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Whole-body retention of α-linolenic acid and its apparent conversion to other *n*-3 PUFA in growing pigs are reduced with the duration of feeding α-linolenic acid

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Abstract

In the present study, fifteen growing pigs were used to determine the whole-body oxidation, retention efficiency (RE) and apparent conversion (AC) of α-linolenic acid (18 : 3*n*-3) to *n*-3 highly unsaturated fatty acids (HUFA), including EPA (20 : 5*n*-3) and DHA (22 : 6*n*-3). The pigs were fed a diet containing 10 % flaxseed for 30 d. Whole-body fatty acid composition was determined at initial (27·7 (SE 1·9) kg), intermediate (day 15; 39·2 (SE 1·4) kg) and final (45·7 (SE 2·2) kg) body weight. On day 12, four pigs were fed 10 mg/kg of uniformly labelled ^{13}C-18 : 3*n*-3 (single-bolus dose) to determine the oxidation of 18 : 3*n*-3. Expired CO_2 samples were collected for 24 h thereafter. The whole-body content of *n*-3 PUFA increased linearly ($P<0·0001$) with time; however, the content of 22 : 6*n*-3 exhibited a quadratic response ($P<0·01$) with a peak occurring at 15 h. As a proportion of intake, the RE of 18 : 3*n*-3 tended to reduce with time ($P=0·098$). The AC of ingested 18 : 3*n*-3 to the sum of *n*-3 HUFA was reduced with time ($P<0·05$; 12·2 *v.* 7·53 % for days 0–15 and days 15–30, respectively). The AC of 18 : 3*n*-3 to 20 : 5*n*-3 or 22 : 6*n*-3 was lower than that to 20 : 3*n*-3, both for days 0–15 ($P<0·05$; 1·14 or 1·07 *v.* 7·06 %) and for days 15–30 ($P<0·05$; 1·51 or 0·33 *v.* 4·29 %). The direct oxidation of 18 : 3*n*-3 was 7·91 (SE 0·98) % and was similar to the calculated disappearance of 18 : 3*n*-3 between days 0 and 30 (8·81 (SE 5·24) %). The oxidation of 18 : 3*n*-3 was much lower than that reported in other species. The AC of 18 : 3*n*-3 to *n*-3 HUFA was reduced over time and that to 20 : 3*n*-3 in the present study was much higher than that reported in other species and should be explored further.

Key words: Fatty acid oxidation: *n*-3 PUFA: Apparent conversion: Pigs

Effective manipulation of the fatty profile in pork products to provide human health benefits of α-linolenic acid (18 : 3*n*-3) and *n*-3 highly unsaturated fatty acids (HUFA) (*n*-3 HUFA and all *n*-3 PUFA minus 18 : 3*n*-3) requires a quantitative understanding of the *n*-3 PUFA metabolism in growing pigs. Most mammalian species, including pigs, are able to metabolise 18 : 3*n*-3 to *n*-3 HUFA such as EPA (20 : 5*n*-3), docosapentaenoic acid (22 : 5*n*-3) and DHA (22 : 6*n*-3)[1]. Previous studies in pigs have shown that the inclusion of 18 : 3*n*-3 from either ground flaxseed (FS, up to 15 % in the diet)[2] or rapeseed oil (10 % in the diet)[3] in pre-slaughter diets represents an effective means to incorporate significant amounts of 18 : 3*n*-3 and *n*-3 HUFA in edible pork tissues, generating value-added healthy pork products for consumers without compromising pork quality[2,3]. In the study of Romans *et al.*[2], it was observed that the incremental retention of 18 : 3*n*-3 and *n*-3 HUFA in pork tissues decreased over time when feeding a 15 % FS-containing diet, suggesting that the retention efficiency (RE) of 18 : 3*n*-3 and apparent conversion (AC) of 18 : 3*n*-3 to *n*-3 HUFA decrease with the duration of feeding 18 : 3*n*-3. However, the RE and AC of 18 : 3*n*-3 on a whole-body basis in growing pigs consuming diets containing FS (as a source of 18 : 3*n*-3) are still unknown. Furthermore, and in contrast to other species[4,5], the oxidation rate of 18 : 3*n*-3, determined directly using isotope tracers, has not been quantified in growing pigs; instead, mass balance calculations based on serial slaughter measurements have been used to indirectly estimate the oxidation (i.e. disappearance) of 18 : 3*n*-3 in growing pigs[6]. Therefore, the objective of the present study was to determine the whole-body RE of 18 : 3*n*-3 and its AC to *n*-3 HUFA in growing pigs fed a 10 % FS-containing diet, utilising the mass balance approach and serial slaughter observations.

Abbreviations: AC, apparent conversion; AID, apparent ileal digestibility; BW, body weight; FA, fatty acid; FS, flaxseed; HUFA, highly unsaturated fatty acids; RE, retention efficiency; U-^{13}C-18 : 3*n*-3, uniformly labelled ^{13}C-18 : 3*n*-3.

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In addition, the oxidation of 18 : 3*n*-3 was measured directly by feeding a single-bolus dose of uniformly labelled ^{13}C-18 : 3*n*-3 (U-^{13}C-18 : 3*n*-3).

Materials and methods

Experimental animals, diets and general design

The University of Guelph Animal Care Committee approved the present experimental protocol. A total of fifteen healthy purebred Yorkshire growing gilts from eight litters and weighing approximately 20 kg body weight (BW; 8–9 weeks old) were obtained from the University of Guelph Arkell Swine Research Station herd and transported to the animal metabolism unit. Before the start of the experiment, the pigs were fed *ad libitum* a maize, wheat and soyabean meal-based pig starter diet, which was assumed to be low in *n*-3 PUFA content. Upon arrival to the unit, the pigs were housed individually in fully slatted floor pens in an environmentally controlled room and were given free access to water via nipple drinkers[7]. The pigs were fed *ad libitum* a FS-free adaptation diet (Table 1) until weighing approximately 27 kg BW (Fig. 1). At the start of the experiment, four pigs from four different litters (initial BW 27·7 (SE 0·96) kg) were slaughtered to determine the initial chemical and physical body composition. The remaining pigs were randomly assigned to one of two different slaughter groups on day 15 (*n* 6; days 0–15) and day 30 (*n* 5; days 15–30). The pigs were fed a maize, wheat and soyabean meal-based diet containing 10 % ground FS (15·5 g/kg of 18 : 3*n*-3). Throughout the experiment, the pigs were fed three times a day (07.00, 15.00 and 23.00 hours); feed intake levels were fixed at 70 % of the voluntary daily digestible energy intake according to the National Research Council[8]. The pigs were weighed weekly to monitor growth rate and to adjust feeding levels. Feed refusals were collected daily and weighed weekly to calculate the actual feed intakes. In a subsample of four pigs, the oxidation of 18 : 3*n*-3 was measured directly based on feeding a single-bolus dose of U-^{13}C-18 : 3*n*-3 on day 12 after the start of the FS-containing diet feeding period.

The experimental diets were prepared at the University of Guelph feed mill. The experimental diets were formulated to contain equal amounts of *n*-6 and *n*-3 PUFA. The dietary contents of essential nutrients, including standardised ileal digestible indispensable amino acids, exceeded the requirements of pigs with high lean gain potentials[8]. The diets were fed in a pelleted form. Titanium oxide was added to the experimental diets as an indigestible marker for the assessment of nutrient digestibility.

