

Whole-body β -oxidation of 18:2 ω 6 and 18:3 ω 3 in the pig varies markedly with weaning strategy and dietary 18:3 ω 3

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Abstract Segregated early weaning (SEW) into a cleaner nursery increases food intake and growth in pigs, presumably because of reduced immune stimulation compared with conventionally reared, nonsegregated pigs (NSW). The aim of the present study was to evaluate the oxidation of linoleic acid (18:2 ω 6) and α -linolenic acid (18:3 ω 3) in SEW and NSW pigs. Pigs consumed a control or high 18:3 ω 3 diet (ω 6 PUFA/ ω 3 PUFA; 21.3 vs. 2.5, respectively) and were weaned at either 14 days old into a SEW nursery or at 21 days old into a conventional NSW nursery. The major acute-phase protein of pigs but not haptoglobin increased in 35-day-old NSW pigs. NSW pigs had 15–25% lower carcass 18:2 ω 6 and 20–30% lower carcass 18:3 ω 3 (% composition) at 49 days old. Between 35- and 49-days-old, NSW pigs had a higher whole-body oxidation of 18:2 ω 6 (40–120%) and 18:3 ω 3 (30–80%). The high 18:3 ω 3 diet decreased the whole-body oxidation of 18:2 ω 6 by 73% and of 18:3 ω 3 by 63% in NSW pigs. **■** We conclude that moderately cleaner housing SEW significantly decreases 18:2 ω 6 and 18:3 ω 3 oxidation in pigs.—Bazinet, R. P., E. G. McMillan, R. Seebaransingh, A. M. Hayes, and S. C. Cunnane. Whole-body β -oxidation of 18:2 ω 6 and 18:3 ω 3 in the pig varies markedly with weaning strategy and dietary 18:3 ω 3. *J. Lipid Res.* 2003. 44: 314–319.

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The eighteen carbon polyunsaturated fatty acids (PUFA), linoleic acid (18:2 ω 6), and α -linolenic acid (18:3 ω 3), are important dietary precursors to longer-chain PUFAs such as arachidonic acid (20:4 ω 6), eicosapentaenoic acid (20:5 ω 3), and docosahexaenoic acid (22:6 ω 3). Several long-chain

PUFAs are eicosanoid precursors and also contribute importantly to membrane structure. Despite their important precursor role, 18:2 ω 6 and 18:3 ω 3 are primarily β -oxidized for energy (1, 2). Although dietary factors like fasting-refeeding and energy restriction are known to significantly increase the β -oxidation of 18:2 ω 6 and 18:3 ω 3, overall little is known about why these two dietarily important fatty acids are mostly β -oxidized.

In this study, we were interested in assessing the effect of weaning strategy and diet on 18:2 ω 6 and 18:3 ω 3 β -oxidation in young growing pigs. Segregated early weaning (SEW) is a management strategy in which pigs are weaned at 10–14 days old instead of the more common 21 days old and are housed in a cleaner environment compared with nonsegregated weaning (NSW) used in conventional commercial units. SEW pigs eat more and grow faster, and there is some evidence that this is due to reduced antigen exposure, i.e., to less demand on their immune system (3–9).

We chose this model to further examine parameters affecting 18:2 ω 6 and 18:3 ω 3 β -oxidation because there are a few, mostly indirect, studies suggesting a stimulated immune system could contribute significantly to the body's oxidation of fatty acids. For instance, during severe inflammation, energy expenditure is increased and the respiratory quotient is decreased, indicating an increase in whole-body fat oxidation (10–14). The extent to which PUFA such as 18:2 ω 6 contributes to this inflammation-

Abbreviations: 20:4 ω 6, arachidonic acid; 18:2 ω 6, linoleic acid; 18:3 ω 3, α -linolenic acid; 22:6 ω 3, docosahexaenoic acid; pigMAP, major acute phase protein of pigs; MUFA, monounsaturated fatty acid; NSW, nonsegregated weaning; 18:1 ω 9, oleic acid; 16:0, palmitic acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; SEW, segregated early weaning; 18:0, stearic acid.

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TABLE 1. Fatty acid profile of the control and high 18:3 ω 3 pig feeds^a

Fatty Acid	Control	High 18:3 ω 3
Sum SFA	14.5	14.3
Sum MUFA	21.0	21.6
18:3 ω 3	1.9	17.8
Sum of other ω 3 PUFA	<0.1	<0.1
18:2 ω 6	61.9	45.4
Sum of other ω 6 PUFA	<0.1	<0.1
Total Fat (mg/g)	76	76

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

^aPercentage of total fatty acids >12 carbons.

induced increase in fat oxidation is not known. In a rat model of sepsis caused by puncture of the cecum, ¹⁴C₂ recovery 6 h after an oral dose of [¹⁴C]18:2n-6 was shown to be 1.5–2 times higher than in controls (15). As well, rats infused with tumor necrosis factor- α (TNF α) have 10% less 18:2 ω 6 in carcass triglyceride, suggesting that 18:2 ω 6 may be mobilized under these conditions (16). Hence, we postulated that β -oxidation of 18:2 ω 6 and 18:3 ω 3 may decrease in animals undergoing a reduced immune challenge.

