Fatty acid supply of growing pigs in Central Vietnam

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Vetzuurvoorziening van vleeswarkens in Centraal Vietnam

(met een samenvating in het Nederlands)

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FOREWORD

This thesis describes research on the intake of essential fatty acids in relation to growth performance of growing-finishing pigs kept on small-holder farms in Central Vietnam. I wish to thank the Utrecht Scholarship Programme and The Netherlands Foundation for Nutrition and Health Research for supporting my research.

This thesis is based on the efforts of many people. In the first place I would like to thank all those persons who contributed to obtaining the data and to the texts of the various chapters. Without their help this book would never have been written. Especially, I would like to express my sincere thanks to Professor Anton C. Beynen, who always opens the gates to scientific research. I am grateful for his supervision and guidance of my research and also for his hospitality while staying in Utrecht. I would like to express heartfelt thanks to Dr. Henk Everts, who supervised the data analysis. Thanks are due to Robert Hovenier and Jan van der Kuilen for chemical analyses. I am grateful to Nguyen Hong Duong, Ha Thi Hue and Phan Van Thanh for their assistance with carrying out the experiments on the farms in Vietnam. I thank Max Nuijens for his contribution to Chapter 2 and Anneke Schellingerhout, Kyung-Woo Lee and Keum-Hee Yeom for helping me to find literature data.

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I sincerely hope I have not forgotten anyone who has contributed to the completion of this thesis; my apologies if I did.

Nguyen Quang Linh

Scope of the thesis

Growing pigs in Central Vietnam are typically kept on small holdings. It is considered necessary to improve their growth performance and health status. This thesis focusses on the intake of polyunsaturated fatty acids (PUFAs) by growing pigs. Linoleic acid (LA) and α-linolenic acid (ALA) are essential PUFAs and they are the parent compounds of the so-called families of n-6 and n-3 fatty acids. The LA requirement of growing pigs with body weight from 30 to 90 kg has been set at 0.08 g/MJ of metabolizable energy (ME) (National Research Council, 1998). There is no formal recommendation as to the requirement of ALA. Overt LA deficiency in swine not only causes reduced growth, but also causes clinical symptoms such as degenerative changes in somniferous tubules and impaired sperm development (Leskanich & Noble, 1999). Suboptimal intake of LA may lead to impaired growth in pigs (Skelley et al., 1975; Myer et al., 1992). In the body, ALA can be converted into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). There is evidence that high intakes of EPA and DHA may improve disease resistance (Fritsche et al., 1993; Calder 2001). Chapter 1 is a brief literature review on the issue of LA and ALA requirements in relation to growth of growing pigs and on the effect of n-3 PUFAs on disease resistance.

The intake of PUFAs by pigs kept on small holdings may be assessed by simulation of the whole diet and subsequent feed analysis. However, the fatty acid composition of adipose tissue may serve as an objective index of the qualitative fat intake. Various studies have shown that increasing intakes of LA and ALA are associated with increasing contents of these fatty acids in adipose tissue of swine (Enser et al., 2000; Riley et al., 2000; Wiseman et al., 2000; Corino et al., 2002). However, mathematical relationships between the intake of these fatty acids and their contents in swine adipose tissue had not been reported. Such relationships may be used to steer the fatty acid profile of swine adipose tissue. The fatty acid composition of adipose tissue relates to meat quality. The amount of polyunsaturated fatty acids in adipose tissue influences the sensitivity to oxidative breakdown, and the formation of peroxides, and thus the development of rancidity (Corino et al. 2002). The degree of unsaturation of fatty acids in meat fat is associated with the firmness of the tissue (Enser et al., 2000). In addition, there is evidence that the intake of the long-chain n-3 PUFAs, EPA and DHA, is beneficial to the human consumer as it is associated with a decreased risk of cancer and cardiovascular diseases (Simopoulos, 2001). Chapter 2 describes the mathematical relationships between PUFA intake and their contents in adipose tissue of growing pigs.

As mentioned above, this thesis focuses on the effects of dietary LA, ALA, EPA and DHA on growing pigs kept on small holdings in Central Vietnam. The first study, involving 12 small holdings (Chapter 3), was carried out to describe the relation between LA or ALA intake and growth of pigs fed on local feed resources according to the farmers' choice. Further studies were controlled feeding trials that were carried out on the small holdings with emphasis on seeking local feedstuffs with high contents of ALA, EPA and/or DHA. It was anticipated that the results obtained would provide insight into the relations between dietary fatty acid composition and growth performance.

In Chapters 4 to 7, feeding trials are described. The feeding of either fish meal (Chapter 4) or shrimp by-product meal (Chapter 5) instead of specially prepared ruminant

meal has been studied. Chapter 4 shows a direct relationship between the content of LA in adipose tissue and average daily gain. Despite the associated higher intakes of crude fibre and ash, the feeding of shrimp by-product meal versus ruminant meal was found to enhance growth (Chapter 5).

In the study described in Chapter 6 the rations were enriched with ALA in the form of linseed oil or with EPA in the form of fish oil. A ration with coconut oil served as control treatment. It is concluded that higher intakes of ALA rather than of EPA and DHA stimulate growth performance in swine. The effect of the feeding of spinach or sweet-potato leaves on fatty acid composition and daily gain in growing pigs is described in Chapter 7. It is suggested that the extra intake of ALA with each of the vegetables was responsible for the observed increase in growth performance. Finally, the general conclusions of the various studies are listed.

References

Calder, P.C. (2001). Polyunsaturated fatty acids, inflammation, and immunity. Lipids, 36: 1007-1024.

Corino, C., Magni, S., Pagliarini, E., Rossi, R., Pastorelli, G. and Chiesa, L.M. (2002). Effects of dietary fats on meat quality and sensory characteristics of heavy pig loins. Meat Sci., 60, 1-8.

Enser, M., Richardson, R.I., Wood, J.D., Gill, B.P. and Sheard, P.R. (2000). Feeding linseed to increase the n-3 PUFA of pork: fatty acid composition of muscle, adipose tissue, liver and sausages. Meat Sci., 55, 201-212.

Fritsche, K. L., D.W.Alexander, N.A. Cassity and Shu-cai Huang (1993). Maternally supplied fish oil alters piglet immune cell fatty acid profile and eicosanoid production. Lipids, 28: 677-682.

Leskanich, C.O. and R.C. Noble (1999). The comparative roles of polyunsaturated fatty acids in pig neonatal development. Brit. J. Nutr. 81, 87-106.

Myer, R.O., J.W. Lamkey, W.R, Walker, J.H. Brendemuhl, and G.E. Combs (1992). Performance and carcass characteristics of swine when fed diets containing canola oil and added copper to alter the unsaturated:saturated ratio of pork fat. J. Anim. Sci., 70: 1417-1423.

National Research Council (1998). The Nutrient Requirements of Swine. National Academy Press. Washington, D.C., US.

Riley, P.A., Enser, M., Nute, G.R. and Wood, J.D. (2000). Effects of dietary linseed on nutritional value and other quality aspects of pig muscle and adipose tissue. J. Anim. Sci. 71, 483-500.

Simopoulos, A.P. (2001). N-3 Fatty acids and human health: defending strategies for public policy. Lipids, 36, S83-S89.

Skelley, G.C., Borgman, R.F., Handlin, D.L., Acton, J.C., McConnell, J.C., Wardlaw F.B., and Evans, E.J. (1975). Influence of diet on quality, fatty acids, and acceptability of pork. J. Anim. Sci. 41, 1298 – 1304.

Wiseman, J., Redshaw, M.S., Jagger, S., Nute, G.R. and Wood, J.D. (2000). Influence of type and dietary rate of inclusion of oil on meat quality of finishing pigs. J. Anim. Sci., 70, 307-315.

Chapter 1

Dietary polyunsaturated fatty acids in relation to growth and disease resistance in growing pigs:

a brief review

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Abstract

This brief review describes the intakes of n-6 and n-3 polyunsaturated fatty acids (PUFAs) in relation to growth and disease resistance in growing pigs. It is concluded that the current recommended intake of linoleic acid (LA), i.e. 0.08 g/MJ of metabolizable energy (ME), is too low to sustain maximum growth. Intakes of α-linolenic acid (ALA) higher than 0.05 g/MJ may not enhance growth. There are limited studies on dietary PUFAs and indicators of disease resistance in pigs and it appears that in those studies n-3 PUFAs had either non-consistent effects or had effects not related to growth performance. In one study, the n-3 PUFAs, eicosapentaenoic and docosahexaenoic acid, had a protective action on *Mycoplasma hyopneumoniae*-induced lung laesions, but this effect was seen at unrealistically high intakes and a concurrent, unacceptably low intake of LA. There is no evidence to suggest that under conditions of infectious pressure in practice, growth of pigs may be enhanced by the intake of extra n-3 PUFAs, but for optimum growth, the intake of ALA should be at least 0.05 g/MJ ME.

Introduction

Linoleic acid (C18:2 n-6, LA) and α-linolenic acid (C18:3 n-3, ALA) are essential polyunsaturated fatty acids (PUFAs) because they cannot be synthesized in the body. LA and ALA are the parent compounds of the families of n-6 and n-3 fatty acids, respectively. The n-6 and n-3 PUFA families are not metabolically interconvertible in mammals (Calder, 1996). LA and ALA can be desaturated and elongated in the animal body to yield the n-6 fatty acid, arachidonic acid (C20:4 n-6, AA) and the n-3 fatty acid, eicosapentaenoic acid (C20:5 n-3, EPA), which are direct precursors for the synthesis of the eicosanoids, i.e. prostaglandins, thromboxanes and leukotrienes. Eicosanoids are important modulators of humoral and cell-mediated immune responses (Wu and Meydani, 1998; Simopoulos, 2001; James et al., 2000; Calder, 1998). In essence, the eicosanoids synthesized from n-6 and n-3 PUFAs have antagonistic activities. The n-3 PUFAs EPA and docosahexaenoic acid (C22:6 n-3, DHA) are used in the treatment of inflammatory diseases such as rheumatoid arthritis, psoriasis and ulcerative colitis (Wu and Meydani, 1998). There is evidence that the extra intake of EPA and DHA reduce tumor growth (Simopoulos, 2001). In rodents, the intake of high amounts of n-3 PUFAs instead of LA has been shown to reduce sensitivity to various types of infections (Blok et al., 1996).

Fortification of the diet with n-3 PUFAs can be done in the form of either ALA or EPA. Oils of plant origin generally contain up to 10% ALA. Linseed oil is extremely rich in ALA, the content being about 55%. On the other hand, oils such as corn oil, soybean oil and sunflowerseed oil typically contain 50-60% of LA. Feeding of extra EPA may be realized by inclusion of fish oil in the diet; fish oil may contain up to 15% EPA. When fortifying diets with either ALA or EPA, the background level of n-6 PUFAs, especially that of LA, is a key issue. There are competitive interactions between LA and ALA that determine their conversion into AA and EPA, respectively. Higher tissue levels of EPA will be reached after ingestion of ALA when the diet has a low LA content. The interactions between ALA and LA relate to the fact that the conversion of the two PUFAs, through desaturation and elongation, is catalyzed by the same enzymes (James et al., 2000).

This brief review focuses on the importance of n-6 and n-3 PUFAs in the diet of growing pigs. An attempt is made to quantify the requirements of LA and ALA when using growth as criterion. In addition, literature data are discussed to address the question whether increased intake of either ALA or EPA can improve disease resistance of growing pigs. It is assumed that an improved disease resistance may enhance growth, at least under conditions of high infectious pressure.

PUFA requirements of growing pigs

The LA requirement of growing pigs has been set at 0.08 g/MJ of metabolizable energy (ME) (National Research Council, 1998). There are no formal recommendations as to the requirement of ALA. Figure 1 shows the relationship between the intake of LA and growth of pigs as based on 8 different publications.

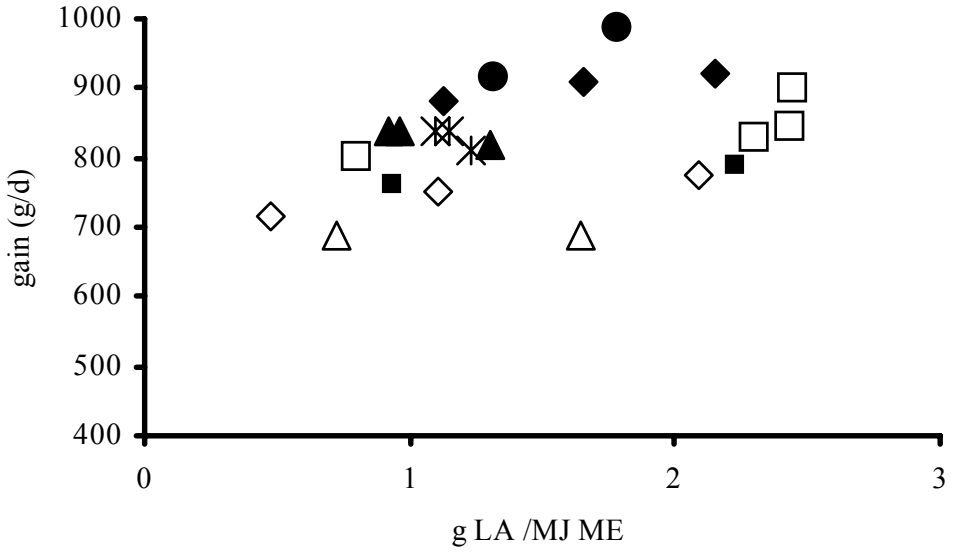

Figure 1. Relationship between dietary LA level (g LA/MJ ME) and daily weight gain (g/day) in growing pigs as based on data published by Lauridsen et al., 1999 (•); Morgan, 1992 (□); Warrants et al., 1996 (♠); Kouba and Mourot, 1999 (□); Myer et al., 1992 (◆); Flachowsky et al., 1997 (□); Leskanich et al., 1997 (()); Fontanillas et al., 1997 (▲). Data points with identical symbols correspond to the same publication.