Serial slaughter procedure and nutrient analysis

The pigs were killed using sodium pentobarbital on day 0, 15 or 30 after the start of the FS-containing diet feeding period. On days 15 and 30, representative digesta samples were quickly collected from the last metre of the distal ileum, by gently squeezing the isolated gut segment, and frozen at −80°C until later analyses[9]. Slaughter procedures and

Table 1. Ingredient composition and nutrient content of the adaptation diet (fed between 20 and 27 kg body weight (BW)) and the grower diet (fed for 30 d between 28 and 45 kg BW)

	Adaptation diet	Grower diet
Ingredient composition (%, as fed)		
Maize	40·00	34·39
Wheat	20·00	20·00
Soyabean meal	35·00	31·50
Ground flaxseed	–	10·00
Fat, animal and vegetable blend	1·00	–
L-Lys HCl	0·20	0·20
DL-Met	0·12	0·13
L-Thr	0·13	0·15
Lincomycin* 44	0·10	0·10
Limestone	1·15	1·15
Dicalcium phosphate	1·30	1·22
Salt	0·40	0·45
Swine vitamin and mineral mix†	0·60	0·60
Titanium dioxide	0·10	0·10
Vitamin E‡	–	0·01
Calculated nutrient content§		
Digestible energy (MJ/kg)	14·6	14·7
Crude protein (%)	22·6	22·6
Crude fat (%)	3·61	5·69
Total Lys (%)	1·40	1·35
Digestible Lys (%)‖	1·25 (100)	1·21 (100)
Digestible Met + Cys (%)	0·76 (61)¶	0·77 (64)
Digestible Thr (%)	0·85 (68)	0·86 (71)
Digestible Trp (%)	0·27 (21)	0·27 (22·5)
Ca (%)	0·80	0·80
P (%)	0·70	0·70
Na (%)	0·18	0·20
Analysed nutrient content (%)		
DM	90·9	92·0
Crude protein	21·6	22·0
Crude fat	4·00	5·93
Ash	6·39	6·96
Ca	0·78	0·69
P	0·65	0·63
Na	0·17	0·18
Fatty acid content (g/kg, as fed)		
Myristic acid (14 : 0)	0·28 (0·93)	0·09 (0·20)**
Palmitic acid (16 : 0)	5·45 (18·3)	4·47 (9·74)
Palmitoleic acid (9*c*-16 : 1)	0·29 (0·97)	0·08 (0·17)
Stearic acid (18 : 0)	2·43 (8·13)	2·13 (4·64)
Oleic acid (9*c*-18 : 1)	7·82 (26·2)	9·63 (21·0)
Linoleic acid (18 : 2 *n*-6)	11·9 (39·8)	13·7 (29·8)
Arachidonic acid (20 : 4 *n*-6)	0·19 (0·64)	ND
α-Linolenic acid (18 : 3 *n*-3)	0·99 (3·31)	15·5 (33·8)
EPA (20 : 5 *n*-3)	0·03 (0·09)	ND
DHA (22 : 6 *n*-3)	0·11 (0·38)	ND
n-6:*n*-3 PUFA ratio	10·7	0·89
ΣSFA	8·25	6·81
ΣMUFA	8·26	9·82
ΣPUFA	13·3	29·3
ΣSFA/ΣPUFA	0·619	0·232

ND, not detected.
* Supplied 44 mg/kg diet as lincomycin hydrochloride.
† Supplemented by kg of diet: vitamin A, 1.2 mg; vitamin D_3, 10 μg; vitamin E, 16 mg; vitamin K, 1 mg; choline, 200 mg; pantothenic acid, 6 mg; riboflavin, 2 mg; folic acid, 0·8 mg; niacin, 10 mg; thiamin, 0·6 mg; vitamin B_6, 0·6 mg; biotin, 0·08 mg; vitamin B_{12}, 0·01 mg. Supplemented by kg of diet: Na, 1800 mg; Cu (from $CuSO_4$), 15 mg; Zn (from ZnO), 100 mg; Fe (FeO), 100 mg; Mn ($MnSO_4$), 20 mg; I (KI), 0·3 mg; Se (Na_2SeO_3), 0·3 mg.
‡ Concentration of vitamin E: 500 mg/g of product.
§ Based on the National Research Council[8].
‖ Represents digestible standardised ileal digestibility.
¶ 100 × ratio to Lys.
** Values in parentheses are fatty acid profile, given as percentage of analysed fat content.

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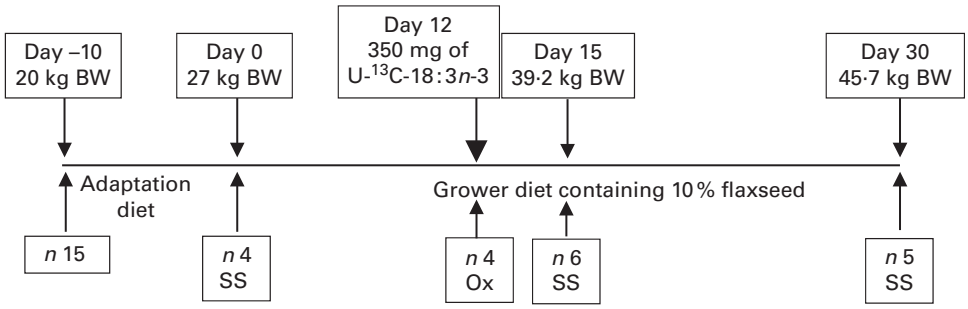

Fig. 1. Schematic representation of the experimental design. Feed intake was fixed at 70 % of the voluntary feed intake based on the National Research Council[8]. On day 12, 10 mg of uniformly labelled ^{13}C-18 : 3*n*-3 (U-^{13}C-18 : 3*n*-3)/kg body weight (BW) were fed to the pigs to measure the direct oxidation (Ox) of 18 : 3*n*-3. For serial slaughter (SS) and whole-body fatty acid composition analyses, the pigs were killed on days 0, 15 and 30. *n* represents the number of observations.

carcass and viscera management for determining the chemical and physical body composition have been outlined in detail previously[7]. In short, at slaughter, blood was collected quantitatively, weighed and discarded, whereas visceral organs (kidneys, spleen, liver, lungs and heart) were weighed individually. The full gastrointestinal tract was weighed, emptied and re-weighed to determine gut fill. Thereafter, the individual segments of the gastrointestinal tract (stomach and small and large intestines) were weighed. The empty gastrointestinal tract was added to the visceral organs, placed in a plastic bag and frozen at −20°C. The empty carcass (which included head, feet, hair, nails and skin) was weighed, placed in plastic bags and stored at −20°C.

Sample preparation and chemical analysis were carried out according to previous procedures in our laboratory[7]. Freeze-dried subsamples (carcass, pooled viscera and diets) were ground and homogenised with liquid N_2 in a conventional coffee grinder. Duplicate samples were then used to determine analytical DM content according to the Association of Official Analytical Chemists[10]. N content in the diet, carcass, viscera and digesta samples was determined in triplicate utilising an induction furnace and thermal conductivity N_2 analyser (Leco FP-528; Leco Corporation). Ash content was determined in duplicate according to the Association of Official Analytical Chemists[10]. Crude fat content in the carcass, viscera, diet and digesta samples was determined by extraction using a fat analyser (ANKOM XT20, method 2; Ankom Technology Corporation) without prior acid hydrolysis. Freeze-dried digesta and diet samples were analysed for titanium dioxide content using standard procedures[10].

For fatty acid (FA) analyses, lipids were extracted from the carcass, viscera, diet and digesta samples using chloroform–methanol (2:1, v/v) extraction according to the method of Bligh & Dyer[11] in the presence of the internal standard, tridecanoic acid (13 : 0; Nu Chek Prep). During sample processing, efforts were made to minimise 13 : 0 losses; aliquots of the lipid extract were dried under N_2 and subjected to trans-methylation using boron trichloride in methanol and heating the methylation tubes in a boiling water-bath[12]. The resulting FA methyl esters were analysed on a Varian 3400 gas–liquid chromatograph (Varian) equipped with a 60 m DB-23 capillary column (0·32 mm internal diameter). GLC and FA peak identification procedures have been described previously[13].

Direct measurement of α-linolenic acid oxidation

Uniformly labelled ^{13}C-α-linolenic acid ethyl ester (>99 atom%) was purchased from Cambridge Isotopes Laboratories, Inc. This preparation contained 95 % of U-^{13}C-18 : 3*n*-3 and other FA made up the remainder. The oxidation of U-^{13}C-18 : 3*n*-3 was measured in the pigs when their BW was approximately 35 kg in an open-circuit respiration calorimetry system[14], with real-time measurements of air flow and CO_2 content in the exhaust air (Qubit Systems). Fresh air was drawn through the system at approximately 50 l/min using a large air pump (Model 0523-101Q-G588EDX; Gast Manufacturing, Inc.). Exhaust air flow was quantified using an air mass flow meter (Alicat M series; Alicat Scientific, Inc.). After quantification with the air flow meter, the air flow was split into two streams. The small stream (about 1 % of total air flow and controlled by air pump Model F360 and air flow meter Model P650) was used for real-time measurements of CO_2 content (Model S155, Infrared CO_2 Analyzer; Qubit Systems) at 5 s intervals (C407 – Data Acquisition Software version 2.0; Qubit Systems) and for trapping CO_2 for subsequent isotopic enrichment analyses. A drierite column was inserted into the small air stream for the removal of water just before the measurement of CO_2 content. From the small air stream, CO_2 was trapped quantitatively over 0·5 h periods for 24 h in a solution of 1 M-NaOH. Background CO_2 samples were collected before feeding the tracer dose between −0·5 and 0 h. Containers containing trapped CO_2 and NaOH were immediately re-vacuumed and stored at room temperature until later analysis.

