British Journal of Nutrition (2009), **102**, 69–81
doi:10.1017/S0007114508137874

Fatty acid metabolism (desaturation, elongation and β-oxidation) in rainbow trout fed fish oil- or linseed oil-based diets

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(Received 11 July 2008 – Revised 17 October 2008 – Accepted 20 October 2008 – First published online 5 January 2009)

In consideration of economical and environmental concerns, fish oil (FO) substitution in aquaculture is the focus of many fish nutritionists. The most stringent drawback of FO replacement in aquafeeds is the consequential modification to the final fatty acid (FA) make-up of the fish fillet. However, it is envisaged that a solution may be achieved through a better understanding of fish FA metabolism. Therefore, the present study investigated the fate of individual dietary FA in rainbow trout (*Oncorhynchus mykiss*) fed a FO-based diet (rich in 20 : 5*n*-3) or a linseed oil-based diet (LO; rich in 18 : 3*n*-3). The study demonstrated that much of the 18 : 3*n*-3 content from the LO diet was oxidised and, despite the significantly increased accretion of Δ-6 and Δ-5 desaturated FA, a 2- and 3-fold reduction in the fish body content of 20 : 5*n*-3 and 22 : 6*n*-3, respectively, compared with the FO-fed fish, was recorded. The accretion of longer-chain FA was unaffected by the dietary treatments, while there was a greater net disappearance of FA provided in dietary surplus. SFA and MUFA recorded a net accretion of FA produced *ex novo*. In the fish fed the FO diet, the majority of dietary 20 : 5*n*-3 was accumulated (53·8 %), some was oxidised (14·7 %) and a large proportion (31·6 %) was elongated and desaturated up to 22 : 6*n*-3. In the fish fed the LO diet, the majority of dietary 18 : 3*n*-3 was accumulated (58·1 %), a large proportion was oxidised (29·5 %) and a limited amount (12·4 %) was bio-converted to longer and more unsaturated homologues.

Aquaculture: Fatty acid metabolism: Fish oil replacement: Whole-body fatty-acid-balance method

n-3 Long-chain PUFA (*n*-3 LCPUFA), particularly EPA (20 : 5*n*-3) and DHA (22 : 6*n*-3), are reportedly beneficial to human health[1]. It is commonly accepted that the only readily available and edible source of *n*-3 LCPUFA for human health is fish and other seafoods[2] and, in consideration of this realisation, global fish consumption is on the rise[3]. However, present exploitation trends of wild fishery stocks are considered unsustainable by most fishery and conservation scientists[4]. Consequently, aquaculture is challenged, and optimistically expected, to fill the increasing gap between demand and supply of fish[3,5]. However, the actual aquaculture impact on world food supplies has been questioned and the dependence of aquaculture on fishery-derived products (fishmeal and fish oil (FO)) for aquafeed production is at the core of a heated global debate[6].

In this context, the situation of FO (the only widely available source of 20 : 5*n*-3 and 22 : 6*n*-3) is particularly exacerbated in consideration of increased demand, declining production and rising commodity price[3,5]. The replacement of dietary FO in aquafeeds with readily available and more economical terrestrial alternatives, such as vegetable oils and animal by-product fats, is consequently a highly investigated research topic and an approach increasingly being adopted by feed-mill companies. However, the most important and stringent drawback of FO replacement in aquafeeds is the resultant unavoidable modification to the final *n*-3 LCPUFA make-up of the fish fillet[7]. Therefore, for the production of the *n*-3 LCPUFA-rich farmed fish, a direct source of dietary *n*-3 LCPUFA is required and this, in a vicious circle, is presently derived only from wild fisheries (FO).

Fish are theoretically capable of biosynthesising 22 : 6*n*-3 via the desaturation and elongation of α-linolenic acid (18 : 3*n*-3; found in some vegetable oils). However, the lipid metabolism of fish has adapted to an abundance of dietary 22 : 6*n*-3 and, as a result of this, the capability for the effective utilisation of the *n*-3 biosynthetic capability has been rendered dormant[7].

Consequently, the fatty acid (FA) elongase and desaturase metabolism capabilities of farmed fish are attracting significant research attention[8,9], and it is envisaged that a solution to the crisis surrounding FO shortages will be realised via a better understanding of fish FA metabolism. Therefore, the aim of the present study was to investigate the FA metabolism, through the monitoring of the fate of individual dietary FA, in rainbow trout (*Oncorhynchus mykiss*) fed with a FO-based diet, rich in 20 : 5*n*-3, or a linseed oil-based diet, rich in 18 : 3*n*-3.

Abbreviations: FA, fatty acids; FO, fish oil; LCPUFA, long-chain PUFA; LO, linseed oil.

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Experimental methods

Animals, husbandry and experimental diets

Rainbow trout, *O. mykiss* (initial mean weight about 90 g), were obtained from DPI Victoria (Snob's Creek, VIC, Australia). Before the experimentation, the fish were acclimatized to the new environmental conditions for 4 weeks. The feeding experiment was conducted in a closed-loop, twelve-tank (600 litre capacity) recirculating system with a physical and biological filtration plant. The system was maintained on a 12 h light–12 h dark cycle at 12·0 ± 1·0°C. Two semi-purified experimental diets (Table 1) containing 220 g/kg lipid in the form of FO or linseed oil (LO) were formulated and prepared as described previously[10].

At the commencement of the experiment, a sample of nine fish was culled for analysis. Sixty individually weighed and measured rainbow trout were randomly distributed among six tanks (ten per tank) and assigned one of the two experimental diets (triplicate groups). The fish were fed to apparent satiation with the dietary treatments twice daily (09.00 and 16.00 hours) for a period of 72 d. In the morning following the final day of feeding, the fish were anaesthetised and faecal samples were collected from the fish by gently stripping. Following collection, faeces were freeze-dried and stored at −20°C until analysed. The following day, the fish were weighed and measured, and random samples of eighteen fish (six per tank) were euthanised for analysis.

Table 1. Ingredient and proximate composition of the experimental diets (g/kg dry diet) and the growth and feed consumption of rainbow trout reared on the different dietary treatments

(Mean values with their standard errors; *n* 3)

	Dietary treatments			
	FO		LO	
	Mean	SEM	Mean	SEM
Diet formulation (g/kg)				
Casein*	304		304	
Gelatin*	72		72	
Dextrin*	92		92	
Fishmeal†	70		70	
Defatted soyabean meal†	70		70	
Wheat flour‡	80		80	
FO†	220		–	
LO§	–		220	
Mineral and vitamin mix‖	50		50	
α-Cellulose*	40		40	
Cr_2O_3¶	2		2	
Proximate composition (g/kg)				
Moisture	3·2		3·1	
Crude protein	43·40		43·16	
Crude lipid	22·1		21·6	
Ash	4·81		4·93	
Nitrogen-free extract	26·49		27·21	
Growth parameters				
Initial weight (g)	89·1	3·05	88·4	2·92
Final weight (g)	359·2	9·19	332·2	13·25
Feed consumption (g per fish)	287·5	6·24	284·5	3·40
Weight gain (%)	304·5	20·98	275·6	5·02
SGR (% per d)**	1·4	0·05	1·4	0·01
FCR††	1·0	0·03	1·1	0·05
Whole-body lipid content (g/kg)	182·9	6·03	180·3	9·80

FO, fish oil; LO, linseed oil.
* Sigma-Aldrich, Inc., St Louis, MO, USA.
† Ridley Agriproducts – Aquafeed, QLD, Australia.
‡ Bi-Lo Pty. Ltd, Tooronga, VIC, Australia.
§ Nature First, Cheltenham, VIC, Australia.
‖ As reported previously[10].
¶ BDH Laboratory Supplies, Poole, UK.
** Specific growth rate.
†† Feed conversion ratio.

Proximate and fatty acid analysis

The nutrient composition of the experimental diets and whole-fish samples was determined by proximate composition analysis according to standard procedures described previously[10]. FA analysis was performed on triplicate subsamples of each of the experimental diets, and on three pooled whole-body samples from each of the replicates. Following the lipid extraction[11], FA were esterified into methyl esters using the acid-catalysed methylation method and analysed by GC as described in detail previously[10].

Digestibility analysis

Chromium oxide in the diets and faeces was estimated according to the method of Furukawa & Tsukahara[12]. Estimates of FA digestibility (ADC_{FA}) were calculated using a standard formula: $ADC_{FA} = 100 - (100\ (Cr_2O_3 \text{ in diet}) \div (Cr_2O_3 \text{ in faeces}) \times ((\%\ FA \text{ in faeces}) \div (\%\ FA \text{ in feed}))$. In consideration of the relatively small sample size obtained, replicate faecal samples collected from each treatment were pooled.