An experiment using young pigs with average daily weight gain less than 400 g/day (Cera et al., 1989) was excluded. There appears to be a weak, direct relationship between dietary LA and growth. The regression equation is y = 752 (± 41, SD) + 51 (± 26) x LA (g/MJ ME) (P = 0.068) with linear correlation coefficient of 0.39. The data in Figure 1 indicate that the LA requirement set by the National Research Council (1998) might be too low to sustain maximum growth. However, it should be noted that in 4 out of the 8 studies, the extra intake of LA was associated with an extra intake of fat and thus higher ME content of the diet. A higher energy density of the diet by itself may be associated with a higher growth rate. Thus, further controlled studies are necessary to establish the LA requirement of growing pigs.

The literature data on dietary ALA and weight gain are illustrated in Figure 2. There is no significant regression coefficient (P = 0.166). Thus, Figure 2 indicates that at ALA intakes higher than about 0.05 g/MJ ME there may be no ALA effect on growth in pigs.

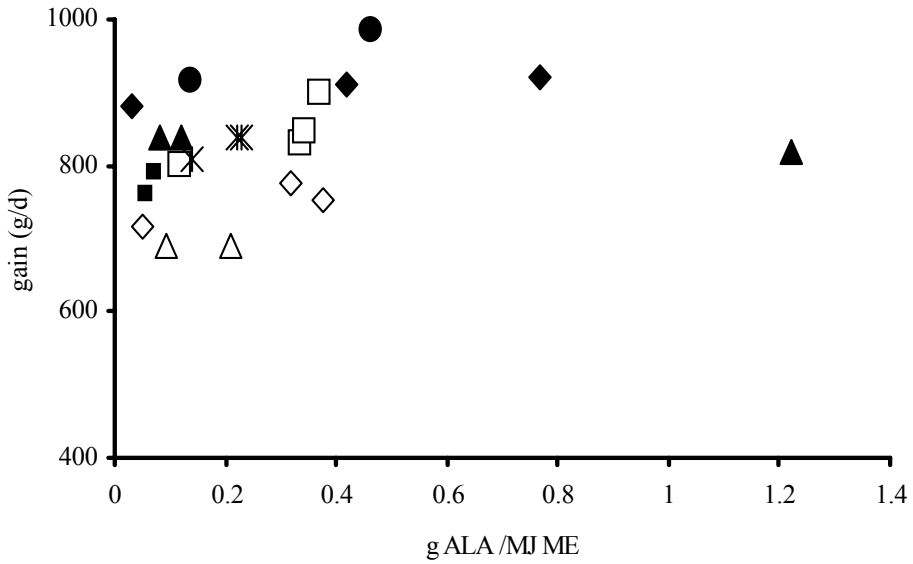

Figure 2. Relationship between dietary ALA level (g LA/MJ ME) and daily weight gain (g/day) in growing pigs as based on data published by Lauridsen et al., 1999 (•); Morgan, 1992 (□); Warrants et al., 1996 (♠); Kouba and Mourot, 1999 (□); Myer et al., 1992 (◆); Flachowsky et al., 1997 (□); Leskanich et al., 1997 (()); Fontanillas et al., 1997 (▲). Data points with identical symbols correspond to the same publication.

In 4 studies, the diets used did not contain EPA and DHA. In the 4 other studies, the maximum content of EPA plus DHA was 0.25 g/MJ. In the various studies, the n-6:n-3 ratio ranged between 1.1 and 38.4. It seems unlikely that there is an interaction between the n-6:n-3 ratio and the intake of ALA as to weight gain. Even at very high n-6:n-3 ratios in the diet, an ALA content higher than 0.05 g/MJ may not stimulate growth.

PUFA intake and disease resistance in growing pigs

As mentioned above, there is evidence from studies with laboratory rodents that the feeding of EPA and DHA instead of LA improves disease resistance and is protective against bacterial challenges under controlled conditions. Here, the effect of PUFA intake and disease resistance in growing pigs is described on the basis of literature data. Attention is focused on the fatty acid composition of the diets used and on the endpoints of disease or indicators of disease resistance. Underlying mechanisms are beyond the scope of this paper and are addressed elsewhere (Calder, 1996; Wu and Meydani, 1998).

Murray and Zhang (1997) have fed to male pigs, weighing 20-25 kg, enteral formulations enriched with either corn oil (diet A), fish oil (diet B) or a mixture of fish and

borage oil (diet C). After 20 days of feeding the experimental diets, blood samples were taken and platelets isolated to measure the release of thromboxane B_2. The baseline release of the eicosanoid and that upon stimulation with A_{23187} was determined. Table 1 shows that thromboxane B_2 release was suppressed in platelets from donor pigs fed the diets containing EPA and DHA (diets B and C), when compared with the diet rich in LA (diet A).

Table 1. Assessed fatty acid content of experimental diets and thromboxane B_2 release from platelets

	Diet		
	A	B	C
Fatty acid (g/MJ ME)			
LA	8.45	3.19	2.36
GLA	0	0	0.66
ALA	0.16	0.74	0.52
EPA	0	0.77	0.81
DHA	0	0.34	0.35
Thromboxane B_2 release (pg/ml medium)			
Baseline	175	38	88
Stimulation	325[a]	50[b]	125[b]

Means (n = 8 / treatment) with different superscripts are significantly different (P< 0.05).

The degree of suppression was less for diet C, i.e. when the diet rich in EPA and DHA also contained a high level of γ-linolenic acid (C18:3 n-6, GLA). It appears that the feeding of n-3 versus n-6 PUFAs inhibited thromboxane B_2 release by isolated platelets. Thromboxane B_2 is a metabolite of thromboxane A_2 which is synthesized from the n-6 PUFA, AA. Thromboxane A_2 stimulates platelet aggregation and vasoconstriction. It is thus difficult to see that consumption of EPA and DHA at the expense of LA, through inhibition of the production of thromboxane A_2, has a favourable influence on growth performance.

Murray et al. (1993) fed pigs, weighing 20-25 kg, on a commercial weaner diet without added oil (C) or mixed with either 10% of corn oil (CO) or menhaden oil (MO). After 9 days on the diets, the pigs were injected intravenously with endotoxin derived from *Escherichia coli* and their response was measured. Table 2 illustrates that in pigs fed extra EPA and DHA instead of LA (diet MO), the group-mean pulmonary vascular resistance, blood pressure and cardiac output were higher when determined after application of the endotoxin.

The feeding of n-3 PUFAs (diet MO) reduced the plasma levels of thromboxane B_2. The pre-endotoxin values of the four variables were not different for the experimental diets. Thus, the results indicate that the general endotoxin-induced profile was more favourable when the pigs were pre-fed on the diet rich in n-3 PUFAs. However, the differences between dietary corn oil (diet CO) and menhaden oil (diet MO) often did not reach statistical significance. The endotoxin-induced arterial oxygen pressure was equally raised by corn oil and menhaden oil in the diet (Table 2).

Table 2. Assessed fatty acid content of the experimental diets and measures at various intervals after intravenous administration of endotoxin from *Escherichia coli*

	Diet		
	C	CO	MO
Fatty acid (g/MJ ME)			
LA	2.15	5.38	1.57
ALA	0.26	0.22	0.23
EPA	0.02	0.02	1.15
DHA	0.01	0.01	0.74
Arterial O_2 pressure (mm Hg)	65^a	78^b	79^b
Pulmonary vascular resistance (Dynes/s/cm^5)	800^a	300^b	500^b
Blood pressure (mm Hg)	80^a	100^{ab}	120^b
Cardiac output (L/min)	1.8^a	2.8^b	3.2^b
Plasma thromboxane B_2 (pg/ml)	8300^a	4200^b	1200^c

Means (n = 6 or 8 / treatment) with different superscripts are significantly different (P < 0.05).

In another paper, Murray et al. (1995) reported the effects of dietary fatty acids on acute lung injury. Pigs, weighing 15-25 kg, were fed enteral diets containing corn oil (A), fish oil (B) or fish and borage oil (C). After 8 days on the diets, acute lung injury was induced by treatment with lipopolysacharide (LPS) from *Escherichia coli*. The values for LPS-induced pulmonary vascular resistance were not systematically influenced by diet (Table 3).

Table 3. Assessed fatty acid content of the experimental diets and measures at various intervals after the induction of endotoxemia

	Diet		
	A	B	C
Fatty acid (g/MJ ME)			
LA	8.45	3.19	2.36
GLA	0	0	0.66
ALA	0.16	0.74	0.52
EPA	0	0.77	0.81
DHA	0	0.34	0.35
Pulmonary vascular resistance (Dynes/s/cm^5)			
After 20 min	850^a	425^b	400^b
After 2 hours	550^a	550^a	425^b
Arterial O_2 pressure (mm Hg)	62^a	77^b	73^{ab}
Plasma thromboxane B_2 (pg/ml)	5000^a	2000^b	1800^b

Means (n = 12 / treatment) with different superscripts are significantly different (P < 0.05).

The diets rich in n-3 PUFAs and low in n-6 PUFAs (diets B and C) produced a relatively high LPS-induced arterial pressure. Plasma levels of thromboxane B_2 after LPS administration were lower for the two diets containing EPA and DHA (diets B and C) than for the LA-rich diet (diet A). When taken together the studies of Murray and Zhang (1997) and Murray et al. (1993, 1995), it can be concluded that a high intake of EPA and DHA consistently reduced the stimulated production of thromboxane B_2. The endotoxin-mediated drop in arterial oxygen pressure was attenuated by high intake of EPA and DHA. However, in one study (Table 2), there was no difference in arterial oxygen pressure for diets containing either corn oil or menhaden oil, whereas in another study (Table 3), there was.

Turek et al. (1994) have fed different diets to pigs for 28-30 days, and then macrophages were isolated from alveolar cells. At the beginning of the experiment, the piglets weighed about 7 kg. They were fed either a commercial, natural-ingredient diet (C) or purified diets to which either corn oil (CO), menhaden oil (MO), linseed oil (LO) or 1 : 1 oil mixtures (LO/CO or MO/CO) were added to a final concentration of 10.5%. Different characteristics of the macrophages were measured such as TNFα-like activity and nitrite production after LPS stimulation, phagocytosis of fluorescent microspheres and production of T-cell growth factors after incubation with Con A. Table 4 shows that the two experimental diets with added EPA and DHA (diets MO and MO/CO) had no systematic effects on the various macrophage characteristics, and neither had the diets rich in ALA (diets LO and LO/CO). Likewise, the only diet rich in LA and without added n-3 PUFAs (diet CO) did not have an unique effect.

Table 4. Assessed fatty acid content of the experimental diets and characteristics of isolated alveolar macrophages

	Diet					
	C	CO	MO	LO	LO/ CO	MO/ CO
Fatty acid (g/MJ ME)						
LA	n.g.	3.82	0.08	1.23	2.52	1.95
ALA	n.g.	0.07	0.07	3.00	1.53	0.66
EPA	n.g.	-	0.98	-	-	0.49
DHA	n.g.	-	0.97	-	-	0.48
TNFα-like activity (pg TNF/mg DNA)	11000	8000^{a}	11000^{a}	9000^{a}	25000^{b}	21000^{b}
Nitrite production (nmol/mg DNA)	122	143^{c}	154^{c*}	60^{cd}	330^{c}	33^{d}
Phagocytosis (% of macrophages with 1 bead)	9	8	13	14	6	8
T-cell growth factors (unit IL-2/% lymphocytes x 100)	34	47^{ab}	18^{a}	16^{a}	34^{ab}	54^{b}

n.g.: not given
Means (n = 6 / treatment) with different superscripts are significantly different (P < 0.05).

Thies et al. (1999) used piglets with body weight of about 7.5 kg and fed them for 40 days on one of five diets. The diets either contained 5% of soybean oil (C), high-oleic sunflower oil (HOSO), sunflower oil (SO), canola oil (CO) or fish oil (FO). Amongst others, the authors determined the uptake of *Escherichia coli* by isolated blood macrophages, natural killer cell activity of lymphocytes and prostaglandin E_2 production by cultured mononuclear cells isolated from either blood or lymph nodes. Table 5 documents that pre-feeding of the diet with high contents of EPA and DHA (diet FO) induced the lowest uptake of *E. coli* by macrophages and lowest natural killer cell activity of lymphocytes. The dietary level of LA had no systematic effect. Prostaglandin E_2 production by blood mononuclear cells was lowest when the donor piglets had been fed the diet with fish oil (diet FO), but in the case of mononuclear cells isolated from lymph nodes, the role of dietary fat type was not clear.

