

Deposition of dietary fatty acids and of *de novo* synthesised fatty acids in growing pigs: effects of high ambient temperature and feeding restriction

Maela Kloareg, Laurent Le Bellego†, Jacques Mourot, Jean Noblet and Jaap van Milgen*

Unité Mixte de Recherche sur le Veau et le Porc, INRA, 35590 Saint-Gilles, France

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Predicting aspects of pork quality becomes increasingly important from both a nutritional and a technological point of view. Little information is, however, available concerning the quantitative relation between nutrient intake and fatty acid (FA) deposition at the whole-animal level. In this study, eight blocks of five littermate barrows were used in a comparative slaughter trial. At 24 kg body weight (BW), one pig from each litter was slaughtered to determine the initial FA composition. The other littermates were assigned to one of four feeding levels (ranging from 70 % to 100 % of intake *ad libitum*) and were given a diet containing 0.36 g/kg lipid and 0.22 g/kg FA. The temperature for each block was maintained at either 23 or 30°C. At 65 kg, the pigs were slaughtered and the body lipid and FA composition was determined. Seventy per cent of the digested *n*-6 FA and 50 % of the *n*-3 FA were deposited. The average composition of *de novo* synthesised FA corresponded to 1.7, 30.3, 2.4, 19.7 and 45.9 % for 14:0, 16:0, 16:1, 18:0 and 18:1 FA, respectively. At 23°C and for feeding *ad libitum*, 33 % of 16:0 FA was deposited, 1.7 % shortened to 14:0, 63 % elongated to 18:0 and 2.8 % unsaturated to 16:1. Twenty-eight per cent of 18:0 FA was deposited and 72 % unsaturated to 18:1. At 30°C, 18:0 FA desaturation was reduced by 3.5 %. Feed intake and temperature independently affected the elongation of 16:0 FA. A reduction in feed intake increased the elongation rate, whereas the increase in temperature reduced the elongation rate.

Lipid deposition: Fatty acid composition: Pig: Model

Predicting aspects of pork quality becomes increasingly important from both a technological and a nutritional point of view. The lipid content and fatty acid (FA) profile in the tissues have an impact on the technological transformation (i.e. a high content of PUFA increases the risk of oxidation) and affect the nutritional and organoleptic quality (e.g. intramuscular lipid content, saturated FA content, and the *n*-3 : *n*-6 ratio).

Lipid and FA deposition in pigs is strongly affected by factors including genotype, sex, age, live weight, environmental temperature and nutrition (e.g. Wood, 1984; Lebret & Mourot, 1998; Le Dividich *et al.* 1998). Although numerous studies have studied the relation between nutrition and FA composition of the tissues (e.g. Miller *et al.* 1990; Madsen *et al.* 1992; Wiseman & Agunbiade, 1998; Gatlin *et al.* 2002; Ostrowska *et al.* 2003), these relations are often limited to a single tissue (typically backfat). Consequently, for predictive purposes, this information can only be exploited using empirical relationships. A more mechanistic representation of the relation between nutrition and FA composition is desirable in order to define nutritional strategies that modulate the FA profile of the tissues. Lizardo *et al.* (2002) developed a model to predict pork quality based on relatively simple hypotheses concerning the fate of dietary FA, the FA profile of the *de novo* synthesised FA and the partitioning of lipids and FA between different tissues. Model development was, however, hampered by a lack of information concerning the relation between nutrient intake and FA deposition at the whole-animal

level (Lizardo *et al.* 2002). In fact, a more mechanistic approach requires accounting for all inputs and outputs of FA metabolism. Measuring these at the whole-animal level is laborious and costly.

Le Bellego *et al.* (2002) studied the effect of feed intake and ambient temperature on protein and lipid deposition at the whole-animal level using the comparative slaughter technique. The objective of the present study is to exploit these data further in order to estimate the key elements of FA metabolism at the whole-animal level. These include the deposition (*v.* catabolism or further metabolism) of dietary FA, the composition of *de novo* synthesised FA and the distribution between different anatomical sites.

Material and methods

Experimental design

Details concerning the experimental design and methods can be found in Le Bellego *et al.* (2002). In short, eight blocks of five or six Piétrain × (Landrace × Large White) littermate barrows were used in a factorial design including two ambient temperatures (23°C for thermoneutrality and 30°C for the high temperature) and four feeding levels (100 %, 90 %, 80 % and 70 % of feed intake *ad libitum*) at each temperature. A 1-week period was used prior to the experiment to allow the pigs to adapt to the experimental conditions (housing, diet and temperature). At the end of the adaptation period, one pig per block was slaughtered at body weight

Abbreviations: BW, body weight; FA, fatty acid; FL, feeding level; L(T), litter within temperature.

* **Corresponding author:** Dr Jaap van Milgen, fax +33 2 23 48 50 80, email jaap.vanmilgen@rennes.inra.fr

† Present address: Ajinomoto Eurolysine, 153, rue de Courcelles, 75 817 Paris cedex 17, France.

(BW) approximately 24 kg (initial group). The remaining pigs were offered feed at one of the four feeding levels (within a temperature). When available (i.e. for four blocks), the additional littermate was allocated to the *ad libitum* treatment. These pigs were slaughtered at approximately 65 kg (Le Bellego *et al.* 2002).

Animals were offered a diet based on wheat (35%), corn (35%) and soyabean meal (26%) without additional oils or fats, and containing 18.5% crude protein. The chemical composition of the diet is given in Table 1. A representative feed sample was obtained by regularly taking samples of the distributed feed. The DM content of the distributed ration was measured weekly. Feed refusals (if any) were collected and weighed daily, and were sampled to measure the DM content.