Whole-body fatty-acid-balance calculations

The *in vivo* assessment of FA metabolism of rainbow trout was deduced using the whole-body FA-balance method, described in detail by Turchini *et al.* [13]. Briefly, the computation of the whole-body FA-balance method is best dealt with in four steps. The first step requires that individual concentrations of FA in the diets, faeces and the initial and final carcass are expressed in mg per fish. Following this, the individual FA intake, excretion and accumulation are calculated. The difference between FA accumulation, intake and excretion results in an overall appearance or disappearance of any given FA.

The second step of the method involves the computation of the SFA and MUFA, *n*-3 and *n*-6 PUFA balances. The amount of FA represented in their respective metabolic pathway needs to be converted from milligrams to micromoles of appeared/disappeared FA per animal. Then, following a backward calculation along the FA metabolic pathways (Fig. 1), the number of micromoles of longer chain or more unsaturated FA that appeared is subtracted from the number of micromoles of the previous FA in the specific FA elongation/desaturation pathway. For instance, the mathematical model used to describe the SFA and MUFA pathways can be described by the following equations (where ε is the total specified FA converted (desaturated or elongated) and δ is the number of micromoles of the specified FA appeared or disappeared; when δ is a negative number (FA disappearance = oxidised),

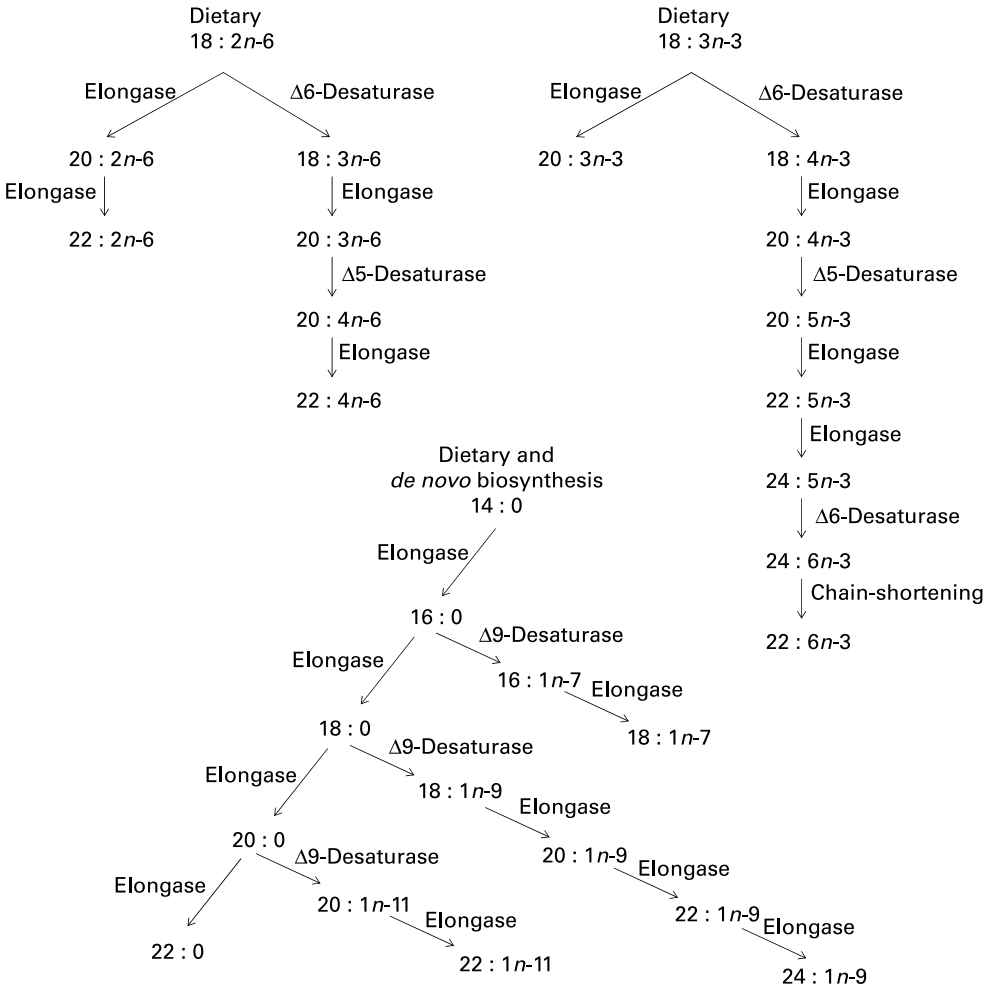

Fig. 1. A schematic of the fatty acid elongation and desaturation pathways of saturated, monounsaturated, *n*-6 and *n*-3 PUFA, modified after Turchini *et al.*[13], Nakamura & Nara[22] and Ackman & Kean-Howie[41]. Only the pathways of the fatty acids used in the present study for the computation of the whole-body fatty-acid-balance method are reported.

then $\delta = 0$ for the following computation):

$$\varepsilon(20:1n\text{-}11) = \delta(22:1n\text{-}11)$$

$$\varepsilon(20:0) = \delta(22:0) + \delta(20:1n\text{-}11) + \varepsilon(20:1n\text{-}11)$$

$$\varepsilon(22:1n\text{-}9) = \delta(24:1n\text{-}9)$$

$$\varepsilon(20:1n\text{-}9) = \delta(22:1n\text{-}9) + \varepsilon(22:1n\text{-}9)$$

$$\varepsilon(18:1n\text{-}9) = \delta(20:1n\text{-}9) + \varepsilon(20:1n\text{-}9)$$

$$\varepsilon(18:0) = \delta(20:0) + \varepsilon(20:0) + \delta(18:1n\text{-}9) + \varepsilon(18:1n\text{-}9)$$

$$\varepsilon(16:1n\text{-}7) = \delta(18:1n\text{-}7)$$

$$\varepsilon(16:0) = \delta(18:0) + \varepsilon(18:0) + \delta(16:1n\text{-}7) + \varepsilon(16:1n\text{-}7)$$

$$\varepsilon(14:0) = \delta(16:0) + \varepsilon(16:0).$$

Detailed descriptions of the models for the whole-body FA-balance computations for the *n*-3 and *n*-6 biosynthetic pathways have been described previously[13,14] and a schematic of the three pathways is shown in Fig. 1.

At this point (the third step of the method), it is possible to quantify the amount of each individual FA (μmol of FA per gram of fish per day) that has been bio-converted (i.e. elongated and/or desaturated) and the net FA appearance/disappearance. If after the backward computations along each of the possible pathways an appearance of 10 μmol of 18 : 1*n*-9 was recorded (quantity given solely as an example), an accretion of 10 μmol of Δ-9 desaturated 18 : 0 would be considered. Similarly, if an appearance of 5 μmol of 20 : 0 was recorded, an accretion of 5 μmol of elongated 18 : 0 would be considered. Likewise, if after all the backward computations along each of the possible pathways a reduction of 15 μmol of 18 : 0 was recorded, a net disappearance of 15 μmol of 18 : 0 would be considered. The *ex novo* production was estimated by the total appearance of 14 : 0 after all the backward computations along all possible pathways were calculated. The fate of individual FA can also be calculated as a percentage of total FA net intake plus total FA *ex novo* production

using the following equations:

$$\%\ \textit{Ex novo}\ \text{production} = (\textit{Ex novo}\ \text{production}) \times (\text{Net intake} + \textit{Ex novo}\ \text{production})^{-1} \times 100$$

$$\%\ \text{Body accumulation} = (\text{Body accumulation}) \times (\text{Net intake} + \textit{Ex novo}\ \text{production})^{-1} \times 100$$

$$\%\ \text{Bio-conversion} = (\text{Bio-conversion}) \times (\text{Net intake} + \textit{Ex novo}\ \text{production})^{-1} \times 100$$

$$\%\ \beta\text{-Oxidation} = (\beta\text{-Oxidation}) \times (\text{Net intake} + \textit{Ex novo}\ \text{production})^{-1} \times 100$$

Ultimately, with the fourth step of the whole-body FA-balance method, it is possible to quantify the total net disappearance of FA, the total accretion of longer chain FA, the total accretion of Δ-9 desaturated FA, the total accretion of Δ-6 desaturated FA, the total accretion of Δ-5 desaturated FA and the net accretion of FA produced *ex novo*; all expressed as μmol of product per gram of fish (average fish weight) per day[13].