Table 5. Assessed fatty acid content of experimental diets and indicators of disease resistance

	Diet				
	C	HOSO	SO	CO	FO
Fatty acid (g/MJ ME)					
LA	2.92	1.61	2.87	1.69	1.43
ALA	0.36	0.19	0.17	0.42	0.24
EPA	0.04	0.11	0.06	0.04	0.33
DHA	0	0.04	0.01	0.01	0.17
Uptake of E. coli by blood macrophages (% positive)					
After 5 min.	59.6^{c}	58.4^{c}	53.9^{bc}	62.9^{c}	42.9^{b}
After 10 min.	78.8^{c}	70.9^{bc}	71.9^{bc}	76.8^{c}	59.9^{b}
Natural killer cell activity of lymphocytes (% cytolysis)	67.5^{bc}	63.5^{bc}	67.5^{bc}	75.3^{bc}	57.1^{b}
Prostaglandin E_2 production by cultured mononuclear cells (pg/ml medium)					
Blood	363^{c}	313^{bc}	281^{bc}	248^{b}	209^{b}
Lymph node	172^{c}	84^{b}	114^{c}	71^{b}	72^{b}

Means (n = 12 / treatment) with different superscripts are significantly different (P < 0.05).

Turek et al. (1996) have studied the effect of dietary fatty acid composition on the sensitivity of piglets to *Mycoplasma hyopneumoniae* infection. Piglets with initial, mean body weight of 7.7 kg were fed different diets for 4 weeks and were then inoculated intratracheally with *M. hyopneumoniae* and killed 3 weeks later. The diets were a commercial natural-ingredient diet (C) or purified diets containing either 10.5% of corn oil (CO), menhaden oil (MO), linseed oil (LO) or 1 : 1 mixtures of the oils (MO/CO or LO/CO). It is clear from Table 6 that the MO diet, which was rich in EPA and DHA and low in LA, produced the lowest degree of lung laesions, when compared with the other purified diets.

Table 6. Assessed fatty acid content of the experimental diets, lung laesions in the *Mycoplasma hyopneumoniae*-infected pigs and TNFα-like activity by alveolar macrophages

	Diet					
	C	CO	MO	LO	MO/CO	LO/CO
Fatty acid (g/MJ ME)						
LA	n.g.	3.82	0.08	1.23	1.95	2.52
ALA	n.g.	0.07	0.07	3.00	0.66	1.53
EPA	n.g.	-	0.98	-	0.49	-
DHA	n.g.	-	0.97	-	0.48	-
Lung laesions						
(% of lung surface)	7	14^{ab}	8^{a}	14^{ab}	16^{b}	20^{b}
(total laesion score)	4.2	5.1^{b}	3.2^{a}	5.0^{b}	4.7^{b}	5.1^{b}
TNFα-like activity (pg TNF/mg DNA)	3000	4000^{b}	1900^{ab}	3800^{b}	800^{a}	2400^{b}

n.g.: not given
Means (n =9 / treatment) with different superscripts are significantly different (P < 0.05).

However, the commercial diet was equally effective. When half of the menhaden oil was replaced by corn oil (diet MO/CO), the protective effect had disappeared. Fortification of the diet with ALA in the form of linseed oil (diet LO) had no suppressing effect on the development of lung laesions. TNFα-like activity by alveolar macrophages from the infected pigs was lowest when the diet contained the MO/CO mixture and highest when it contained corn oil (diet CO). The diet effects on TNFα-like activity do not correspond with those seen in another study of the same group of investigators (Table 4).

Conclusions

The limited number of controlled studies on dietary PUFAs and indicators of disease resistance in pigs cannot be easily interpreted. The outcomes were not consistent and/or cannot be related to health and performance of growing pigs. In one study (Turek et al., 1996), it was found that a diet rich in EPA and DHA protected against the development of lung laesions as induced by infection with *Mycoplasma hyopneumoniae.* However, that diet had a LA content of 0.08 g/MJ ME, which may be suboptimal as to sustaining maximum growth. In addition, the EPA and DHA contents were unrealistically high; the contents were 0.98 and 0.97 g/MJ, respectively. In practice, EPA and DHA contents of swine diets are generally not higher than 0.06 and 0.04 g/MJ, respectively. Furthermore, the commercial, natural-ingredient diet with unknown fatty acid composition, was equally effective in reducing *M. hyopneumoniae*-induced lung laesions as was the diet rich in EPA and DHA (Table 6). Thus, there is no evidence to suggest that under conditions of infectious pressure in practice, growth of pigs is enhanced by the intake of extra n-3 PUFAs. At this stage, an optimum dietary level and composition of n-3 PUFAs cannot be advised, but the diet should contain at least 0.05 g ALA/MJ ME.

Acknowledgements

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References

Blok, W.L., M.B. Katan and J.W.M. Van der Meer (1996). Modulation of inflammation and cytokine production by dietary (n-3) fatty acids. J. Nutr. 126: 1515-1533.

Calder, P.C. (1996). Effects of fatty acids and dietary lipids on cells of the immune system. Proc. Nutr. Soc. 55: 127-150.

Calder, P.C. (1998). Dietary fatty acids and the immune system. Nutr. Rev. 56: S70-S83.

Cera, K.R., D.C. Mahan and G.A. Reinhart (1989). Postweaning swine performance and serum profile responses to supplemental medium-chain free fatty acids and tallow. J. Anim. Sci. 67: 2048-2055.

Flachowsky, G., F. Schone, G. Schaarmann, F. Lubble and H. Bohme (1997). Influence of oil seeds in combination with vitamin E supplementation in the diet on backfat quality of pigs. Anim. Feed Sci. Technol. 64: 91-100.

Fontanillas, R., A. Barroeta, M.D. Baucells and R. Codony (1997). Effect of feeding highly cis-monounsaturated, trans, or n-3 fats on lipid composition of muscle and adipose tissue of pigs. J. Agricul. Food Chem. 45: 3070-3075.

James, J.M., R.A. Gibson and L.G. Cleland (2000). Dietary polyunsaturated fatty acids and inflammatory mediator production Am. J. Clin. Nutr. 71: 343S-348S.

Kouba, M. and J. Mourot (1999). Effect of high linoleic acid diet on lipogenic enzyme activities and on the composition of the lipid fraction of fat and lean tissues in the pig. Meat Sci. 52: 39-45.

Lauridsen, C., S. Hojsgaard and M.T. Sorensen (1999). Influence of dietary rapeseed oil, vitamin E, and copper on the performance and the anti-oxidative and oxidative status of pigs. J. Anim. Sci. 77: 906-915.

Leskanich, C.O., K.R. Matthews, C.C. Warkup, R.C. Noble and M. Hazzledine (1997). The effect of dietary oil containing (n-3) fatty acids on the fatty acid, physicochemical, and organoleptic characteristics of pig meat and fat. J. Anim. Sci. 75: 673-683.

Morgan, C.A., R.C. Boble, M. Cocchi and R. McCartney (1992). Manipulation of the fatty acid composition of pig meat lipids by dietary means. J. Sci. Food Agric. 58: 357-368.

Murray, M.J. and T. Zhang (1997). The incorporation of dietary n-3 polyunsaturated fatty acids into porcine platelet phospholipids and their effects on platelet thromboxane A_2 release. Prostaglandins, Leukotrienes and Essential Fatty Acids 56: 223-228.

Murray, M.J., B.A. Svingen, T.L. Yaksh and R.T. Holman (1993). Effects of endotoxin on pigs prefed ω-3 vs. ω-6 fatty-acid enriched diets. Am. J. Physiol. 265: E920-E927.

Murray, M.J., M. Kumar, T.J. Gregory, P.L. Banks, H.D. Tazelaar and S.J. DeMichele (1995). Select dietary fatty acids attenuate cardiopulmonary dysfunction during acute lung injury in pigs. Am. J. Physiol. 269: H2090-H2099.

Myer, R.O., J.W. Lamkey, W.R. Walker, J.H. Brendemuhl and G.E. Combs (1992). Performance and carcass characteristics of swine when fed diets containing canola oil and added copper to alter the unsaturate:saturated ratio of pork fat. J. Anim. Sci. 70: 1417-1423.

National Research Council (1998). Nutrient Requirements of Swine. Tenth revised edition, National Academy Press, Washington DC.

Simopoulos, A.P. (2001). N-3 fatty acids and human health: defending strategies for public policy. Lipids 36: S83-S89.

Thies, F., L.D. Peterson, J.R. Powell, G. Nebe-von-Caron, T.L. Hurst, K.R. Matthews, E.A. Newsholme and P.C. Calder (1999). Manipulation of the type of fat consumed by growing pigs affects plasma and mononuclear cell fatty acid compositions and lymphocyte and phagocyte functions. J. Anim. Sci. 77: 137-147.

Turek, J.J., I.A. Schoenlein, B.A. Watkins and W.G. Van Alstine (1994). Dietary polyunsaturated fatty acid effects on immune cells of porcine lung. J. Leukoc. Biol. 56: 599-604.

Turek, J.J., I.A. Schoenlein, B.A. Watkins, W.G. Van Alstine, L.K. Clark and K. Knox (1996). Dietary polyunsaturated fatty acids modulate immune responses of pigs to *Mycoplasma hyopneumoniae* infection. J. Nutr. 126: 1541-1548.

Warnants, N., M.J. Van Oeckel and Ch.V. Boucqué (1996). Incorporation of dietary polyunsaturated fatty acids in pork tissue and its implications for quality of the end products. Meat Sci. 44: 125-144.

Wu, D. and S.N. Meydani (1998). N-3 polyunsaturated fatty acids and immune function. Proc. Nutr. Soc. 57: 503-509.

Chapter 2

Mathematical relationships between the intake of n-6 and n-3 polyunsaturated fatty acids and their contents in adipose tissue of growing pigs

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Abstract

To establish the relationships between the fatty acid composition of adipose tissue in growing pigs and the intake of fatty acids, we performed a feeding trial and did a literature survey. Six groups of pigs were fed diets with variable combinations of corn, linseed and fish oil. After 38 days, biopsies of adipose tissue were analyzed for their contents of linoleic, α-linolenic, eicosapentaenoic and docosahexaenoic acid. For the four fatty acids, intake data and adipose tissue levels were also collected from the literature. Linear correlations were computed for the intake of each polyunsaturated fatty acid and its level in adipose tissue, the data set consisting of either the original results only or combined with literature figures. The observed strong correlations between dietary and fat tissue polyunsaturated fatty acids indicate that the fatty acid composition of the diet may be used as an index of the fatty acid composition of the adipose tissue, and vice versa. The regression equations presented can be used to steer the fatty acid composition of adipose tissue of growing pigs by the fatty acid composition of their diet.

Introduction

It is well known that the fatty acid profile of adipose tissue of pigs reflects the fatty acid composition of their diet. Adipose tissue fatty acids are derived from either de novo synthesis or from the diet. Linoleic acid (C18:2 n-6) and α-linolenic acid (C18:3 n-3) are the parent fatty acids of the so-called n-6 and n-3 families of polyunsaturated fatty acids, respectively. Linoleic and α-linolenic acid cannot be synthesized by pig tissues so that the intake of these fatty acids will determine their contents in swine adipose tissue. Indeed, various studies have shown that increasing intakes of linoleic and α-linolenic acid are associated with increasing contents of these fatty acids in adipose tissue of swine. However, mathematical relationships between the intake of these fatty acids and their contents in swine adipose tissue have not been reported yet. Such relationships may be used to steer the fatty acid profile of swine adipose tissue. The fatty acid composition of adipose tissue relates to meat quality. The amount of polyunsaturated fatty acids in adipose tissue influences the sensitivity to oxidative breakdown, and the formation of peroxides, and thus the development of rancidity (Corino, Magni, Pagliarini, Rossi, Pastorelli & Chiesa, 2002; Gray, Gomaa & Buckley, 1996). The degree of unsaturation of fatty acids in meat fat is associated with the firmness of the tissue (Davenel, Riaublanc, Marchal & Gandemer, 1999). In addition, there is evidence that the intake of the long-chain n-3 polyunsaturated fatty acids, eicosapentaenoic acid (C20:5 n-3) and docosahexaenoic acid (C22:6 n-3), is beneficial to the human consumer as it is associated with a decreased risk of cancer and cardiovascular diseases (Simopoulos, 2001). These n-3 fatty acids are abundant in fish oil and oily foodstuffs from fish (Newton, 1998), but can also be produced by mammalian tissues from their natural precursor α-linolenic acid. In addition, the intake of fish oil by mammals such as swine, also results in the incorporation of eicosapentaenoic and docosahexaenoic acid in their adipose tissue (Taugbol, 1993). Fish oil and oily fish are not consumed by certain populations so that long-chain n-3 polyunsaturated fatty acids have to be provided with other foodstuffs, for example pork. As far as we know, there is no literature on the mathematical relationships between the intake of n-3 polyunsaturated fatty acids and the contents of eicosapentaenoic and docosahexaenoic acid in adipose tissue of swine.

The present study focuses on the contents of linoleic, α-linolenic, eicosapentaenoic and docosahexaenoic acid in adipose tissue of swine fed diets with different amounts of these fatty acids. To establish mathematical relationships, literature data and the results of an original feeding trial were used. In the current trial, growing swine were fed diets with different combinations of corn, linseed and fish oil so that the literature data could be complemented.

Materials and methods

Animals

Forty-eight pigs (Dutch Landrace x German Pietrain) were fed the experimental diets for a period of 38 days. There were 24 barrows and 24 gilts. The pigs were divided into six groups of 8 animals each so that the distribution of barrows and gilts was identical for each dietary group. The animals of each group were housed together in pens located in one compartment of a building. The average weight of the six groups ranged from 22.1 kg (SD = 0.78) to 24.3 kg (SD = 1.56) at the beginning of the trial. The groups were fed

twice daily, except on Sunday when they were fed once. Pigs were weighed at the beginning and end of the trial. Two pigs died during the experimental period on days 17 and 38 from a circo-virus infection.

