in membrane-rich fractions such as mitochondria and microsomes, and it is possible by dietary means to further increase the concentration (Lauridsen and Jensen, 2012). Pigs are not able to synthesize α-tocopherol or essential fatty acids, and in terms of vitamin E, feed is therefore commonly supplemented with the commercially available form of vitamin E;

Abbreviations: ACC, acetyl CoA carboxylase; TTP, tocopherol transfer protein; D6D, Δ6-desaturase; FAS, fatty acid synthase; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; SCD, stearoyl-CoA-desaturase; SE, standard error; SREBP-1, sterol regulatory element-binding protein 1; UNS, unstaturated fatty acids.

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all-rac-α-tocopheryl acetate. Importantly, synthetic α-tocopherol is an equimolar mixture of 8 isomers (VERIS, 1999), only one of which is identical to the natural occurring stereoisomer, RRR-α-tocopherol. We have shown that sows were able to discriminate between RRR- and all-rac-α-tocopherols (Lauridsen et al., 2002), and moreover that the bioavailability of α-tocopherol stereoisomers in rats depends on dietary doses of all-rac-α-tocopheryl acetate (Jensen et al., 2006). Recently it was claimed (Traber and Atkinson, 2007) that virtually all of the variation and scope of vitamin E's biological activity could be seen and understood in the light of protection of PUFA and membrane qualities that PUFA bring about. However, in terms of animal as well as human studies concerning dietary vitamin E supplementation, little emphasis has been given to dietary fatty acid composition although former (Buttriss and Diplock, 1988) and recent (Atkinson et al., 2010) research have reported the importance of considering fatty acid composition in relation to the deposited proportion of α-tocopherol in biological membranes where it is most needed. It was concluded (Buttriss and Diplock, 1988) that the protective effect of each molecule of α-tocopherol must be exerted toward a large number of molecules of membrane unsaturated fatty acids simultaneously.

Dietary PUFA, especially of the n-3 and n-6 series, have called for attention in relation to their stimulation of gene expression, as these FA families have well-known physiological impact (Jump, 2002). The liver is the most important organ for the intermediary metabolism of lipids and energy and hence, regulation of hepatic gene expression may play a central role in the adaptive response to altered nutrient digestion and metabolism by changing the capacity of enzymes in relevant metabolic pathways. Likewise, the liver is playing an essential role in the vitamin E metabolism due to the presence of the α-tocopherol transfer protein, α-TTP, whereby the α-tocopherol, rather than β-,γ-,δ-tocopherols, is maintained in human plasma and tissues. In the present study we hypothesized that different dietary fatty acid sources and vitamin E supplementation would affect tissue composition of stereoisomer forms of α-tocopherol and of fatty acids. Furthermore, that hepatic expression of TTP and genes involved in fatty acid metabolism in the liver would be modulated by dietary vitamin E supplementation and/or by varying dietary fatty acid composition. The purpose of the present study was to evaluate the impact of dietary n-6 (from sunflower oil) and n-3 (from fish oil) fatty acids, and increasing levels of all-rac-α-tocopheryl acetate on the composition of fatty acids and stereoisomer forms of α-tocopherol, and on hepatic expression of genes involved in the β-oxidation and metabolism of these nutrients in tissues of young pigs.

2. Materials and methods

2.1. Animals and housing

A total of 7 litters of crossbred ([Landrace × Yorkshire] × Duroc) pigs weaned at 28 ± 1 days of age were used in this experiment. Pigs were obtained from sows which had been provided a lactation feed containing a high content of vitamin E (250 mg all-rac-α-tocopheryl acetate/kg feed), and during the last 2 weeks of the suckling period, piglets received creep feed containing 70 mg all-rac-α-tocopheryl acetate/kg feed. Nine pigs of each litter were allotted to nine dietary treatments in a 3 × 3 factorial arrangement of treatments in a randomized complete block design, which was conducted in 7 replicates (blocks). Pigs were housed individually in 1.5 × 1.8 m pens. The floor comprised two parts: a concrete, heated floor (located just behind the trough) and a manure area with a plastic net. Pigs had access to treatment diets on *ad libitum* basis and one nipple waterer per pen. Environmental temperatures were initially established at 32 °C, but were adjusted downward at weekly intervals. The experiment complied with the guidelines of the Danish Ministry of Justice with respect to animal experimentation and care of animals under study.

2.2. Diets

The first factor of the design evaluated the effect of fat source, which was either tallow (from the local supermarket), sunflower oil (BAST International I/S, Copenhagen, Denmark) or fish oil from Marocco, primarily of the fish species Pilchart and Sardine (FF of Denmark, Skagen, Denmark), and the second factor evaluated the effect of vitamin E (85, 150, or 300 IU/kg) provided as all-rac-α-tocopheryl acetate (DSM Nutritional Products a/s). The content of vitamin E (α-tocopherol) in each fat source was measured, and was found to be 23, 542, and 65 mg/kg fat in tallow, sunflower oil, and fish oil, respectively. The differences in the content of vitamin E between the oils were adjusted by mixing vitamin E (RRR-α-tocopherol (Natur E-Micelle), obtained from Pharmalett a/s) with the fat before addition to the ground feed. The basic feed was composed of barley (31.2%), wheat (31.2%), soybean meal (21.7%), calcium carbonate (0.92%), monocalcium phosphate (0.91%), sodium chloride (0.29%), vitamin- and mineral premix (0.4%), fish meal (8%), L-lysine (0.39%), DL-methionine (0.04%), and fat (5%), and was mixed at Research Centre Foulum, Aarhus University, and provided as mash. The basic diet contained 85 mg of vitamin E (all-rac-α-tocopheryl acetate), and other details on the content of the vitamin- and mineral premix and analyzed composition are presented by Møller and Lauridsen (2006). Dietary lipids were extracted according to Stoldt (1952) using petroleum ether, and the long-chain fatty acids (>8C) were determined by GLC (capillary) after saponification and methylation as described by Rothenberg and Andersen (1980), with substitution of hexane with heptane, and with C17:0 as internal standard. The α-tocopherol concentration in dietary oils and final experimental diets were determined by HPLC after saponification and extraction into heptane as described by Jensen et al. (1999).