The objective of the present study was therefore to assess whole-body PUFA balance in NSW and SEW pigs consuming a conventional compared with an increased level of dietary 18:3 ω 3. Whole-body PUFA balance was chosen as the method to study 18:2 ω 6 and 18:3 ω 3 oxidation because, with this method, β -oxidation is determined in relation to the intake of 18:2 ω 6 or 18:3 ω 3 over several days to weeks, thus giving a long term comparison between PUFA utilization as fuels versus incorporation into tissue membrane structure or as long chain PUFA precursors (1). We hypothesized that SEW pigs would have lower whole-body 18:2 ω 6 and 18:3 ω 3 β -oxidation as a result of less immune stimulation due to being housed in a cleaner environment with lower antigen exposure. In view of the anticipated higher oxidation of 18:3 ω 3 in the NSW pigs, we wanted to assess whether raised 18:3 ω 3 intake would influence 18:2 ω 6 and 18:3 ω 3 balance, especially in the NSW pigs.

In the pig, the plasma acute-phase proteins, haptoglobin, and major acute-phase protein of pigs (pig-MAP), are sensitive indicators of inflammation (17). Therefore, in the present study, haptoglobin and pig-

MAP were determined at various times before and after weaning.

MATERIALS AND METHODS

Experimental animals and treatments

Pregnant Yorkshire-Landrace sows (Maple Leaf Foods Agresearch, Burford, ON) were randomly fed either a control diet or a diet high in 18:3 ω 3 from ~1 week prior to parturition until weaning. The diets consisted of base commercial pig feed (Shur-Gain Feed # 694, Guelph, ON) containing 477 g/kg of corn, 270 g/kg of soy bean meal, 75 g/kg of wheat middlings, 74 g/kg of whey powder, 36.2 g/kg of fish meal, 10 g/kg of limestone, 7 g/kg of dicalcium phosphate, 3.3 g/kg of salt, and 17.6 g/kg of a vitamin/mineral supplement. The control diet consisted of the base pig feed supplemented with 50 g/kg of sunflower oil, while the high 18:3 ω 3 diet consisted of the same base pig feed supplemented with 15 g/kg of sunflower oil and 35 g/kg of flaxseed oil. The fatty acid profile of the two diets is shown in Table 1. The piglets in the present study suckled from their respective sows consuming either the control or high-18:3 ω 3 diet. At 14 days old, 12 suckling pigs per dietary treatment were randomly allocated to either the SEW or NSW treatment. SEW pigs were weaned at 14 days old into a "clean" nursery while NSW pigs were weaned at 21 days old into a conventional nursery. The SEW nursery was made cleaner by segregating pigs in this study from all other pigs except for littermates, while NSW pigs remained in the same nursery as the sow and nonlittermates (3, 9). After weaning, pigs consumed diets with the same 18:3 ω 3 content as their respective sows until the end of the study. Pigs were anesthetized and killed at 14, 35, or 49 days old for tissue proximate and fatty acid analysis. Blood samples were anticoagulated with citrate. Organ distribution of fatty acids was evaluated in liver, brain, viscera, and carcass. Liver, brain, viscera, and carcass were completely homogenized four times using a Hobart Grinder with 0.12 cm dye. An 80 g sample of the carcass homogenate was freeze dried for proximate analysis. A 5 g sample of the liver, brain, viscera, and carcass homogenate were stored at -20°C for fatty acid analysis. The term "whole-body" refers to the carcass, brain, liver, and viscera combined, while "carcass" refers to whole-body minus the liver, brain, and viscera.

Tissue composition

Carcass dry matter content was determined by weighing before and after 2 h at 100°C in a drying oven. Dried samples were then analyzed for fat, protein, and ash, according to the American Oil Chemists' Society method Ba 3–38 and the Association of Official Analytical Chemists methods 990.03 and 925.23, respectively (18, 19).