At slaughter, blood was collected, weighed and sampled. As the average lipid deposition in the blood was less than 0.05 g/d (Le Bellego *et al.* 2002), its contribution to lipid and FA deposition was ignored in the analysis. The viscera (kidneys, liver, heart and lungs, spleen and diaphragm), combined with the head, tail and leaf fat, were considered as a single compartment (C1), whereas the two half-carcasses were considered as another compartment (C2). The two compartments were ground separately, minced and homogenised. Two samples of each compartment were taken for further chemical analysis (Le Bellego *et al.* 2002).

Chemical analyses

The DM, ash, crude protein, starch, ether extract and crude fibre contents in the diet, and the DM, ash, crude protein, ether extract

contents in the compartment samples, were measured according to Association of Official Analytical Chemists (1990). Prior to FA determination, lipids were extracted according to the method of Folch *et al.* (1957). This method extracts lipids with a chloroform and methanol mixture at room temperature for 2 h. The FA were transmethylated according to Morrisson & Smith (1964), and the FA profile was obtained by gas chromatography on a 30 m long, 0.25 mm wide capillary column.

Calculations

In addition to FA, extracted lipids also contained glycerol, phospholipids and other chloroform- or methanol-soluble components. The FA content of the two carcass components was calculated as the lipid content multiplied by their respective analysed FA profile.

Deposited FA originate from digested dietary FA and, for non-essential FA, from *de novo* synthesised FA. Ileal digestibility values for FA were not determined but were supposed to correspond to the means of those measured by Jørgensen *et al.* (1993) for five diets, comprising a basal diet containing 0.5% crude fat and different levels of added soyabean oil (from 0% to 3%). These values were 90.9%, 90.7%, 85.3%, 86.0%, 93.9%, 96.2% and 94.4% for myristic acid (14:0), palmitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3), respectively. Not all digested dietary FA are deposited as some may be oxidised (or metabolised further) by the animal. Because of the *de novo* synthesis, it is not possible to determine the oxidation rate for non-essential dietary FA with the comparative slaughter technique. Deposited essential FA originate only from the diet, and their deposition rate may be used to estimate the oxidation rate of non-essential FA. Essential FA may, however, also be used for the synthesis of other essential FA. For example, 18:2 FA is a precursor for other *n*-6 FA such as dihomolinolenic acid (20:3) and arachidonic acid (20:4). Similarly, 18:3 is a precursor of such other *n*-3 FA as eicosapentaenoic acid (20:5), docosapentaenoic acid (22:5) and docosahexaenoic acid (22:6).

Thus, in addition to the balance of individual essential FA, the balance for the *n*-6 FA family was calculated (no *n*-3 FA other than 18:3 being analysed). FA 20:3 and 20:4 were expressed as the molar 18:2 equivalents required to synthesise these FA. In order to calculate the *de novo* synthesis of non-essential FA during the 24–65 kg period, the oxidation rate (i.e. the complement of the deposition rate) of dietary non-essential FA was supposed to be equivalent to that of *n*-6 FA. The *de novo* synthesis of non-essential FA was then calculated as the difference between the total deposited FA and the deposited FA originating from dietary FA. Because the quantities of long-chain FA synthesised *de novo* are very small relative to those of other FA (see Results), only 14:0, 16:0, 16:1, 18:0 and 18:1 FA were taken into account in calculating *de novo* synthesis.

Statistical analyses

Results were submitted to a covariance analysis using temperature and litter within temperature (L(T)) as main effects, and feeding level (FL, % of feeding *ad libitum*) as covariable (SAS, 2000). Because data concerning FA concentration are inherently correlated (i.e. a reduction of one FA will have consequences on the concentrations of all other FA), a data analysis model was

Table 1. Chemical composition and nutritional values of the experimental diet (adjusted for a dry matter content of 87.3%)

Chemical composition (%)	
Ash	5.2
Crude protein	18.7
Starch	45.6
Lipid content	3.6
Crude fibre	3.5
Gross energy (MJ/kg)	15.92
Nutritional values	
DE (MJ/kg)*	13.93
ME (MJ/kg)	13.43
NE (MJ/kg)†	9.97
Digestible lysine (g/MJ NE)‡	0.94
Fatty acid composition (mg/100 g)	
14:0	2
16:0	345
16:1	4
18:0	47
18:1	458
18:2	1262
18:3	68
20:0	7
20:1	7
20:2	2
20:3	0
20:4	1
22:0	5
Total fatty acids	2208

DE, Digestible energy; ME, Metabolisable energy; NE, net energy.

* Measured in a digestibility trial including five barrows weighing approximately 47 kg body weight (Le Bellego *et al.* 2002).

† Mean of NEg4 and NEg7 using equations of Noblet *et al.* (1994).

‡ Calculated from the measured amino acid content and standardised digestibility coefficients (according to Eurolysine and ITCF, 1995).

developed based on the biochemical conversions between 14:0, 16:0, 16:1, 18:0 and 18:1 FA. This model is schematically represented in Fig. 1. Each FA (represented as an intermediate pool) is partitioned between different destinies. The net dietary supply of an FA (digestible supply minus oxidation) results in an input to the pool, whereas deposition and further metabolism are outputs. Palmitic acid is an intermediate for all *de novo* synthesised FA. Precursors such as glucose will therefore transit through the 16:0 pool. In addition to deposition, 16:0 can be shortened to 14:0, elongated to 18:0 and unsaturated to 16:1. Similarly, 18:0 (synthesised from 16:0) can be deposited and unsaturated to 18:1.