Statistical analysis

All data were expressed as means with their standard errors (three fish pooled per tank, three tanks per replicate; n 3). An independent t test was applied to determine significant differences between the two dietary treatments (FO and LO; significance reported as $P < 0{\cdot}05$, $P < 0{\cdot}01$ and $P < 0{\cdot}001$). The data relative to the utilisation of individual FA within the same dietary treatment were analysed with the one-way ANOVA at a significance level of 0·05 following the confirmation of normality and homogeneity of variance. Where significant differences were detected by ANOVA, the data were subjected to a Student–Newman–Keuls *post hoc* test for homogeneous subsets. All statistical analyses were computed using SPSS version 14.0 (SPSS Inc., Chicago, IL, USA).

Results

During the experiment, the fish tripled their body weight and no significant differences were recorded in growth and feed efficiency parameters of trout fed the two experimental diets (Table 1). The whole-body lipid content increased from 67·5 g/kg at the commencement of the experiment to 182·9 and 180·3 g/kg in the fish fed the FO and LO diets, respectively. At the end of the feeding trial, the fish fed the LO diets were slightly, although not significantly, smaller and leaner. The two experimental semi-purified diets were iso-proteic and iso-lipidic (Table 1) and, as expected, their FA composition was largely influenced by the oil source utilised in their formulation (Table 2). In particular, 10·6 mg/g lipid of α-linolenic acid (18 : 3n-3) was recorded in the FO diet, while, in the LO diet, its content was markedly higher with a value of 607·5 mg/g lipid. The dietary content of n-3 LCPUFA was noticeably different between diets, with the FO diet containing 126·0, 20·2 and 77·0 mg/g lipid and the LO diet containing 1·9, 1·1 and 4·0 mg/g lipid of EPA (20 : 5n-3), docosapentaenoic acid (22 : 5n-3) and DHA (22 : 6n-3), respectively.

The whole-body FA composition of trout was significantly modified by the dietary treatment with the differences between diets mirrored in the fish body. Significant differences were observed between all twenty-seven isolated and identified FA, with the exclusion of 20 : 0, 22 : 0, 18 : 1n-9, 20 : 1n-9 and 18 : 3n-6. A dramatic increase in the 18 : 2n-6 and 18 : 3n-3 contents and a simultaneous decrease in the 20 : 4n-6, 20 : 5n-3, 22 : 5n-3 and 22 : 6n-3 contents were recorded in the fish receiving the LO diet (Table 2).

The results of the first step in the computation of the whole-body FA-balance method are reported in Tables 3 and 4. Initial total FA content of the fish (expressed as mg of FA per fish) was not different between the two treatments, while the net intake and final body content was clearly affected by the FA composition of the two experimental diets. In the fish fed the FO diet, 20 : 5n-3 and 14 : 0 recorded the highest disappearance (−0·750 (SEM 0·027) and −0·273 (SEM 0·038) μmol/g per d, respectively), while, in the LO-fed fish, 18 : 3n-3 and 18 : 2n-6 were the highest in disappearance (−3·967 (SEM 0·839) and −0·694 (SEM 0·228) μmol/g per d, respectively; Table 4). In the FO-fed fish, 18 : 1n-9 was the FA recording the highest appearance, followed by 22 : 6n-3 and 16 : 0 (0·768 (SEM 0·109), 0·435 (SEM 0·026) and 0·278 (SEM 0·120) μmol/g per d, respectively), while, in the LO-fed fish, the FA appearing in the greatest abundance was 22 : 6n-3, followed by 18 : 1n-9 and 16 : 0 (0·300 (SEM 0·028), 0·263 (SEM 0·269) and 0·168 (SEM 0·113) μmol/g per d, respectively; Table 4).

In Fig. 2, the total FA net disappearance, the total accretion of longer chain FA, the total accretion of desaturated FA and the total net accretion of FA produced *ex novo* in rainbow trout fed the two dietary treatments are reported. Despite a relatively large numerical difference between the total FA net disappearance, which varied from 0·499 (SEM 0·105) and 3·621 (SEM 1·285) μmol/g per d in the fish fed the FO and LO diets, respectively, no statistical significance was observed given the high variability recorded for the total fatty net disappearance in the LO-fed fish. Large differences were observed in the individual FA net disappearance between the two treatments (Fig. 3). In the fish fed the LO diet, the FA recording the greatest rates of net disappearance were 18 : 3n-3 and 18 : 2n-6, while, in the FO-fed fish, the FA with the greatest disappearance were 20 : 5n-3 and 18 : 4n-3.

With regard to the individual accretion of elongated FA (Fig. 4), the two treatments followed similar trends. Independent of the dietary treatment, elongated FA recording the highest rate of accretion, in decreasing order, were 14 : 0, 16 : 0, 20 : 5n-3 and 22 : 5n-3. In the LO-fed fish, elongated 18 : 3n-3, 18 : 2n-6, 18 : 4n-3 and 18 : 3n-6 recorded a higher rate of accretion ($P<0{\cdot}05$) compared with the FO-fed fish, while the accretion of elongated 20 : 5n-3, 22 : 5n-3, 16 : 1n-7 and 22 : 1n-9 was higher in the FO-fed fish (Fig. 4).

The total accretion of Δ-6 desaturated FA was significantly different between the treatments, with the highest accretion (1·281(SEM 0·117) μmol/g per d) recorded in the LO-fed fish. The individual accretion of (Δ-9, Δ-6 or Δ-5) desaturated

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Table 2. Fatty acid composition of the experimental diets (mg/g lipid), the whole-body fatty acid composition of juvenile rainbow trout (expressed in mg/g lipid) and the fatty acid digestibility (%)

(Mean values with their standard errors; *n* 3)

	Diet (mg/g)		Whole body (mg/g)						Digestibility (%)	
			Initial		FO		LO			
	FO	LO	Mean	SEM	Mean	SEM	Mean	SEM	FO	LO
14 : 0	88·1	1·9	29·6	0·27	53·1***	1·08	6·0	0·19	77·3	90·8
16 : 0	192·5	57·5	146·8	1·37	152·6***	4·55	85·7	0·33	65·5	94·7
18 : 0	33·5	23·8	37·5	0·99	31·8*	0·63	28·7	0·33	55·3	92·4
20 : 0	2·6	2·0	1·8	0·46	1·5	0·16	1·1	0·12	55·9	90·9
22 : 0	1·3	1·3	1·6	0·55	1·0	0·19	0·9	0·11	48·1	79·3
16 : 1*n*-7	96·8	1·7	44·4	0·04	90·9***	1·32	16·2	0·53	95·8	93·7
18 : 1*n*-7	26·2	8·6	31·0	0·28	32·4***	0·24	13·6	0·67	92·3	96·9
18 : 1*n*-9	75·5	112·6	326·8	2·94	151·2	4·87	170·7	6·43	93·4	96·1
20 : 1*n*-9	8·9	1·8	9·7	0·40	8·9	1·58	5·2	0·12	87·1	87·2
22 : 1*n*-9	1·9	0·2	2·2	0·31	1·6***	0·03	0·8	0·03	73·8	89·0
24 : 1*n*-9	4·1	3·4	5·0	0·57	4·5*	0·28	2·6	0·38	72·4	92·4
20 : 1*n*-11	1·4	0·7	8·3	1·01	1·1***	0·03	0·4	0·07	85·1	96·6
22 : 1*n*-11	5·3	0·8	5·9	0·18	3·4***	0·02	0·8	0·04	83·7	87·3
18 : 2*n*-6	16·8	152·4	76·0	1·36	21·1***	0·35	121·9	2·51	95·3	97·1
18 : 3*n*-6	2·4	0·1	2·3	0·36	1·8	0·17	1·8	0·07	97·8	97·9
20 : 2*n*-6	1·7	1·3	4·4	0·23	1·8***	0·08	4·1	0·11	74·9	99·4
20 : 3*n*-6	2·1	0·4	3·9	0·83	2·5*	0·02	2·2	0·07	93·6	93·5
20 : 4*n*-6	10·7	0·5	8·0	0·48	9·1***	0·08	1·5	0·14	97·7	84·1
22 : 2*n*-6	5·4	0·3	2·9	0·10	5·4***	0·19	0·4	0·11	96·3	100†
22 : 4*n*-6	2·1	0·3	1·3	0·17	2·2***	0·21	0·4	0·08	95·4	95·3
18 : 3*n*-3	10·6	607·5	11·1	0·30	6·9***	0·11	378·9	10·63	96·2	98·0
18 : 4*n*-3	17·7	3·4	5·8	0·85	9·9***	0·16	25·9	0·57	98·5	93·3
20 : 3*n*-3	1·0	0·7	1·3	0·84	0·9***	0·03	16·6	0·35	88·5	92·3
20 : 4*n*-3	7·7	0·0	4·7	0·25	8·4**	0·22	14·0	0·79	97·2	97·4
20 : 5*n*-3	126·0	1·9	38·3	1·72	68·4***	0·37	13·8	0·33	99·1	95·2
22 : 5*n*-3	20·2	1·1	14·4	0·61	26·5***	0·61	6·4	0·10	97·0	78·2
22 : 6*n*-3	77·0	4·0	95·8	2·22	116·6***	1·98	38·3	0·39	97·7	87·1

FO, fish oil; LO, linseed oil.

