British Journal of Nutrition (2009), **101**, 709–715
doi:10.1017/S0007114508030742

Echium oil is better than rapeseed oil in enriching poultry meat with *n*-3 polyunsaturated fatty acids, including eicosapentaenoic acid and docosapentaenoic acid

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(Received 28 March 2008 – Revised 2 June 2008 – Accepted 3 June 2008 – First published online 11 July 2008)

α-Linolenic acid (ALA; 18 : 3*n*-3) and stearidonic acid (SDA; 18 : 4*n*-3) are on the biosynthetic pathway of EPA (20 : 5*n*-3) and DHA (22 : 6*n*-3). The *n*-3 fatty acid in rapeseed oil is ALA while Echium oil contains both ALA and SDA. To determine the comparative efficacy of ALA- and SDA-rich oils in enriching broiler meat with *n*-3 PUFA, we offered diets supplemented with rapeseed oil (rapeseed group) or Echium oil (Echium group) for 35 d to two groups of chicks (age 21 d). There were no differences in carcass weight (2·20 (SEM 0·06) *v.* 2·23 (SEM 0·05) kg), boned, skinless thigh muscle (494 (SEM 20·5) *v.* 507 (SEM 16·7) g), boned, skinless breast muscle (553 (SEM 13·4) *v.* 546 (SEM 11·6) g) or organ weights (heart, liver and gizzard) between the two groups. The total intramuscular fat (IMF) percentage of thigh (8·0 (SEM 0·64) *v.* 8·1 (SEM 0·62) %) and breast muscles (2·3 (SEM 0·24) *v.* 2·0 (SEM 0·19) %) were also similar between the groups. In contrast, the concentrations of most of the individual *n*-3 fatty acids (ALA, SDA, EPA and docosapentaenoic acid) were all higher in the Echium than the rapeseed group ($P<0·05$). However, differences in DHA concentrations were significant in breast but not thigh muscle IMF. The total *n*-3 yields/100 g serve thigh muscle were 265 and 676 mg for the rapeseed and Echium groups, respectively ($P<0·0001$). The corresponding values for equivalent breast muscles were 70 and 137 mg, respectively ($P<0·01$). We conclude that Echium oil is a better lipid supplement than rapeseed oil in changing the concentration and yield of *n*-3 fatty acids, except DHA, in broiler meat.

Echium oil: Stearidonic acid: Rapeseed oil: Poultry meat: *n*-3 Polyunsaturated fatty acids

Cardiovascular diseases are one of the major sources of morbidity and mortality across the globe[1,2]. It is now widely accepted that dietary long-chain ($\geq C_{20}$) *n*-3 PUFA (*n*-3 LC-PUFA) play a significant role in minimising the risk of CVD[3,4]. The American Heart Association recommends an average intake of about 500 mg EPA and DHA/d for CVD risk reduction[5]. The estimated population average daily *n*-3 LC-PUFA intake in 1995 in Australia, at 246 mg/d, was less than half of this target, especially considering 71 mg of that estimated intake was from docosapentaenoic acid (DPA)[6], which is not included in the American Heart Association figure. Fish and seafood are the richest sources of EPA and DHA[7], but there are significant amounts of *n*-3 PUFA in pasture-fed livestock products[6]. From Australia's 1995 National Nutrition Survey, Howe *et al.* [6] estimated that 43 % of the *n*-3 LC-PUFA consumed by adults originated from meat, poultry and game while 48 % originated from fish and seafood.

Meat is a major component of the Western-style diet. For instance, York & Gossard[8] estimated the annual per capita meat and fish consumptions for Australia at 110 and 18 kg, respectively. A survey of European diets indicated meat and meat products provide 21 % of the total fat intake[9]. The trend in developing nations is also for increased meat consumption with increasing affluence[10]. Myers & Kent[10] estimated that between 1997 and 2020 developing countries as a whole would increase their demand for meat by 92 % and that the great bulk of this increase would be to serve new consumers. The estimates for poultry meat consumption growth rate in Organisation for Economic Co-operation and Development (OECD) and non-OECD countries between 2003 and 2013 is 1·7 and 2·5 % per annum, respectively[11]. In the UK, poultry meat accounts for 41 % of the consumption of meat and meat products[12].

It can be reasonably argued therefore that increasing the *n*-3 LC-PUFA content of meat needs to be part of the global strategy to minimise the impact of chronic diseases. In previous studies, we have shown that the concentration of EPA and DHA in lamb can be tripled by supplementing lambs with protected tuna oil[13]. Similarly, various authors have shown that poultry meat can also be enriched with EPA and DHA by supplementing their diets with marine-based *n*-3 LC-PUFA sources[12]. However, the use of seafood products and by-products in livestock feed will not be a sustainable strategy if the

Abbreviations: ALA, α-linolenic acid; CSIRO, Commonwealth Scientific and Industrial Research Organisation; DPA, docosapentaenoic acid; *n*-3 LC-PUFA, *n*-3 long-chain PUFA; SDA, stearidonic acid.