The model was constructed so that the sum of the input flows equalled the sum of output flows for each intermediate compartment. This model of FA partitioning was analysed as a nested, multivariate model using the NLIN (non linear models) procedure of SAS (2000), as described by van Milgen & Noblet (1999). As a multivariate model, the FA balances of all FA were analysed simultaneously. For a given temperature and FL, the model for the *de novo* synthesis of FA included four parameters related to desaturation of 16:0 (to 16:1), chain-shortening (to 14:0), elongation (to 18:0) and desaturation of 18:0 (to 18:1). Total deposition rates can be calculated from this partitioning (e.g. deposition of 16:0 is calculated as 1–16:0 desaturation – elongation – chain shortening).

Because lipids and FA are deposited in compartments C1 and C2, a partitioning rule has to be included in the model. For reasons of simplicity, a single partitioning rule was assumed for all treatments and FA. With two temperatures and four FL, the most complete model would therefore include 33 parameters ($2 \times 4 \times 4 + 1$). The effect of FL or temperature on FA partitioning was evaluated using the extra-sum-of-squares principle (Ratkowsky, 1983). We attempted to progressively simplify the complete model so that the final model was not significantly different from the complete model. For example, FL can be included as a fixed effect within temperature (using eight degrees of freedom) or as a linear effect relative to feeding *ad libitum* (using two degrees of freedom).

Results

Results concerning performance and protein and lipid deposition are reported by Le Bellego *et al.* (2002), and some of these results are given in Table 2 for reference. In brief, high ambient tempera-

ture reduced feed intake and daily gain. The temperature and FL independently affected the partitioning of ingested energy between protein and lipid deposition. Protein deposition was limited by heat stress, and the upper level of protein deposition was 143 g/d at 30°C v. 165 g/d at 23°C. Moreover, a strong restriction in feed intake at thermoneutrality reduced protein deposition more than the same restriction at a high ambient temperature. As anticipated, total lipid deposition was reduced when feed intake was restricted. Compared with the lipid deposition and feed intake for animals fed *ad libitum* at 23°C, the reduction in lipid deposition was always proportionally greater than the reduction in feed intake (Table 2). The ratio between the reduction in lipid deposition and the reduction in feed intake was the smallest for the pigs undergoing the most severe feed restrictions. It is not the purpose of the present paper to elucidate the mechanisms of energy partitioning between protein and lipid deposition. We will focus on FA metabolism and the effect of temperature and FL on this metabolism. For this, the observed lipid deposition was used as an input from which hypotheses concerning FA metabolism were formulated.

Lipid and fatty acid deposition

Most lipids are present in the carcass (C2), with relatively few lipids in the viscera (including leaf fat), head and tail (C1). At 65 kg, 92% of the extracted lipids were present in C2, whereas at 24 kg, 89% of the lipids were present in C2. The proportion of FA in lipid differed significantly between the two compartments and between the initial and final pigs ($P < 0.001$), whereas temperature and FL had no effect. On average, lipids in C1 and C2 contained 76% and 79% FA, respectively, at 65 kg BW. At 24 kg BW, these values were 70% and 75%, respectively. Quantitatively, the major FA are 18:1, 16:0, 18:0, 18:2 and 16:1, averaging 41%, 27%, 15%, 10% and 2.5%, respectively, of the deposited FA at 65 kg for the two anatomical compartments combined.

Both total lipid and FA deposition in C1 and C2 were reduced when feed intake was restricted. The FA deposition in C1 ranged from 10 to 20 g/d, whereas that in C2 ranged from 92 to 188 g/d. On average, 9.1% of the total FA were deposited in C1 and 90.9% in C2. Reducing FL increased ($P = 0.05$) the fraction of FA deposited in C1, especially at 30°C. The FL affected the chemical composition of the compartments. In C2, the FA content of the weight gain decreased linearly with feed intake (Fig. 2). In C1, the FA content of the weight gain was 16% for pigs fed *ad libitum* at 23°C, whereas it was 11% for all others ($P < 0.0001$; Fig. 2).

The fraction of total FA deposited in C1 was slightly higher for 18:0 and 18:3 FA (10.2% and 9.7% respectively) and lower for 18:1 and 18:2 FA (8.6% and 8.9% respectively). The partitioning of 20:4 FA was very different from that of the other FA: 42.5% of the 20:4 deposition was deposited in C1. As indicated earlier, a single estimate was used to estimate the partitioning between C1 and C2 for the main non-essential FA (14:0, 16:0, 16:1, 18:0 and 18:1); 9.7% of the deposited FA were deposited in C1.

Deposition of dietary fatty acids and *de novo* synthesis

For non-essential FA, deposition exceeded intake of digestible FA 9–60-fold (data not shown). For essential FA, approximately 67% of the estimated digestible 18:2 FA was deposited compared with 48% for 18:3 FA. Part of the 18:2 FA will be used for synthesis of the other *n*-6 FA. Approximately 69% of

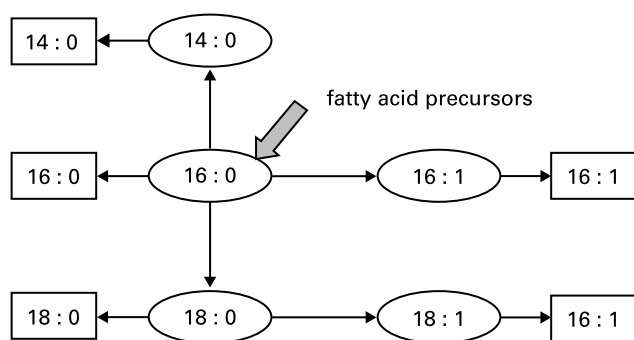


Fig. 1. Representation of *de novo* fatty acid synthesis, deposition and oxidation in growing pigs. Ovals, intermediate fatty acids undergoing deposition or further metabolism; rectangles, deposited fatty acid.