Mean value was significantly different from that of the LO group: *$P<0·05$, **$P<0·01$, ***$P<0·001$ (independent *t* test). Statistical analysis was not performed on the initial sample, diet composition or digestibility data.

† Not detected in the faeces.

FA is reported in Fig. 5. Irrespective of the dietary treatment, there was a greater accretion of Δ-9 desaturated 18 : 0 compared with the accretion of Δ-9 desaturated 16 : 0. In the FO-fed fish, the most abundant desaturated FA accretion was recorded on 18 : 0 (Δ-9 desaturated), followed by 24 : 5*n*-3 (Δ-6 desaturated) and 16 : 0 (Δ-9 desaturated). Within the LO-fed fish, the most abundant desaturated FA accretion was recorded on 18 : 3*n*-3 (Δ-6 desaturated), followed by 20 : 4*n*-3 (Δ-5 desaturated), 18 : 0 (Δ-9 desaturated) and 16 : 0 (Δ-9 desaturated; Fig. 5).

The fate of individual FA, expressed as a percentage of total FA net intake plus *ex novo* production, is reported in Tables 5 and 6. There was a greater ($P<0·05$) *ex novo* production of SFA and MUFA in rainbow trout receiving the LO treatment. Alternatively, there was a significantly higher *ex novo* production of 18 : 1*n*-9 and 24 : 1*n*-9 in rainbow trout fed the FO diet (Table 5). Among the *n*-6 and *n*-3 PUFA, with the exception of 22 : 2*n*-6 and 22 : 4*n*-6, there was a greater ($P<0·05$) *ex novo* production of all other FA in the fish fed the LO treatment, with the obvious exclusion of 18 : 2*n*-6 and 18 : 3*n*-3, which cannot be produced *ex novo* in fish (Table 6). In rainbow trout fed the FO diet, the highest *ex novo* production of FA was recorded, in decreasing order, for 18 : 0, 22 : 5*n*-3, 22 : 1*n*-9, 14 : 0, 16 : 0 and 22 : 6*n*-3, varying, respectively, from 78·2 to 32·8 %.

All dietary 22 : 6*n*-3 were accumulated in rainbow trout across both the treatments, while 82·9 and 92·1 % of the total dietary intake plus *ex novo* production of 20 : 4*n*-6 was deposited in the FO and LO fed fish, respectively. The fate of total dietary intake plus *ex novo* production of 20 : 5*n*-3 was markedly different between the treatments. In the fish receiving the FO diet, 14·7 % of 20 : 5*n*-3 was β-oxidised and 31·6 % was bio-converted to longer and more unsaturated homologues, while, in the LO-fed fish, 72·7 % of 20 : 5*n*-3 was bio-converted up to 22 : 5*n*-3 and 22 : 6*n*-3. No β-oxidation of 20 : 5*n*-3 was recorded in the fish receiving the LO diet.

A small percentage of dietary 18 : 2*n*-6 was elongated and/or desaturated (1·8 and 3·1 % for the FO- and LO-fish, respectively), while larger percentages were oxidised (7·1 and 26·2 %, respectively). In the fish fed the FO diet, no bio-conversion of dietary 18 : 3*n*-3 was recorded and 40·4 % of its dietary intake was oxidised, while in the fish fed the LO diet, 12·4 % was bio-converted to longer and/or more unsaturated homologues and 29·5 % was used for energy production (Table 6).

Discussion

Rainbow trout responded equally well to both dietary treatments and gained approximately 300 % of their initial body weight in the 72 d of the feeding trial with no mortality.

Table 3. Total fatty acid content of fish at the beginning and the end of the experiment and total fatty acid net intake (mg of fatty acid per fish)
(Mean values with their standard errors; *n* 3)

	Initial				Final				Net intake			
	FO		LO		FO		LO		FO		LO	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
14 : 0	178	6·1	177	5·8	3498***	218·4	360	22·8	4320***	93·7	112	1·3
16 : 0	883	30·2	877	29·0	10 048**	686·4	5152	474·5	8002***	173·6	3591	43·0
18 : 0	226	7·7	224	7·4	2096	128·2	1730	176·0	1176**	25·5	1451	17·4
20 : 0	11	0·4	10	0·3	97**	5·2	63	3·9	94**	2·0	115	1·4
22 : 0	10	0·3	9	0·3	67	15·2	54	6·2	40***	0·9	69	0·8
16 : 1*n*-7	267	9·1	265	8·8	5974***	297·7	976	95·7	5883***	127·7	105	1·3
18 : 1*n*-7	186	6·4	185	6·1	2132***	118·4	821	87·3	1536***	33·3	548	6·6
18 : 1*n*-9	1967	67·2	1952	64·5	9952	667·00	10 304	1173·3	4472***	97·0	7145	85·5
20 : 1*n*-9	59	2·0	58	1·9	580	97·8	313	26·2	489***	10·6	103	1·2
20 : 1*n*-9	13	0·5	13	0·4	107**	7·0	48	2·9	91***	2·0	9	0·1
24 : 1*n*-9	30	1·0	30	1·0	298**	23·7	150	7·3	187*	4·0	208	2·5
20 : 1*n*-11	50	1·7	50	1·6	70***	4·4	22	2·1	77***	1·7	43	0·5
22 : 1*n*-11	35	1·2	35	1·2	222***	10·3	47	3·3	284***	6·2	49	0·6
18 : 2*n*-6	458	15·6	454	15·0	1387**	71·8	7355	826·3	1018***	22·1	9776	117·0
18 : 3*n*-6	14	0·5	14	0·4	117	16·0	108	6·3	150***	3·2	7	0·1
20 : 2*n*-6	27	0·9	26	0·9	121*	3·3	249	28·2	79*	1·7	85	1·0
20 : 3*n*-6	23	0·8	23	0·8	161*	6·4	129	9·0	124***	2·7	22	0·3
20 : 4*n*-6	48	1·7	48	1·6	597***	24·2	88	1·8	661***	14·3	27	0·3
22 : 2*n*-6	17	0·6	17	0·6	352***	5·3	20	4·7	330***	7·2	21	0·2
22 : 4*n*-6	8	0·3	8	0·2	143**	16·3	27	7·1	126***	2·7	18	0·2
18 : 3*n*-3	67	2·3	66	2·2	455***	28·7	22 902	2731·1	650***	14·1	39 295	470·3
18 : 4*n*-3	35	1·2	35	1·1	652**	38·1	1560	171·6	1108***	24·0	215	2·6
20 : 3*n*-3	8	0·3	8	0·3	57***	4·7	999	106·6	56***	1·2	45	0·5
20 : 4*n*-3	28	1·0	28	0·9	552*	22·2	842	98·5	474***	10·3	0	0·0
20 : 5*n*-3	230	7·9	229	7·6	4496***	220·5	828	64·8	7923***	171·9	117	1·4
22 : 5*n*-3	87	3·0	86	2·9	1743***	72·8	387	40·2	1240***	26·9	55	0·7
22 : 6*n*-3	576	19·7	572	18·9	7658***	279·2	2302	210·7	4774***	103·6	229	2·7

FO, fish oil; LO, linseed oil.
Mean values was significantly different from that of the LO group: *$P<0·05$, **$P<0·01$, ***$P<0·001$ (independent *t* test).