Experimental diets

A basal diet was used that consisted of (g/97 g): barley, 30.5; maize, 5.6; wheat, 25; peas, 3; soybean meal, 20; sunflower-seed meal, 4; molasses, 2; tapioca, 1.2; beet pulp, 2; toasted soybeans, 1; vitamins/minerals, 2.7. The experimental diets were formulated by adding different oils, and mixtures of the oils, to the basal diet. The experimental diets contained 3 % of added oil; Table 1 shows the various combinations. According to chemical analysis, the basal diet had the following composition (g/100 g diet): dry matter, 90.2; crude protein, 16.7; crude fiber, 4.7; crude fat, 4.7; ash, 4.2. The calculated energy content of the experimental diets was 13.7 MJ of metabolizable energy (ME)/kg.

Table 1. Composition of the experimental diets

	Diet					
Ingredient	1	2	3	4	5	6
	g/100 g of air dry diet					
Basal diet	97	97	97	97	97	97
Corn oil	3	-	-	1	2	-
Linseed oil	-	3	-	-	1	2
Fish oil	-	-	3	2	-	1

The basal diet and the oils were mixed freshly every day. The diets were fed as porridge. The basal diet and the oils were mixed in the trough and then water was added while stirring the mixture. After 30 minutes, the pigs were given access to the trough. A restricted feeding regimen was used. On day 1 of the experiment, the pigs received, per pen per day, 6.4 kg of air-dry feed mixture and 5.0 kg water (per meal) per pen. The amount supplied was gradually increased each week and was equal to 14.4 kg of feed mixture and 11.4 kg of water on day 35. In the event of feed left-overs, the amount of feed to be supplied the next meal was somewhat lowered so that the left-overs would be consumed. The amount of feed consumed was registered.

Collection of samples

Fat biopsies were taken on days 0 and 38. On day 0, biopsies were taken from two animals per group (1 male, 1 female). On the last day, biopsies were taken from all animals. The animals were sedated by an intramuscular injection of 1 ml/10 kg body weight of Stressnill (Janssen-Cilag, Tilburg, the Netherlands) and 1 ml/25 kg body weight of ketamine (Nimatek®, AUV, Cuyck, The Netherlands). The animals had been withheld from food for 16 hours before sedation. A 30-40 mm incision was made in the left inguinal region and about 1 g of adipose tissue was removed. The skin wounds were not closed. No complications occurred. The biopsies were stored at –20 °C until they were analyzed. Samples of the oils were taken on the first day of the experiment and were stored in a dark container at 4 °C. Samples of the basal diet were also taken.

Analytical methods

Fatty acid analyses were carried out on samples of the basal diet, oils and adipose tissue. The diet and oil samples were extracted with the chloroform:methanol procedure (Folch, Lees & Sloane Stanley 1957). Further procedures were carried out according to Metcalfe, Schmitz & Pekka (1966). The samples were then analyzed by gas chromatography using the Chrompack 9002 (Varian, Middelburg, The Netherlands) with FFAP column (25m x 0.32mm, Chrompack) and a temperature program. The composition of the basal diet was determined by the Weende analyses.

Data collection

The literature used was collected with the help of ERL (keywords: pigs, fatty acid, adipose tissue, linseed oil, maize/corn oil, fish oil) and through references given by the literature found. The literature used (Table 2) was restricted to pigs, and the fatty acid composition of their diet and adipose tissue.

Table 2. Characteristics of the literature data used

Authors	Number of dietary treatments	Number of animals per treatment	Duration of treatment	Weight at beginning of treatment	Sex
Enser et al. (2000)	2	40	90 days	25 kg	M/F
Riley et al. (2000)	4	16	75 days	46 kg	M/F
Riley et al. (2000)	2	8	27 days	87 kg	M/F
Otten et al. (1993)	2	6	13 weeks	29 kg	CM
Wiseman et al. (2000)	10	16	55 days	55 kg	M/F
M. Kouba & J. Mourot (1999)	2	8	80 days	40 kg	CM
Fontanillas et al. (1997)	3	10	82 days	26 kg	CM
Leskanich et al. (1997)	3	150	65 days	52 kg	M/F
Smith et al. (1996)	6	7	35 days	8 kg	M/F
Warnants et al. (1996)	5	22	130 days	25 kg	M/F
Morgan et al. (1992)	4	10	60 days	25 kg	F
Cava et al. (2000)	5	5	56 days	60 kg	CM
Warnants et al. (1999)	10	14	130 days	25 kg	M/F
Van Oekel et al. (1996)	3	26	135 days	35 kg	M/F
Wiseman & Agunbiade (1998)	3	30	variable	25 kg	M
Corino et al. (2002)	3	10	180 days	25 kg	CM
Irie & Sakimoto (1992)	4	4	28 days	81 kg	M/F
Romans et al. (1995)	5	8	25 days	114 kg	F/CM
Brooks (1971)	2	20	30 days	18 kg	M/F
Current study	6	8	38 days	23 kg	M/F

F: female, M: male, CM: castrated male

The dietary energy levels were given in some of the articles, and for the others it was calculated on the basis of the given composition of the diet by using the raw material compendium (Novum). The dietary amounts of linoleic, α-linolenic, eicosapentaenoic and docosahexaenoic acid are expressed as g per MJ of ME. When only the relative percentage of fatty acids in the whole diet was given in the published articles, it was assumed that the dietary crude fat contains 95% of its weight in the form of fatty acids.

Statistics

The linear regression equations were calculated with the following model. $Y = aX + b$, where Y = adipose tissue fatty acid (weight % of total fatty acids) and X = dietary fatty acid (g fatty acid/MJ ME). Linear correlations were computed only for linoleic, α-linolenic, eicosapentaenoic and docosahexaenoic acid. The calculations were made with the statistical computer program SPSS. For each experiment, group mean values for adipose tissue fatty acids were used and no corrections were made for the number of animals represented by each value. Group means can be considered the best estimate of the diet-induced fatty acid profile of adipose tissue while the degrees of freedom are not inflated.

Results

Feeding trial

The fatty acid composition of the experimental diets, as calculated by using the analyzed fatty acid composition and fat content of the basal diet and that of the added oils, is given in Table 3. Data are expressed as g fatty acid methyl ester / MJ ME of the diet. When considering the individual pigs as experimental units, it was found that there were no significant differences (Student's t test, $P < 0.05$) in body-weight gain between the pigs fed different diets. The overall, mean weight gain of the pigs was 25.4 kg (n=46; SD= 0.459). Overall mean feed conversion was 1.99 kg air dry feed/kg weight gain. Table 4 shows the relative contents of all the fatty acids measured in the fat biopsies. Diet 1 had produced the highest level of linoleic acid and diet 2 the highest level of α-linolenic acid. The adipose tissue contents of eicosapentaenoic and docosahexaenoic acid were highest in pigs fed diet 3.

Table 3. Fatty acid composition of the experimental diets

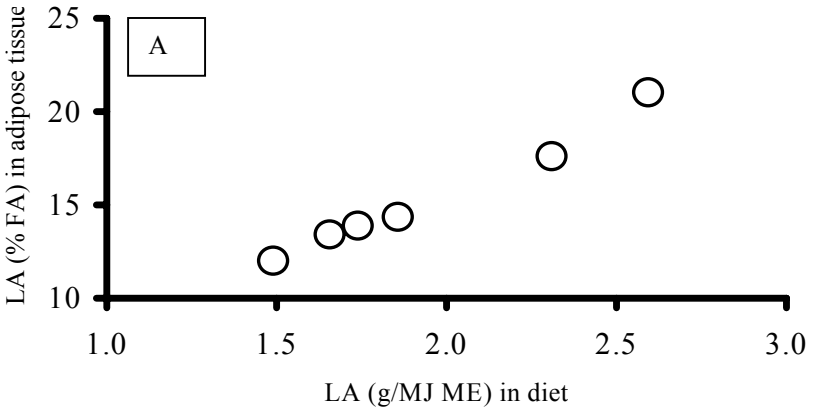
Fatty acid	Diet					
	1	2	3	4	5	6
	g fatty acid/MJ ME					
C14:0	0.026	0.026	0.136	0.100	0.026	0.063
C15:0	0.000	0.000	0.007	0.005	0.000	0.002
C16:0	0.809	0.706	0.889	0.863	0.775	0.767
C16:1	0.026	0.026	0.171	0.123	0.026	0.075
C17:0	0.006	0.006	0.011	0.010	0.006	0.008
C18:0	0.181	0.206	0.205	0.197	0.189	0.206
C18:1 n-9	1.375	1.154	1.124	1.207	1.301	1.144
C18:1 n-7	0.067	0.069	0.118	0.101	0.067	0.085
C18:2 n-6	2.730	1.830	1.568	1.955	2.430	1.743
C18:3 n-6	0.000	0.000	0.005	0.003	0.000	0.002
C18:3 n-3	0.147	1.160	0.150	0.149	0.484	0.823
C18:4 n-3	0.000	0.000	0.037	0.025	0.000	0.012
C20:0	0.017	0.013	0.012	0.014	0.016	0.013
C20:1	0.021	0.022	0.130	0.094	0.022	0.058
C20:2 n-6	0.000	0.000	0.007	0.004	0.000	0.002
C20:3 n-6	0.000	0.000	0.004	0.003	0.000	0.001
C20:4 n-6	0.000	0.000	0.012	0.008	0.000	0.004
C20:5 n-3	0.020	0.020	0.209	0.146	0.020	0.083
C22:0	0.015	0.015	0.012	0.013	0.015	0.014
C22:1 n-9	0.000	0.000	0.013	0.009	0.000	0.004
C22:5	0.009	0.008	0.082	0.058	0.009	0.033
C22:6 n-3	0.000	0.000	0.237	0.158	0.000	0.079
C24:1	0.024	0.024	0.024	0.024	0.024	0.024
Unknown	0.024	0.211	0.333	0.230	0.087	0.252

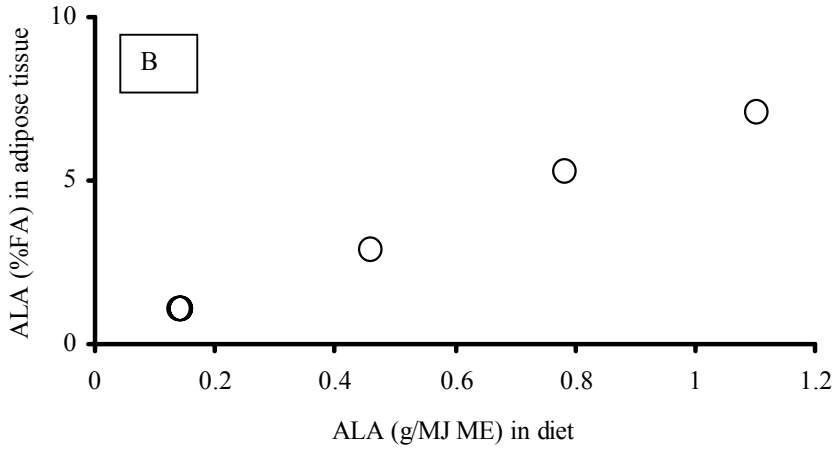
Table 4. Fatty acid composition of adipose tissue biopsies from pigs fed the experimental diets (g fatty acid methylester/ 100 g methylesters)

		Diet					
Fatty acid	Day 0	1	2	3	4	5	6
C14:0	1.42	1.18	1.23	1.44	1.42	1.24	1.27
	(0.11)	(0.09)	(0.11)	(0.12)	(0.08)	(0.07)	(0.07)
C16:0	23.14	22.99	22.74	23.94	24.21	22.94	23.10
	(0.98)	(1.35)	(1.37)	(0.95)	(1.01)	(0.72)	(1.38)
C16:1	3.30	1.79	2.04	2.66	2.52	2.02	2.03
	(0.45)	(0.31)	(0.29)	(0.33)	(0.32)	(0.34)	(0.24)
C17:0	0.24	0.24	0.18	0.23	0.21	0.22	0.25
	(0.08)	(0.03)	(0.08)	(0.10)	(0.09)	(0.04)	(0.04)
C18:0	8.72	11.77	11.78	12.47	12.34	11.37	12.81
	(0.91)	(1.74)	(1.15)	(0.71)	(1.11)	(0.88)	(1.81)
C18:1 n-9	40.43	34.66	34.27	35.80	35.40	35.53	33.83
	(1.26)	(0.97)	(1.81)	(2.10)	(2.03)	(1.53)	(1.31)
C18:1 n-7	3.14	2.20	2.37	3.06	2.79	2.42	2.45
	(0.20)	(0.19)	(0.28)	(0.12)	(0.19)	(0.25)	(0.18)
C18:2 n-6	14.86	21.02	13.90	12.01	14.37	17.63	13.44
	(1.15)	(1.90)	(1.10)	(1.13)	(1.23)	(2.13)	(1.80)
C18:3 n-3	1.35	1.06	7.08	1.09	1.11	2.90	5.27
	(0.10)	(0.10)	(0.63)	(0.09)	(0.08)	(0.37)	(0.72)
C20:0	0.04	0.19	0.16	0.15	0.16	0.18	0.18
	(0.08)	(0.08)	(0.07)	(0.10)	(0.07)	(0.02)	(0.08)
C20:1	0.69	0.67	0.59	1.15	0.90	0.66	0.74
	(0.05)	(0.07)	(0.08)	(0.13)	(0.06)	(0.06)	(0.05)
C20:2 n-6	0.46	0.65	0.43	0.39	0.42	0.55	0.40
	(0.04)	(0.08)	(0.07)	(0.05)	(0.05)	(0.07)	(0.03)
C20:4 n-6	0.30	0.27	0.15	0.16	0.20	0.25	0.20
	(0.03)	(0.05)	(0.07)	(0.11)	(0.08)	(0.03)	(0.05)
C20:3 n-3	0.01	0.03	0.62	0.03	0.03	0.28	0.42
	(0.04)	(0.05)	(0.11)	(0.06)	(0.05)	(0.04)	(0.04)
C20:5 n-3	0.17	0.02	0.17	0.81	0.58	0.11	0.42
	(0.11)	(0.04)	(0.08)	(0.14)	(0.08)	(0.07)	(0.07)
C22:5	0.33	0.22	0.33	1.10	0.82	0.29	0.64
	(0.11)	(0.03)	(0.04)	(0.10)	(0.07)	(0.03)	(0.07)
C22:6 n-3	0.60	0.31	0.31	1.56	1.12	0.35	0.75
	(0.07)	(0.02)	(0.03)	(0.19)	(0.11)	(0.04)	(0.09)
Unknown	0.77	0.66	1.62	1.85	1.34	0.97	1.74
	(0.19)	(0.28)	(0.11)	(0.73)	(0.51)	(0.20)	(0.38)

Means and SD's (in parentheses) for 12 pigs (Day 0) or 6 pigs per dietary group.