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current rates of harvest remain. Worm *et al.* [14] predicted that seafood resources would face total collapse mid this century. Although their drastic predictions have been challenged[15], it is generally accepted that increasing demand for aquatic feed and human consumption has brought into question the sustainability of capture fisheries[16]. In recognition of this future imbalance in supply and demand for *n*-3 LC-PUFA, some authors advocate the production of *n*-3 LC-PUFA in land plants through plant biotechnology[17,18]. Some early results in enriching plants with *n*-3 PUFA through transgenesis have been reported in *Arabidopsis* [19], soyabean[20] and rapeseed[17]. In the short term though, existing vegetable oils offer some scope for enriching meat with *n*-3 PUFA as most of them contain α-linolenic acid (ALA; 18 : 3*n*-3). Many studies have considered the quantitative conversion of dietary ALA to *n*-3 LC-PUFA but the most reliable estimates of these conversions in man come from stable-isotope tracer studies[21–24]. These tracer studies have considered overall[21–24] conversion as well as compartmental analysis[23] and modelling[24] to quantify conversion into *n*-3 LC-PUFA. It was estimated that up to 60 % of dietary ALA in man is oxidised to carbon dioxide[21], with the majority of the remainder serving as acetate precursor for synthesis of saturates and monounsaturates[21], which left very little to be stored as ALA or conversion to EPA and DHA. The estimates for the percentage of dietary ALA that is converted to EPA and DHA is 7 and <1 %, respectively[24]. Plourde & Cunnane[25] put the estimated ALA conversion to DHA in man at less than 0·5 %. This low-level conversion is also supported by the in-depth compartmental analysis reported by Pawlosky *et al.* [23], which showed that only about 0·2 % of the plasma ALA in healthy individuals was destined for synthesis of EPA. Hence, there seems to be very limited opportunity for using ALA-containing oils to influence EPA and DHA levels in tissues. For health outcomes from dietary ALA itself, there is a comprehensive recent review by Burdge & Calder[26], which concluded that from the perspectives of both conversions to *n*-3 LC-PUFA and amelioration of cardiovascular risk factors, there was very little evidence for arguing for increased consumption of ALA.

Stearidonic acid (SDA) or octadecatetraenoic acid (18 : 4*n*-3) is in the same family of *n*-3 PUFA as ALA, EPA, DPA and DHA. For more background on the metabolism and nutritional importance of SDA, the reader is referred to a very recent review by Guil-Guerrero[27]. In the present study our focus was on the relative efficiency of ALA- and SDA-containing oils in enriching poultry meat with *n*-3 LC-PUFA. Since SDA lies in a more advanced position than ALA in the *n*-3 biosynthetic pathway, there is a justifiable ground for hypothesising that oils containing this fatty acid would be better precursors for EPA and DHA than ALA-containing oils. Recently, Miller *et al.* [28] reported results of a study where salmon were supplemented with oil extracted from *Echium plantagineum*, which has common names in Australian including Patterson's Curse, Salvation Jane, Riverina Bluebell, Blue Weed and Purple Bugloss. It is a broadleaf temperate pasture weed introduced into Australia from Europe during the mid-19th century. Miller *et al.* [28] showed that salmon parr fed on Echium oil-supplemented diets had more EPA and DHA than those fed on rapeseed oil-supplemented feeds. Furthermore, the authors noted that the amounts (mg/g) of EPA and DHA in the Echium-supplemented groups were comparable with those supplemented with fish oil. We investigated if these findings would hold true in domestic livestock, and help us develop animal products as the vehicle for supply of *n*-3 LC-PUFA. Therefore, the aim of the present study was to compare the level of enrichment of broiler meat with *n*-3 PUFA, including the health-benefiting *n*-3 LC-PUFA, when broilers were supplemented with rapeseed or Echium oil.

Materials and methods

All the animal housing, feeding and sampling procedures undertaken in the present experiment were approved by the Commonwealth Scientific and Industrial Research Organisation's (CSIRO's) Animal Ethics Committee according to the guidelines set out by the National Health and Medical Research Council for the ethical care and handling of animals under experimental conditions[29].

Animals, diet and design of experiment

We obtained twenty-four broilers (age 21 d) of mixed sex from a commercial poultry farm in Western Australia (Ingham Enterprises Pty Ltd, Perth, Australia). The broilers were randomly allocated into two groups of twelve chicks, balanced for sex and live weight. We had two blocks of twelve pens in our facility and we randomly assigned six chicks from each treatment group to individual pens in each block. Our experimental design was a randomised complete block design with two treatments, two blocks and six chicks per block (*n* 24). The pen dimensions for each animal were 40 cm high by 45 cm wide and 50 cm deep, which was equivalent to 0·09 m^3 per bird (three times that provided in commercial farms). Each pen had two separate bowls for food and water secured to the gate from the inside. The pens were designed to allow air flow on all sides except at the base, which was solid and covered with sawdust. Fresh sawdust was provided on Mondays and Thursdays when the chicks were removed for weighing. Each broiler received a weighed amount of feed at 08.00 hours after the residue from the previous day was removed and weighed. The feeding plan used was adopted from Ingham Enterprises Pty Ltd, Perth, Australia. The recommended feed offer/bird per d is shown in Fig. 1. Ingredient and lipid compositions of the diets are shown in Table 1. The treatment diets only differed in the type of vegetable oil added (Table 1). The Echium oil used in the present study was supplied by Croda Australia (product name Crossential SA14; product code SR03959/SAMP; batch 0000154257; Croda Australia, Villawood, NSW, Australia). Rapeseed oil was obtained from the local supermarket in Perth (WA, Australia). All animals were weighed twice per week on Mondays and Thursdays.