To determine the recovery of CO_2 in the respiration system, CO_2 was released from a 2·24 M-$NaHCO_3$ solution (1 litre) that was placed in the empty respiration system. The quantity of $NaHCO_3$ was chosen to simulate CO_2 output from a 50 kg BW pig over a 2 h period and consuming approximately 1·8 kg of feed daily. To release CO_2, HCl was continuously infused into the solution at 265 ml/h for 2 h. The recovery of CO_2 was 96·9 (SE 1·01) % (*n* 6), demonstrating complete CO_2 recovery.

The pigs were fasted overnight (9 h) before the direct measurement of 18 : 3*n*-3 oxidation. The pigs were placed in the calorimetry system at approximately 07.00 hours, and measurements were started the next day at approximately 07.00 hours. At 07.30 hours, the pigs were fed a single-bolus

dose of U-^{13}C-18 : 3*n*-3 (10 mg/kg BW) mixed with 200 g of the experimental diet. The pigs consumed the tracer-containing feed within 5 min; thereafter, the remainder of the morning meal was given. At 6, 12 and 18 h after the start of CO_2 collection, CO_2 analysers were calibrated using a two-point calibration using a custom-made span gas containing 4 % CO_2 and N_2 (grade 4·8 representing 0 % CO_2) (BOC gas).

Enrichment of CO_2 in the exhaust air samples was determined in duplicate for each 0·5 h period by continuous flow isotope ratio MS[15]. The analyses were carried out at the Department of Earth Sciences, University of Waterloo (200 University Avenue West, Waterloo, ON, Canada N2L 3G1). The CO_2 enrichment and whole-body CO_2 excretion values were used to estimate the proportion of administered FA that was oxidised and excreted with breath as CO_2 over 24 h[14].

Calculations and statistical analyses

The apparent ileal digestibility (AID) of the ingested crude fat, 18 : 3*n*-3 and linoleic acid (18 : 2*n*-6) was calculated as described elsewhere[16], and it is expressed as a percentage.

The RE of 18 : 3*n*-3 and 18 : 2*n*-6 were calculated as follows:

$$\text{FA RE (\%)} = (\text{FA retention}/\text{FA intake}) \times 100, \quad (1)$$

where FA retention represents the difference between the final and initial whole-body 18 : 3*n*-3 and 18 : 2*n*-6 masses at defined stages of growth; FA intake for each individual FA was also calculated for defined stages of growth.

The AC of 18 : 3*n*-3 to *n*-3 HUFA was calculated as follows:

$$\begin{aligned}\text{AC of 18 : 3}n\text{-3 to individual } n\text{-3 HUFA (\%)} &= (\%)\\ &= ((\text{individual } n\text{-3 HUFA retention}\\ &\quad - \text{total individual } n\text{-3 HUFA intake})/\\ &\quad \text{total 18 : 3}n\text{-3 take)) } \times 100, \quad (2)\end{aligned}$$

where individual *n*-3 HUFA retention represents the whole-body retention of 18 : 3*n*-3 intermediary products such as stearidonic acid (18 : 4*n*-3), eicosatrienoic acid (20 : 3*n*-3), eicosatetraenoic acid (20 : 4*n*-3), 20 : 5*n*-3, docosatetraenoic acid (22 : 4*n*-3), 22 : 5*n*-3 and 22 : 6*n*-3 at defined stages of growth. Similar calculations were used to estimate the AC of 18 : 2*n*-6 to individual *n*-6 HUFA (e.g. all *n*-6 PUFA minus 18 : 2*n*-6). The intakes of 18 : 3*n*-3 and 18 : 2*n*-6 were calculated for the two subsequent 15 d periods.

The calculated mean disappearance of 18 : 3*n*-3 (expressed as a proportion of 18 : 3*n*-3 intake), an indirect measure of oxidation, was calculated[14] as follows:

$$\begin{aligned}\text{Disappearance of 18 : 3}n\text{-3 (\%)} &= (\text{AID 18 : 3}n\text{-3 intake} - (\text{RE 18 : 3}n\text{-3} + \text{AC of 18 : 3}n\text{-3 to }n\text{-3 HUFA}))/\text{total 18 : 3}n\text{-3 intake}. \quad (3)\end{aligned}$$

Direct oxidation of 18 : 3*n*-3 was calculated from the cumulative recovery of CO_2 with CO_2 in the exhaust air 24 h after feeding the tracer, and it is expressed as a proportion of the administered ^{13}C dose from U-^{13}C-18 : 3*n*-3. Cumulative recovery was calculated either directly from the air flow measurements and determined CO_2 enrichment excess for each 0·5 h period or from the estimated cumulative CO_2 recovery using alternative mathematical functions. No attempt was made to adjust values for the retention of CO_2 within bicarbonate pools in the pigs' body and faecal and urinary losses.

To fit the hourly recovery of ^{13}C in the expired air as a percentage of administered ^{13}C, four mathematical functions were applied: γ[17]; Ali & Schaeffer[18]; Wilmink[19]; Dijkstra *et al.*[20]. Similarly, to fit the cumulative recovery of ^{13}C in the expired air as a percentage of administered ^{13}C, five alternative mathematical growth functions were applied: monomolecular; Gompertz; Richards; Michaelis–Menten; von Bertalanffy[21–23]. The Solver Module of Excel (Excel; Microsoft Corporation) was used for the optimisation of numerical algorithms[24]. The model with the lowest values of Akaike's information criterion was considered the most appropriate model.

Cumulative FA intake and whole-body FA mass are expressed in g per pig. The various measures of FA utilisation were calculated using pigs as experimental units. For the various response variables, statistical analyses were carried out using ANOVA according to the MIXED procedure of SAS (version 9.2; SAS Institute, Inc.). The pigs were used as a random effect. The effect of litter (*n* 8) was first explored as a source of variation, but it was found to be not significant for the various response variables ($P > 0{\cdot}10$) and was therefore removed from the statistical model. Period effects on the RE and AC were assessed using the Tukey–Kramer honestly significant difference test. Linear and quadratic contrast analyses were carried out to assess changes in whole-body chemical components (protein, fat, ash and water) and whole-body FA over time. $P < 0{\cdot}05$ was considered significant, whereas $0{\cdot}05 < P < 0{\cdot}10$ was considered a trend and $P > 0{\cdot}10$ was considered non-significant.

Results

General observations, growth performance and body composition

The analysed contents of protein, crude fat, Ca, P, Na, 18 : 3*n*-3 and 18 : 2*n*-6 and the 18 : 2*n*-6/18 : 3*n*-3 ratio in the experimental diets were similar to the anticipated values (Table 1), indicating that the diets were prepared properly. The pigs appeared to be healthy, readily consumed their feed allowances and did not exhibit abnormalities in behaviour. Similarly, no problems were encountered with the direct measurement of 18 : 3*n*-3 oxidation using the isotope tracer. Growth performance data (Table 2) can be considered typical for this relatively low level of feed intake[25]. Growth rate and feed efficiency were similar between phase I and phase II ($P > 0{\cdot}10$), whereas daily feed intake increased with BW ($P < 0{\cdot}002$) as expected.

Across all the observations, the sum of chemical body constituents (protein, lipid, ash and water) contributed to 99·5 (SE 0·41) % of empty BW (Table 3), confirming the adequacy of the sampling and analytical procedures.