Table 2. Lipid content and lipid and fatty acid deposition in pigs as a function of ambient temperature and feeding level

Temperature (°C)	23			23			30			Statistical analysis				
	23	30	Initial pigs	100	90	80	80	70	80	73	68	62	Residual sd	Effects
Number of animals	5	4	4	6	4	4	6	4	6	4	4	4		
Final live weight (kg)	23	24	66	65	66	66	66	65	66	66	67	66	1.6	
Feed intake (g/day)	2406	2118	1908	2406	2118	1908	1929	1682	1929	1764	1646	1485	NA	
Empty body weight gain (g/d)	1052	1016	951	1052	1016	951	898	806	898	855	815	760	34	T** FL(T)** L(T)**
Lipid content (%)	11.8	10.8	17.0	20.8	18.1	17.0	18.3	17.5	18.3	16.9	16.2	15.2	1.5	T** FL(T)** L(T)**
Lipid gain (g/d)	262	206	180	262	206	180	196	156	196	164	151	128	20	T*** FL(T)**
viscera, head and tail	26	18	17	26	18	17	15	14	15	16	14	13	2.5	T*** FL(T)**
carcass	236	189	163	236	189	163	181	143	181	148	137	115	19	T*** FL(T)**
Total fatty acid gain (g/d)	209	165	144	209	165	144	156	125	156	131	121	103	15	T*** FL(T)**
Composition of FA	% total FA			% total FA gain										
14:0	2	2	1	1	1	1	1	1	1	2	2	1	0.3	
16:0	25	26	27	28	27	26	28	26	28	28	28	27	1.3	
16:1	4	5	2	2	2	2	2	2	2	2	2	2	0.4	
18:0	11	10	15	15	15	16	17	16	17	17	18	17	1.0	T** L(T)*
18:1	42	41	43	42	43	42	40	43	40	39	39	39	1.5	T***
18:2	12	13	8	8	8	10	9	9	9	10	10	11	0.6	T*** FL(T)** L(T)**
18:3	0.7	0.7	0.4	0.3	0.4	0.4	0.3	0.3	0.3	0.4	0.3	0.4	0.1	L(T)*
20:0	0.2	0.2	0.3	0.4	0.3	0.3	0.2	0.2	0.3	0.2	0.2	0.2	0.1	
20:1	1.1	0.9	1.3	1.1	1.3	1.2	1.0	1.1	1.0	1.1	1.2	1.0	0.2	T*
20:2	0.6	0.5	0.7	0.5	0.8	0.8	0.5	0.6	0.5	0.6	0.7	0.7	0.1	
20:3	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	
20:4	0.8	0.8	0.2	0.3	0.2	0.4	0.2	0.2	0.2	0.3	0.3	0.3	0.1	L(T)*
22:0	0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	

FA, fatty acid; FL(T), effect of feeding level within temperature; L(T), effect of litter within temperature; T, effect of temperature; NA, not applicable.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† Expressed as percentage of intake *ad libitum* at 23°C.

‡ Expressed as percentage of total empty body weight (blood, carcass and viscera, head, feet and tail compartment).

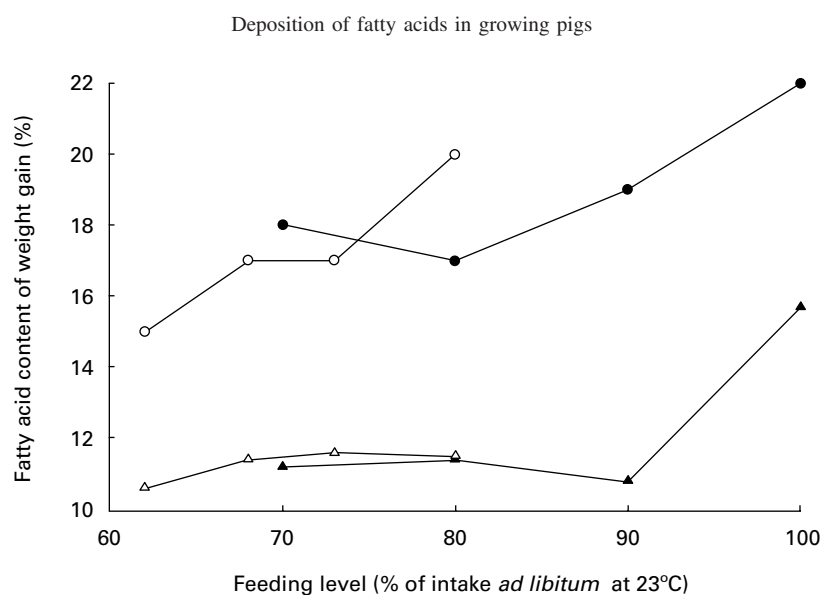


Fig. 2. Effect of feeding level and ambient temperature on the fatty acid content in carcass and viscera (% of total weight gain). (○, △) 30°C; (●, ▲) 23°C; (○, ●) carcass; (△, ▲) viscera, head, feet and tail.

the digestible *n*-6 family of FA was deposited (Table 3). A high ambient temperature increased the retention of digestible dietary *n*-6 FA (72% at 30°C *v.* 67% at 23°C). The results for the *n*-3 FA concern only 18:3 and therefore underestimate the retention of *n*-3 FA. As these results are similar to the whole-body oxidation rates of medium-chain FA and essential PUFA observed in rats by Leyton *et al.* (1987), it was assumed for the remainder of the study that 70% of the digestible non-essential dietary FA were deposited (i.e. approximately equal to the oxidation of *n*-6 FA); the remainder was supposed to be oxidised. Owing to the low lipid content of the diet (relative to lipid deposition by the pigs), this hypothesis has little influence on further calculations.