Slaughter and carcass sampling

All broilers were slaughtered when they reached the age of 56 d, as per commercial farm practices in the region. Each bird was de-feathered in an automated plucking device, the internal organs removed, and total carcass yield weighed. The thigh and breast muscles were weighed separately after de-boning, and samples were taken from each muscle for

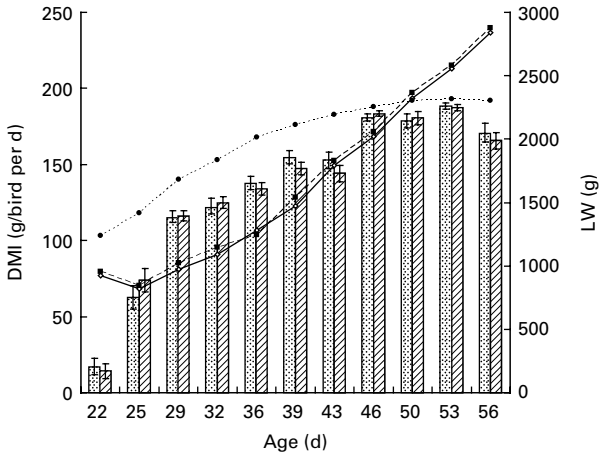

Fig. 1. Daily DM intake (DMI) and live weight (LW) of broilers offered finisher rations supplemented with rapeseed or Echium oil. (▨), DMI of rapeseed oil-supplemented birds; (▩), DMI of Echium oil-supplemented birds; (–◇–), LW of rapeseed oil-supplemented birds; (--■--), LW of Echium oil-supplemented birds; (---●---), recommended feed offer used by Ingham Enterprises Pty Ltd, Perth, Australia. Values are means, with standard deviations represented by vertical bars.

total intramuscular fat and fatty acid analysis. The heart, liver and gizzard of each animal were also weighed separately.

Lipid analysis

The total intramuscular fat in each muscle sample was determined using a SoxTec™ 2050 auto-extraction unit following hydrolysis with a SoxCap™ 2047 according to Foss application note AN 3904 (Foss Pacific Pty Ltd, Sydney, Australia). Briefly, each sample was hydrolysed for 1 h in 4 M-HCl then rinsed with water until the pH was 6·5–7·0. It was then dried in a microwave oven for 15 min or until a constant weight was achieved. The fat was then extracted with petroleum ether on a SoxTec™ 2050 using a boiling time of 30 min, rinsing time of 45 min, evaporation time of 10 min and pre-drying time of 1 min. The extraction cups containing the fat were then dried in an oven at 103°C for 30 min or until constant weight was reached.

Table 1. Ingredients, total fat content and fatty acid composition of treatment diets

	Treatment diets	
	Rapeseed oil	Echium oil
Ingredients (kg/100 kg)		
Wheat	42·9	42·9
Barley	27·0	27·0
Soyabean meal	11·0	11·0
Limesand	1·80	1·80
Dicalcium phosphate	1·00	1·00
Salt	0·50	0·50
Lupin kernel	12·0	12·0
Lysine	0·40	0·40
Methionine	0·30	0·30
Rapeseed oil	3·00	–
SDA-rich oil	–	3·00
Lipid composition		
Total fat (g/100 g DM)	5·30	5·00
Fatty acids (% total fatty acids)		
16 : 0	11·4	12·1
18 : 0	2·17	2·74
18 : 1*n*-9 (oleic)	38·7	18·1
18 : 2*n*-6 (LA)	38·7	36·6
18 : 3*n*-6 (GLA)	0·25	5·20
18 : 3*n*-3 (ALA)	5·94	17·0
18 : 4*n*-3 (SDA)	0·00	6·30
Total SFA	15·0	15·9
Total *n*-3	5·98	23·4
Total *n*-6	39·1	41·9

SDA, stearidonic acid; LA, linoleic acid; GLA, γ-linolenic acid; ALA, α-linolenic acid.

Fatty acid methyl esters were prepared by published methods[14]. Briefly, lipid was extracted using chloroform–methanol (2 : 1, v/v). The lipid-containing fraction was recovered and evaporated to dryness under N_2. Fatty acid methyl esters were prepared using 1 % H_2SO_4 in methanol on a dry block at 50–60°C for 1·5 h, and were extracted into petroleum ether and transferred to a GC vial for analysis. The gas chromatograph was a Perkin-Elmer Autosystem GC (PerkinElmer Life and Analytical Sciences Pty Ltd, Melbourne, Vic, Australia) fitted with a flame ionization detector and a split injector, and BPX70 column (SGE Analytical Science Pty Ltd, Melbourne, Vic, Australia). We created a 110 m capillary column by connecting 60 m and 50 m BPX70 columns both with internal diameters of 0·32 mm and film thicknesses of 0·25 μm. The carrier gas was He. The injector and detector temperatures were 210 and 250°C, respectively. The initial oven temperature was 150°C and it was ramped up at 1°C/min up to 223°C, when the rate was changed to 45°C/min to 250°C which was held for 13·96 min. The internal standard used during extraction and the standard fatty acid methyl esters mixture used for peak identifications were sourced from Sigma-Aldrich Pty Ltd. (Sydney, NSW, Australia). Fatty acid methyl ester standards were a C_4–C_{24} mix (Supelco product no. 18 919; Sigma-Aldrich Pty Ltd), methyl all-*cis*-7,10,13,16,19-docosapentanoate (Sigma product no. D5679; Sigma-Aldrich Pty Ltd) and methyl stearidonate (Fluka product no. 43 959; Sigma-Aldrich Pty Ltd). The internal standard used with each extraction was tridecanoic acid (Sigma product no. T0502; Sigma-Aldrich Pty Ltd).