Fig. 1 shows the relationships between the content of a selected polyunsaturated fatty acid in the diet, expressed as g/MJ ME, and that in the adipose tissue, expressed as percentage of total fatty acids. The data points correspond with the six group mean values. For linoleic acid (panel A), α-linolenic acid (panel B), eicosapentaenoic acid (panel C) and docosahexaenoic acid (panel D), the correlations found are equally strong. The regression formulas are shown in Table 5.





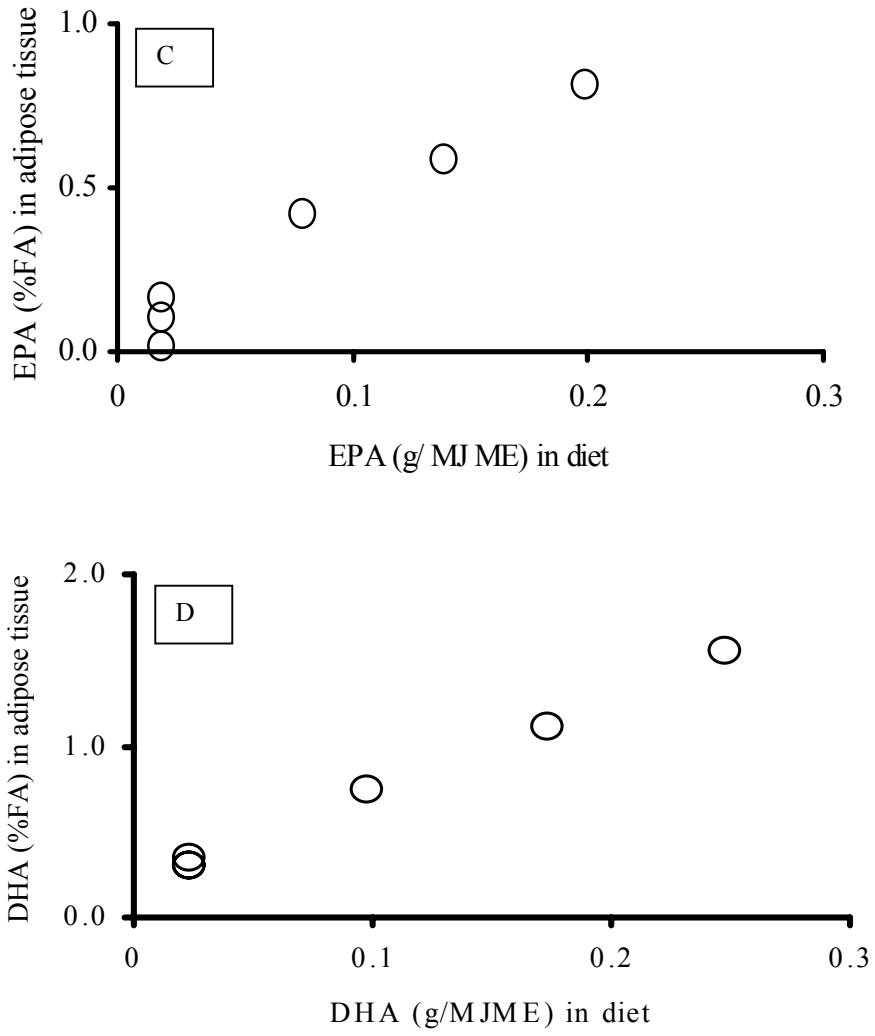


Fig. 1A-D. Relationship between polyunsaturated fatty acid intake and group mean content in adipose tissue of the corresponding fatty acid for pigs in the present feeding trial. Panel A: linoleic acid (LA); panel B: α-linolenic acid (ALA); panel C: eicosapentaenoic acid (EPA); panel D: docosahexaenoic acid (DHA).

Combination of literature and original data

Based on the literature data, including our own group mean data, the scattergrams shown in Figures 2-5 could be computed. Dietary fatty acids are expressed as g/MJ ME and adipose tissue fatty acids as percentage of total fatty acids. For linoleic acid (Fig. 2), α-linolenic acid (Fig. 3), eicosapentaenoic acid (Fig. 4) and docosahexaenoic acid (Fig. 5) the correlations were equally strong. Table 5 shows the regression equations.

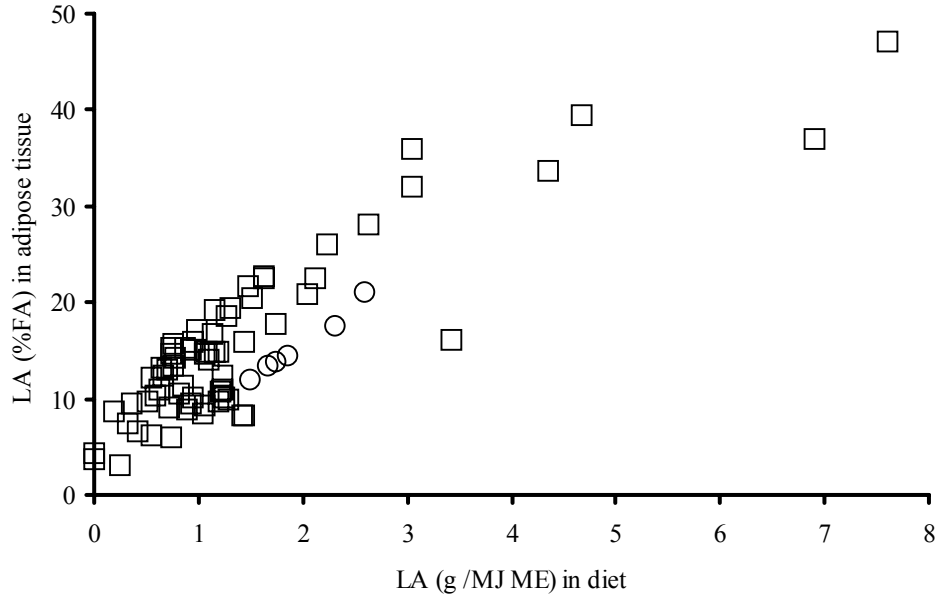


Fig. 2. Relationship between dietary linoleic acid and adipose tissue linoleic acid in growing pigs. The data points are group means derived from the literature (squares) and the present feeding trial (circles).

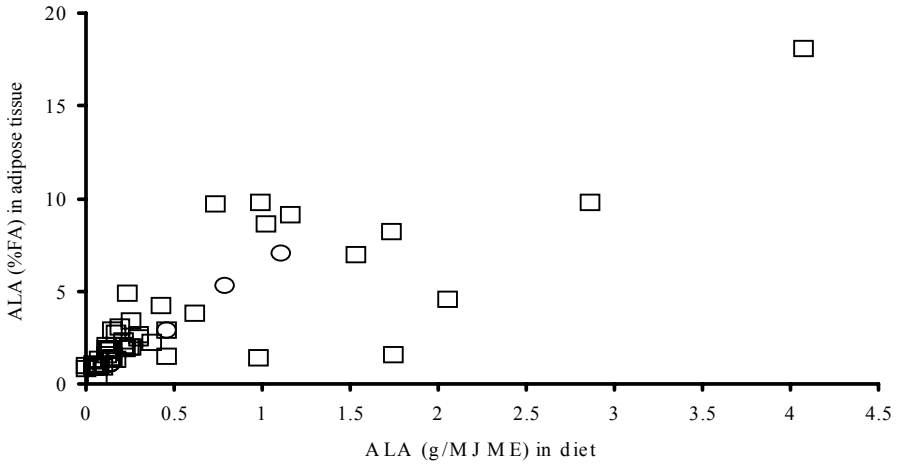


Fig. 3. Relationship between dietary α-linolenic acid and adipose tissue α-linolenic acid in growing pigs. The data points are group means derived from the literature (squares) and the present feeding trial (circles).

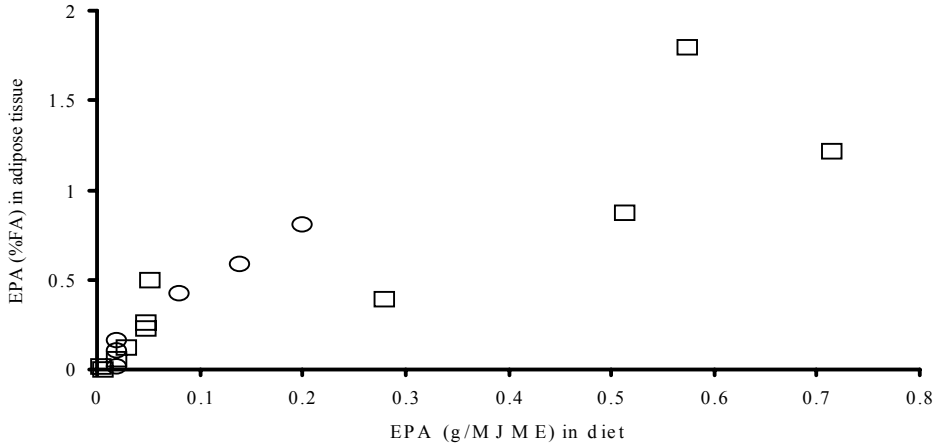


Fig. 4. Relationship between dietary eicosapentaenoic acid and adipose tissue eicosapentaenoic acid in growing pigs. The data points are group means derived from the literature (squares) and the present feeding trial (circles).

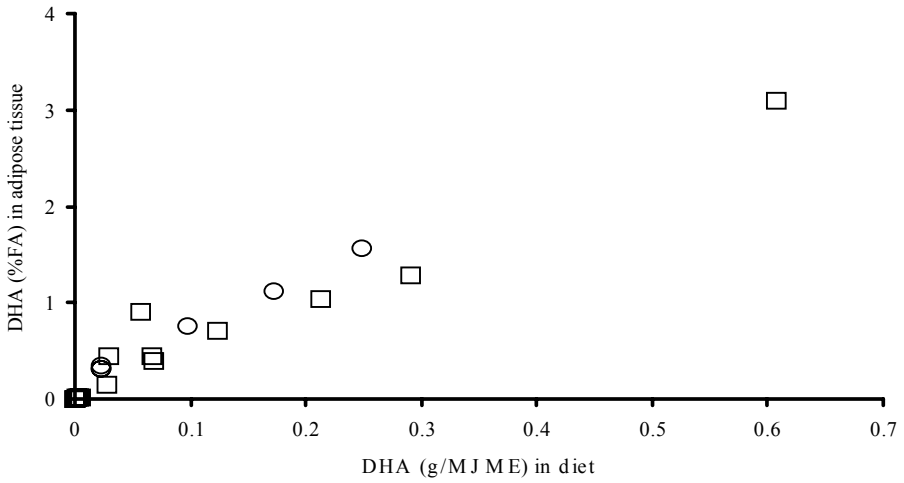


Fig. 5. Relationship between dietary docosahexaenoic acid and adipose tissue docosahexaenoic acid in growing pigs. The data points are group means derived from the literature (squares) and the present feeding trial (circles).

Table 5. The regression formulas (Y = Intercept + Slope • X) for the relations between dietary and adipose tissue polyunsaturated fatty acids

Data source	Fatty acid	Number		R^2	Intercept	SE	F probability	Slope	SE	F probability
		Data points	Expt.							
Literature + trial	C18:2 n-6	73	17	0.750	7.437	0.737	< 0.001	5.577	0.378	< 0.001
Literature + trial	C18:3 n-3	53	16	0.682	1.414	0.318	< 0.001	3.603	0.340	< 0.001
Literature + trial	C20:5 n-3	17	5	0.770	0.132	0.071	0.085	1.937	0.262	< 0.001
Literature + trial	C22:6 n-3	20	6	0.941	0.139	0.049	0.012	4.871	0.278	< 0.001
Trial	C18:2 n-6	6	1	0.979	0.280	1.010	0.797	7.791	0.508	< 0.001
Trial	C18:3 n-3	6	1	0.998	0.177	0.080	0.095	6.312	0.136	< 0.001
Trial	C20:5 n-3	6	1	0.956	0.037	0.040	0.410	3.992	0.383	< 0.001
Trial	C22:6 n-3	6	1	0.998	0.200	0.014	< 0.001	5.440	0.104	< 0.001

Y = adipose tissue fatty acid (% of total fatty acids).
X = dietary fatty acid (g/MJ ME).