The *de novo* synthesis of the main five non-essential FA (14:0, 16:0, 16:1, 18:0 and 18:1) represented 98% of the total *de novo* synthesised FA and 92% of the total deposited FA. The average composition of *de novo* synthesised FA corresponded to 1.7%, 30.3%, 2.4%, 19.7% and 45.9% for 14:0, 16:0, 16:1, 18:0 and 18:1 FA, respectively. Values for the four FL and two temperatures are given in Table 4. Both temperature and FL strongly affected the composition of the *de novo* FA. On average, the 18:1 content was 3.2% lower at 30°C than at 23°C, whereas the 16:0 and 18:0 FA contents were respectively 1.2% and 2% greater at 30°C. Reducing feed intake reduced the 16:0 FA content, whereas it increased that of 18:0. Although no significant interaction between temperature and FL was observed,

the effect of FL appeared numerically more important at 23°C than at 30°C.

De novo synthesis rates

The statistical analysis model illustrated in Fig. 1 allows estimation of the metabolic partitioning of *de novo* synthesised FA. At 23°C and for pigs offered feed *ad libitum*, 1.7% of 16:0 FA was shortened to 14:0, 63% was elongated to 18:0, 2.8% was unsaturated to 16:1 and, by difference, 33% of 16:0 was deposited. Subsequently, 72% of 18:0 FA (synthesised from 16:0) was unsaturated to 18:1 and 28% deposited. Parameter estimates for the partitioning of *de novo* synthesised FA for all FLs and both temperatures are given in Fig. 3. These data suggest that especially temperature affected the elongation and desaturation of 18:0 to 18:1 FA.

Hypotheses that FL and temperature affected FA partitioning were tested by successively eliminating parameters from the full model (parameters of the full model are given in Fig. 3), without affecting the precision of the model. Results of this procedure are given in Table 5. For example, the fraction of 16:0 FA used for 14:0 synthesis is small and appears not to be affected by FL or temperature (Fig. 3). Simplifying chain-shortening as a single phenomenon across temperature and FL (compared with using the eight rates for the eight treatments) did not affect the precision of the model ($P=0.63$). Similarly, desaturation of 16:0 to 16:1

Table 3. Ratio between deposited *n*-3 and *n*-6 fatty acids and digestible *n*-3 and *n*-6 fatty acid intake in growing pigs as a function of ambient temperature and feeding level†

Temperature (°C)	23				30				Average	Statistical analysis	
	100	90	80	70	80	73	67	62		Residual sd	Effects
<i>n</i> -6	67	63	71	65	70	74	70	75	69	7	T*
<i>n</i> -3	46	49	51	44	47	57	43	49	48	10	L(T)*

L(T), effect of litter within temperature; T, effect of temperature.

* $P < 0.05$.

† Estimated ileal digestibility according to Jørgensen *et al.* (1993); *n*-6 = C18:2 + C20:3 + C20:4; *n*-3 = C18:3.

‡ Expressed as percentage of intake *ad libitum* at 23°C.

Table 4. Composition of *de novo* synthesised fatty acids (FA) in growing pigs as a function of ambient temperature and feeding level

Temperature (°C)	23				30				Statistical analysis	
	100	90	80	70	80	73	67	62	Residual SD	Effects
Feeding level (%)†										
FA deposition (g/d)	209	165	144	125	156	131	121	103	15	FL*** L(T)**
<i>De novo</i> FA deposition (g/d)‡	175	135	117	102	129	105	98	82	14	FL*** L(T)**
<i>De novo</i> synthesised FA (% total)										
14:0	1.7	1.6	1.5	1.6	1.6	1.9	2.0	1.8	0.3	T* FL*
16:0	31.1	30.0	29.1	28.7	30.7	31.2	31.0	30.6	1.4	L(T)*
16:1	2.9	2.6	2.2	2.4	2.4	2.1	2.1	2.5	0.5	T* FL*
18:0	17.7	18.2	19.3	19.4	20.0	21.0	21.4	20.4	1.2	L(T)*
18:1	46.6	47.5	47.9	47.9	45.3	43.9	43.4	44.8	1.8	T**

FL(T), effect of feeding level (covariable) within temperature; L(T), effect of litter within temperature; T, effect of temperature.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† Expressed as percentage of intake *ad libitum* at 23°C.

‡ The *de novo* synthesis of 14:0, 16:0, 16:1, 18:0 and 18:1 FA is based on the assumption that the β -oxidation (or further metabolism) of fatty acid equals 30% (see p. for details).

FA was not affected by temperature and FL and indicated that 2.5% of 16:0 FA was desaturated to 16:1. Desaturation of 18:0 was strongly affected by temperature ($P < 0.01$) but not by FL. Consequently, two 18:0 desaturation rates were required (one for each temperature) to maintain the accuracy of the model.