Table 2. Growth performance of pigs fed a grower diet containing 10 % ground flaxseed for 30 d*

Items	Days 0–15	Days 15–30	SEM	*P*
Number of pigs	11	5		
Initial BW (kg)	28·5	36·4		
Final BW (kg)	37·9	45·7		
Average daily feed intake (g/d)	1307	1452	25·6	0·002
Average daily gain (g/d)	604	630	38·0	0·655
Gain/feed (g/g)	0·463	0·430	0·249	0·378

BW, body weight.
* Pigs were fed restrictedly at 70 % of the voluntary daily digestible energy intake according to the National Research Council[8]. On day 15, six pigs were killed to determine chemical and physical body composition.

Across the slaughter BW, gut fill represented 5·03 (SE 0·36) % of live BW. Empty BW and main physical and chemical body component masses increased linearly over time ($P<0·01$). Relationships between body protein, body ash and body water masses were similar across the slaughter BW ($P>0·10$).

Fatty acid mass, apparent ileal digestibility and apparent conversion

The whole-body content of individual FA including SFA, MUFA, *n*-3 and *n*-6 PUFA increased linearly over time ($P<0·01$; Table 4). Time had no effect on the AID of FA and crude fat ($P>0·10$); across the two digesta sampling times, AID was 77 (SE 3·60) % for crude fat, 78·2 (SE 3·38) % for 18 : 2*n*-6 and 83·6 (SE 2·24) % for 18 : 3*n*-3. Expressed as a proportion of intake, RE declined with time (days 0–15 *v.* days 15–30) for both 18 : 3*n*-3 (68·4 *v.* 52·6 %; $P=0·098$) and 18 : 2*n*-6 (107 *v.* 53·2 %; $P=0·031$). Similarly, the AC of 18 : 3*n*-3 to eicosatrienoic acid (20 : 3*n*-3; 7·51 *v.* 4·05 %), eicosatetraenoic acid (20 : 4*n*-3; 0·62 *v.* 0·31 %) and 22 : 6*n*-3 (1·16 *v.* −0·004 %) was reduced over time ($P<0·05$; Fig. 2), whereas that of 18 : 3*n*-3 to 20 : 5*n*-3 (1·22 *v.* 0·83 %; $P=0·141$) and 22 : 5*n*-3 (2·52 *v.* 1·64 %; $P=0·110$) did not change over time. The AC of 18 : 2*n*-6 to *n*-6 HUFA such as γ-linolenic acid (18 : 3*n*-6; 0·34 *v.* 0·05 %), eicosadienoic acid (20 : 2*n*-6; 4·66 *v.* 1·04 %), dihomo-γ-linolenic acid (20 : 3*n*-6; 0·74 *v.* 0·16 %), arachidonic acid (20 : 4*n*-6; 2·32 *v.* 0·18 %), adrenic acid (22 : 4*n*-6; 0·63 *v.* −0·003 %) and *n*-6 docosapentaenoic acid (22 : 5*n*-6; 0·15 *v.* −0·01 %) was reduced over time ($P<0·05$; Fig. 3). Within the periods, total AC was higher for *n*-3 HUFA than for *n*-6 HUFA (13·1 *v.* 6·9 % and 8·8 *v.* 1·4 % for days 0–15 and 15–30, respectively; $P<0·01$; Figs. 2 and 3).

Oxidation and disappearance of α-linolenic acid

The appearance of CO_2 after feeding the single-bolus dose of U-^{13}C-18 : 3*n*-3 is shown in Figs. 4 and 5 for each individual pig. The excretion of ^{13}C in the expired air followed a similar pattern for all the pigs. The enrichment of ^{13}C in the exhaust air (breath CO_2) was detected 1 h after the administration of U-^{13}C-18 : 3*n*-3 and was found to reach peak levels at 4·5 h (7·25 (SE 0·95) % of the total ^{13}C exhaled or 0·554 (SE 0·06) % administered dose/h) and decrease to approximately half maximum levels by 9 h (3·08 (SE 0·34) % of the total ^{13}C

Table 3. Chemical and physical body composition of growing pigs fed a grower diet containing 10 % ground flaxseed for 30 d

Items	Day 0	Day 15	Day 30	SEM	*P*	Linear	Quadratic
Number of pigs	4	6	5				
Days	0[a]	15·8[b]	30[c]	0·566	<0·001	<0·001	0·232
Empty BW (kg)	25·1[a]	37·3[b]	44·1[c]	0·717	<0·001	<0·001	0·008
PB (%)*	16·8	17·0	17·3	0·313	0·524	0·266	0·991
LB (%)†	10·0[a]	13·5[b]	13·3[b]	0·695	0·009	0·008	0·039
LB/PB	0·599[a]	0·798[b]	0·774[b]	0·053	0·053	0·047	0·101
AB (%)‡	2·83	2·60	2·85	0·094	0·130	0·851	0·051
WB (%)§	70·3[a]	66·1[b]	66·1[b]	0·485	<0·001	<0·001	0·004
WB/PB$^{0.855}$	5·17	5·08	5·13	0·068	0·652	0·729	0·388
AB/PB	0·169	0·153	0·165	0·007	0·235	0·737	0·098
PVisc/PB	15·5[a]	11·00[b]	10·6[b]	0·543	<0·001	<0·001	0·007
PCar/PB	80·2[a]	84·6[b]	84·8[b]	0·701	0·001	0·001	0·023
LVis/LB	9·04[a]	5·34[b]	6·15[b]	0·327	<0·001	<0·001	<0·001
LCar/LB	90·7[a]	94·5[b]	93·7[b]	0·331	<0·001	<0·001	<0·001

BW, body weight; PB, body protein; LB, body lipid; AB, body ash; WB, body water; PVisc, viscera protein mass; PCar, carcass protein mass; LVis, viscera lipid mass; LCar, carcass lipid mass.
[a,b,c] Mean values with unlike superscript letters were significantly different ($P<0·05$).
* Whole PB mass, expressed as a percentage of empty BW.
† Whole LB mass, expressed as a percentage of empty BW.
‡ Whole AB mass, expressed as a percentage of empty BW.
§ Whole WB mass, expressed as a percentage of empty BW.

Table 4. Whole-body fatty acid (FA) mass in growing pigs fed a grower diet containing 10 % ground flaxseed for 30 d*