Table 6. The regression formulas (Y = Intercept + Slope • X) for the relations between dietary and intramuscular fat polyunsaturated fatty acids

Fatty acids	Number		R^2	Intercept	SE	F probability	Slope	SE	F probability
	Data points	Expt.							
C18:2 n-6	30	5	0.787	5.46	1.04	< 0.001	4.256	0.403	< 0.001
C18:3 n-3	10	4	0.640	0.124	0.263	0.648	3.138	0.724	0.002

Based on literature data.
Y = intramuscular fatty acid (% of total fatty acids).
X = dietary fatty acid (g/MJ ME)

Discussion

As has been described for other animal species and humans (Feunkes, Van Staveren, De Vries, Burema & Hautvast, 1993; Lin, Connor & Spenler, 1993; Mills, Searle & Evans, 1979; Van Niel & Beynen, 1997), the relative percentage of linoleic, α-linolenic, eicosapentaenoic and docosahexaenoic acid in the adipose tissue of growing pigs was found to be a good index of the amount of these fatty acids in the diet. For all of the four fatty acids, we found high correlations between dietary intake and the levels in the adipose tissue. The linear correlation coefficients found for all data combined were 0.80 or higher. The correlation coefficients within the current trial were higher than 0.98. Thus, the correlations were stronger within our study than those found for pooled data that were derived from different studies. When using data from different experiments, the variation is increased due to differences in experimental design and conditions which leads to a decrease in the correlation coefficient.

The slopes of the regression equations for the relationships between dietary and adipose tissue fatty acids can be considered a measure of the efficiency of incorporation of the dietary fatty acids into adipose tissue. Both for the pooled data and for the data from the present trial, the slope was steepest for linoleic acid and most shallow for eicosapentaenoic acid. It would follow that proportionally more eicosapentaenoic acid, when compared with the other three polyunsaturated fatty acids, is converted into metabolites or is stored at sites other than adipose tissue. Similar observations have been made earlier in cats (Van Niel & Beynen 1979). It has been shown in rats (Sheppard & Herzberg 1992) and in rabbits (Lin, Connor & Spenler 1993) that dietary eicosapentaenoic acid is preferentially stored in organs rather than in adipose tissue. It is interesting to note that docosahexaenoic acid is more efficiently incorporated into adipose tissue than is eicosapentaenoic acid. The difference between the two fatty acids probably relates to the fact that eicosapentaenoic acid, and not docosahexaenoic acid, can be converted into eicosanoids.

For the quality of meat, the fatty acid composition of intramuscular fat may be more important than that of adipose tissue. Figures 6 and 7 show the correlations between dietary linoleic and ∝-linolenic acid and their percentages in intramuscular fat. Table 6 shows the regression formulas. It is clear that the regression coefficient for linoleic acid is smaller for intramuscular fat than for adipose tissue. This points at preferential incorporation of linoleic acid into adipose tissue. The intake of linoleic acid is much higher than the amount required for membrane synthesis so that a high proportion of the intake is channelled into the site of storage, i.e. adipose tissue. For ∝-linolenic acid the opposite may be suggested. It was found that α-linolenic acid is more efficiently incorporated into intramuscular fat than in adipose tissue.

In conclusion, with the given regression formulas, we can predict the fatty acid composition of the adipose tissue as based on the fatty acid composition of the swine diet. The use of the formulas thus allows to steer the quality of swine meat as related to the fatty acid composition. In broilers, there is a direct relationship between the fatty acid composition of adipose tissue and that of consumable meat, and also between fatty acid intake and the slippoint of adipose tissue (Bavelaar & Beynen, 2002). It is also possible to use the fatty acid profile of adipose tissue of swine as a biomarker of fatty acid intake, which could be helpful in studies on range-fed swine kept by small holders in developing countries (Nguyen, Everts & Beynen, 2002).

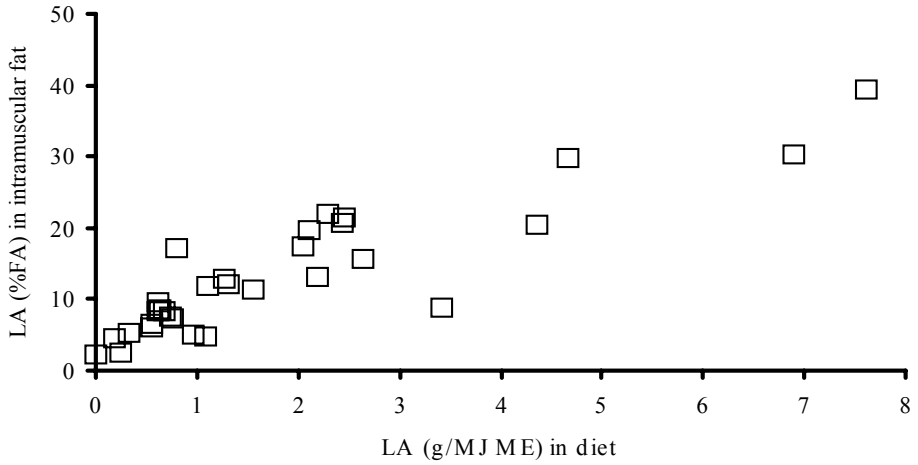


Fig. 6. Relationship between dietary linoleic acid and intramuscular fat linoleic acid in growing pigs. The data points are derived from the literature.

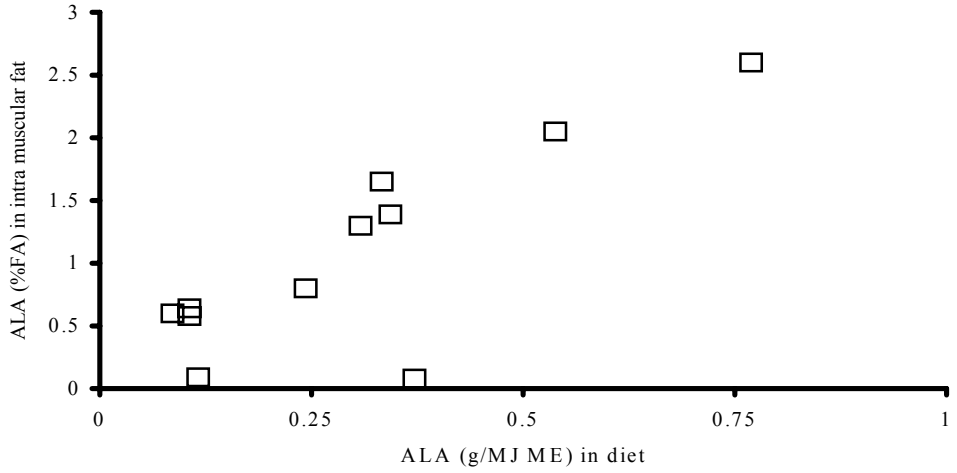


Fig 7. Relationship between dietary α-linolenic acid and intramuscular fat α-linolenic acid in growing pigs. The data points are derived from the literature.

Acknowledgements
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References

Bavelaar, F.J., & Beynen, A.C. (2002). Relationships between dietary fatty acid composition and either melting point or fatty acid profile of adipose tissue in broilers. *Meat Science* (in press).

Brooks, C.C. (1971). Fatty acid composition of pork lipids as affected by basal diet, fat source and fat level. *Journal of Animal Science* 33: 1224-1231.

Cava, R., Ventanas, J., Tejeda, J.F., Antequera, J.R.T. (2000). Effect of free-range rearing and α-tocopherol and copper supplementation on fatty acid profiles and susceptibility to lipid oxidation of fresh meat from Iberian pigs. *Food Chemistry*, 68, 51-59.

Corino, C., Magni, S., Pagliarini, E., Rossi, R., Pastorelli, G., & Chiesa, L.M. (2002). Effects of dietary fats on meat quality and sensory characteristics of heavy pig loins. *Meat Science, 60*, 1-8.

Davenel, A., Riaublanc, A., Marchal, P., & Gandemer, G., (1999). Quality of pig adipose tissue: relationship between solid fat content and lipid composition. *Meat Science, 51,* 73-79.

Enser, M., Richardson, R.I., Wood, J.D., Gill, B.P., & Sheard, P.R. (2000). Feeding linseed to increase the n-3 PUFA of pork: fatty acid composition of muscle, adipose tissue, liver and sausages. *Meat Science*, 55, 201-212.

Feunekes, G.I., Van Staveren, W.A. De Vries, J.H., Burema, J., & Hautvast, J.G.A.J. (1993). Relative and biomarker-based validity of a food-frequency questionnaire-estimating intake of fats and cholesterol. *American Journal of Clinical Nutrition,* 58, 489-496.

Flachowsky, G., Schone, F., Schaarmann, G., Lubbe, F., & Bohme, H. (1997). Influence of oilseeds in combination with vitamin E supplementation in the diet on backfat quality of pigs. *Animal Feed Science Technology,* 64, 91-100.

Folch, J., Lees, M., & Sloane Stanley, G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry,* 226, 497-509.

Fontanillas, R., Barroeta, A., Baucells, M.D., & Codony, R. (1997). Effect of feeding highly cis-monounsaturated, trans, or n-3 fats on lipid composition of muscle and adipose tissue of pigs. *Journal of Agricultural Food Chemistry,* 45, 3070-3075.

Gray, G.I., Gomaa, E.A., & Buckley, D.J., (1996). Oxidative quality and shelf life of meats. *Meat Science, 43,* S111.

Irie, M., & Sakimoto, M.. (1992). Fat characteristics of pigs fed fish oil containing eicosapentaenoic and docosahexaenoic acids. *Journal of Animal Science,* 70, 470-477.

Kouba, M., & Mourot, J. (1999). Effect of high linoleic acid diet on lipogenic enzyme activities and on the composition of the lipid fraction of fat and lean tissues in the pig. *Meat Science*, 52, 39-45.

Larick, D.K., Turner, B.E., Schoenherr, W.D., Coffey, M.T., & Pilkington, D.H. (1992). Volatile compound content and fatty acid composition of pork as influenced by linoleic acid content of the diet. *Journal of Animal Science*, *70*, 1397-1403.

Leskanich, C.O., Matthews, K.R., Warkup, C.C, Noble, R.C., & Hazzledine, M. (1997). The effect of dietary oil containing (n-3) fatty acids on the fatty acid, physicochemical and organoleptic characteristics of pig meat and fat. *Journal of Animal Science,* 75, 673-683

Lin, D.S., Connor, W.E., & Spenler, C.W. (1993). Are dietary saturated, monounsaturated and polyunsaturated fatty acids deposited to the same extent in adipose tissue of rabbits. *American Journal of Clinical Nutrition, 58*, 174-179

Metcalfe, L.D., Schmitz A.A., & Pekka J.R. (1966). Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Analytical Chemistry, 18,* 514-516.

Miller, M.F., Sjackelford, S.D., Hayden, K.D., & Reagan, J.O. (1990). Determination of the alternation in fatty acid profiles, sensory characteristics and carcass traits of swine fed elevated levels of monounsaturated fats in the diet. *Journal of Animal Science, 1990,* 1624-1631.

Mills, S.C., Searle, T.W., & Evans, R. (1979). Long-term effects of feeding protected sunflower seed supplements on the composition of body fat in growing sheep. *Australian Journal of Biological Science, 32,* 457-462

Morgan, C.A., Noble, R.C., Cocchi, M., & McCartney, R. (1992). Manipulation of the fatty acid composition of pig meat lipids by dietary means. *Journal of the Science of Food and Agriculture* 58, 357-368.

Newton, I.S. (1998). Land based animal food products and their health effects. *World Review of Nutrition and Dietetics, 83,*199-209

Nguyen, L.Q., Everts, H., & Beynen, A.C. (2002). Intake of essential fatty acids by growing-finishing pigs kept on small holdings in Central Vietnam. *Tropical Animal Health and Reproduction* (in press).

Van Niel, M.H.F., & Beynen, A.C. (1997). The intake of polyunsaturated fatty acids by cats is reflected in their adipose tissue. *The Veterinary Quarterly, 19,* 150-153.

Van Oeckel, M.J.V., Casteels, M., Warnants, N., Van Damme, L., & Boucque, Ch. V. (1996). Omega-3 fatty acids in pig nutrition: Implications for intrinsic and sensory quality of the meat. *Meat Science, 44,* 55-63.

Otten, W., Wirth, C., Iaizzo, P.A., & Eichinger, H.M. (1993). A high omega-3 fatty acid diet alters fatty acid composition of heart, liver, kidney, adipose tissue and skeletal muscle of swine. *Annals of Nutrition and Metabolism, 37,* 134-141.

Romans, J.R., Wulf, D.M., Johnson, R.C., Libal, G.W. and Costello, W.J. (1995). Effects of ground flaxseed in swine diets on pig performance and on physical and sensory characteristics and omega-3 fatty acid content of pork. II. Duration of 15 % dietary flaxseed. *Journal of Animal Science* 73: 1987-1999.