The fraction of 18:0 desaturated to 18:1 was 3.5% lower at 30°C than at 23°C. The reduced desaturation of 18:0 FA at 30°C only partly resulted in an increased deposition of 18:0 as the elongation rate of 16:0 to 18:0 was also reduced by increased temperature ($P < 0.01$). The effect of FL on the elongation rate was mainly apparent at 23°C (Fig. 3). Ignoring the effect of FL on elongation (model B in Table 5) resulted in a slightly less accurate model compared when the complete model ($P = 0.09$). When it was assumed that both temperature and FL affected the elongation rate, no difference ($P = 0.29$) in accuracy was observed between the complete model and the reduced model (model A in Table 5). Despite the statistical difference, the magnitude of the difference in elongation rates was small: a 40% reduction in feed intake increased the elongation rate by only 2.5%.

Discussion

Lipid and fatty acid deposition

In this experiment, there was a difference in FA composition between the initial and the experimental pigs. In general, the non-essential FA content was greater and the essential FA content lower in experimental pigs. The difference in FA composition between animals at the start and end of the balance period was due to the diets fed before and during the experiment. The diet offered to the pigs before the experiment contained some vegetable oil, whereas the experimental diet contained no added sources of fat or oil. Owing to the contribution of *de novo* synthesised FA during the experimental period, the proportion of essential FA in the carcass was mainly reduced by dilution.

A difference was observed between the FA profiles of the two anatomical compartments. The structural FA (those which are part of membranes and nervous tissues) were proportionally more important in the viscera, head and tail than in the carcass. Relative to the deposition of other FA, 20:4 FA was preferentially deposited in C2. Compared with the FA of lipid reserves in adipose

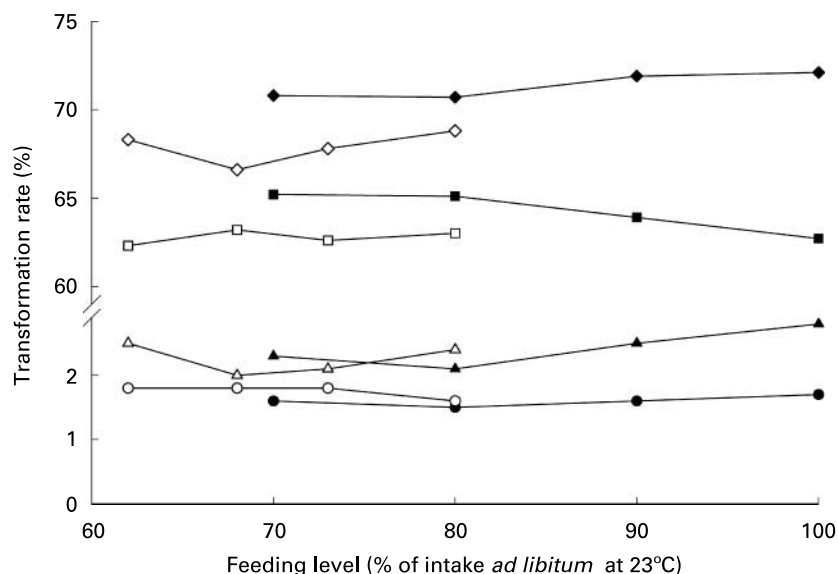


Fig. 3. Estimates of the rate of 16:0 to 14:0 fatty acid chain-shortening (●), 16:0 to 16:1 fatty acid desaturation (▲), 16:0 to 18:0 fatty acid elongation (■) and 18:0 to 18:1 fatty acid desaturation (◆) in growing pigs as a function of ambient temperature and feeding level (○, △, □, ◇, 30°C; ●, ▲, ■, ◆, 23°C).

Table 5. Parameter estimates (%) describing fatty acid (FA) partitioning in the body and metabolism of *de novo* synthesised fatty acids†

Parameter				E0		deltaE	D9		Probability <i>P</i>
	V	S	D7	23	30		23	30	
Model A	9.7	1.6	2.5	63.1	61.3	0.063	71.6	68.1	0.288
ASE of estimate	0.19	0.04	0.08	0.33	0.64	0.022	0.40	0.56	
Model B	9.7	1.6	2.5	63.7	62.8	0	71.6	68.2	0.095
ASE of estimate	0.19	0.04	0.08	0.26	0.32	0	0.40	0.51	

ASE, asymptotic standard error; V, FA deposited in viscera, head, feet and tail (% total FA deposition); S, percentage of *de novo* synthesised 16:0 FA shortened to 14:0; D7, percentage of *de novo* synthesised 16:0 FA desaturated to 16:1; E0, percentage of *de novo* synthesised 16:0 FA elongated to 18:0 with feeding *ad libitum*; deltaE, change in percentage of 16:0 FA elongated to 18:0 owing to a change in feeding level (i.e. percentage of *de novo* synthesised 16:0 FA elongated to 18:0 equals $E_0 + \text{deltaE} \times (100 - FL)$); D9, percentage of *de novo* synthesised 18:0 FA desaturated to 18:1.

† See text and Fig. 1 for details.

tissues, structural lipids typically contain more essential FA (Mervyn & Leat, 1983). As the contribution of adipose tissue to total lipid mass increases with increasing age or BW, this may also contribute to the observation that the FA composition in the initial pigs differed from that in the other pigs.