Items	Day 0	Day 15	Day 30	SEM	*P*	Linear	Quadratic
Number of pigs	4	6	5				
Live BW (kg)†	26·2	39·2	45·7	0·733	<0·001	<0·001	0·001
Empty BW (kg)	25·1^{a}	37·3^{b}	44·1^{c}	0·717	<0·001	<0·001	0·008
Body lipid (% of empty BW)	10·0^{a}	13·5^{b}	13·3^{b}	0·695	0·009	0·008	0·039
FA (g in empty BW)							
SFA							
14 : 0	27·6^{a}	48·1^{b}	55·0^{b}	4·68	0·004	0·002	0·213
16 : 0	439^{a}	771^{b}	846^{b}	69·5	0·004	0·002	0·122
18 : 0	169^{a}	325^{b}	392^{b}	45·0	0·015	0·005	0·406
20 : 0	2·22^{a}	4·46^{b}	4·93^{b}	0·697	0·041	0·020	0·276
22 : 0	0·05	0·07	0·08	0·019	0·897	0·743	0·750
24 : 0	0·06	0·05	0·07	0·244	0·812	0·854	0·545
ΣSFA	638^{a}	1148^{b}	1298^{b}	113	0·005	0·002	0·190
MUFA							
7*c*-14 : 1	0·83	0·99	0·88	0·127	0·629	0·794	0·364
9*c*-16 : 1	77·9	110	98·9	10·2	0·096	0·185	0·076
9*c*-18 : 1	847^{a}	1561^{b}	1645^{b}	111	<0·001	<0·001	0·025
11*c*-20 : 1	20·1^{a}	33·9^{b}	32·5^{a,b}	2·89	0·010	0·013	0·036
13*c*-22 : 1	0·56	0·69	0·19	0·242	0·307	0·281	0·270
15*c*-24 : 1	0·01^{a,b}	0·01^{a}	0·04^{b}	0·012	0·249	0·336	0·171
ΣMUFA	947^{a}	1707^{b}	1777^{b}	120	<0·001	<0·001	0·024
n-6 PUFA							
18 : 2*n*-6	261^{a}	583^{b}	748^{c}	53·7	<0·001	0·001	0·084
18 : 3*n*-6	1·03^{a}	2·05^{b}	2·22^{b}	0·159	<0·001	<0·001	0·028
20 : 2*n*-6	11·4^{a}	25·5^{b}	28·7^{b}	2·63	<0·001	<0·001	0·025
20 : 3*n*-6	2·55^{a}	4·78^{b}	5·29^{b}	0·321	<0·001	<0·001	0·019
20 : 4*n*-6	7·30^{a}	14·6^{b}	15·0^{b}	0·978	<0·001	<0·001	0·005
22 : 4*n*-6	2·39^{a}	4·31^{b}	4·30^{b}	0·341	0·002	0·002	0·021
22 : 5*n*-6	0·10^{a}	0·56^{b}	0·55^{b}	0·121	0·042	0·035	0·123
Σ*n*-6 PUFA	286^{a}	634^{b}	804^{c}	54·8	<0·001	<0·001	0·069
n-3 PUFA							
18 : 3*n*-3	15·2^{a}	247^{b}	431^{c}	22·6	<0·001	<0·001	0·357
18 : 4*n*-3	1·61	1·69	1·90	0·215	0·683	0·438	0·713
20 : 3*n*-3	2·05^{a}	27·5^{b}	41·5^{c}	2·71	<0·001	<0·001	0·135
20 : 4*n*-3	0·50^{a}	2·65^{b}	3·75^{c}	0·301	<0·001	<0·001	0·135
20 : 5*n*-3	0·717^{a}	4·86^{b}	7·55^{c}	0·582	<0·001	<0·001	0·350
22 : 5*n*-3	1·67^{a}	10·2^{b}	16·0^{c}	1·14	<0·001	<0·001	0·291
22 : 6*n*-3	2·39^{a}	6·45^{b}	6·50^{b}	0·632	<0·001	<0·001	0·015
Σ*n*-3 PUFA	24·2^{a}	300^{b}	509^{c}	26·9	<0·001	<0·001	0·273
Σ*n*-6 PUFA/*n*-3 PUFA	11·8^{a}	2·11^{b}	1·59^{c}	0·156	<0·001	<0·001	<0·001
ΣPUFA	310^{a}	935^{b}	1313^{c}	93·6	<0·001	<0·001	0·115
ΣAnalysed FA	1894^{a}	3791^{b}	4388^{b}	278	<0·001	<0·001	0·058

BW, body weight.
^{a,b,c} Mean values with unlike superscript letters were significantly different ($P<0·05$).
* Pigs were fed a common diet for 30 d. Cumulative 18 : 2*n*-6 intakes for days 0–15, 15–30 and 0–30 were 299 (SE 4·42), 302 (SE 21·6) and 567 (SE 16·4) g/pig, respectively, whereas cumulative 18 : 3*n*-3 intakes for days 0–15, 15–30 and 30 were 339 (SE 5·01), 343 (SE 24·5) and 643 (SE 18·6) g/pig, respectively.
† Live BW.

exhaled or 0·247 (SE 0·04) % administered dose/h). At 15 h, 95·2 (SE 2·61) % of the 24 h cumulative expired ^{13}C was recovered. Between 15 and 17 h, the enrichment of ^{13}C in the exhaust air returned to baseline levels for all the pigs. Based on Akaike's information criterion (Table 5), the Dijkstra function was the best mathematical function to fit the hourly ^{13}C recovery in the exhaust air (Fig. 4), whereas the Richards function was the most appropriate function to represent the cumulative recovery of ^{13}C in the exhaust air as a function of time (Fig. 5). The estimated cumulative oxidation of U-^{13}C-18 : 3*n*-3 according to the Richards, Gompertz, Michaelis–Menten, von Bertalanffy and monomolecular functions was 7·88 (SE 0·97), 7·89 (SE 0·98), 7·77 (SE 0·95), 7·88 (SE 0·98) and 7·67 (0·93) %, respectively, whereas the directly determined cumulative recovery of CO_2 in the exhaust air 24 h after feeding the tracer was 7·91 (SE 0·98) %, ranging from 5·14 to 9·68 % between the pigs.

Based on the mass balance approach, the calculated disappearance of 18 : 3*n*-3 tended to increase over time (2·0 *v.* 23·7 % of the available intake; $P=0·07$). The calculated disappearance of 18 : 3*n*-3 over the entire 30 d period (8·81 (SE 5·24) %) was similar to the value obtained with the direct oxidation method.

Discussion

The purpose of the present study was to determine changes over time in the RE of 18 : 3*n*-3 and its AC to 20 : 5*n*-3, 22 : 5*n*-3 and 22 : 6*n*-3 and intermediary *n*-3 HUFA metabolites on a whole-body basis in growing pigs fed a diet containing

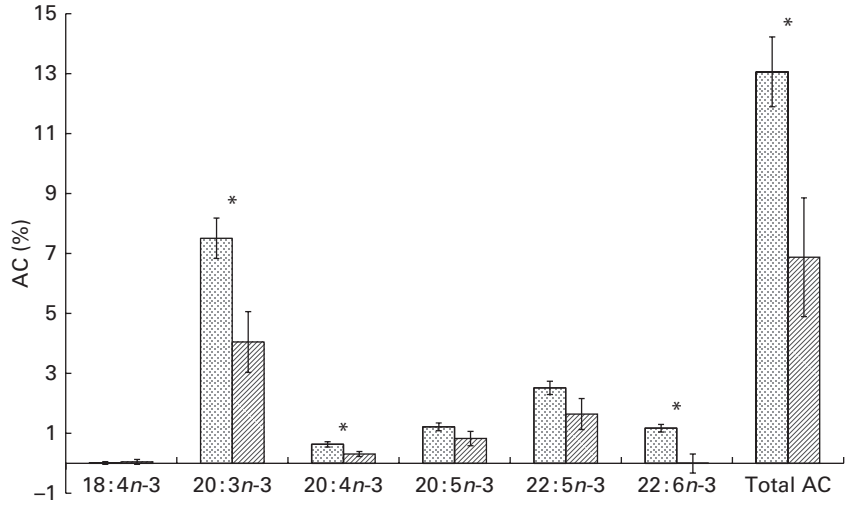

Fig. 2. Apparent conversion (AC) of α-linolenic acid to 18 : 4*n*-3, stearidonic acid; 20 : 3*n*-3, eicosatrienoic acid; 20 : 4*n*-3, eicosatetraenoic acid; 20 : 5*n*-3, EPA; 22 : 5*n*-3, docosapentaenoic acid; and 22 : 6*n*-3, DHA, during different time periods (days 0–15 (▨) and 15–30 (▧)) in growing gilts fed a grower diet containing 10 % ground flaxseed for 30 d. * Values were significantly different between days 0–15 and days 15–30 ($P<0·05$).

10 % FS. Using the serial slaughter technique, measurements were made over two consecutive 15 d periods. Moreover, the oxidation of 18 : 3*n*-3 was determined using both a direct isotope method and a mass balance approach (e.g. calculated disappearance). In previous studies, the effect of feeding diets containing either 1·44 or 13·5 g/kg 18 : 3*n*-3 on whole-body RE was determined in weanling pigs (14 or 21 d old) raised in two environments (segregated and non-segregated early weaning) without determining the AC of 18 : 3*n*-3 to *n*-3 HUFA[6]. Similarly, Kloareg *et al.*[26] determined the RE of 18 : 3*n*-3 and its AC to *n*-3 HUFA in pigs weighing between 90 and 150 kg BW and consuming a diet containing 1·5 g/kg 18 : 3*n*-3. In these two studies, the AID of 18 : 3*n*-3 was not determined.

The design of the present experiment is in contrast to that of human studies in which no attempt was made to adapt the subjects to the experimental diet before determining the oxidation of 18 : 3*n*-3[27–29]. Moreover, in most of the human studies, breath is not sampled continuously[27,30]. The latter can lead to inaccuracies in the estimates of CO_2 production, and consequently FA oxidation, especially when breath is sampled during periods that are less than 5 min in duration and at 2 or 3 h intervals[29,31].

