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Enriching milk fat with *n*−3 polyunsaturated fatty acids by supplementing grazing dairy cows with ruminally protected *Echium* oil

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ABSTRACT

Echium oil is a naturally rich source of stearidonic acid (SDA; C18:4*n*−3), an *n*−3 polyunsaturated fatty acid (*n*−3 PUFA) which is a precursor to the long chain (LC) *n*−3 polyunsaturated fatty acids (LC *n*−3 PUFA) eicosapentaenoic (EPA, C20:5*n*−3) and docosahexaenoic (DHA, C22:6*n*−3). The latter are LC *n*−3 PUFA for which there is accumulating evidence for positive cardiovascular health claims in human consumers. To determine the extent to which SDA supplementation can enrich milk fat with EPA and DHA, we supplemented 5 dairy cows on irrigated pasture with *Echium* oil for 10 d. The oil supplement was ruminally protected against biohydrogenation by using a protein–aldehyde matrix. Milk samples were collected during supplementation and 1, 2 and 30 d after supplementation ceased. *Echium* oil supplementation had no effect on levels of milk crude protein, fat, lactose and solids-not-fat. Average daily milk yield gradually declined as the period of supplementation progressed, and returned to pre-supplementation period levels after withdrawal of the supplement. The proportions of α-linolenic acid (ALA, 18:3*n*−3), SDA, EPA and total *n*−3 PUFA in milk fat increased in response to supplementation. The initial (Day 1) and final (Day 10) concentrations (in mg/l) of ALA, SDA, EPA and DPA in whole milk were 463 ± 29.2 *versus* 877 ± 63.1, 38 ± 8.6 *versus* 144 ± 12.4, 13 ± 6.2 *versus* 76 ± 9.0 and 45 ± 4.5 *versus* 65 ± 4.3, respectively. ALA, SDA, EPA and total *n*−3 PUFA concentrations increased linearly with increasing days of supplementation, while increases in DPA concentrations were curvilinear with a 3–4 d delay in their rise. DHA was not detected in milk fat. In terms of fatty acid yield/cup (i.e., 250 ml) of whole milk, enrichment of milk with EPA amounted to an increase from around 3.1–13.9 mg. As there was no change in DHA content, the long-chain *n*−3 PUFA content based on EPA alone was less than half of the cut-off point for omega-3 "source" claim (30 mg EPA + DHA per human serving) for foods in Australia. However, the total *n*−3 PUFA content of milk increased from 559 ± 41.0 to 1162 ± 82.4 mg/l. Data suggest that this oil containing SDA enriched milk with EPA, but not DHA. Dose response and large scale studies are needed to determine the optimal dietary inclusion rate and the commercial feasibility of SDA-containing oils as a means of increasing *n*−3 fatty acids in dairy cow milk.

Abbreviations: ALA, α-linolenic acid; CP, crude protein; DHA, docosahexaenoic acid; DM, dry matter; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FAME, fatty acid methyl esters; FSANZ, Food Standards Australia and New Zealand; GC, gas chromatograph; LA, linoleic acid; NHMRC, National Health and Medical Research Council; MUFA, mono-unsaturated fatty acids; PEO, ruminally protected *Echium* oil; PTO, ruminally protected tuna oil; PUFA, polyunsaturated fatty acids; SDA, stearidonic acid; SFA, saturated fatty acids.

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1. Introduction

Cardiovascular diseases (CVD) are one of the major sources of morbidity and mortality in humans (Mitka, 2004; Yach et al., 2004). A number of eminent international bodies such as the World Health Organisation (WHO) and The International Society for Study of Fatty Acid and Lipids (ISSFAL), as well as several national heart foundations and associations recommend that people consume long chain (≥C20) *n*−3 polyunsaturated fatty acids (LC *n*−3 PUFA) to reduce CVD risk. In most cases these recommendations specifically advise consumption of LC *n*−3 PUFA, eicosapentaenoic acid (EPA, C20:5*n*−3) and docosahexaenoic acid (DHA, C22:6*n*−3). These fatty acids are commonly sourced from oily fish and seafood. In Australia, the Suggested Dietary Targets (SDT) for adult men and women are 610 and 430 mg of EPA plus DHA/d (NHMRC, 2006). The average value is consistent with the American Heart Association's recommended value of 500 mg of EPA plus DHA/d (Kris-Etherton et al., 2002). Such recommendations of consumption of LC *n*−3 PUFA for cardiovascular health, and additional evidence for the roles of LC *n*−3 PUFA in modulation of inflammatory disorders (Calder, 2006), mood disorders (Colangelo et al., 2009; Lucas et al., 2009) and positive effects on visual acuity and cognition early (Jacobson et al., 2008) and late (Connor and Connor, 2007) in life, have resulted in sustained growth of consumer demand for LC *n*−3 PUFA ingredients over the last decade (Bimbo, 2009). It has been recognised for some time now that this continuing increased consumer demand for LC *n*−3 PUFA may not be matched by the diminishing supply of wild catch fish due to the deterioration of the marine ecosystems (Worm et al., 2006a,b). In their recent review, Brunner et al. (2009) called for urgent policy action to address this dichotomy between increasing human demand for fish and seafood, and the declining marine ecosystem health.

