



Influence of feeding stearidonic acid (18:4n-3)-enriched soybean oil, as compared to conventional soybean oil, on tissue deposition of very long-chain omega-3 fatty acids in meat-type chickens

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ABSTRACT

In chickens, the desaturation of α -linolenic acid (ALA; 18:3n-3) to stearidonic acid (SDA; 18:4n-3) is considered to be rate-limiting for the hepatic conversion of ALA to very long-chain (VLC; i.e. >20 C) n-3 polyunsaturated fatty acids (PUFAs). Thus, we hypothesized that feeding broilers SDA plus ALA, as compared to ALA alone, would bypass this inefficient metabolic step and enrich meat with greater amounts of VLC n-3 PUFAs. Female Ross \times Heritage broilers were fed mash diets containing 50 g/kg of conventional soy oil (CON) from hatch until d 28. On d 29, they were divided into two groups and fed diets containing either 50 g/kg CON or 50 g/kg of SDA-enriched oil derived from the genetic modification of the soybean (SDASOY) until d 42. Final (42 d) body weights, as well as weight gains and feed conversion values from 29 to 35 d and 36 to 42 d, were not different ($P > 0.05$) between treatments. Compared to the CON treatment, dietary SDASOY increased ($P < 0.01$) total VLC n-3 PUFA contents of skinless and boneless breasts, tenders, and thighs by almost 3-fold. However, the SDASOY diet also contained more total n-3 fatty acids (ALA + SDA) than the CON diet (ALA only), and it was estimated that ALA and SDA were metabolized to VLC n-3 PUFAs and deposited into breast, tenders, and thigh meat with equal efficiency. Docosapentaenoic acid (DPA; 22:5n-3) was the predominant VLC n-3 PUFA in all three muscles, suggesting that another control point downstream of the initial hepatic $\Delta 6$ -desaturase reaction was rate-limiting in the biosynthesis of DHA from ALA. Alternately, since broilers have the capability to convert ALA to DHA in the liver, it is likely that the capacity of the VLC n-3 PUFA biosynthetic pathway is simply not great enough to allow for the deposition of DHA into muscle at levels equal to those attained by direct dietary supplementation. It is also possible that, rather than undergoing elongation and desaturation, some of the ALA and SDA pool underwent β -oxidation in the liver, as suggested by others, while a large portion of each fatty acid was not metabolized and was transported out of the liver to other tissues, such as adipose. However, the relative hepatic expression of genes whose protein products are involved in fatty acid oxidation (as well as in desaturation and elongation or lipogenesis) were not significantly affected by dietary treatment or age.

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Abbreviations: ALA, α -linolenic acid; BW, body weight; CON, conventional soybean oil diet; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; GLA, γ -linoleic acid; LA, linoleic acid; PUFA, polyunsaturated fatty acid; SDA, stearidonic acid; SDASOY, oil derived from soybeans genetically modified to produce relatively high concentrations of SDA; VLC, very long-chain.

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1. Introduction

Findings from numerous epidemiologic studies and randomized controlled trials in humans have provided convincing evidence that high intakes of very long-chain (VLC; i.e. >20 C) n-3 polyunsaturated fatty acids (PUFAs), primarily eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3), are beneficial in the context of lowering cardiovascular disease morbidity and mortality (Walker et al., 2013; Maehre et al., 2015). Dietary VLC n-3 supplementation may also improve inflammatory conditions, such as rheumatoid arthritis (Calder, 2006; Spite, 2013), cognitive function (Flock et al., 2013; Huhn et al., 2015), and visual acuity of preterm children (Molloy et al., 2012). Other conditions in which patients may benefit from the consumption of VLC n-3 PUFAs were recently reviewed by Fares et al. (2014).

The VLC n-3 PUFA content of fish varies greatly according to the species, their body fat content, and their diet (Strobel et al., 2012). Cold water oily marine fish (e.g., herring, tuna, salmon, mackerel, and sardines) are the main sources of EPA and DHA in the human diet; however, the health risks posed by methylmercury in the flesh and oil of these species, the sustainability of global fish stocks, and the environmental effects of aquaculture – all concerns pointed out by Nesheim and Nestle (2014) – suggest that new sources of VLC n-3 PUFAs must be found to meet an increasing worldwide demand. In this regard, microalgae oils (Lenihan-Geels et al., 2013; Ryckebosch et al., 2014) and plant-based fish oil substitutes produced by transgenic technology (Venegas-Calderón et al., 2010; Mansour et al., 2014) hold promise; however, costly and timely regulatory hurdles, as well as the lack of acceptance of genetically modified (GM) foods by a segment of consumers, represent impediments of the latter to commercialization. Yet, as pointed out by Venegas-Calderón et al. (2010), many economies in the global marketplace are not antipathic toward GM foods. Moreover, an alternate way to introduce GM plant oils into the human food chain is via animal feedstuffs.