TABLE 2. Pig body weights and feed intake

Age	SEW		NSW		Significance
	Control	High 18:3 ω 3	Control	High 18:3 ω 3	
Body weight ^a					
14 days	4.6 \pm 0.7	4.5 \pm 0.7	4.6 \pm 0.7	4.5 \pm 0.7	NS
35 days	9.1 \pm 0.9	10.5 \pm 1.8	10.5 \pm 1.7	9.0 \pm 2.0	DXW
49 days	17.1 \pm 1.7	17.2 \pm 3.0	14.7 \pm 3.9	14.3 \pm 0.9	W
Feed intake ^b	750 \pm 121	786 \pm 140	493 \pm 207	485 \pm 185	W

SEW, segregated early weaning; NSW, nonsegregated weaning; NS, not significant; D, diet effect; W, weaning effect; DXW, diet by weaning interaction. $P < 0.05$.

^aKg, mean \pm SD; n = 8 pigs/group at 14 days old and n = 6 pigs per group at 35 and 49 day old.

^bFeed intake from 35 to 49 days old (g/day/pig).

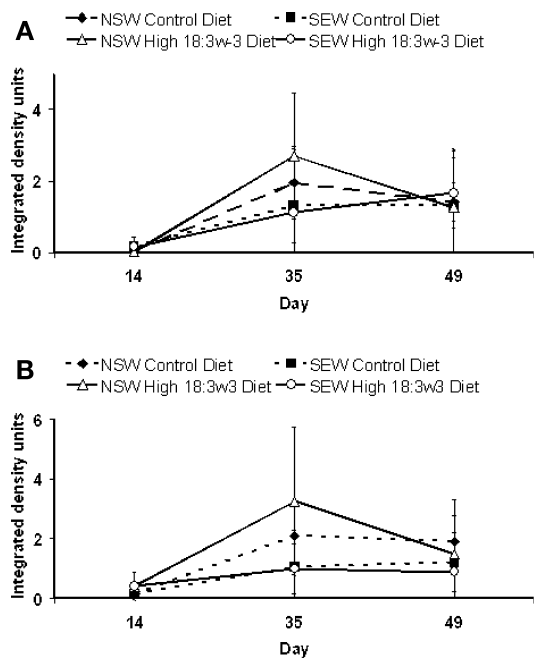


Fig. 1. Plasma haptoglobin (A) and pigMAP (B) levels at 14, 35, and 49 days old. There was a significant effect of weaning at 35 day old for pigMAP only ($P < 0.05$).

Lipid extraction and fatty acid analysis

Total lipids of plasma and homogenized samples of liver, carcass, viscera, and brain were extracted into chloroform-methanol (2:1, v/v). Nonesterified heptadecanoic acid (Sigma, St. Louis, MO) was added as an internal standard to an aliquot of the total fatty acid extract to quantitate total lipids. Total lipids were then recovered and saponified in methanolic potassium hydroxide (60 g potassium hydroxide/L-methanol) for 1 h at 100°C and the fatty acids were converted to fatty acid methyl esters using 14% boron trifluoride-methanol at 100°C for 30 min (Sigma) under nitrogen (20). Fatty acid methyl esters were analyzed by gas liquid chromatography using a capillary column (JandW Scientific DB-23, 30 m × 0.25 mm, ID), in a Hewlett-Packard 5890A gas liquid chromatograph (Palo Alto, CA) with automated sample delivery and injection (Hewlett-Packard 7671A) and peak integration (Hewlett-Packard 3393 inte-

grator). Total lipid fatty acids were quantified on the basis of the proportion in each chromatogram of the corresponding internal standard added to each sample.

Acute-phase proteins

Haptoglobin and pigMAP were separated with one-dimensional reducing SDS-PAGE on 5–12% acrylamide gels stained with Coomassie Brilliant Blue. Bands for pig haptoglobin (~42–44 kDa) and pigMAP (~115 kDa) were first identified by immunoblot with goat anti-human haptoglobin (Sigma-Aldrich) and rabbit anti-pigMAP (gift of courtesy of Dr. F. Lampreave, University of Zaragoza, Spain). These bands could be readily distinguished and quantified in Coomassie Blue stained gels, which were used to quantify each protein. The density of the respective bands was determined from digital images (using the public domain NIH Image program developed at the National Institutes of Health and available on the Internet at <http://rsb.info.nih.gov/nih-image/>) and expressed as integrated density units. Integrated density units were correlated directly with milligrams of stained protein in the gel loading conditions used, as derived from a standard curve of integrated pixel density versus known amounts of BSA as a reference (21).

Whole-body fatty acid oxidation

Whole-body fatty acid oxidation (disappearance) was determined as previously described according to equation 1:

$$\text{disappearance} = \text{intake} - (\text{accumulation} + \text{excretion}). \quad (\text{Eq. 1})$$

Actual fatty acid excretion was not measured, but a reference value of 3% of fatty acid intake was used (1). Food intake and fatty acid accumulation were measured between 35 and 49 days old.