Riley, P.A., Enser, M., Nute, G.R., & Wood, J.D. (2000). Effects of dietary linseed on nutritional value and other quality aspects of pig muscle and adipose tissue. *Journal of Animal Science, 71*, 483-500.

Sheppard, K., & Herzberg, G.R. (1992). Triacylglycerol composition of adipose tissue, muscle and liver of rats fed diets containing fish oil or corn oil. *Nutrition Research* 12, 1405-1418.

Simopoulos, A.P. (2001). N-3 Fatty acids and human health: defining strategies for public policy. *Lipids, 36, S83-S89*.

Smith, D.R., Knabe, D.A., & Smith, S.B. (1996). Depression of lipogenesis in swine adipose tissue by specific dietary fatty acids. *Journal of Animal Science, 74,* 975-983.

Taugbol, O. (1993). Omega-3 fatty acid incorporation in fat and muscle tissue of growing pigs, fed supplements of fish oil. *Journal of Veterinary Medicine A, 40,* 93-101.

Warnants, N., Van Oeckel, M.J., & Boucqué, Ch.V. (1996). Incorporation of dietary polyunsaturated fatty acids in pork tissue and its implications for quality of the end products. *Meat Science,* 44, 125-144.

Warnants, N., Van Oeckel, M.J., & Boucqué, Ch.V. (1998). Effect of incorportation of dietary polyunsaturated fatty acids in pork backfat on the quality of Salami. *Meat Science, 49*, 435-445.

Warnants, N., Van Oeckel, M.J., & Boucque, Ch.V. (1999). Incorportation of dietary polyunsaturated fatty acids into pork fatty tissues. *Journal of Animal Science, 77,* 2478-2490.

Wiseman, J., & Agunbiade, J.A. (1998). The influence of changes in dietary fat and oils on fatty acid profiles of carcass fat in finishing pigs. *Livestock Production Science* 54, 217-227.

Wiseman, J., Redshaw, M.S., Jagger, S., Nute, G.R., & Wood, J.D. (2000). Influence of type and dietary rate of inclusion of oil on meat quality of finishing pigs. *Journal of Animal Science ,70,* 307-315.

Chapter 3

Intake of essential fatty acids by growing-finishing pigs kept on small holdings in Central Vietnam

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Abstract

The intake of linoleic acid (LA) and α-linolenic acid (ALA) in relation to average daily gain (ADG) was studied in growing-finishing pigs kept on small-holdings in Central Vietnam. Groups of 3 piglets each were randomly assigned to 12 farms where they were fed on local feedstuffs according to the farmer's choice, but were given a restricted amount of dry matter according to a preset feeding regimen. On arrival on the farms, the pigs weighed 10.4 ± 0.9 kg (mean ± SD, n=36) and at 130 days of age they weighed 45.4 ± 9.6 kg. Dietary LA concentration ranged from 1.34 – 2.41 g/ MJ metabolizable energy (ME) and ALA from 0.06-0.33 g/ MJ ME. On a farm level, dietary LA and ALA concentrations were significantly correlated with their concentrations in adipose tissue, both correlation coefficients being 0.63. Dietary protein concentration and protein:energy ratio were significantly correlated with ADG; the correlation coefficients were 0.68 and 0.64. For individual piglets there were significant correlations between either LA or ALA in adipose tissue and ADG, the correlation coefficients being 0.37 and 0.45. Dietary protein concentration or protein:energy ratio was correlated with the dietary contents of LA and ALA. It is uncertain whether LA and ALA intake had a causal relationship with ADG. Because dietary LA levels were above the LA requirement, LA intake may not have limited growth. It is suggested tentatively that, through enhanced disease resistance, supplementation with ALA of the diets on the farms studied might have a positive influence on ADG.

Introduction

Linoleic acid (LA) and α-linolenic acid (ALA) are essential polyunsaturated fatty acids (PUFA) because they cannot be synthesized in the body. LA and ALA are the parent compounds of the families of n-6 and n-3 fatty acids, respectively. They can be desaturated and elongated in the animal body to yield the n-6 fatty acid, arachidonic acid (AA) and the n-3 fatty acid, eicosapentaenoic acid (EPA), which are direct precursors for the synthesis of prostaglandines and leukotrienes. The LA requirement of growing pigs weighing up to 30 kg or weighing from 30 to 90 kg has been set at 0.08 g/ MJ of metabolizable energy (ME), (National Research Council, 1998). There are no formal recommendations as to the requirement of ALA.

Overt LA deficiency in swine not only causes reduced growth, but also clinical symptoms such as degenerative changes in somniferous tubules and impaired sperm development (Leskanich & Noble, 1999). Suboptimal intake of LA may lead to impaired growth in pigs (Skelley et al., 1975; Myer et al., 1985; McDonald et al., 1995; Romans et al., 1995; Smith et al., 1996; Soler-Velasquez et al., 1998). High intakes of EPA and docosahexaenoic acid (DHA) may improve disease resistance in pigs (Calder, 1996; Jolly et al., 1997; Irie and Sakimoto, 1992), which in turn may beneficially affect growth performance. It could be suggested that in those practical situations where swine rations are composed without consciously taking into account the supply of PUFA, growth performance could be limited by insufficient PUFA intake. We have tested our suggestion in small holdings in Central Vietnam. On 12 farms, using different ingredient compositions of the swine rations, we have assessed fatty acid intake, determined growth performance and analysed the fatty acid composition of swine subcutaneous adipose tissue. The objective was to look for a relationship, if any, between LA and ALA intake and growth. In a field setting, LA and ALA intake cannot be accurately determined (Beynen et al., 1986). The LA and ALA contents of adipose tissue can be considered a valid index of the long-term intake of these fatty acids (Leskanich et al., 1997; Romans et al., 1995; Soler-Velasquez et al., 1998; Fritsche et al., 1993; Smith et al., 1996; Myer et al., 1992 & 1997; Fontanillas et al., 1998). Thus, we determined LA and ALA in adipose tissue collected at slaughter and correlated their concentrations with growth performance. The information obtained from this study could contribute to optimize the diet of growing-finishing pigs.

Material and methods

Pigs and diets

For the field experiment, 36 castrated, male weanling piglets ((male) Large White x (female) Mong cai) were purchased from a breeding pig farm. The piglets were 70 days old and weighed 10.4 ± 0.9 kg (mean ± SD). Groups of 3 piglets each were randomly assigned to 12 small-holdings. Each piglet had an ear-cut number for identification. Pigs were fed on different local feedstuffs, which were mixed on the farms according to the farmer's choice, but they were fed a restricted amount of dry matter according to a pre-set feeding regimen (Table 1).

Table 1. Feeding schedule that was applied on each farm

	Bodyweight (kg)									
	10	*20*	*30*	*40*	*50*	*60*	*70*	*80*	*90*	*100*
Feed intake (kg DM/day)	*0.67*	*1.00*	*1.25*	*1.50*	*1.75*	*2.0*	*2.25*	*2.42*	*2.58*	*2.75*

The farmers were individually instructed by B.Sc students of animal science who were present on the farms. The students recorded what pigs were fed by the farmers and watched over the feeding schedule. On each farm, the three pigs were housed together in one pen. The pigs were fed 2 times/day. Water was available ad libitum through nipples that were situated besides the trough in each pen. During the experiment, between 70 and 76 days after the start, the pigs could not be fed properly because there was a dramatic flood in Central Vietnam (November 1999). The pigs were weighed on arrival at the farm, at 130 days of age and at slaughter.

Sample collection

The students recorded the ingredient composition of the ration, weighed the ingredients for each meal on each farm, and took samples of the feedstuffs, which were stored at –20^{0}C. Any feed left-overs were collected and weighed. At slaughter of the pigs, subcutaneous adipose tissue samples were collected from the belly. The samples were stored at - 20 ^{0}C until analysis.

Fatty acid analysis

Total lipids in feedstuffs were extracted with chloroform:methanol 2:1 (Folch et al. 1957). The lipids were transesterified with 12% BF_3 in methanol at 80^{0}C. The methyl esters were then extracted with water and petroleum ether, taken to dryness under nitrogen, redissolved in heptane and separated and quantified by gas liquid chromatography (GC). The fat in adipose tissue was saponified, methylesters of fatty acids were formed and subjected to GC.

Statistical analysis

Linear regression analysis was done for farm mean data and for individual data. The level of statistical significance was pre-set at $P < 0.05$.

Results

Diet composition

Dry matter (DM) was measured in one representative sample of each type of feedstuff. Metabolizable energy (ME), crude protein, crude fat, crude fibre, ash, calcium (Ca) and phosphorus (P) contents of each feedstuff were taken from tables composed by Sumilin and Nguyen (1992). The commercial feed was based on the guaranteed analysis panel provided by the manufacturer.

The ingredient composition of the diets is shown in Table 2. On all farms, rice bran

and rice formed at least 60% of the rations. The amounts and type of the other ingredients were variable. Means and ranges of energy values and nutrient concentrations of the diets are given in Table 3. The diets on farms 1 and 4 had the lowest and highest energy values, respectively. For crude protein, the dietary concentration on farm 5 was lowest and on farm 3 it was highest. The diet on farm 3 had the highest contents of LA and ALA, whereas the LA content was lowest on farm 12 and that of ALA was lowest on farm 10. The amounts of dietary fatty acids are expressed on an energy basis because fat and energy contents of the diets were different. Fatty acid intake for the different diets was related to fat content and DM intake, but for a comparison of farms it is appropriate to express fatty acid intake on an energy basis.

Growth performance

Pigs had reached a final weight of 89.2 ± 13.3 kg when they were slaughtered at 180 to 190 days of age. The average daily gain (ADG) and feed conversion ratio were measured from 70 to 130 days of age and varied between 0.29 and 0.82 kg and 2.23 and 4.61 kg of feed/kg of weight gain, respectively. Body weight at the age of 130 days was 45.4 ± 9.6 kg.

Table 3. Nutrient composition of the pig rations, expressed as average for the entire feeding period

	Mean for the 12 farms	Range
ME, MJ/kg DM	13.9	13.5 - 14.4
Crude protein, g/kg DM	173.4	144.6 - 190.3
Crude fat, g/kg DM	79.9	70.3 - 94.0
Crude fibre, g/kg DM	60.0	42.9 - 69.1
Ash, g/kg DM	115.7	100.4 - 145.6
Ca, g/kg DM	6.98	3.48 - 12.26
P, g/kg DM	11.89	10.37 - 13.69
LA, g/kg DM	1.56	1.28 - 2.29
ALA, g/kg DM	0.11	0.06 - 0.33

Fatty acid composition of adipose tissue

There was considerable variation between the farm averages for the three pigs with respect to fatty acid composition of adipose tissue. LA varied between 6.87 (farm 10) to 14.25 % (farm 3) of total fatty acids (TFA). ALA varied between 0.44 (farm 8) and 0.86 % (farm 3). EPA was not detectable and DHA varied between 0.1 (farm 8) and 0.5 % (farm 12) of adipose tissue TFA.

Correlations between fatty acids in diet, adipose tissue and growth

For farm means of ADG and both dietary and adipose tissue fatty acids (LA, ALA, DHA, and ALA + EPA + DHA) linear correlation coefficients were calculated. The correlation coefficients were equal to or lower than 0.56 so that none of the correlations reached statistical significance. However, there were significant linear correlations between the levels of LA and ALA in the diet and those in adipose tissue (Fig. 1).

Table 2. Feedstuff composition of the pig rations, expressed as an average for the entire feeding period

	Feedstuffs (% of total dietary DM)									
Farm no.	Rice bran	Rice	Fresh fish	Fish meal	Groundnut cake	Soybean byproduct	Commercial feed	Alcohol byproduct	Beer byproduct	Vegetables
1	40.7	30.7		8.8		13.3	2.5		3.9	0.1
2	33.6	31.9		6.8		13.7	16.5		7.4	0.1
3	34.5	37.1	2.2			26.1				0.1
4	42.5	37.2	3.4	11.5				3.4		2.0
5	36.6	39.5	2.4			2.0	10.5	8.6		0.4
6	39.3	39.1	0.1	11.0		1.9	8.5			0.1
7	30.8	43.5	4.4		8.4		7.7		5.1	0.1
8	33.7	45.4	4.4		10.5	1.2	4.9			0.1
9	39.7	45.2	6.2	2.1			6.7		0.1	0.1
10	33.8	46.7	3.1	9.5			5.7	1.1		0.1
11	37.6	40.3	4.9				5.7		3.4	0.1
12	28.5	40.6	15.3		8.0		7.6		7.9	0.1