Statistical analysis

All data analyses were performed by using ANOVA for a randomised complete block design[30] with two treatments, two blocks and six observations per block. The statistical software used was GenStat® version 4.2 (Lawes Agricultural Trust, Rothamsted, Herts, UK)[31].

Results

Intake, live-weight gain, carcass yield and organ weights

Daily DM intake and live-weight gain of the two groups of broilers were nearly identical throughout the 5-week feeding period (Fig. 1). Under both treatments the broilers adapted to their feed within week 1 and were consuming more than 80 % of the recommended feed offer after week 1 (Fig. 1). The similarity in daily DM intake was also reflected in the similarity in the rate of live-weight change and final live weight achieved under both treatments (data not shown). This was also true of the comparative magnitudes of boned muscle yield and organ weights (Table 2).

Table 2. Boned muscle yield and organ weights of broilers supplemented with rapeseed or Echium oil*

(Means values and standard errors of difference)

Tissue/organ	Rapeseed oil	Echium oil	SED
Boned thigh muscle (g)	494	507	19
Boned breast muscle (g)	553	546	13
Heart (g)	13·8	13·4	0·4
Liver (g)	45·8	43·2	1·1
Gizzard (g)	32·0	29·7	1·0

* None of the differences were significant at $P<0·05$.

Fatty acid composition of muscles

In thigh muscle, Echium oil supplementation resulted in a significantly greater increase in concentration of all *n*-3 fatty acids, except DHA (0·32 *v.* 0·37 %; $P>0·05$; Table 3). The contrast between the two diets was similar for breast muscle except that the difference between the treatments in DHA concentration was statistically significant ($P<0·01$). In both thigh and breast muscles we found traces of SDA (0·21 and 0·10 %, respectively) in birds that were in the rapeseed oil treatment groups. Among all *n*-3 PUFA, the biggest difference between the rapeseed- and Echium oil-supplemented birds was in SDA concentration (0·21 *v.* 1·69 % for thigh and 0·10 *v.* 1·45 % for breast muscle, respectively). The total *n*-3 concentration of both muscles more than doubled when the birds were supplemented with Echium oil compared with rapeseed oil. The content of EPA plus DHA in the thigh muscle from Echium oil-fed birds was 53 % higher than those fed rapeseed oil (49 *v.* 32 mg/100 g). The *n*-6 : *n*-3 ratio in muscle from Echium oil-supplemented groups was less than half that from birds supplemented with rapeseed oil. Echium oil supplementation did not alter the total SFA from that observed with rapeseed oil supplementation. The same was true of the concentration of total *n*-6 PUFA despite the nearly 10-fold increase in γ-linolenic acid (18 : 3*n*-6) in both muscles (Table 3).

Intramuscular fat and yield of selected fatty acids in thigh and breast muscle

Table 4 shows the fatty acid yield based on the total intramuscular fat extracted (TAG and phospholipids) from a sample from each animal against the percentage of each fatty acid in that animal. There was more than twice as much intramuscular fat in thigh muscle than in breast muscle (Table 4). In terms of individual fatty acid yields (100 g × intramuscular fat % × fatty acid %), the use of Echium oil was more effective in enriching both muscles with *n*-3 PUFA. The magnitude of difference between the two treatments for thigh muscle ranged from almost two-fold with DPA (47 *v.* 85 mg/100 g) to almost 40-fold for SDA (4·1 *v.* 141 mg/100 g) (Table 4). Consequently, the total *n*-3 PUFA yield/100 g muscle in Echium-supplemented birds was more than twice than in those supplemented with rapeseed oil. There was no treatment difference in the DHA yield/100 g muscle in either muscle tissue. This was also true of the total SFA yield and total *n*-6 PUFA yield (Table 4).

Discussion

For both the rapeseed and Echium oil-fed groups, the mean final live weight was close to the 3·0 kg industry target in the region, where tallow is the most common lipid supplement.

Table 3. Fatty acid composition (g/100 g total fatty acids) of muscle tissues of broilers supplemented with rapeseed or Echium oil

(Means values and standard errors of difference)