Data analysis

All data are expressed as means ± SD. Statistical comparisons were made using two-way ANOVA with weaning (SEW and NSW) and diet (control and high 18:3w3) as the main independent variables and sow as a nested variable. Two-way ANOVA was used to test the independent effects of weaning, diet, and weaning by diet interactions. All comparisons were made pair-wise using Tukey's test. Correlations were calculated using Pearson's test. A probability of less than 0.05 was consid-

TABLE 3. Fatty acid composition of 49-day-old pig plasma total lipids

Fatty Acid	SEW		NSW		Significance
	Control	High 18:3w3	Control	High 18:3w3	
Sum SFA	28.3 ± 2.1 ^a	29.6 ± 1.9	31.1 ± 3.6	29.0 ± 0.8	NS
Sum MUFA	14.9 ± 0.6	15.9 ± 1.0	15.7 ± 1.3	17.4 ± 2.7	D
18:3w3	0.7 ± 0.1	5.4 ± 1.1	0.5 ± 0.1	6.0 ± 0.2	D
20:5w3	0.4 ± 0.1	2.9 ± 0.3	0.4 ± 0.1	2.7 ± 0.4	D
22:6w3	1.3 ± 0.3	1.3 ± 0.2	1.3 ± 0.2	1.3 ± 0.1	NS
Sum ω3 PUFA ^b	3.3 ± 0.4	10.7 ± 1.2	2.7 ± 0.5	11.3 ± 0.6	D
18:2w6	43.4 ± 3.7	38.6 ± 0.8	40.9 ± 4.5	37.1 ± 2.6	D
20:4w6	8.4 ± 1.1	4.2 ± 0.6	8.0 ± 1.2	3.9 ± 0.6	D
Sum ω6 PUFA ^b	53.5 ± 2.8	43.8 ± 0.9	50.5 ± 4.1	42.1 ± 2.9	D

SEW, segregated early weaning; NSW, nonsegregated weaning; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; NS, not significant; D, diet effect; W, weaning effect. $P < 0.05$.

^aPercentage of total fatty acids >12 carbons, $n = 6$ /group, mean ± SD.

^bSum of fatty acids includes more than those shown.

TABLE 4. Fatty acid composition of 49-day-old pig liver total lipids

Fatty Acid	SEW		NSW		Significance
	Control	High 18:3 ω 3	Control	High 18:3 ω 3	
Sum SFA	36.8 \pm 2.3 ^a	36.9 \pm 1.2	28.1 \pm 5.9	36.3 \pm 2.4	D, W, DXW
Sum MUFA	10.9 \pm 0.5	10.9 \pm 1.1	11.7 \pm 2.4	12.1 \pm 1.4	NS
18:3 ω 3	0.4 \pm 0.1	3.0 \pm 0.7	0.6 \pm 0.2	3.4 \pm 0.6	D
20:5 ω 3	0.5 \pm 0.1	5.6 \pm 0.4	0.6 \pm 0.2	5.0 \pm 0.9	D
22:6 ω 3	4.7 \pm 0.5	4.4 \pm 0.9	4.9 \pm 1.0	4.4 \pm 0.3	NS
Sum ω 3 PUFA ^b	6.7 \pm 0.6	15.7 \pm 1.0	7.4 \pm 1.6	15.7 \pm 1.3	D
18:2 ω 6	25.7 \pm 1.9	23.4 \pm 1.2	29.8 \pm 3.1	22.8 \pm 2.3	D, DXW
20:4 ω 6	17.4 \pm 1.5	11.3 \pm 1.3	20.5 \pm 3.7	11.3 \pm 1.3	D
Sum ω 6 PUFA ^b	45.8 \pm 2.3	36.6 \pm 1.3	52.8 \pm 5.5	36.0 \pm 1.7	D, W
Total Fatty Acids (mg/g) ^c	20.0 \pm 2.3	20.3 \pm 1.8	28.1 \pm 9.8	18.9 \pm 2.1	DXW

SEW, segregated early weaning; NSW, nonsegregated weaning; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; NS, not significant; D, diet effect; W, weaning effect; DXW, diet by weaning interaction. $P < 0.05$.

^aPercentage of total fatty acids $>12C$, $n = 6$ per group, mean \pm SD.

^bSum of fatty acids includes more than those shown.

^cTotal extracted fatty acids based on liver wet weight.

ered to be statistically significant for main effects, interactions, and correlations. All statistics were performed using the Statistical Analysis System, version 8.01 (SAS Institute, Cary, NC).