Although the experimental diets were iso-proteic and iso-lipidic, trout fed the LO diet exhibited a slight growth reduction in comparison with trout fed the FO diet (7·5 % lighter in final body weight), while the percentage of weight gain decreased from 304·5 to 275·6 % in the FO- and LO-fed fish, respectively. This is in accordance with the previously reported results for rainbow trout fed low-fishmeal-content diets, in which the dietary FO was replaced by terrestrial alternatives[15].

FA metabolism can be evaluated using a variety of *ex vivo* and *in vivo* methods. The intrinsic advantages and/or limitations of each of these methods have recently been critically reviewed by Brown[16]. In the present study, the whole-body FA-balance method[13], implemented previously in fish models[14,17], was utilised given its capability to track the individual fate of dietary FA.

A variety of methods exist for the assessment of FA metabolism *ex vivo* and *in vivo,* typically with the utilisation of radio-, ^{2}H- or stable isotope-labelled FA. The whole-body FA-balance method, utilised in the present study, employed an *in vivo* approach and is based on a theoretical model, and, admittedly, as with all models, is based on some assumptions that can potentially simplify the actual biological processes. However, the whole-body FA-balance method is a robust model capable of estimating the fate (β-oxidation, bio-conversion towards longer or more unsaturated FA and *ex novo* production) of all dietary FA, a goal not easily achieved using the other methods in consideration of the practical/technical difficulties of individually labelling each dietary FA. Nevertheless, there are certain limitations that can restrict the accuracy and applicability of the method, which need to be clearly spelled out and carefully considered.

One variable that the method does not take into consideration is the allowance of eicosanoid production. However, as previously reported[13], the extent of conversion of 20 : 4*n*-6 and 20 : 5*n*-3 is minimal, probably having little impact on the total FA balance. Similarly, other methods employing the utilisation of labelled FA are commonly implemented with the same assumption, as labelled eicosanoids are not usually quantified.

A second assumption of the whole-body FA-balance method is that the bio-conversion of FA proceeds solely in the normal direction of its specific pathway (from shorter to longer and from less unsaturated to more unsaturated FA) and not the opposite. Consequently, a second variable that the method does not take into consideration is the possibility of chain-shortening and oxidation of FA previously elongated and desaturated. For example, if a given amount of 18 : 2*n*-6 is desaturated to 18 : 3*n*-6 and successively oxidised, it will be considered as an oxidation process of 18 : 2*n*-6. This, as previously reported[13], is also a possible occurrence, and hence a limit of other methods that employ labelled FA. If, for example, 1-^{14}C-labelled 18 : 2*n*-6 is employed, the radioactive acid-soluble FA oxidation products determined to quantify

Table 4. Total fatty acid appearance/disappearance during the experiment expressed as mg of fatty acid per fish and as μmol/g per d (*n* 3)

(Mean values with their standard errors)

	Appearance/disappearance (mg/fish)				Appearance/disappearance (μmol/g per d)			
	FO		LO		FO		LO	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
14 : 0	−1000***	123·5	71	17·6	−0·273**	0·0383	0·020	0·0044
16 : 0	1163	503·7	684	456·3	0·278	0·1200	0·168	0·1133
18 : 0	694*	99·6	55	174·2	0·151*	0·0198	0·010	0·0411
20 : 0	−7**	7·1	−62	5·1	−0·001**	0·0014	−0·013	0·0011
22 : 0	17	14·7	−24	6·9	0·003	0·0026	−0·005	0·0014
16 : 1*n*-7	−177*	168·8	606	87·6	−0·044*	0·0425	0·156	0·0179
18 : 1*n*-7	410	85·0	89	85·8	0·089	0·0173	0·019	0·0202
18 : 1*n*-9	3514	535·4	1208	1151·8	0·768	0·1094	0·263	0·2690
20 : 1*n*-9	32	103·3	152	24·8	0·006	0·0204	0·032	0·0043
22 : 1*n*-9	3**	5·2	26	2·5	0·001*	0·0010	0·005	0·0003
24 : 1*n*-9	81**	20·1	−88	7·0	0·014***	0·0033	−0·016	0·0007
20 : 1*n*-11	−57	4·1	−71	3·6	−0·011*	0·0010	−0·015	0·0002
22 : 1*n*-11	−97***	4·9	−37	2·7	−0·018**	0·0012	−0·007	0·0008
18 : 2*n*-6	−89*	43·4	−2875	836·9	−0·020*	0·0101	−0·694	0·2284
18 : 3*n*-6	−46**	12·8	87	5·9	−0·010***	0·0031	0·021	0·0007
20 : 2*n*-6	16*	4·0	137	27·7	0·003**	0·0008	0·029	0·0050
20 : 3*n*-6	14**	3·8	84	8·3	0·003***	0·0007	0·018	0·0012
20 : 4*n*-6	−113***	9·9	13	2·0	−0·023***	0·0025	0·003	0·0005
22 : 2*n*-6	5*	3·0	−18	5·2	0·001*	0·0006	−0·004	0·0009
22 : 4*n*-6	9	15·8	1	6·8	0·002	0·0029	0·000	0·0013
18 : 3*n*-3	−262**	15·6	−16460	2823·9	−0·058**	0·0046	−3·967	0·8390
18 : 4*n*-3	−491***	14·0	1311	171·3	−0·110***	0·0049	0·311	0·0299
20 : 3*n*-3	−8***	3·7	946	106·5	−0·002***	0·0008	0·203	0·0157
20 : 4*n*-3	50**	16·5	814	97·5	0·010**	0·0032	0·176	0·0159
20 : 5*n*-3	−3657***	71·6	482	57·9	−0·750***	0·0275	0·104	0·0091
22 : 5*n*-3	415	55·0	245	37·6	0·078	0·0090	0·049	0·0058
22 : 6*n*-3	2308*	168·2	1501	193·1	0·435*	0·0259	0·3001	0·028

FO, fish oil; LO, linseed oil.

Mean value was significantly different from that of the LO group: *$P<0·05$, **$P<0·01$, ***$P<0·001$ (independent *t* test).

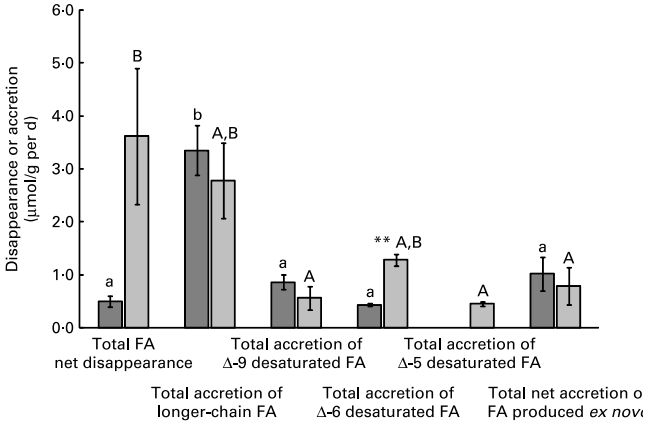

Fig. 2. Total fatty acid (FA) net disappearance, total accretion of longer-chain FA, total accretion of desaturated FA and total net accretion of FA produced *ex novo* in rainbow trout fed two different dietary treatments FO, fish oil-based diet (■); LO, linseed oil-based diet (□) for 72 d. The data are reported as means with their standard errors (*n* 3). **Statistically significant differences between the two dietary treatments ($P < 0·01$; independent *t* test). Within each dietary treatment, different letters indicate statistically significant differences (ANOVA and Student–Newman–Keuls *post hoc* test).

β-oxidation activity can be derived from 1-^{14}C-labelled 18 : 2*n*-6 that has been directly oxidised, but also from 1-^{14}C-labelled 18 : 2*n*-6 previously desaturated to 1-^{14}C-labelled 18 : 3*n*-6 and successively oxidised. As such, the present method does not differ from other methodologies with regard to these assumptions and limitations.

All methods used to estimate enzyme activity require a specific incubation time during which the activity of the enzyme is estimated relative to total enzyme product production (over that given time) and expressed as average enzyme activity (velocity). As such, the principal difference of the present method, when compared directly with other methods used for FA metabolism estimation, is the time frame. The whole-body FA-balance method estimates the fate of individual FA over a longer time frame and, consequently, the actual instantaneous enzyme velocity can differ from the average value computed with this method.