	Thigh IMF			Breast IMF		
Fatty acid	Rapeseed	Echium	SED	Rapeseed	Echium	SED
12 : 0	0·11	0·09	0·009	nd	nd	–
14 : 0	0·53	0·57	0·015	0·46	0·50	0·07
16 : 0	23·1	24·0	0·382	23·9	25·7	0·93
16 : 1	4·79	4·61	0·311	4·88	4·66	0·26
18 : 0	7·31	7·45	0·293	5·92	7·22*	0·34
18 : 1*n*-9	38·3	30·7***	0·58	43·7	36·0***	0·60
18 : 2*n*-6 (LA)	17·8	17·1	0·474	16·6	17·3	0·58
18 : 3*n*-6 (GLA)	0·13	1·54***	0·067	0·19	1·44***	0·093
18 : 3*n*-3 (ALA)	2·33	6·50***	0·219	2·84	6·40***	0·317
18 : 4*n*-3 (SDA)	0·21	1·69***	0·076	0·10	1·45***	0·081
20 : 3*n*-6	0·31	0·56***	0·025	0·08	0·29***	0·022
20 : 4*n*-6 (AA)	2·14	1·50*	0·181	0·30	0·48***	0·037
20 : 5*n*-3 (EPA)	0·07	0·24***	0·008	0·03	0·17***	0·016
22 : 1*n*-13	0·23	0·60**	0·035	0·02	0·30***	0·014
22 : 5*n*-3 (DPA)	0·57	1·09***	0·068	0·12	0·33***	0·025
22 : 6*n*-3 (DHA)	0·32	0·37	0·033	0·03	0·08**	0·012
Total SFA	31·0	32·1	0·70	30·0	33·1	1·2
Total *n*-3	3·35	9·89***	0·35	3·12	8·43**	0·56
Total *n*-6	20·5	20·8	0·81	17·11	19·50	0·73

IMF, intramuscular fat; nd, not detected; LA, linoleic acid; GLA, γ-linolenic acid; ALA, α-linolenic acid; SDA, stearidonic acid; AA, arachidonic acid; DPA, docosapentaenoic acid.

Mean value was significantly different (within a muscle tissue) from that of the rapeseed oil-supplemented birds: *$P<0·05$, **$P<0·01$, ***$P<0·001$.

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Table 4. Total intramuscular fat (IMF) and fatty acid yield of thigh and breast muscle from broilers fed rations with rapeseed or Echium oil as a lipid supplement

(Means values and standard errors of difference)

	Thigh muscle			Breast muscle		
	Rapeseed	Echium	SED	Rapeseed	Echium	SED
IMF (g/100 g)	8·0	8·1	0·63	2·3	2·0	0·17
Fatty acid yield (mg/100 g)						
14 : 0	42	46	3·8	5·2	4·1	1·67
16 : 0	1871	1939	152	553	504	48
16 : 1	388	377	46	112	91	10
18 : 0	594	592	47	137	141	13
18 : 1*n*-9 (oleic)	3057	2467*	195	1008	745*	95
18 : 2*n*-6 (LA)	1415	1396	118	382	343	35
18 : 3*n*-6 (GLA)	8·3	129***	9·7	4·4	29***	2·5
18 : 3*n*-3 (ALA)	185	542***	48	66	126***	11
18 : 4*n*-3 (SDA)	4·1	141***	73	2·8	29***	2·9
20 : 4*n*-6 (AA)	178	117*	18	6·9	9·5	1·08
20 : 5*n*-3 (EPA)	6·0	19·9***	1·25	0·6	3·2***	0·39
22 : 5*n*-3 (DPA)	47	85***	6·7	2·8	6·4**	0·53
22 : 6*n*-3 (DHA)	26	29	3·35	0·9	1·6	0·28
EPA + DHA	32	49*	4·23	1·5	4·8**	0·58
Total SFA	2506	2578	197	696	649	62
Total *n*-3	265	676***	48	70	137**	12
Total *n*-6	1618	1557	135	391	358	37

LA, linoleic acid; GLA, γ-linolenic acid; ALA, α-linolenic acid; SDA, stearidonic acid; AA, arachidonic acid; DPA, docosapentaenoic acid.

Mean value was significantly different (within a muscle tissue) from that of the rapeseed oil-supplemented birds: *$P<0·05$, **$P<0·01$, ***$P<0·001$.

Hence both supplements showed no adverse growth performance or carcass yield outcome. The present results clearly demonstrated that Echium oil was more effective than rapeseed oil in increasing EPA. The results also showed that although the improvements in *n*-3 LC-PUFA in breast muscle were statistically significant, the absolute changes (mg/100 g) were of little, if any, nutritional significance. Therefore, we will focus our discussion to the contrasts in enrichment of thigh muscles between the treatment groups. The total *n*-3 PUFA in the Echium group was more than double that in the rapeseed group (265 *v.* 676 mg/100 g), but the change in EPA plus DHA was less pronounced (32 *v.* 49 mg/100 g), because of lack of change in DHA. In relation to the recommended daily intake, 100 g of thigh muscle from the rapeseed and Echium groups provided approximately 6 and 10 %, respectively, of the recommended 500 mg EPA plus DHA[5]. It is worthwhile noting that the thigh muscle EPA and DHA content from the Echium group (49 mg/100 g serve) will meet the 'source' category of claims for *n*-3 LC-PUFA content of foods under standards set by Food Standards Australia and New Zealand[32]. The label claim categories for 'source' and 'good source' reflect the degree to which a meal contributes to the consumer achieving daily values for a nutrient[32]. Food Standards Australia and New Zealand's cut off points for 'source' and 'good source' claims for *n*-3 LC-PUFA are 30 and 60 mg/serve, respectively. Under the US Food and Drug Administration guidelines meals which provide 10–19 % of the recommended daily intake (for example, 500 mg for *n*-3 LC-PUFA) can be called a 'good source', while those which provide 20 % or more can be called 'high', 'rich in' or 'excellent source'[33]. In addition to enabling product differentiation, the 17 % increase in EPA plus DHA achieved by using Echium oil instead of rapeseed oil would be a nutritionally significant improvement as part of a normal diet, and we contend that both supplements would produce chicken meat with a better *n*-3 PUFA profile than that produced locally where tallow is the most prevalent lipid supplement in large commercial operations. The enrichment with ALA achieved using Echium oil, at 540 mg/100 g muscle, takes the thigh muscle tissues within 30 % of the 1·5 to 3 g/d recommended ALA intake[5].