RESULTS

Feed intake and body weight

SEW pigs consumed 57% more feed between 35 and 49 days old than NSW pigs, a difference reflected in the 15% heavier body weight of SEW pigs at 49 days old ($P < 0.05$; Table 2).

Plasma acute-phase proteins

There was no effect of diet on plasma haptoglobin or pigMAP at 14 days old (Fig. 1). At 35 days old, SEW pigs had lower levels of pigMAP ($P < 0.05$) but not haptoglobin ($P = 0.06$). There were no differences in haptoglobin or pigMAP at 49 days old.

Proximate composition

At 35 days old, SEW pig carcasses contained 7% more water, but 28% less fat, 12% less protein, 11% less ash,

15% less calcium, and 15% less phosphorus ($P < 0.05$ vs. NSW; data not shown). There were no differences in the proximate composition of 49-day-old pigs.

Plasma and tissue fatty acids. At 49 days old, weaning strategy had no significant effect on plasma total lipid fatty acid profiles. However, NSW and SEW pigs consuming the high 18:3 ω 3 diet had higher levels of plasma 18:3 ω 3 and 20:5 ω 3 but not 22:6 ω 3, and had lower levels of 18:2 ω 6 and 20:4 ω 6 (Table 3). At 49 days old, NSW pigs consuming the control diet (lower 18:3 ω 3 intake) had higher total fatty acids in liver total lipids (Table 4). Pigs consuming the high 18:3 ω 3 diet had higher carcass 18:3 ω 3 and 20:5 ω 3 but lower 18:2 ω 6 and 20:4 ω 6 relative to pigs consuming the control diet. At 49 days old, SEW pig carcasses contained more ω 3 and ω 6 PUFA but less saturated and monounsaturated fatty acids than NSW pigs (Table 5). As well, SEW pigs consuming the high 18:3 ω 3 diet had higher ω 3 PUFA but lower 18:2 ω 6 in the carcass. Plasma 20:4 ω 6 was positively correlated with pigMAP ($r^2 = +0.46$, $P < 0.001$) and haptoglobin ($r^2 = 0.55$, $P < 0.001$), while plasma 20:5 ω 3 was negatively correlated with pigMAP ($r^2 = -0.29$, $P < 0.05$) and haptoglobin ($r^2 = -0.28$, $P < 0.05$; data not shown).

TABLE 5. Fatty acid composition of 49-day-old pig carcass total lipids

Fatty Acid	SEW		NSW		Significance
	Control	High 18:3 ω 3	Control	High 18:3 ω 3	
Sum SFA	26.3 \pm 2.3 ^a	27.6 \pm 2.4	28.7 \pm 0.7	30.9 \pm 3.8	W
Sum MUFA	36.6 \pm 2.4	35.7 \pm 1.9	42.6 \pm 1.5	39.2 \pm 2.8	D, W
18:3 ω 3	1.0 \pm 0.2	9.8 \pm 1.6	0.8 \pm 0.1	7.0 \pm 1.1	D, W, DXW
20:5 ω 3	0.1 \pm 0.1	0.31 \pm 0.1	0.04 \pm 0.1	0.2 \pm 0.1	D, W
22:6n-3	0.2 \pm 0.1	0.3 \pm 0.1	0.1 \pm 0.1	0.2 \pm 0.1	D, W
Sum ω 3 PUFA ^b	1.5 \pm 0.3	10.8 \pm 1.6	1.1 \pm 0.1	7.7 \pm 1.3	D, W
18:2 ω 6	34.2 \pm 3.7	24.8 \pm 1.6	25.8 \pm 2.2	20.9 \pm 2.5	D, W, DXW
20:4 ω 6	0.8 \pm 0.4	0.7 \pm 0.1	0.8 \pm 0.2	0.6 \pm 0.2	NS
Sum ω 6 PUFA ^b	35.6 \pm 4.1	26.0 \pm 1.7	27.7 \pm 1.8	22.2 \pm 2.7	D, W

^aPercentage of total fatty acids $>12C$, $n = 6$ per group, mean \pm SD.

^bSum of fatty acids includes more than those shown SEW. $P < 0.05$.