Retention efficiency and apparent conversion of n-3 fatty acids

In the present study, the whole-body masses of SFA, MUFA, and *n*-3 and *n*-6 PUFA increased over time in the pigs when fed modest amounts of FS. These results are in agreement with those of other studies in which the contents of SFA,

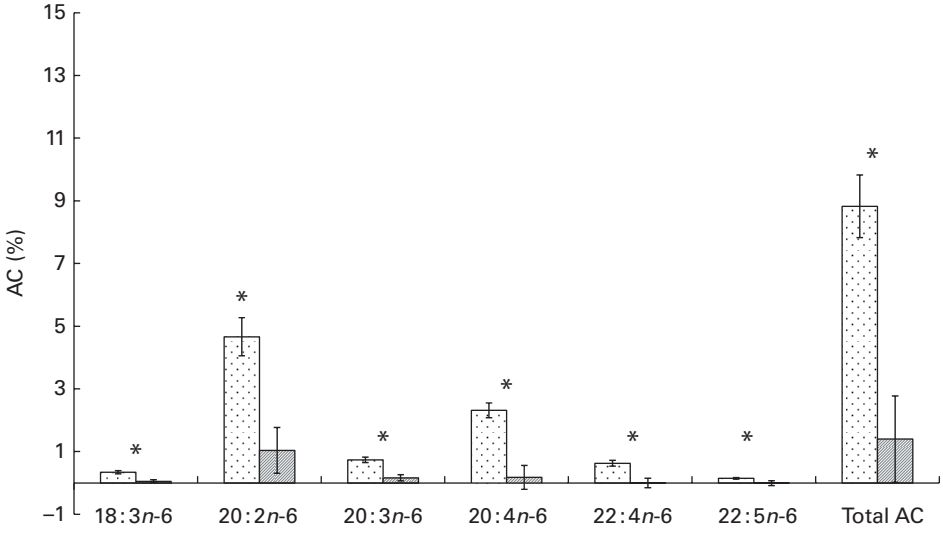

Fig. 3. Apparent conversion (AC) of linoleic acid to 18 : 3*n*-6, γ-linoleic acid; 20 : 2*n*-6, eicosadienoic acid; 20 : 3*n*-6, dihomo-γ-linoleic acid; 20 : 4*n*-6, arachidonic acid; 22 : 4*n*-6, adrenic acid; and 22 : 5*n*-6, docosapentaenoic acid, during different time periods (days 0–15 (□) and 15–30 (▩)) in growing gilts fed a grower diet containing 10 % ground flaxseed for 30 d. * Values were significantly different between days 0–15 and days 15–30 ($P<0·05$).

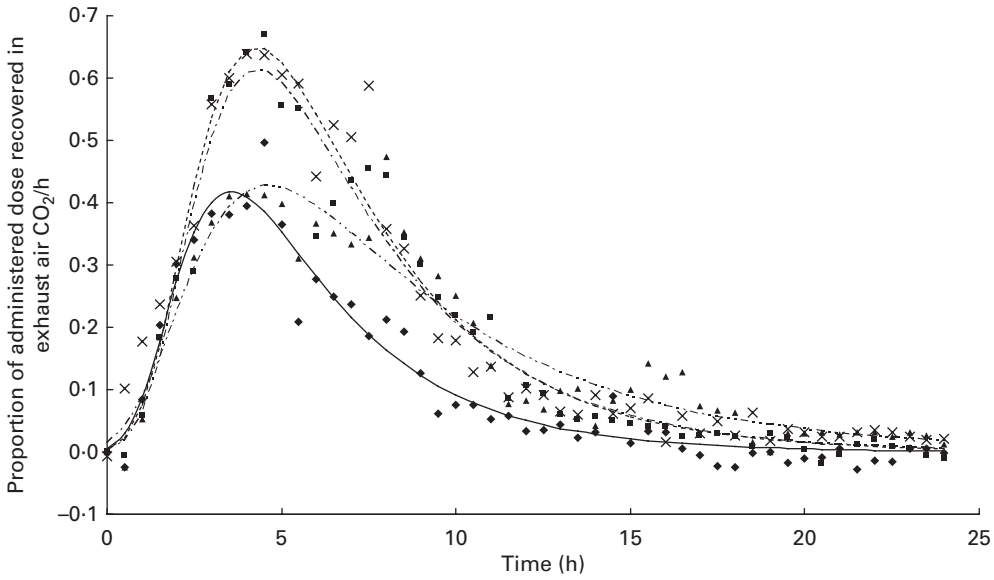

Fig. 4. Hourly ^{13}C recovery in the exhaust air expressed as a proportion of the administered tracer dose of uniformly labelled ^{13}C-α-linolenic acid 24 h immediately following the oral administration of a single bolus of the tracer to growing gilts that were fed a grower diet containing 10 % flaxseed (*n* 4). Oxidation study was conducted on day 12 of feeding the experimental diets. The Dijkstra[20] function used to fit the present data set was as follows: $Y = a \times \exp(k1 \times (1 - \exp\{-k2 \times t\})/k2 - c \times t)$, where '*a*', '*c*' and '*t*' represent the theoretical initial CO_2 production at time '0', rate of disappearance of ^{13}C in the exhaust air (per h) and time since the start of sample collection, respectively, whereas *k*1 and *k*2 represent the rate of ^{13}C production and the decay parameter constant, respectively. ◆, Pig 03; —, predicted 03; ■, Pig 10; ----, predicted 10; ▲, Pig 02; ----, predicted 02; ×, Pig 06; ---- , predicted 06.

MUFA, and *n*-3 and *n*-6 PUFA in specific pork tissues[2,32,33] or in pigs' whole body[6] increased over time when feeding FS-containing diets. In general, the whole-body masses of *n*-3 or *n*-6 PUFA are proportional to dietary *n*-3 or *n*-6 PUFA intakes.

In the present study, the RE of 18 : 3*n*-3 in pigs weighing between 27 and 45 kg BW was approximately 58·7 %, whereas Kloareg *et al.*[26] observed that the RE of 18 : 3*n*-3 in pigs weighing between 90 and 150 kg BW was 42·4 %, much lower than that observed in the present study. In younger pigs aged between 14 and 49 d, Bazinet *et al.*[6] observed that the RE of 18 : 3*n*-3 in pigs fed high 18 : 3*n*-3 (13·5 g/kg) content varied between 60 and 84 % of total 18 : 3*n*-3 intake. The latter suggests that the RE of *n*-3 PUFA decreases with age and BW. However, the dietary levels and cumulative intake of 18 : 3*n*-3, or whole-body 18 : 3*n*-3 content, may also affect the RE of *n*-3 PUFA. Additional observations are required to be made to further investigate this phenomenon in growing pigs.

In pigs weighing between 90 and 150 kg BW and consuming low amounts of 18 : 3*n*-3, the whole-body AC of 18 : 3*n*-3 to *n*-3 HUFA was found to be approximately 15 %[26], which was similar to that observed in the present study. In other studies, the AC of 18 : 3*n*-3 to *n*-3 HUFA in blood was measured using isotope tracers (^{14}C-, ^{13}C- or ^{2}H-18 : 3*n*-3) and at one point in time; in these studies, the effects of the level and duration of feeding 18 : 3*n*-3 on the RE and AC of 18 : 3*n*-3 to *n*-3 HUFA over time were not explored[27,34,35]. In the present study, the AC of 18 : 3*n*-3 to *n*-3 HUFA was largest for 20 : 3*n*-3, followed by 22 : 5*n*-3 and 20 : 5*n*-3, respectively. The latter is in contrast to that observed in human studies and, based on observations in blood, indicates that 20 : 5*n*-3 and then 22 : 5*n*-3 are the main HUFA derived from 18 : 3*n*-3[27,36]. It can be argued that *n*-3 PUFA profiles in blood do not represent whole-body *n*-3 PUFA profiles[37–39]; therefore, measurements based on blood *n*-3 PUFA profiles should be interpreted with caution.