The lack of substantiation of epidemiological claims for the health benefits of ALA[34] with direct cause–effect relationship between ALA intake and cardiovascular risk[26], and the emerging nature of evidence for the benefits of SDA[27,35], forces us to look at the value of vegetable oils in terms of their contribution to *n*-3 LC-PUFA synthesis. Furthermore, current dietary strategies aimed at increasing the *n*-3 PUFA content of human diets are targeted at the marine-based LC-PUFA – EPA and DHA[5]. Echium oil supplementation to chickens did not significantly improve the content of DHA (mg/100 g muscle) in either muscle any more than rapeseed oil did. Since Echium oil contained about 14 % SDA, which is one step closer to DHA than ALA, we anticipated that SDA-supplemented birds would contain more DHA in their muscle than ALA-supplemented birds. The present results do not concur with the observations of Miller *et al.* [28] on salmon, where SDA supplementation was just as effective as fish oil supplementation in increasing DHA in muscle. Harris *et al.* [36] supplemented adult male Beagle dogs with a range of doses of SDA (21, 64 and 193 mg/kg body weight) and observed significant changes in erythrocyte and heart tissue DHA at the highest dose. However, when averaged across all time points (across 12 weeks) and treatment groups the authors noted that DHA

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levels were unaffected in their study. In all cases (including the present study) there was conclusive evidence that SDA supplementation is consistent in its effectiveness in improving tissue EPA concentrations. Hence, our findings on SDA supplementation agree with previous ALA supplementation studies in chickens where it was also noted that there was a weak relationship between dietary ALA and the edible tissue content of EPA, and no relationship between dietary ALA and edible tissue DHA (for a review, see Rymer & Givens[12]).

Our diets did not include any fishmeal or other seafood by-products. Therefore, we propose that the content of EPA, DPA and DHA in all the tissues resulted from biosynthetic conversion of dietary ALA and SDA. In the past, the conversion of ALA to EPA and DHA in man was suggested to be relatively more efficient when the background diet is low in *n*-6 fatty acids[37]. Recent evidence has shown that conversion of ALA is related to the absolute amount of ALA rather than its ratio to linoleic acid[38]. Furthermore, a recent UK workshop which analysed the usefulness of the *n*-6 : *n*-3 ratio in understanding *n*-3 metabolism or addressing cardiovascular risk concluded that the focus should be on the absolute amounts rather than the ratio[39]. Therefore, we exclude the background *n*-6 : *n*-3 ratio as the cause of lack of treatment difference in DHA response to ALA or SDA supplementation.

Although SDA supplementation did not enrich broiler muscles with DHA, the enrichment achieved in terms of the SDA content itself may be of some health benefit to consumers. For instance, studies on human and rat microsomal tissues showed that SDA plays an inhibitory role in 5α-reductase activity[40], which is a prostate cancer risk biomarker. Other health claims for SDA include an anti-inflammatory role through inhibition of the 5-lipoxygenase pathway which was comparable with EPA when tested *in vitro*[41]. There are also claims for anti-thrombotic activity for SDA through competition with arachidonic acid metabolism[42]. Perhaps a stronger evidence for SDA health benefit that was based on human studies is that reported by Surette *et al.*[43]. The authors showed that Echium oil supplementation to hypertriacylglycerolaemic human subjects significantly increased plasma and neutrophil long-chain *n*-3 PUFA and decreased serum TAG by about 30 % from baseline. The caveat regarding that study is that it was a single-centre, open-label design with no placebo groups. Nonetheless, we calculated the doses used in that study to be in the order of 1875 mg SDA/d offered in fifteen capsules/d (five capsules at each meal time). Our enriched thigh muscle yielded 141 mg SDA/100 g, which is about 8 % of that dose. Hence, it remains to be determined what the magnitude of benefit in reduced cardiovascular risk factors would be from eating our enriched poultry meat. Without discounting the above caveats and considering the health benefits of SDA reported from short-term studies[27,35], we propose that Echium oil supplementation creates a poultry meat with an improved *n*-3 fatty acid profile and, importantly, *n*-3 LC-PUFA that will benefit the consumer. We also suggest that SDA-containing oils are more effective supplements for enriching poultry meat with SDA and EPA than vegetable oils that only contain ALA.

In terms of recommendation for using Echium oil in animal feed for improving the health benefits of animal-derived foods, the current price per tonne is prohibitive unless cheaper sources are made available in land crops through plant biotechnology. The Echium plant naturally found in Australia is toxic (pyrrolizidine alkaloids) to grazing livestock[44] and the Croda product is a purified 'food-grade' product intended for human consumption. In the past, plant breeders have been able to create safer varieties of crops from wild types containing toxic alkaloids (for example, lupins), but the feasibility of use of plant biotechnology from a technical standpoint has already been demonstrated in rapeseed and soyabean, and involves the insertion of Δ-6 desaturase in ALA-producing plants[17,18,20]. In contrast, producing DHA in land plants requires an assembly of a number of desaturase and elongase genes[19]. Even under the ideal situation where we have ample and cheap SDA-containing oils, there needs to be further large-scale and rigorous studies to confirm the health benefits for human consumers from SDA itself, as the conversion to DHA seems very little at best.