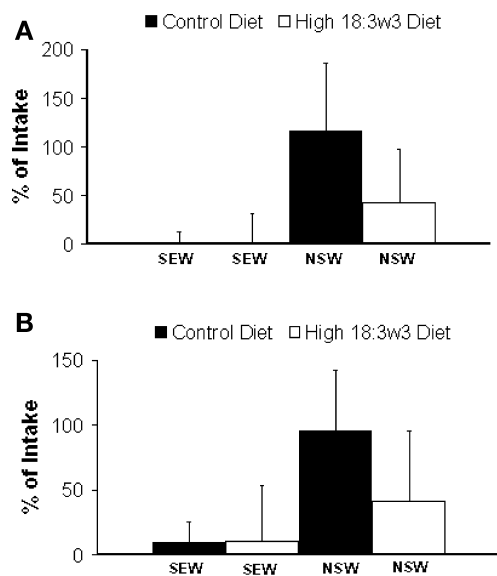


Fig. 2. Whole-body 18:2ω6 (A) and 18:3ω3 (B) disappearance (oxidation) as a percentage of intake in pigs between 35 and 49 day old. Disappearance of both fatty acids was significantly affected by weaning method and by a diet-by-weaning interaction ($P < 0.05$).

Whole-body fatty acid disappearance (oxidation)

As a percentage of intake, whole-body disappearance of 18:2ω6 and 18:3ω3 between 35 and 49 days old was lower in the SEW pigs by 80% and 59%, respectively. The high 18:3ω3 diet reduced whole-body disappearance of 18:2ω6 and 18:3ω3 only in the NSW pigs (**Fig. 2**). Whole-body disappearance of 18:2ω6 but not 18:3ω3 ($P = 0.06$) was positively correlated with plasma pigMAP ($r^2 = 0.44$, $P < 0.05$; data not shown) but not with haptoglobin ($r^2 = 0.23$, $P = 0.23$).

DISCUSSION

The present study demonstrates that NSW pigs appear to oxidize all the 18:2ω6 and 18:3ω3 they consume after weaning and that SEW markedly reduces 18:2ω6 and 18:3ω3 oxidation. SEW probably contributed to lower whole-body PUFA oxidation in part by being a cleaner environment and presumably by reducing immune stimulation, as indicated by the lower plasma pigMAP in SEW pigs at 35 days old (**Fig. 1**). The high 18:3ω3 diet also reduced whole-body oxidation of both 18:2ω6 and 18:3ω3 but only in the NSW pigs.

SEW raised feed intake (**Table 2**), which probably explains the heavier body weights in this group also reported elsewhere (3, 5, 9). Higher feed intake after weaning may have prolonged effects on body weight, even though the difference in plasma pigMAP was transient and disappeared by the time the pigs were 49 days old. Although not measured in the present study, cytokines such as TNFα would be expected to be lower in the SEW com-

pared with NSW pigs, which would increase appetite and growth in the former group (22).


The 40% higher total fatty acid content of the liver in NSW pigs on the control (low 18:3ω3) diet is consistent with other animal models of infection (23, 24). Higher 18:3ω3 intake significantly reduced total fatty acid accumulation in NSW pig livers, mostly by lowering ω6 PUFA, (**Table 4**), an effect consistent with reports showing that raised intake of 20:5ω3 inhibits fatty acid accumulation in the liver of rats infused with lipopolysaccharide and *Escherichia coli* (23, 24).

SEW pigs had higher carcass levels of 18:2ω6 and 18:3ω3, but lower levels of saturates and monounsaturates (**Table 5**), reflecting differences in both weaning strategy and ω3 PUFA intake. Thus, compared with NSW, SEW preserved carcass PUFA, thereby contributing to markedly reduced whole-body disappearance (oxidation) of 18:2ω6 and 18:3ω3. Perhaps surprisingly, higher 18:3ω3 in NSW pigs helped reduce 18:2ω6 and 18:3ω3 oxidation (**Fig. 2**). It seems likely that some of the PUFA released from the carcass stores contributed to PUFA accumulation in the liver of the NSW pigs. This appears to be similar to the process described as “triglyceride recycling” in models of infection (25).

One potential mechanism for the lower carcass PUFA in NSW pigs could relate to the action of hormone sensitive lipase. Hormone sensitive lipase is responsible for releasing free fatty acids from adipose triglycerides into the circulation. In vitro studies with adipocytes have shown that when stimulated with stress hormones, hormone sensitive lipase preferentially releases free PUFA into the medium (26).

From the present study it is not clear how higher 18:3ω3 intake modified the effects of SEW on PUFA metabolism. 18:3ω3 did not decrease the acute-phase protein response at any time point measured (**Fig. 1**). However, plasma 20:4ω6 was positively correlated and 20:5ω3 was negatively correlated with the levels of haptoglobin and pigMAP (see Results). 20:5ω3 has potent anti-inflammatory properties that are thought to arise from its ability to decrease T-cell proliferation as well by competing with 20:4ω6 for eicosanoid production (27, 28, 29). 20:5ω3 is also a ligand for peroxisome proliferator-activated receptors regulating the transcription of many proteins involved in fat metabolism (30, 31). 18:3ω3 is slowly converted to 20:5ω3, which may possibly explain some of the anti-inflammatory properties of 18:3ω3 (32, 33).