In light of the above, it is believed that the whole-body FA-balance method, though previously used to tentatively estimate enzyme activity, would be more appropriately implemented by reporting the FA production values, such as the FA net disappearance (as an indication of β-oxidation activity), the accretion of longer chain FA (as an indication of elongase activity), the accretion of desaturated FA (as an indication of desaturase enzyme activity, Δ-9, Δ-6 or Δ-5) and the net accretion of FA produced *ex novo* (as an

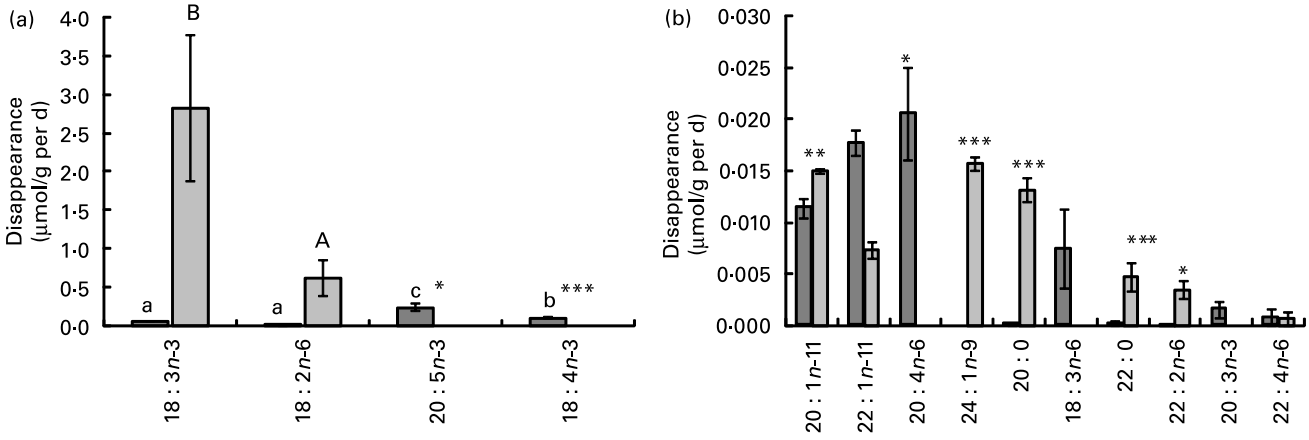

Fig. 3. The individual fatty acid net disappearance in rainbow trout fed two different dietary treatments (FO, fish oil-based diet (■); LO, linseed oil-based diet (□) for 72 d. The data are reported as mean values with their standard errors (n 3). Statistically significant differences between the two dietary treatments: *$P<0{\cdot}05$, **$P<0{\cdot}01$ and ***$P<0{\cdot}001$ (independent t test). Within each dietary treatment, different letters indicate statistically significant differences (ANOVA and Student–Newman–Keuls *post hoc* test). The data are reported in (a) and (b) with different y-axis scales for clarity. The data reported in (b) are not significantly different from the ANOVA test, and all these should be considered as indicating the letter a or A for the FO and LO treatments, respectively.

indication of FA *neogenesis*). Thus, the whole-body FA-balance method is useful as it offers an estimate of an organism's overall capacity to metabolise FA within the context of an integrated system over a relatively long time.

It is well documented that in fish, the substitution of dietary FO with alternative lipid sources lacking in n-3 LCPUFA is responsible for increased elongase and desaturase activity and transcription rate[8,14,17–20]. However, it has also been shown that this metabolic effort is insufficient to compensate for the decreased n-3 LCPUFA intake, resulting in a significant reduction in the n-3 LCPUFA tissue levels. Accordingly, the present study demonstrated that rainbow trout fed a vegetable oil-based diet had a marked enhancement in the accretion of Δ-6 and Δ-5 desaturated FA. However, this was insufficient in preserving the 20 : 5n-3 and 22 : 6n-3 contents of the whole body, which were, respectively, 5·0- and 3·0-fold lower than the fish fed the FO-based diet.

The percentage of total dietary 18 : 2n-6 intake subsequently bio-converted to longer and more unsaturated homologues or β-oxidised was relatively limited in both the treatments, underlining once more that this FA is not well utilised by fish[21], and, consequently, dietary 18 : 2n-6 is preferentially deposited.

The most elongated FA were, irrespective of the dietary treatment, 14 : 0, 16 : 0, 20 : 5n-3 and 22 : 5n-3. However, in fish fed the FO diet, a significantly higher accretion of elongated 20 : 5n-3 was recorded, suggesting that, despite an abundance of dietary 22 : 6n-3, the fish were actively attempting to bio-convert dietary 20 : 5n-3 to the longer and more unsaturated homologues.

Trout fed the LO diet demonstrated a high accretion rate of elongated 18 : 4n-3, which was 62-fold higher than that of fish fed the FO diet. In the fish receiving the FO diet, 18 : 4n-3 was preferentially oxidised (91 %) in contrast to elongation (9 %),

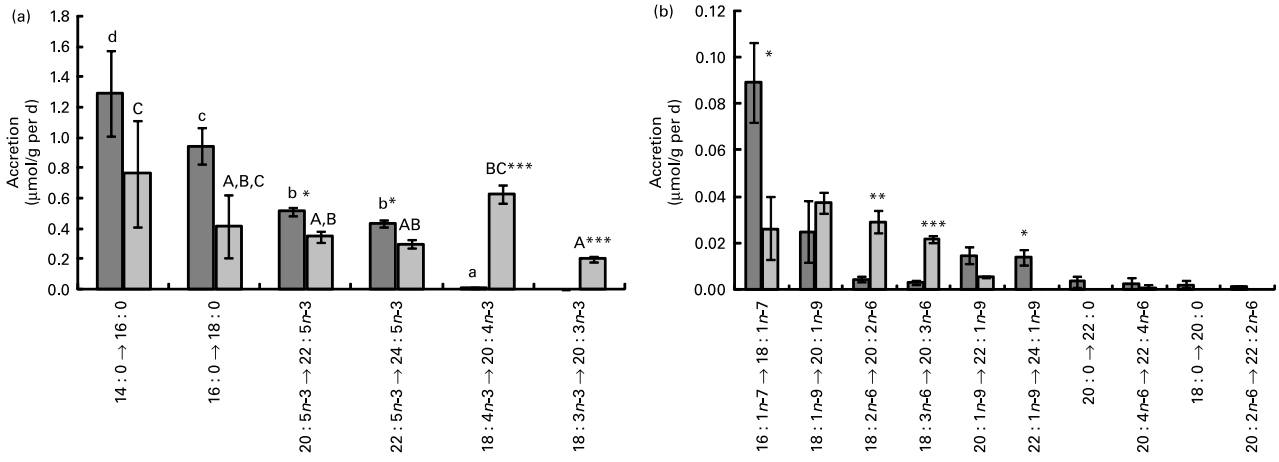

Fig. 4. The individual accretion of elongated fatty acids in rainbow trout fed two different dietary treatments (FO, fish oil-based diet (■); LO, linseed oil-based diet (□) for 72 d. The data are reported as mean values with their standard errors (n 3). Statistically significant differences between the two dietary treatments: *$P<0{\cdot}05$, **$P<0{\cdot}01$ and ***$P<0{\cdot}001$ (independent t test). Within each dietary treatment, different letters indicate statistically significant differences (ANOVA and Student–Newman–Keuls *post hoc* test). The data are reported in (a) and (b) with different y-axis scales for clarity. The data reported in (b) are not significantly different from the ANOVA test, and all these should be considered as indicating the letter a or A for the FO and LO treatments, respectively.