Relatively few experiments[32,33] have reported increasing contents of 20 : 3*n*-3 in tissues after feeding FS to pigs. Moreover, to our knowledge, the AC of 18 : 3*n*-3 to 20 : 3*n*-3 in mammals has not been reported in the literature. In the present experiment, 20 : 3*n*-3 was the largest contributor to total *n*-3 HUFA in the body (8 %), which deserves further attention. Pigs fed FS appear unique in their ability to elongate 18 : 3*n*-3 to 20 : 3*n*-3 via chain elongation process, which differs from previous views of FA metabolism[40]. In mammals, 20 : 3*n*-3 is generally considered to be an end product of *n*-3 PUFA metabolism, in part because its conversion to 20 : 4*n*-3 has not been unequivocally established[41]. Recently, it has been determined that Δ8-desaturate is rather active in mammals and is able to convert 20 : 3*n*-3 to 20 : 4*n*-3[42], which in turn can enter the Sprecher pathway. This is of particular interest because 20 : 3*n*-3 can help to maintain an adequate supply of *n*-3 HUFA for physiologically active eicosanoids and different membrane structures. Whether this alternative pathway is highly active in pigs deserves further investigation.

In agreement with studies involving human subjects and small mammals[27,43], the AC of 18 : 3*n*-3 to 22 : 6*n*-3 was low in the present study. It has been suggested that the initial conversion of 18 : 3*n*-3 to stearidonic acid (18 : 4*n*-3) by the action of Δ6-desaturase and the conversion of 20 : 4*n*-3 to 20 : 5*n*-3 by the action of Δ5-desaturase are the two main rate-limiting reactions in the Sprecher pathway[40].

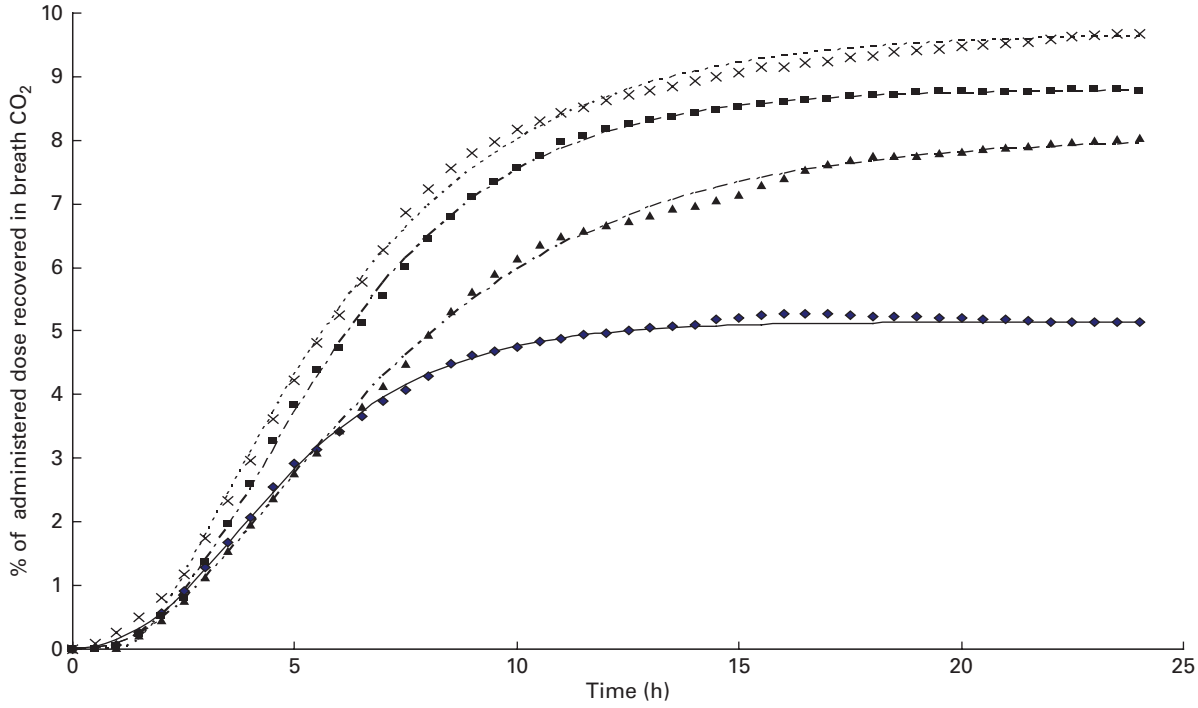

Fig. 5. Cumulative ^{13}C recovery in the exhaust air expressed as a proportion of the administered tracer dose of uniformly labelled ^{13}C-α-linolenic acid over 24 h following administration at hour 0 (*n* 4). Growing gilts were fed a grower diet containing 10 % flaxseed for 30 d. Oxidation study was conducted on day 12 of feeding the experimental diets. The Richards function used to fit the present data set was as follows: $Y = A + (H - A)/(1 + t \times \exp\ [-B \times \{ x - M \}])^{(1/t)}$, where '*A*', '*H*' and '*B*' represents the initial ^{13}C concentration, final ^{13}C concentration and ^{13}C production rate, whereas '*t*' and '*M*' are constants representing the asymptote of maximum ^{13}C production and *t* of maximum production, respectively. ◆, Pig 03; —, predicted Pig 03; ■, Pig 10; ----, predicted Pig 10; ×, Pig 06; ·····, predicted Pig 06; ▲, Pig 02; ----, predicted Pig 02. (A colour version of this figure can be found online at http://www.journals.cambridge.org/bjn).

However, based on feeding 18 : 4*n*-3 and 20 : 5*n*-3 to human subjects, it has been shown that the concentrations of both 20 : 5*n*-3 and 22 : 5*n*-3 in plasma phospholipids can be increased and that no significant change occurs in the concentration of 22 : 6*n*-3[44,45]. The latter suggests that the main constraint for the appearance of 22 : 6*n*-3 occurs after the synthesis of 22 : 5*n*-3, which supports the lower content of 22 : 6*n*-3 than of 22 : 5*n*-3 in body fat in the present experiment. These results suggest that the potential rate-limiting reactions, involving the conversion of 22 : 5*n*-3 to 22 : 6*n*-3, might be found in the last steps of the pathway and in the conversion via the intermediary tetracosapentaenoic acid (24 : 5*n*-3). This is consistent with the observation that 22 : 6*n*-3 cannot be synthesised from 22 : 5*n*-3 in the heart of rats, due to the absence of elongase-2 expression, which is critical for the conversion of 22 : 5*n*-3 to 24 : 5*n*-3[46]. While elongase-5, which elongates FA that are sixteen to twenty-two carbons long, is regulated by PPAR (PPARα), elongase-2 is regulated by sterol regulatory element-binding protein-1[46]. These two different genetic control mechanisms might explain the tissue-selective conversion of 18 : 3*n*-3 to 22 : 6*n*-3 and other *n*-3 PUFA metabolites.

The observed reduction in the AC of 18 : 3*n*-3 to *n*-3 HUFA with the duration of feeding 18 : 3*n*-3 has not been fully explored. Matsuzaka *et al.*[47] demonstrated that the expression of Δ6-desaturase and Δ5-desaturase mRNA in rat hepatocytes, controlled by sterol regulatory element-binding protein-1, decreased over time in the presence of 18 : 2*n*-6; similar but smaller effects were observed in the presence of 20 : 5*n*-3, 22 : 5*n*-3 or 22 : 6*n*-3, whereas feeding a fat-free diet, stearic acid or oleic acid did not affect the expression of these desaturases. The latter suggests that feeding 18 : 2*n*-6 or 18 : 3*n*-3 might, over time, reduce the AC of 18 : 3*n*-3 to *n*-3 HUFA and that *n*-3 HUFA, in turn, can down-regulate the expression of Δ6-desaturase, Δ5-desaturase and elongase-2. This is consistent with the observed reduction over time in the AC of both 18 : 3*n*-3 and LA to their metabolites in the present study.

According to Sprecher *et al.*[40], *n*-3 and *n*-6 FA share a common pathway for their elongation and desaturation, implying interdependence of the metabolism of these two PUFA groups. In the present experiment, equal amounts of *n*-3 and *n*-6 PUFA were included in the diets, which resulted in higher rates of AC of *n*-3 PUFA than of *n*-6 PUFA. Similar results were obtained by others[43,48]. In the present study, 18 : 3*n*-3 appears to be a stronger suppressor of *n*-6 HUFA conversion than 18 : 2*n*-6 is of *n*-3 HUFA conversion.