In summary, SEW resulted in faster growth, less total fat in the liver, higher PUFA in the carcass, and a marked decrease in the whole-body disappearance of both 18:2ω6 and 18:3ω3. By reducing whole-body oxidation of 18:2ω6 and 18:3ω3, dietary 18:3ω3 mimicked some of the metabolic changes in PUFA metabolism induced by SEW. The mechanism by which this occurred is not known. We conclude that moderately cleaner housing after weaning (SEW) markedly reduces PUFA oxidation. This effect on PUFA metabolism is probably linked to less immune stimulation in the SEW compared with

NSW nursery but confirmation of this awaits further study. 

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REFERENCES

1. Cunnane S. C., and M. J. Anderson. 1997. The majority of dietary linoleate in growing rats is β -oxidized or stored in visceral fat. *J. Nutr.* **127**: 146–52.
2. Leyton J., P. J. Drury, and M. A., Crawford. 1987. Differential oxidation of saturated and unsaturated fatty acids in vivo in the rat. *Br. J. Nutr.* **57**(3): 383–393.
3. Bassaganya-Riera J., R. Hontecillas-Magarzo, K., Bregendahl, M. J. Wannemuehler, and D. R. Zimmerman. 2001. Effects of dietary conjugated linoleic acid in nursery pigs of dirty and clean environments on growth, empty body composition, and immune competence. *J. Anim. Sci.* **79**: 1714–1721.
4. Patience J. F., H. W. Gonyou, D. L. Whittington, E. Beltranena, C. S. Rhodes, and A. G. Van Kessel. 2000. Evaluation of site and age of weaning on pig growth performance. *J. Anim. Sci.* **78**(7): 1726–31.
5. Dritz, S. S., M. M. Chengappa, J. L. Nelssen, M. D. Tokach, R. D. Goodband, J. C. Nietfeld, and J. J. Staats. 1996. Growth and microbial flora of non-medicated, segregated, early weaned pigs from a commercial swine operation. *J. Am. Vet. Med. Assoc.* **208**: 711–715.
6. Williams, N. H., T. S. Stahly, and D. R. Zimmerman. 1997. Effect of chronic immune system activation on the rate, efficiency, and composition of growth and lysine needs of pigs fed from 6 to 27 kg. *J. Anim. Sci.* **75**: 2463–2471.
7. Williams, N. H., T. S. Stahly, and D. R. Zimmerman. 1997. Effect of level of chronic immune system activation on body nitrogen retention, partial efficiency of lysine utilization, and lysine needs of pigs. *J. Anim. Sci.* **75**: 2472–2480.
8. Donham, K. J. 1991. Association of environmental air contaminants with disease and productivity in swine. *Am. J. Vet. Res.* **52**: 1723–1730.
9. Schinckel, A. P., L. K. Clark, G. Stevenson, K. E. Knox, J. Nielsen, A. L. Grant, D. L. Hancock, and J. Turek. 1995. Effects of antigenic challenge on growth and composition of segregated early-weaned pigs. *J. Swine Health Prod.* **3**: 228–234.
10. Frankenfield, D. C., C. E. Wiles, 3rd, S. Bagley, and J. H. Siegel. 1994. Relationships between resting and total energy expenditure in injured and septic patients. *Crit. Care Med.* **22**: 1796–1804.
11. Hwang, T. L., S. L. Huang, and M. F. Chen. 1993. The use of indirect calorimetry in critically ill patients—the relationship of measured energy expenditure to Injury Severity Score, Septic Severity Score, and APACHE II Score. *J. Trauma.* **34**: 247–251.
12. Al-Jaouni, R., X. Hebuterne, I. Pouget, and P. Rampal. 2000. Energy metabolism and substrate oxidation in patients with Crohn's disease. *Nutrition.* **16**: 173–178.
13. Stoner, H. B., R. A. Little, K. N. Frayn, A. E. Elebute, J. Tresadern, and E. Gross. 1983. The effect of sepsis on the oxidation of carbohydrate and fat. *Br. J. Surg.* **70**: 32–35.
14. Nanni, G., J. H. Siegel, B. Coleman, P. Fader, and R. Castiglione. 1994. Increased lipid fuel dependence in the critically ill septic patient. *J. Trauma.* **24**: 14–30.
15. Iriyama, K., N. Kusaka, H. Nishiwaki, T. Teranishi, H. Mori, and H. Suzuki. 1986. Metabolism of non-protein energy-substrates in septic rats receiving parental nutrition. *Int. Surg.* **71**: 5–8.