There was a marked difference in FA content of the weight gain of C1 between the feeding level *ad libitum* at 23°C and the other feeding levels (see Fig. 2). This observation suggests that there is a minimum FA content of weight gain (about 11% FA in weight gain) in viscera, head and tail, probably related to a need for structural lipids. A minimum FA content was not apparent for the carcass, at least not within the range of observations. The concept of a minimum lipid content in gain has been exploited in some growth models for pigs (e.g. Whittemore & Fawcett, 1976) as a minimum lipid/protein deposition ratio. The possible existence of a minimum FA content in weight gain for C1 also implies that, with decreasing feed intake, an increasing fraction of the FA deposition will be deposited in C1. The pigs used in this experiment were slaughtered at 60 kg and were thus relatively lean. Moreover, internal lipids develop late relative to other lipid depots (Kouba *et al.* 1999a). This may explain the observation that the FA content of the weight gain in C1 exceeded the minimum values only for the animals fed *ad libitum* at 23°C (see Fig. 2).

It is well known that temperature and FL affect lipid deposition and fat distribution in the carcass. The results of this experiment indicated that high ambient temperature increased carcass fatness only when the feed intake was high (at identical feed intake for both temperatures; see Fig. 1 in Le Bellego *et al.* 2002). At lower feed intake (less than 80% of intake *ad libitum*), pigs were leaner at 30°C than at 23°C. Kouba *et al.* (2001) observed that a high ambient temperature increased the lipid content of backfat. The exposure of pigs to a high ambient temperature enhances the lipid metabolism in both liver and adipose tissue, which results in greater fatness (Kouba *et al.* 2001).

There are also several indications in the literature that temperature and FL affect the distribution of body lipid and FA. Under hot environmental conditions, there is a redistribution of fat from external fat towards internal fat (i.e. viscera, leaf fat; Rinaldo & Le Dividich, 1991; Le Dividich *et al.* 1998). Similarly, backfat becomes more saturated with increasing temperature (Rinaldo & Le Dividich, 1991; Le Dividich *et al.* 1998). In the present study, no clear effect of environmental temperature of distribution of body fat was found. This may be due to the fact that

we used relatively young pigs, which have less external fat and are less sensitive to heat stress than heavier animals. Nevertheless, the results illustrated in Fig. 2 earlier suggest that carcass lipids are more 'mobile' than internal lipids. The observed redistribution of lipids between tissues as a function of temperature may simply be the result of a necessity to maintain minimum lipid content in certain tissues.

Fatty acid oxidation

Essential FA cannot be synthesised by the pig and can therefore be used as natural markers to study the partitioning between oxidation and deposition. In this study, 70% of the digestible *n*-6 FA were retained by the animal. The complement of this value (30%) provided an estimate of the net oxidation during the growth phase of the animal. This net oxidation can be seen as the result of two processes: postprandial oxidation and oxidation due to turnover of the lipid mass. Ingested dietary lipids (triacylglycerols) are hydrolysed during digestion and metabolism. The free FA originating from this hydrolysis can be oxidised to synthesise ATP (e.g. in muscle). Similarly, deposited lipids undergo repeated hydrolysis and re-esterification, and free FA are potentially exposed to oxidation during this process. The comparative slaughter technique does not allow a distinction between postprandial oxidation and oxidation due to lipid turnover. Using a simple compartmental model of lipid deposition and lipid oxidation, and simulating the conditions of the present experiment, an overall oxidation rate of 30% of dietary FA can also be obtained by assuming a fractional oxidation rate of deposited FA of 1.5%/d.

Quantitative information on FA turnover in literature is scarce. Anderson *et al.* (1972) estimated the half-life of 18:3 FA in 8–12-month-old pigs fed a diet containing 20% linseed oil at a level to maintain a constant body weight. The estimated half-life was 300 d in subcutaneous adipose tissue, 175 d in diethyl ether-extractable muscle lipids and 47 d in muscle membrane lipids. In rats, 1.7% of the glycerol esters of epididymal fat is hydrolysed and re-synthesised every 24 h, giving a half-life for glycerol bonds of 41 d and for fatty acids of 141 d (Gorin & Shafir, 1963). In human subjects, the half-life of FA has been estimated at between 350 and 700 d (Hirsh *et al.* 1960). These estimates suggest that FA turnover is relatively low.

On the other hand, Danfaer (1999) used data from Dunshea *et al.* (1992a,b) and calculated a fractional lipid turnover rate of

0.9%/d in 60 kg pigs. However, this turnover applied to lipolysis within the adipocyte. According to Danfaer (1999), 59% of the free FA were re-esterified in the adipocyte and 41% were exported to the bloodstream, after which they may have undergone oxidation. The results from these calculations show that lipid turnover, from a perspective of whole-animal metabolism, would not exceed 0.35%/d.

In this study, the fraction of digested 18:2 FA directly deposited (67%) was higher than that found by Flanzy *et al.* (1970; 50%), who also used the comparative slaughter technique. The current value is nevertheless smaller than the one presumed in the model of Lizardo *et al.* (2002; 85%). Chwalibog *et al.* (1992) concluded, based on calorimetric data, that all digestible dietary lipids are stored. The retention of *n*-6 FA is much higher than that of *n*-3 FA (70% *v.* 48%). Although, in this study, no FA that could be synthesised from 18:3 FA were measured, 18:3 FA appears to be a relative good substrate of β -oxidation compared with 18:2 (Hovik & Osmundsen, 1987; Leyton *et al.* 1987). In contrast, Crespo & Esteve-Garcia (2003) did not observe important differences in oxidation rates between *n*-6 and *n*-3 FA in chicken; these rates varied between 6.5% and 29.6%. The quantity of *n*-3 FA is much smaller in the carcass than the quantity of *n*-6 FA (0.4% of total FA *v.* 10.5%). Consequently, the calculation of the *n*-3 FA balance will be less precise.