Conclusions

Birds fed Echium oil-supplemented rations had more tissue ALA, SDA, EPA and DPA than those fed similar rations supplemented with rapeseed oil. The total *n*-3 PUFA content of edible meat from birds supplemented with Echium oil was more than double that of those supplemented with rapeseed oil. Echium oil was more effective than rapeseed oil in changing the EPA levels in chicken meat, but the two vegetable oil sources were similar in that they both had no impact on the amount of DHA in edible tissues.

Acknowledgements

We are grateful to Mr Michael Carthew for his assistance during the feeding experiment and the slaughter of the chickens. Ingham Enterprises Pty Ltd, Perth, Australia, kindly supplied the 21-d-old chicks for this experiment. Croda Australia supplied the Echium oil used in the study. This project was wholly funded by the CSIRO Food Futures Flagship. The animal and laboratory facilities were provided by CSIRO Livestock Industries. S. M. K. developed the proposal, designed the experiment, analysed the data and drafted the manuscript. P. Y. managed the feeding experiment, conducted all the laboratory analyses and helped with the design and analysis of the data. Both authors have no conflict of interest to disclose.

References

1. Mitka M (2004) Heart disease a global health threat. *JAMA* **291**, 2533.
2. Yach D, Hawkes C, Gould CL & Hofman KJ (2004) The global burden of chronic diseases: overcoming impediments to prevention and control. *JAMA* **291**, 2616–2622.
3. Kris-Etherton PM, Harris WS & Appel LJ (2002) Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation* **106**, 2747–2757.
4. Ruxton CHS, Reed SC, Simpson MJA & Millington KJ (2007) The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. *J Hum Nutr Diet* **20**, 275–285.
5. Gebauer SK, Psota TL, Harris WS & Kris-Etherton PM (2006) *n*-3 Fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. *Am J Clin Nutr* **83**, Suppl. 6, 1526S–1535S.