Oxidation of α-linolenic acid

In the present study, the direct measurement of oxidation, using isotope tracers, and the mass balance approach (calculated disappearance, indirect means to estimate oxidation) yielded very similar estimates of 18 : 3*n*-3 oxidation, which suggests that both methods may be suitable to assess the oxidation of 18 : 3*n*-3 in growing pigs.

Table 5. Mean square prediction error (MSPE)* and Akaike's information criterion (AIC)† values for alternative mathematical functions to represent either cumulative or hourly excretion of CO_2 in the expired air following the ingestion of a bolus of uniformly labelled ^{13}C-18 : 3*n*-3 (U-^{13}C-18 : 3*n*-3) for individual pigs (Figs. 3 and 4)

		Pig			
Model		2	3	6	10
Hourly excretion of CO_2 in the expired air expressed as a proportion of the administered dose of tracer					
Dijkstra	MSPE	0·002	0·001	0·002	0·002
	AIC	−104·7	−139·9	−104·0	−109·7
Gamma	MSPE	0·003	0·003	0·006	0·007
	AIC	−85·00	−94·12	−54·09	−45·40
Wilmink	MSPE	0·004	0·006	0·012	0·011
	AIC	−67·35	−50·08	−17·90	−24·20
Ali–Schaeffer	MSPE	0·002	0·002	0·006	0·005
	AIC	−96·42	−105·8	−54·02	−61·70
Cumulative excretion of CO_2 in the expired air expressed as a proportion of the administered dose of tracer					
Richards	MSPE	0·009	0·005	0·019	0·004
	AIC	−30·84	−59·80	2·874	−72·16
Gompertz	MSPE	0·020	0·005	0·034	0·007
	AIC	6·463	−56·97	33·02	−44·49
Michaelis–Menten	MSPE	0·025	0·016	0·025	0·024
	AIC	18·21	−4·80	18·21	15·85
Monomolecular	MSPE	0·435	0·190	0·535	0·666
	AIC	155·9	115·3	166·0	176·8
von Bertalanffy	MSPE	0·009	0·005	0·019	0·004
	AIC	−32·62	−60·77	4·120	−73·18

* MSPE = residual sum of squares between the actual and estimated cumulative oxidation of 18 : 3*n*-3/number of observations within treatment.
† AIC = 2 × (number of parameters of function) + total observations within treatment × (Ln (residual sum of squares between the actual and estimated cumulative oxidation of 18 : 3*n*-3)).

The appearance of ^{13}C in the exhaust air, and thus oxidation of 18 : 3*n*-3, followed similar patterns when compared with those observed in human studies[28,29,31]. In the present study, several alternative mathematical functions were used to fit the patterns of hourly and actual cumulative CO_2 excretion following the oral administration of a bolus dose of U-^{13}C-18 : 3*n*-3. A detailed discussion of the biological meaning of parameters in these mathematical functions has been published elsewhere[20–22,49]. Based on Akaike's information criterion, the Dijkstra function was the best mathematical function to fit the hourly ^{13}C recovery in the exhaust air (Fig. 4). Because of the complexity of the data set, the γ, Wilmink and Ali–Schaeffer functions were not sufficiently flexible to represent the hourly recovery of CO_2. Furthermore, various growth functions accurately fit the cumulative recovery of CO_2 (Fig. 5 and Table 5). Although the estimated cumulative oxidation of 18 : 3*n*-3 was identical for the Richards, Gompertz and von Bertalanffy functions, Akaike's information criterion values indicated that the Richards and von Bertalanffy functions were the most suitable mathematical functions to represent the actual cumulative oxidation pattern observed in the present study (Fig. 5). These results indicate that the estimated cumulative oxidation of 18 : 3*n*-3, using the mathematical models, compared quite closely to the actual cumulative oxidation of 18 : 3*n*-3. Modelling patterns of hourly and actual cumulative CO_2 excretion will help us to assess the dynamics of 18 : 3*n*-3 oxidation in growing pigs and can provide an accurate estimate of the partitioning of 18 : 3*n*-3 towards oxidation.

The observed oxidation of 18 : 3*n*-3 appears to be lower in the present study (approximately 8 %) when compared with observations in other mammalian species, including adult humans[28,36,50]. In human studies[27,36], the oxidation of 18 : 3*n*-3 accounts for 20–38 % of the administrated tracer dose during 24 h. The oxidation of 18 : 3*n*-3 in guinea pigs was found to be approximately 40 %[5], whereas in rats it was found to range between 60 and 78 % of the administrated dose[4,43]. Differences among studies might be related to inherent factors such as age of subjects, adaptation to the experimental diet, sex, body composition and rates of body fat gain[51–54]. These highly variable results highlight the need to better understand animal and dietary factors that influence 18 : 3*n*-3 utilisation in growing pigs and other species. Moreover, the experimental methodology should also be considered carefully, including between-animal variability and analytical procedures to quantify FA contents in diets and animal tissues. Using deuterated palmitic acid as an indicator of FA oxidation, Westerterp *et al.*[54] determined that the oxidation of dietary fat was inversely related to body fatness (r −0·66). As growing pigs have a higher capacity for lipid deposition (< 200 g/d)[6] and body fatness (13·5 % of body fatness; Table 3) than adult humans, the lower oxidation rate of 18 : 3*n*-3, observed in the present experiment, appears to be consistent with the results of Westerterp *et al.*[54].

In the present study, feeding 15·5 g/kg of 18 : 3*n*-3 to pigs during a 30 d period induced increasing rates of oxidation of 18 : 3*n*-3 over time. To our knowledge, quantitative information concerning the increasing rates of oxidation of

18 : 3*n*-3 with age is not available for either pigs or humans. Data on the incorporation of 18 : 3*n*-3 in select tissues of pigs fed 15 % FS for 7, 15, 21 and 28 d before slaughter at 136 kg BW appear consistent with the findings of the present study[2]. In the present study, it was observed that the incorporation of 18 : 3*n*-3 in pork loin was quite efficient during the first 2 weeks of feeding FS; thereafter, the incremental incorporation of 18 : 3*n*-3 diminished, suggesting that the oxidation of *n*-3 PUFA increases over time or increases with increasing whole-body *n*-3 PUFA content. The latter is an important nutritional phenomenon that has not been fully explored in both animal and human studies. It appears that when feeding constant increased dietary levels of *n*-3 PUFA, and 18 : 3*n*-3 in particular, the oxidation of *n*-3 PUFA increases over time. Whether there is a particular time period during pigs' development when the RE and AC of 18 : 3*n*-3 to *n*-3 HUFA are more efficient, as well as the effect of whole-body FA composition on the RE and AC of 18 : 3*n*-3, remains to be explored further.

In conclusion, on a whole-body basis, the RE and AC of dietary 18 : 3*n*-3 to *n*-3 HUFA decreases with the duration of feeding a diet containing 15·5 g/kg of 18 : 3*n*-3 to growing pigs with a low initial whole-body *n*-3 PUFA content. Direct measures of 18 : 3*n*-3 oxidation (7·91 % of tracer dose; based on feeding a single bolus of labelled 18 : 3*n*-3) and indirect estimates of 18 : 3*n*-3 oxidation (8·81 % disappearance of intake; based on mass balance) yield similar values; these values are lower in pigs when compared with those in other mammalian species. Feeding modest amounts of 18 : 3*n*-3 leads to a significant increase in the content of 20 : 5*n*-3, 22 : 5*n*-3 and 22 : 6*n*-3 in the pigs' body, thus providing health benefits to *n*-3 PUFA-enriched pork consumers. Pigs appear to be unique in that they elongate and store 20 : 3*n*-3, an intermediate between 18 : 3*n*-3 and 20 : 5*n*-3, which deserves to be explored further.

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The authors' contributions are as follows: H. R. M.-R. and C. F. M. d. L. conducted the experiment and had the primary responsibility of the final content of the manuscript; J. P. C. assisted in the modelling part of the manuscript; A. K. S. and J. L. A. were responsible for teaching the use of the calorimetric equipment; C. F. M. d. L chaired all the meetings; all the authors contributed to the manuscript concept and result interpretation, wrote parts of the draft version, critically revised the manuscript, and read and approved the final version.

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The authors declare that they have no conflicts of interest to declare.

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