The search for alternative/complementary sources of LC *n*−3 PUFA through land plant biotechnology has ensued in earnest, and groups from Australia (Robert et al., 2005), the USA (Damude and Kinney, 2007) and Canada (Truksa et al., 2009) have reported on the feasibility of production of LC *n*−3 PUFA in substantive quantities in land plants. These approaches, which are based on genetic engineering of oilseed plants, remove the likelihood of heavy metal poisoning of humans potentially associated with fish consumption, especially prenatal exposure in high fish diet populations (Dewailly et al., 2008). While this genetic modification option points to a safe (from methyl mercury poisoning), sustainable, land-based LC *n*−3 PUFA supply over the long term, wide scale development and potential use is yet to overcome agronomic and regulatory issues. In a previous article (Kitessa and Young, 2009), we showed the merits of using precursors of EPA and DHA from vegetable oils in livestock feed in production of animal derived foods with enhanced levels of EPA and DHA.

Alpha linolenic acid (ALA, C18:3*n*−3), which is abundantly found in linseed oil, and stearidonic acid (SDA, C18:4*n*−3), which constitutes 120–140 g/l of *Echium* oil, are on the same biosynthetic pathway as EPA and DHA, and can act as precursors for the latter two. ALA is very inefficiently converted to EPA and DHA, and there are serious reservations about use of increased consumption of ALA for CVD risk reduction (Burdge and Calder, 2006). Evidence for the role of SDA in CVD risk reduction, and other chronic diseases, is at an emerging stage, probably due to the lack of naturally rich sources of SDA. A recent critical review by Whelan (2009), which focused on direct comparison of the biological activities of SDA with other dietary *n*−3 PUFA, concluded that "SDA could become a prominent surrogate for EPA in the commercial development of foods fortified with *n*−3 PUFA." In a recent publication (Kitessa and Young, 2009), we demonstrated that, per unit of supplemental oil, an oil containing ALA and SDA (*Echium* oil) was more effective in enhancing the EPA content of thigh muscle in broiler chickens than that containing ALA alone (i.e., rapeseed oil). In that study, we also observed that, while *Echium* oil supplementation improved the mg EPA and DPA (docosapentaenoic acid, C22:5*n*−3) per human serving in both thigh and breast muscles of chicken, only the change in the thigh muscle (i.e., 6.0 mg with ALA diet *versus* 19.9 mg with SDA diet/100 g muscle) was nutritionally meaningful. The difference in the comparative levels in breast muscle from rapeseed and *Echium* oil supplemented groups, at 0.6 and 3.2 mg/100 g muscle respectively, is of little nutritional consequence. This brought into question the cost effectiveness of using *Echium* oil in poultry diets where only half the product will have meaningful amounts of LC *n*−3 PUFA per human serving. In contrast, using milk as the vehicle for enhanced supply of *n*−3 PUFA to humans does not pose such a problem.

The current study was initiated to determine the degree to which milk can be enriched with LC *n*−3 PUFA using *Echium* oil as a supplement in dairy cows grazing irrigated pasture and if such enrichment can be achieved without a detrimental effect on milk yield and composition.

2. Materials and methods

All animal handling, feeding, and sampling procedures were approved by the Common Wealth Scientific and Industrial Research Organisation's Animal Ethics Committee according to guidelines of the National Health and Medical Research Council (NHMRC) for ethical care and handling of animals under experimental conditions (NHMRC, 2004).

2.1. Supplement preparation and ingredients

The ruminally protected supplement was produced following the method (Scott et al., 1971) used in a previous study (Kitessa et al., 2004). Briefly, a protein–oil emulsion was produced by mixing *Echium* oil and casein in 1 kg batches in a Gifford-Wood colloid mill (Model W200 79, Fallsdell Machinery Pty Ltd., Condell Park, NSW, Australia). The emulsion was treated with formaldehyde and dried using a bench top Fluid Bed Dryer (Extech Equipment Pty. Ltd., Boronia, VIC, Australia). Dried material was pulverised using a household blender and stored at −20 °C until the beginning of the feeding experiment. Two days before the start of feeding the ruminally protected *Echium* oil powder was mixed with vitamin E (3000 IU/kg