Plant sources of n-3 fatty acids typically provide only ALA. A notable exception is a small number of plant families whose oils also contain >10% stearidonic acid (SDA; 18:4 n-3) (Kuhnt et al., 2012) and, of these, the seeds of *Echium* species of the Boraginaceae family were considered to be the most useful for industrial extraction (Guil-Guerrero, 2007). In addition, transgenic soybean, canola, and linseed oils containing SDA have been recently developed (Kuhnt et al., 2014). High levels of γ -linolenic acid (GLA; 18:3 n-6), a Δ 6-desaturation product of linoleic acid (LA; 18:2 n-6) with putative health benefits, are also found in Boraginaceae and other plant families (Kuhnt et al., 2012), as well as in SDA-enriched transgenic soybeans (Eckert et al., 2006; Rymer et al., 2011; Elkin et al., 2015).

Since SDA lies beyond the initial rate-limiting Δ 6-desaturase step in the biosynthesis of ALA to DHA (Gregory et al., 2011), *Echium* oil has been shown to be more effective than ALA-containing plant oils, such as rapeseed oil, in enriching poultry meat with VLC n-3 PUFAs (Kitessa and Young, 2009). However, the breast and thigh contents of EPA, docosapentaenoic acid (DPA; 22:5 n-3), and DHA were much less than those reported in broilers fed fish oil or algal oil (e.g., see Leskanich and Noble, 1997; Gonzalez-Esquerra and Leeson, 2000; Rymer and Givens, 2006; Rymer et al., 2010).

Rymer et al. (2011) fed broilers diets containing either a conventional soybean oil (CON), fish oil, or an oil extracted from GM soybeans engineered to produce SDA (SDASOY). The latter oil contained 24% SDA, which was more than twice that in the *Echium* oil used by Kitessa and Young (2009). Although they observed the highest tissue concentrations of EPA, DPA, and DHA in breast meat, leg meat, and skin of birds fed the fish oil diet, SDASOY-fed broilers had significantly greater tissue contents of SDA, EPA, and DPA (but not DHA) compared to CON-fed birds (except for skinless breast meat). They also concluded that the birds did not convert SDA to VLC n-3 PUFAs any more efficiently than ALA.

The objectives of the present study were three-fold: (1) To confirm the findings of Rymer et al. (2011) with regard to the influence of dietary SDASOY vs. CON on broiler growth performance and meat fatty acid contents; (2) To extend the observations of Rymer et al. (2011) to adipose and liver fatty acid contents in order to gain a better understanding of the incorporation of SDA and its products into metabolically important tissues; and (3) To determine the hepatic expression of key genes whose protein products are involved in fatty acid desaturation and elongation, oxidation, or lipogenesis. Through this, we hoped to glean a more complete understanding of SDA metabolism in meat-type chickens.

2. Materials and methods

2.1. Animals, diets, and management

Sixty, one-d-old female Ross \times Heritage (Perdue) broilers were obtained from a local hatchery, wing-banded, weighed individually, and placed into Petersime wire-floored battery-brooders (76 \times 102 \times 25 cm; w \times d \times h) in groups of 10 birds per pen. Feed (mash) and water were provided in troughs. All birds were fed a corn-soybean meal-based (mash) antibiotic-free broiler starter diet (Wenger Feeds, Rheems, PA) containing 50 g/kg CON for 21 d. The proprietary basal mixture (i.e., the other 95% of the diet) consisted primarily of ground corn (519 g/kg), soybean meal (322 g/kg), and distillers grains with solubles (70 g/kg), with supplemental amino acids, vitamins and minerals, and phytase. Twenty-four h of light were provided daily throughout the experiment, while brooder temperatures were decreased from 35 °C at placement, to 32 °C, 29 °C, and 27 °C on d 7, 14, and 21, respectively. On d 21, all chicks were individually weighed, transferred into Petersime finisher batteries (74 \times 69 \times 36 cm; w \times d \times h), housed 4 birds per pen, and fed a corn-soybean meal-based (mash) antibiotic-free broiler grower diet containing 50 g/kg CON for the next 7 d (Table 1). On d 28, all of the birds were individually weighed and 40 birds were selected in order to provide a uniform (weight-wise) population for a 14-d feeding trial. The remaining 20 birds were no longer used in the study. Of the selected 40 birds, 8 were euthanized by electrical stunning and exsanguination on d

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