Another reason may be put forward to explain the difference in oxidation rates between *n*-6 and *n*-3 FA. In this study, 18:3 FA was the only *n*-3 FA measured. Nevertheless, 18:3 can be converted to other *n*-3 FA (mainly eicosapentaenoic acid and docosapentaenoic acid, and, to a lesser extent, docosahexaenoic acid) and to hormones such as prostaglandins, resulting in an underestimation of the *n*-3 balance. In human adults, the apparent conversion of 18:3 FA to eicosapentaenoic acid is limited (less than 8%), whereas docosahexaenoic acid synthesis is marginal (less than 4%) (Burdge & Wootton, 2002; Burdge *et al.* 2002), but these rates have not yet been estimated in growing pigs.

The partitioning of 70% *v.* 30% between deposition and net oxidation calculated for *n*-6 FA was applied to non-essential dietary FA, although it is acknowledged that there may be a difference in β -oxidation between FA (Hovik & Osmundsen, 1987; Leyton *et al.* 1987; Rioux *et al.* 2000; Raclot, 2003). Leyton *et al.* (1987) estimated whole-body oxidation rates of ^{14}C -labelled medium-chain FA and essential PUFA *in vivo* over a 24 h period in rats. The fastest rate (measured as expired $^{14}\text{CO}_2$) was observed for C18:3 FA (64%), followed by C12:0 (63%), C18:1 (57%), C18:2 (48%), C14:0 (40%), C16:0 (32%), C18:0 (25%) and C20:4 (14%). Although the recovery rates varied between 60% and 86%, these data nevertheless suggest that the oxidation depends on FA chain length and FA saturation. Although the assumption of a constant oxidation rate for all dietary FA is probably invalid, it has little effect on the outcome of this study owing to the low fat content of the diet.

De novo synthesis

The composition of *de novo* synthesised FA in pigs offered feed *ad libitum* and kept under thermoneutral conditions is different from that used in the model of Lizardo *et al.* (2002), particularly for 16:0 (30% here *v.* 24% reported by Lizardo *et al.* 2002) and 18:1 (46% *v.* 54%). As few data were available at the whole-animal level, Lizardo *et al.* (2002) parameterised their model using data from Gerfault

et al. (2000), who employed diets containing different dietary oils and slaughtered the pigs at 100 kg, and data from Flanzy *et al.* (1970), who used a lipid-free diet. Differences between the estimates of *de novo* FA composition in our study and that of Lizardo *et al.* (2002) may be due to the total FA synthesised *de novo*, affected by the age of the pigs (65 kg *v.* 100 kg) and by the lipid content and FA profile of the diet. Several studies show that dietary FA affect *de novo* lipogenesis in adipose tissue (Mourot *et al.* 1994, Smith *et al.* 1996). However, no information is available concerning the effect of the dietary FA composition of *de novo* synthesised FA.

The data analysis model used to partition the *de novo* synthesised FA showed that the quantitatively most important transformations were the elongation of 16:0 to 18:0 and the desaturation of 18:0 to 18:1. The fraction of 18:0 FA desaturated to 18:1 was 26 times greater than the fraction of 16:0 desaturated to 16:1 (72% *v.* 2.8% for feeding *ad libitum* at 23°C) despite the fact that the same enzyme, stearoyl-CoA-desaturase, was involved in this transformation. Noble *et al.* (1969) found the 18:0 desaturation rate to be five times greater than the 16:0 desaturation rate. This may be due to a different affinity of the enzyme for the two FA, or to competition between desaturation, elongation, esterification and β -oxidation. For example, if the elongase has a much greater affinity to 16:0 than the desaturase, partitioning of 16:0 FA between 16:1 and 18:0 (and thus 18:1) will be affected.

The data analysis model used in this study allowed analysis of the effects of temperature and FL on the partitioning of *de novo* synthesised FA. High ambient temperature decreased the fraction of 18:0 FA desaturated to 18:1 by 3.5%, whereas FL had no effect on desaturation. This confirmed results obtained by Kouba *et al.* (1999b) in specific adipose or muscular tissues, showing that, at similar levels of feeding, the activity of stearoyl-CoA-desaturase was lower at 31°C than at 20°C, whereas the feeding level had no effect (Kouba *et al.* 1999b). Although there is information on the structure and activity of the elongase enzymes in different tissues (St John *et al.* 1991; Moon *et al.* 2001; Leonard *et al.* 2004), no explanation of the negative effect of increasing FL on the 16:0 FA elongation rate as observed in this study was found in literature. This observation may suggest the existence of a saturation phenomenon.

Conclusion

The main objective of this study was to estimate the parameters of a mechanistic model that relates nutrition and animal development to FA composition at the animal level. The major elements, obtained by measuring the FA balance, include the deposition rate of dietary FA (relative to oxidation), the composition of *de novo* synthesised fatty acids and the distribution of FA between the carcass and non-carcass components. Approximately 70% of the digested *n*-6 FA was retained by the animal, suggesting that 30% was oxidised. This partitioning between deposition and oxidation was also applied to other dietary FA. A data analysis model was used that allowed estimation of the partitioning of different fluxes of the *de novo* synthesised FA. It was shown that ambient temperature affected the desaturation of 18:0 FA and that FL had a small but significant effect on the elongation of 16:0 FA to 18:0. In conclusion, this study provides the

elementary data needed for building a mechanistic model relating nutrition to FA deposition in growing pigs.

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