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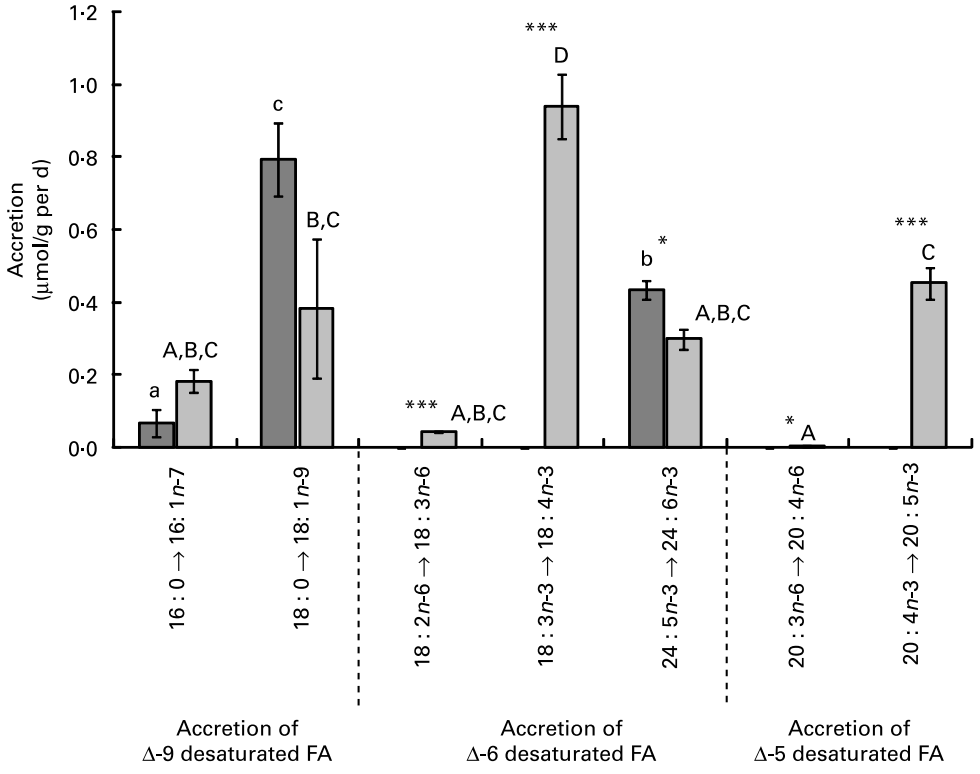

Fig. 5. The individual accretion of (Δ-9, Δ-6 or Δ-5) desaturated fatty acids in rainbow trout fed two different dietary treatments (FO, fish oil-based diet (■); LO, linseed oil-based diet (□) for 72 d. The data are reported as mean values with their standard errors (n 3). Statistically significant differences between the two dietary treatments: *$P<0{\cdot}05$, ***$P<0{\cdot}001$ (independent t test). Within each dietary treatment, different letters indicate statistically significant differences (ANOVA and Student–Newman–Keuls *post hoc* test).

suggesting some form of metabolic recognition of the abundant quantity of dietary 20 : 5n-3, which rendered the elongation of 18 : 4n-3 for the ultimate production of 20 : 5n-3 redundant.

Despite the greater abundance of dietary 16 : 0 compared with 18 : 0 in both the treatments, there was an 11·7- and 2·1-fold higher accretion of Δ-9 desaturated 18 : 0 compared with the accretion of Δ-9 desaturated 16 : 0 in the FO- and LO-fed fish, respectively, showing clearly the presence of a higher affinity of Δ-9 desaturase for 18 : 0.

In the FO-fed fish, the accretion of Δ-5 desaturated FA could not be quantified as the potential appearance of 20 : 5n-3 and 20 : 4n-6 was masked by the abundant concentrations of these FA in the diet. Admittedly, this is a limit of the whole-body FA-balance method as described and discussed previously[14]. Nevertheless, it is likely that a negligible amount of Δ-5 desaturase activity would have been evident in consideration of the high concentration of the enzyme products (20 : 5n-3 and 20 : 4n-6) provided within the diet. In the fish receiving the LO diet, characterised by a limited content of 20 : 5n-3 and 20 : 4n-6, the accretion of Δ-5 desaturated FA was 120-fold greater on 20 : 4n-3 in comparison with 20 : 3n-6. Similarly, the accretion of Δ-6 desaturated FA was 22-fold higher on 18 : 3n-3 compared with 18 : 2n-6, and 7-fold higher on 24 : 5n-3 than on 18 : 2n-6, underlining the higher affinity of the Δ-6 desaturase enzyme towards n-3 FA[7,8,22,23].

The fish fed with the FO-based diet were receiving an abundance of dietary 22 : 6n-3. However, in the present study, it was shown that 22 : 6n-3 was also actively produced via the bio-conversion of dietary 20 : 5n-3. As such, it is conceivable that the optimal diet for rainbow trout would be characterised by higher levels of 22 : 6n-3 and lower levels of 20 : 5n-3 in comparison with the typical composition of FO.

The Δ-6 desaturase has previously been described as the rate-limiting enzyme in the LCPUFA biosynthetic pathway[24,25]. However, in the present study, it has been shown that, on the n-6 FA pathway, the accretion of Δ-6 desaturated 18 : 2n-6 was 11-fold higher than that of Δ-5 desaturated 20 : 3n-6. Likewise, on the n-3 FA pathway, the accretion of Δ-6 desaturated 18 : 3n-3 was 2·1-fold higher than that of Δ-5 desaturated 20 : 4n-3, which in turn was 1·5-fold higher than that of Δ-6 desaturated 24 : 5n-3. Therefore, more than the existence of a 'rate-limiting enzyme', which restricts the LCPUFA biosynthetic pathway, a 'funnel-like', progressively less efficient bio-conversion of FA to more unsaturated homologues seems to occur along the pathway itself.

Little information is available on the potential effects of different dietary lipid sources and β-oxidation activity in fish, and only marginal increases in the β-oxidation capacity in fish fed with high n-3 LCPUFA compared with those fed with diets lacking n-3 LCPUFA have been reported[26–28]. Moreover, it is likely that this effect is primarily due to an increased uptake of FA into the cells[29] or into mitochondria[30] rather than a direct stimulation of the actual β-oxidation system. However, in the present study, total FA net disappearance was 7·2-fold greater in rainbow trout receiving the LO diet (low in n-3 LCPUFA), with the net disappearance

Table 5. The fate of individual fatty acids (SFA and MUFA) as *ex novo* production, body accumulation or depletion, bio-conversion and β-oxidation (*n* 3)†

(Mean values with their standard errors)

	Percentage of *ex novo* production		Percentage of body accumulation		Percentage of bio-conversion		Percentage of β-oxidation	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
14 : 0								
FO	44·0*	8·6	42·4	4·5	57·6	4·5	0	
LO	88·5	8·8	16·2	11·5	83·8	11·5	0	
16 : 0								
FO	39·1	5·3	69·0	2·2	31·0	2·2	0	
LO	39·3	16·3	68·4	10·3	31·6	10·3	0	
18 : 0								
FO	78·2	2·2	34·2	1·8	65·8	1·8	0	
LO	43·9	22·0	53·2	13·8	40·4	20·2	6·4	6·4
20 : 0								
FO	9·3	6·2	84·5*	10·2	14·9	10·4	0·7***	0·7
LO	0		45·9	3·9	0		54·1	3·9
22 : 0								
FO	24·3	15·4	96·8**	3·2	0		3·2*	3·2
LO	0		64·7	9·6	0		35·3	9·6
16 : 1*n*-7								
FO	4·4***	2·3	92·5	0·6	5·9	1·0	1·6	1·6
LO	86·2	3·0	89·1	5·6	10·9	5·6	0	
18 : 1*n*-7								
FO	20·6	3·3	100		0		0·0	
LO	16·1	8·2	95·2	4·8	0		4·8	4·8
18 : 1*n*-9								
FO	44·3*	3·1	98·5	0·8	1·5	0·8	0	
LO	17·6	8·8	93·7	4·4	1·8	0·1	4·5	4·5
20 : 1*n*-9								
FO	18·3*	9·6	83·4	8·5	12·5*	4·5	4·1	4·1
LO	62·4	4·2	91·3	0·2	8·7	0·2	0	
22 : 1*n*-9								
FO	45·0**	5·7	56·6**	5·3	43·4	5·3	0	
LO	74·7	1·9	100		0		0	
24 : 1*n*-9								
FO	29·6**	4·6	100***		0		0***	
LO	0		57·8	3·6	0		42·2	3·6
20 : 1*n*-11								
FO	0		26·1*	6·1	0		73·9***	6·1
LO	0		− 62·7	8·8	0		162·7	8·8
22 : 1*n*-11								
FO	0		65·8**	2·3	0		34·2***	2·3
LO	0		24·1	4·7	0		75·9	4·7

FO, fish oil; LO, linseed oil.
Mean values was significantly different from that of the LO group: *$P<0·05$, **$P<0·01$, ***$P<0·001$ (independent *t* test).
†The data are expressed as a percentage of total fatty acid net intake plus total fatty acid *ex novo* production.

of 18 : 3*n*-3 and 18 : 2*n*-6 accounting for 78 % and 17 %, respectively, of the total FA net disappearance. In the LO diet, 18 : 3*n*-3 and 18 : 2*n*-6 were the two most abundant FA and, subsequently, 30·2 and 27·0 % of the net intake of these FA was oxidised, respectively. Similarly, studies on Atlantic salmon (*Salmo salar*) have indicated that 18 : 3*n*-3 and 18 : 2*n*-6, as well as MUFA (such as 18 : 1*n*-9 and 22 : 1*n*-11), are readily β-oxidised when present in high concentrations in the diet[23,27,31]. It has also been reported that 20 : 5*n*-3 is abundantly β-oxidised when abundantly present in the diet[27]. Consequently, despite the possible existence of a preferential order of oxidation for certain FA, these differences are hidden when dietary FA are in surplus. Accordingly, in the present study, 20 : 5*n*-3 was abundantly oxidised in the fish fed the FO diet. However, the FA of greatest abundance in the FO diet was 16 : 0, which was apparently not utilised for energy production, and, conversely, it was preferentially elongated to 18 : 0 or desaturated to 16 : 1*n*-7. Similarly, 22 : 6*n*-3 was abundantly present in the FO diet, but it was not oxidised and was conversely biosynthesised. It is known, indeed, that the presence of the Δ-4 double bond requires a peculiar mechanism to be removed before β-oxidation, reducing its utilisation for energy production[7]. Accordingly, this resulted in the total amount of 22 : 6*n*-3 deposited in trout body exceeding the total 22 : 6*n*-3 intake.