6. Howe P, Meyer B, Record S & Baghurst K (2006) Dietary intake of long-chain *n*-3 polyunsaturated fatty acids: contribution of meat sources. *Nutrition* **22**, 47–53.
7. Nichols PD, Mooney B, Virtue P & Elliott N (1998) *Nutritional Value of Australian Fish: Oil, Fatty Acid and Cholesterol of Edible Species*, Final report, August 1998. FRDC Project 95/122. Report prepared for the Fisheries Research and Development Corporation. Hobart, Tasmania, Australia: Fisheries Research and Development Corporation.
8. York R & Gossard MH (2004) Cross-national meat and fish consumption: exploring the effects of modernisation and ecological context. *Ecol Econ* **48**, 293–302.
9. Hulshof KFAM, van Erp-Baart MA, Anttolainen M, *et al.* (1999) Intake of fatty acids in Western Europe with emphasis on *trans* fatty acids. *Eur J Clin Nutr* **53**, 143–157.
10. Myers N & Kent J (2003) New consumers: the influence of affluence on the environment. *Proc Natl Acad Sci U S A* **100**, 4963–4968.
11. Anonymous (2004) *OECD Agricultural Outlook 2004–2013*. Paris: OECD Publications.
12. Rymer C & Givens DI (2005) *n*-3 Fatty acid enrichment of edible tissue of poultry: a review. *Lipids* **40**, 121–130.
13. Kitessa SM, Gulati SK, Ashes JR, Scott TW & Fleck E (2001) Effect of feeding tuna oil supplement protected against hydrogenation in the rumen on growth and *n*-3 fatty acid content of lamb fat and muscle. *Anim Feed Sci Technol* **52**, 433–437.
14. Worm B, Barbier EB, Beaumont N, *et al.* (2006) Impacts of biodiversity loss on ocean ecosystem services. *Science* **314**, 787–790.
15. Worm B, Barbier EB, Beaumont N, *et al.* (2006) Response to comments on 'Impacts of biodiversity loss on ocean ecosystem services'. *Science* **316**, 1285d.
16. Food and Agriculture Organization (2006) *State of World Aquaculture: 2006*, *FAO Fisheries Technical Paper* no. 500. Rome: FAO.
17. Ursin VM (2003) Modification of plant lipids for human health: development of functional land-based omega-3 fatty acids. *J Nutr* **133**, 4271–4274.
18. Damude HG & Kinney AJ (2007) Engineering oilseed plants for a sustainable, land-based source of long chain polyunsaturated fatty acids. *Lipids* **42**, 179–185.
19. Robert SS, Singh S, Zhou XR, Petrie JR, Blackburn S, Mansour PM, Nichols PD, Liu Q & Green A (2005) Metabolic engineering of *Arabidopsis* to produce nutritionally important DHA in seed oil. *Funct Plant Biol* **32**, 473–479.
20. Sato S, Xing A, Ye X, Schweiger B, Kinney A, Graef G & Clemente T (2004) Production of γ-linolenic acid and stearidonic acid in seeds of marker-free transgenic soybean. *Crop Sci* **44**, 646–652.
21. Brenna JT (2002) Efficiency of conversion of α-linolenic acid to long chain *n*-3 fatty acids in man. *Curr Opin Clin Nutr Metab Care* **5**, 127–132.
22. Burdge GC & Calder PC (2005) α-Linolenic acid metabolism in adult humans: the effect of gender and age on conversion to longer-chain polyunsaturated fatty acids. *Eur J Lipid Sci Technol* **107**, 426–439.
23. Pawlosky RJ, Hibbeln JR, Novotny JA & Salem N Jr (2001) Physiological compartmental analysis of α-linolenic acid metabolism in adult humans. *J Lipid Res* **42**, 1257–1265.
24. Goyens PLL, Spilker ME, Zock PL, Katan MB & Mensink RP (2005) Compartmental modeling to quantify α-linolenic acid conversion after long term intake of multiple tracer boluses. *J Lipid Res* **46**, 1474–1483.
25. Plourde M & Cunnane SC (2007) Extremely limited synthesis of long chain polyunsaturates in adults: implications for their dietary essentiality and use as supplements. *Appl Physiol Nutr Metab* **32**, 619–634.
26. Burdge GC & Calder PC (2006) Dietary α-linolenic acid and health-related claims: a metabolic perspective. *Nutr Res Rev* **19**, 26–52.
27. Guil-Guerrero JL (2007) Stearidonic acid (18 : 4*n*-3): metabolism, nutritional importance, medical uses and natural sources. *Eur J Lipid Sci Technol* **109**, 1226–1236.
28. Miller MR, Nichols PD & Carter CG (2007) Replacement of dietary fish oil for Atlantic salmon parr (*Salmo salar* L.) with a stearidonic acid containing oil has no effect on omega-3 long-chain polyunsaturated fatty acid concentrations. *Comp Biochem Physiol B Biochem Mol Biol* **146**, 197–206.
29. National Health and Medical Research Council (2004) *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes*, 7th ed. Canberra: Australian Government.
30. Snedcor GW & Cochran WG (1980) *Statistical Methods*, 7th ed. Ames, IA: Iowa State University Press.
31. Lawes Agricultural Trust (2000) *GenStat® Release 4.2. Reference Manual*. Oxford, UK: VSN International Ltd.
32. Food Standards Australia New Zealand (2003) Australia New Zealand Food Standards Code. Standard 1.2.8. Nutrition Information Requirements. Canberra: Food Standards Australia New Zealand (FSANZ). http://www.foodstandards.gov.au/thecode/foodstandardscode.cfm
33. US Food and Drug Administration Center for Food Safety and Applied Nutrition (2008) Guidance for Industry. A Food Labelling Guide. http://www.cfsan.fda.gov/~dms/2lg-toc.html.
34. Brouwer IA, Katan MB & Zock PL (2004) Dietary α-linolenic acid is associated with reduced risk of fatal coronary heart disease, but increased prostate cancer risk: a meta-analysis. *J Nutr* **134**, 919–922.
35. James MJ, Ursin VM & Cleland LG (2003) Metabolism of stearidonic acid in human subjects: comparison with the metabolism of other *n*-3 fatty acids. *Am J Clin Nutr* **77**, 1140–1145.
36. Harris WS, DiRienzo MA, Sands SA, George C, Jones PG & Eapen AK (2007) Stearidonic acid increases the red blood cell and heart eicosapentaenoic acid content in dogs. *Lipids* **42**, 325–333.
37. Chan JK, McDonald BE, Gerrard JM, Bruce VM, Weaver BJ & Holub BJ (1993) Effect of dietary α-linolenic acid and its ratio to linoleic acid on platelet and plasma fatty acids and thrombogenesis. *Lipids* **28**, 811–817.
38. Goyens PLL, Spilker ME, Zock PL, Katan MB & Mensink RP (2006) Conversion of α-linolenic acid in humans is influenced by the absolute amounts of α-linolenic acid and linoleic acid in the diet and not by their ratio. *Am J Clin Nutr* **84**, 44–53.
39. Stanley JC, Elsom RL, Calder PC, Griffin BA, Harris WS, Jebb SA, Lovegrove JA, Moore CS, Riemersma RA & Sanders TAB (2007) UK Food Standards Agency workshop report: the effects of the dietary *n*-6 : *n*-3 fatty acid ratio on cardiovascular health. *Br J Nutr* **98**, 1305–1310.
40. Liang T & Liao S (1992) Inhibition of steroid 5α-reductase activity by specific aliphatic unsaturated fatty acids. *Biochem J* **285**, 557–562.
41. Guichardant M, Traitler H, Spielmann D, Sprecher H & Finot PA (1993) Stearidonic acid, an inhibitor of the 5-lipoxygenase pathway. A comparison with timnodonic and dihomo-γ-linolenic acid. *Lipids* **28**, 321–324.
42. Kockmann V, Spielmann D, Traitler H & Lagarde M (1989) Inhibitory effect of stearidonic acid (18 : 4*n*-3) on platelet aggregation and arachnidonate oxygenation. *Lipids* **24**, 1004–1007.
43. Surette ME, Edens M, Chilton FH & Tramposch KM (2004) Dietary Echium oil increases plasma and neutrophil long-chain (*n*-3) fatty acids and lowers serum triacylglycerols in hypertriglyceridemic humans. *J Nutr* **34**, 1406–1411.
44. Seaman JT, Turvey WS, Ottaway SJ, Dixon RJ & Gilmour AR (1989) Investigations into the toxicity of *Echium plantagineum* in sheep. 1. Field grazing experiments. *Aus Vet J* **66**, 279–285.

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