16. Raina, N., J. Matsui, S. C. Cunnane, and K. N. Jeejeebhoy. 1995. Effect of tumor necrosis factor α on triglyceride and phospholipid content and fatty acid composition of liver and carcass in rats. *Lipids.* **30**: 713–718.
17. Heegaard, P. M., J. Klausen, J. P. Nielsen, N. Gonzalez-Ramon, M. Pineiro, F. Lampreave, and M. A. Alava. 1998. The porcine acute phase response to infection with *Actinobacillus pleuropneumoniae*. Haptoglobin, C-reactive protein, major acute phase protein and serum amyloid A protein are sensitive indicators of infection. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **119**: 365–373.
18. American Oil Chemists' Society 1998 Official methods and recommended practices of the AOCS. 5th ed. American Oil Chemists' Society, Champaign, Ill.
19. Association of Official Analytical Chemists 1995 AOAC Official Methods of Analysis 16th ed. Washington DC, Association of Analytical Chemists.
20. Cunnane, S. C. 1990. Differential utilization of long chain fatty acids during fasting-induced triacylglycerol depletion. III. Comparison of ω 3 and ω 6 fatty acids in rat plasma and liver. *Biochim. Biophys. Acta.* **1036**: 64–70.
21. Simko, E., A. El-Mowafi, B. J. Bettger, V. O. Ostland, H. W. Ferguson, and M. A. Hayes. 1999. Alterations in iron, zinc and major plasma proteins in rainbow trout and brook trout with sterile peritonitis induced by furunculosis vaccines. *J. Fish Diseases.* **22**: 81–90.
22. Stabel, T. J., P. J. Fedorka-Cray, and J. T. Gray. 1995. Tumor necrosis factor- α production in swine after oral or respiratory challenge exposure with live *Salmonella typhimurium* or *Salmonella choleraesuis*. *Am. J. Vet. Res.* **56**: 1012–1018.
23. Tashiro, T., H. Yamamori, K. Takagi, N. Hayashi, K. Furukawa, and N. Nakajima. 1998. n-3 versus n-6 polyunsaturated fatty acids in critical illness. *Nutrition.* **14**: 551–553.
24. Lanza-Jacoby, S., H. Pchetteplace, and R. Tripp. 1995. Enteral feeding of a structured lipid emulsion containing fish oil prevents the fatty liver of sepsis. *Lipids.* **30**: 707–712.
25. Aarsland, A., D. Chinkes, R. R. Wolfe, R. E. Barrow, S. O. Nelson, E. Pierre, and D. N. Herndon. 1996. Beta-blockade lowers lipolysis in burn patients receiving growth hormone. Rate of hepatic very low density lipoprotein triglyceride secretion remains unchanged. *Ann. Surg.* **223**: 777–789.
26. Gavino, V. C., and G. R. Gavino. 1992. Adipose hormone-sensitive lipase preferentially releases polyunsaturated fatty acids from triglycerides. *Lipids.* **27**: 950–954.
27. Shapiro, A. C., D. Wu, and S. N. Meydani. 1993. Eicosanoids derived from arachidonic and eicosapentaenoic acids inhibit T cell proliferative response. *Prostaglandins.* **45**: 229–240.
28. Purasiri, P., A. Mckechnie, S. D. Heys, and O. Eremin. 1997. Modulation in vitro of human natural cytotoxicity, lymphocyte proliferative response to mitogens and cytokine production by essential fatty acids. *Immunology.* **92**: 166–172.
29. Kinsella, J. E., B. Lokesh, S. Broughton, and J. Whelan. 1990. Dietary polyunsaturated fatty acids and eicosanoids: potential effects on the modulation of inflammatory and immune cells: an overview. *Nutrition.* **6**: 24–44.
30. Hwang, D. 2000. Fatty acids and immune responses—a new perspective in searching for clues and mechanism. *Annu. Rev. Nutr.* **20**: 431–456.
31. Devchand, P. R., H. Keller, J. M. Peters, M. Vazquez, F. J. Gonzalez, and W. Wahli. 1996. The PPAR α -leukotriene B4 pathway to inflammation control. *Nature.* **384**: 39–43.
32. Brenna, J. T. 2002. Efficiency of conversion of α -linolenic acid to long chain n-3 fatty acids in man. *Curr. Opin. Clin. Nutr. Metab. Care.* **5**: 127–132.
33. Cunnane, S. C. 1995. Metabolism and function of α -linolenic acid in humans. In: Cunnane SC, Thompson LU. Flaxseed in human nutrition. AOCS Press, Champaign, IL. 99–127.