In both the treatments, 20 : 1*n*-11 and 22 : 1*n*-11 recorded a very high net disappearance. This result is in agreement with the previous findings[32,33], which suggest that MUFA, particularly 20 : 1 and 22 : 1 isomers, are the substrate of choice for mitochondrial β-oxidation in salmonids. These FA are, in fish, commonly derived from the corresponding fatty alcohol abundant in the wax esters of zooplankton[7],

Table 6. The fate of the individual fatty acid (PUFA) as *ex novo* production, body accumulation or depletion, bio-conversion and β-oxidation (*n* 3)†

(Mean values with their standard errors)

	Percentage of *ex novo* production		Percentage of body accumulation		Percentage of bio-conversion		Percentage of β-oxidation	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
18 : 2*n*-6								
FO	–		91·1	4·6	1·8	0·6	7·1	4·3
LO	–		70·6	8·4	3·1	0·4	26·2	8·8
20 : 2*n*-6								
FO	20·1**	5·3	95·4	2·4	4·6	2·4	0	
LO	60·4	5·6	100		0		0	
22 : 2*n*-6								
FO	1·6	0·8	99·9*	0·1	0		0·1*	0·1
LO	0		12·4	24·6	0		87·6	24·6
18 : 3*n*-6								
FO	0***		68·9	9·4	8·6*	2·2	22·6	11·6
LO	96·1	0·3	49·2	1·4	49·2	1·4	0	
20 : 3*n*-6								
FO	10·1***	2·4	100*		0*		0	
LO	18·9	1·6	86·0	3·3	14·0	3·3	0	
20 : 4*n*-6								
FO	0**		82·9	1·9	1·8	1·8	15·3	3·3
LO	38·1	6·9	92·1	7·9	7·9	7·9	0	
22 : 4*n*-6								
FO	7·9	7·8	96·7	3·3	0		3·3	3·3
LO	14·5	14·5	80·7	16·1	0		19·3	16·1
18 : 3*n*-3								
FO	–		59·6	3·2	0***		40·4	3·2
LO	–		58·1	7·0	12·4	1·6	29·5	8·5
20 : 3*n*-3								
FO	0***		86·0	6·8	0		14·0	6·8
LO	95·3	0·6	100		0		0	
18 : 4*n*-3								
FO	0***		55·6**	2·2	4·0***	1·3	40·3***	2·8
LO	94·7	0·7	36·6	0·5	63·4	0·5	0	
20 : 4*n*-3								
FO	9·3***	2·7	100***		0***		0	
LO	100		28·0	0·8	72·0	0·8	0	
20 : 5*n*-3								
FO	0***		53·8***	1·7	31·6***	1·7	14·7*	3·3
LO	94·5	0·8	27·3	0·8	72·7	0·8	0	
22 : 5*n*-3								
FO	68·7***	1·2	41·7**	1·3	58·3	1·3	0	
LO	96·8	0·5	16·6	0·1	83·4	0·1	0	
22 : 6*n*-3								
FO	32·5***	1·2	100		0		0	
LO	86·4	1·7	100		0		0	

FO, fish oil; LO, linseed oil.
Mean values was significantly different from that of the LO group: *$P<0·05$, **$P<0·01$, ***$P<0·001$ (independent *t* test).
†The data are expressed as a percentage of total fatty acid net intake plus total fatty acid *ex novo* production.

and, consequently, it has been suggested that the fish FA metabolism evolved and adapted to preferentially use these FA as an energy source[32,34].

The pathway of FA *ex novo* biosynthesis in fish is fundamentally similar to that operating in mammals by the conventional pathway catalysed by the cytosolic FA synthetase[7,32], and, in the present study, a net appearance of 14 : 0 was recorded. Therefore, trout fed with both the dietary treatments were actively producing FA, achieved obviously via the utilisation of other dietary nutrients such as protein or carbohydrate. Both the diets contained 220 mg/kg lipid and 430 mg/kg protein, values considered to be within the optimal levels for this species[35]. However, in consideration that the fish were actively producing their own FA, it seems plausible that a higher dietary lipid level and, in particular, a higher dietary SFA content would have been beneficial for the overall nutritional balance of the fish, permitting an increased sparing of dietary protein. However, it is known that *ex novo* FA synthesis in fish, in contrast to the processes occurring in mammalians, is only minimally affected by different macronutrient concentrations[32]. Consequently, it is possible to speculate that the recorded active *ex novo* production of SFA and MUFA could also be related to a defensive mechanism against the excessive increased peroxidation hazard due to the increased tissue content of the readily oxidisable 18 : 3*n*-3 or *n*-3 LCPUFA[17,36,37], or as a metabolic mechanism to modulate membrane fluidity, which is well known to be extremely important in poikilothermic animals and easily affected by environmental conditions[36–40].

In summary, the present study has shown that, while the FO diet was characterised by a high *n*-3 LCPUFA content (20 : 5*n*-3 and 22 : 6*n*-3), a large proportion of 20 : 5*n*-3 was used for energy production; a smaller amount was further bio-converted up to 22 : 6*n*-3, and only a limited amount was deposited as is. Therefore, trout seem to require a higher level of 22 : 6*n*-3 in their diets. On the other hand, the LO diet was characterised by an extremely high level of 18 : 3*n*-3. Although the rainbow trout were actively bio-converting this FA up to 22 : 6*n*-3, this metabolic effort was insufficient to compensate for the significant reduced dietary intake of 22 : 6*n*-3. A relatively large fraction of dietary 18 : 3*n*-3 was used for energy production, but a larger part was simply deposited in the fish body. Thus, when making the assumption that the optimal dietary FA composition for a growing animal is the FA composition which minimises *in vivo* bio-conversion while simultaneously providing an optimal substrate for energy production, the findings of the present study suggest that the theoretical optimal dietary FA composition for farmed rainbow trout should be characterised by a high content of 22 : 6*n*-3 and SFA (as these are actively produced by the fish) and a high content of MUFA, particularly 20 : 1 and 22 : 1 isomers (as these FA are the optimal substrate for energy production). Conversely, it appears evident that an excessive dietary content of other PUFA, particularly 18 : 2*n*-6, 18 : 3*n*-3 and/or 20 : 5*n*-3, seems a relatively wasteful practice.

Acknowledgements

The present research was supported under the Australian Research Council's *Discovery Projects* funding scheme (project no. DP0772271). The views expressed herein are those of the authors and are not necessarily those of the Australian Research Council. The authors also wish to thank Mark Porter and Richard Smullen (Ridley Agriproducts – Aquafeed) for kindly providing the raw materials used for diet formulation and Andrew J. Sinclair (School of Exercise and Nutrition Sciences, Deakin University) for his important hints, suggestions and careful manuscript revision. The present study represents the original work that has not been published previously, is not presently being considered by another journal, and that if accepted for the *British Journal of Nutrition* it will not be published elsewhere in the same form, in English or in any other language, without the written consent of the Nutrition Society. All procedures used were approved by the Deakin University Animal Welfare Committee. G. M. T. and D. S. F. contributed equally to the study and manuscript preparation and have seen and approved the contents of the submitted manuscript. There are no financial or other contractual agreements that might cause conflicts of interest or be perceived as causing conflicts of interest. There are no financial arrangements between any author and any company whose product figures in the submitted manuscript.

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