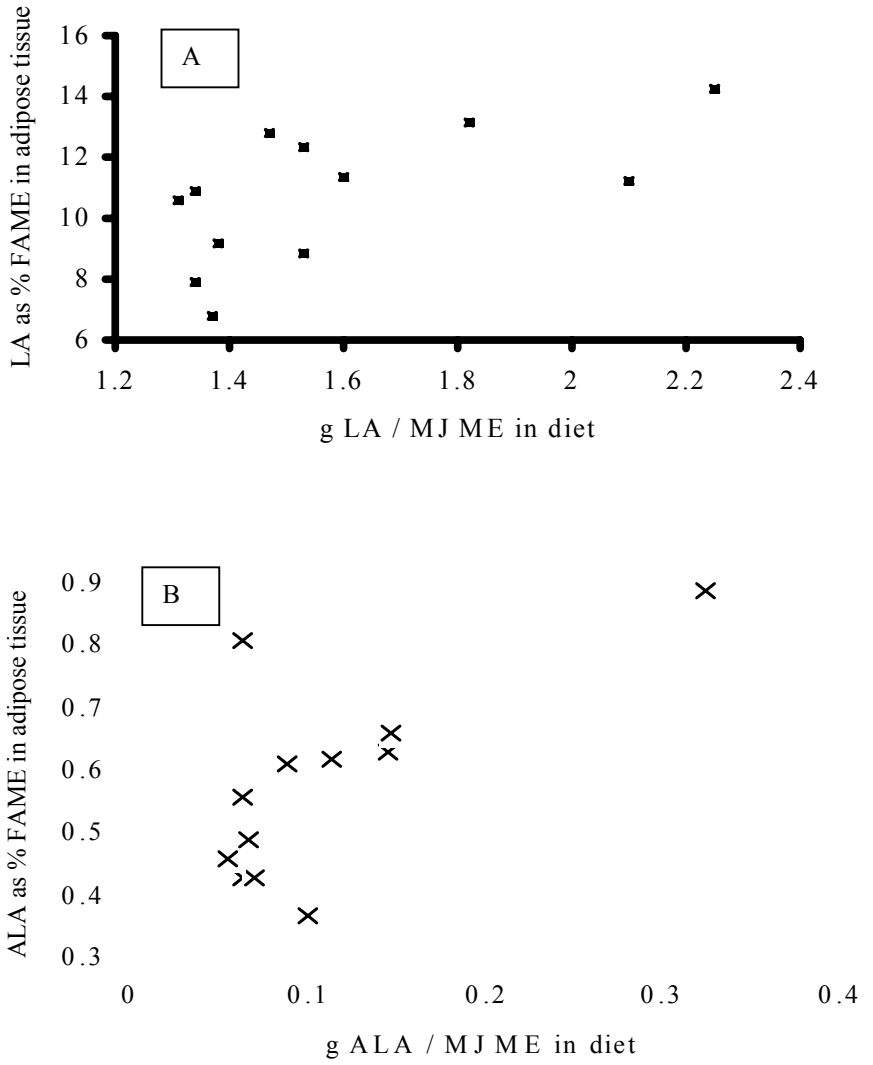

Figure 1. Relationship between LA in diet and that in adipose tissue (panel A) and the relationship for ALA (panel B). The data points refer to farm means. For LA the correlation coefficient is 0.63 (n =12, p = 0.028) and for ALA it is 0.66 (p =0.027).

Likewise, the sum of n-3 fatty acids (ALA + EPA + DHA) in diet and adipose tissue were significantly correlated and the correlation coefficient was 0.77. For individual pigs (n=36) there were significant correlations between ADG during 70 and 130 days of age and either the content of LA or ALA in adipose tissue at 187 days of age (Fig. 2).

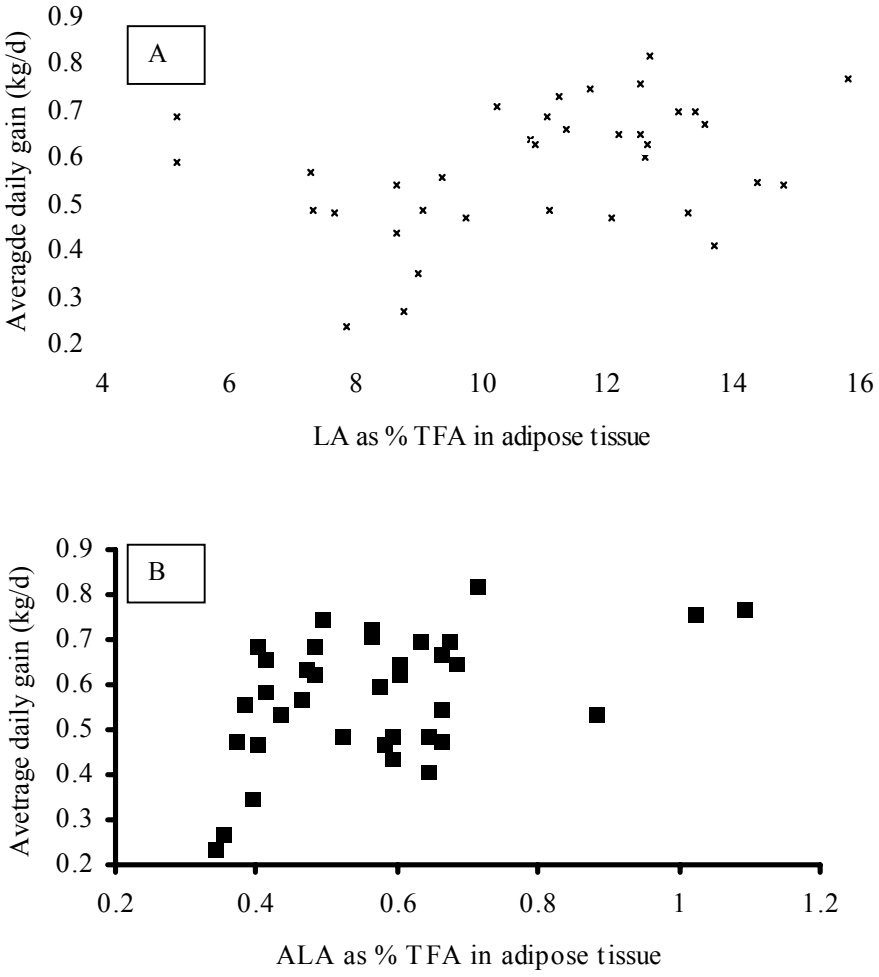

Figure 2. Relationship between either LA (panel A) or ALA (panel B) in adipose tissue and ADG for the period of 70 to 130 days of age. The data points correspond with individual pigs. For LA the correlation coefficient is 0.37 (n = 36, p = 0.027) and for ALA it is 0.45 (n = 36, p = 0.006).

However, the correlation coefficients were only 0.37 and 0.45, indicating that the variation in LA and ALA contents in adipose tissue accounted for only about 14 % of the variation in ADG. There was no correlation between DHA in adipose tissue and ADG (r= 0.075, p = 0.666, n=36).

Discussion

Table 3 shows that dietary crude protein concentration varied between 144.6 and 190.3 g/kg DM. The protein requirement of growing-finishing pigs weighing between 10 and 50 kg has been set at 18 % and for pigs weighing between 50 and 80 kg at 15.5% for feed containing 90% dry matter (National Research Council, 1998). Thus, the protein content of the diets fed on the various farms was not in all cases high enough for growing-finishing pigs. Indeed, there were significant, positive correlations between either dietary crude protein concentration or the protein:energy ratio and ADG (Fig.3). Protein concentration and the protein:energy ratio were correlated with the dietary levels of LA or ALA, but the correlations failed to reach statistical significance. The correlation coefficients for the relation between dietary protein concentration and the amounts of dietary LA and ALA were 0.53 (P = 0.077, n = 12) and 0.41 (P = 0.189), respectively. The protein:energy ratio and dietary LA and ALA contents had correlation coefficients of 0.55 (P = 0.065, n=12) and 0.38 (P = 0.219), respectively. Thus, it is likely that the observed relationships between adipose tissue LA or ALA contents and ADG were surrogates for the relationship between protein intake and ADG. The Ca and P concentrations in the rations were either close to or well above the requirements so that the variation in mineral intake would not have influenced ADG.

There were significant correlations for the amounts of LA and ALA in the diets and those in adipose tissue. In controlled studies with swine it has also been demonstrated that LA and ALA intake are associated with their levels in adipose tissue (Warnants et al., 1998). Dietary intakes were reflected by the back-fat fatty acid composition with correlation coefficients higher than 0.90. It appears that the correlations found in this study are relatively weak. The intake of the two fatty acids was based on chemical analysis of one representative sample of each type of feedstuff. Clearly, within one type of feedstuff there will have been variation in fatty acid composition between farms. As a result, the fatty acid intake for individual farms was not accurately assessed, leading to a low correlation of dietary and adipose tissue fatty acids. Thus, the true correlations must be higher than those observed. The inaccuracy of the dietary LA and ALA levels also explains why there were no significant relationships between fatty acid intake and ADG. However, there were significant, positive relations between either LA or ALA in adipose tissue and ADG. Statistical significance was reached due to the high number of degrees of freedom, but a cause-and-effect relationship might underlie the correlation. It is reasonable to assume that the LA and ALA contents of adipose tissue more accurately reflect the intake of these fatty acids than did the estimated dietary concentration. It would thus appear that among the 12 farms studied the intakes of LA and ALA were positively related with growth performance. The dietary LA levels on the 12 farms varied between 1.34 and 2.41 g/ MJ ME, while the requirement has been set at 0.08 g/ MJ ME (National Research Council, 1998). It can be concluded that LA intake did not impair growth performance. The observed positive relationship between ALA in adipose tissue and ADG might indicate that ALA intake was

too low to sustain maximum growth. The data from this study would support the notion that supplementation of the farm rations with ALA could be beneficial. However, it cannot be excluded that the observed correlation between adipose tissue ALA and ADG is a surrogate relationship for that between protein intake and ADG. Supplementation of the farm rations with either ALA or EPA may prove or disprove a causal relationship between the intake of these fatty acids and ADG. In practice, the rations may be enriched with soybean byproducts as a source of ALA. Fortification of the diets with fish byproducts would raise the contents of EPA, the desaturation and elongation product of ALA.

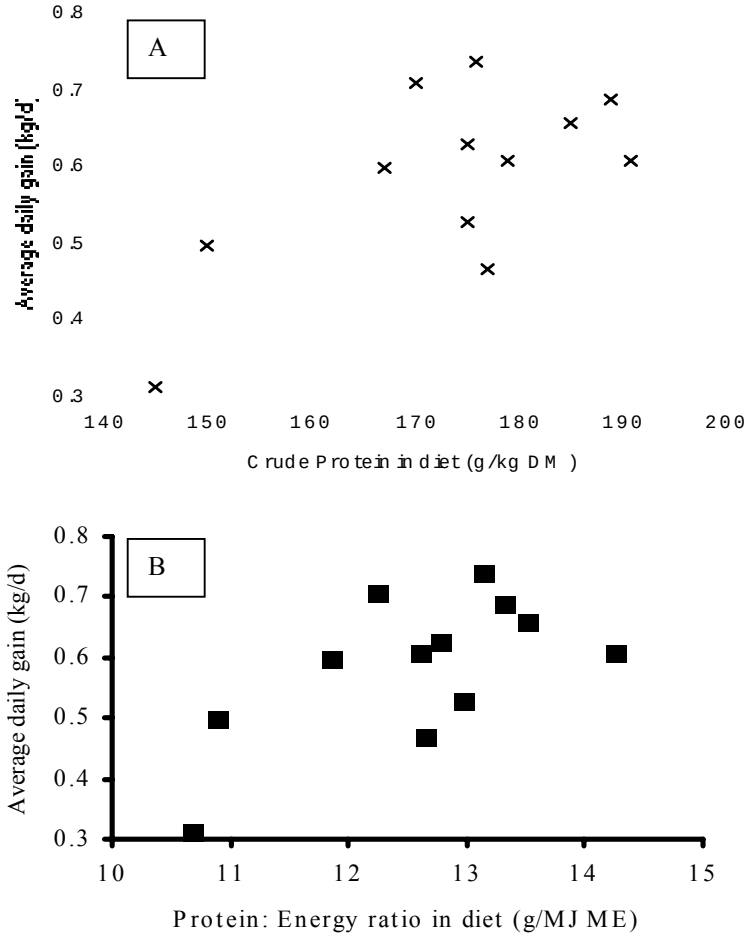

Figure 3. Relationship between crude protein concentration in diet and ADG (panel A) and the relationship for protein: energy (ME) ratio (panel B). The data points refer to farm

means. For protein the correlation coefficient is 0.68 (n=12, P = 0.015) and for protein:

energy ratio it is 0.64 (P = 0.026)

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References

Beynen, A.C., Katan, M.B. and Van Staveren W.A., 1986. The linoleic acid content of subcutaneous adipose tissue as a valid index of the intake of linoleic acid by individuals. *Fette Seifen Anstrichmittel,* **88**, 579-581.

Calder, P.C., 1996. Immunomodulatory and anti-inflammatory effects of n-3 polyunsaturated fatty acids. *Proceedings of the Nutrition Society,* 55:737-774.

Folch, J., Lees, M. and Sloan-Stanley, G.N., 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, **226,** 497-509.

Fontanillas, R., Barroeta, A., Baucells, M.D. and Guardiola, F., 1998. Backfat fatty acid evolution in swine fed diets high in cis-monounsaturated, trans, or (n-3) fats. *Journal of Animal Science*, **76,** 1045-1055.

Fritsche, K.L., Huang, S.C. and Cassity, N.A., 1993. Enrichment of omega-3 fatty acids in suckling pigs by maternal dietary fish oil supplementation. *Journal of Animal Science,* **71**, 1841-1847.

Irie, M. and Sakimoto, M., 1992. Fat characteristics of pigs fed fish oil containing eicosapentaenoic and docosahexaenoic acids. *Journal of Animal Science,* **70,** 470-477.

Jolly, C.A., Jiang, Y-H., Chaptin, R.S. and McMurray, D.N., 1997. Dietary (n-3)polyunsaturated fatty acids suppress murine lymphoproliferation, interleukin-2 secretion, and the formation of diacylglycerol and ceramide. *Journal of Nutrition,* **127,** 37-43.

Leskanich, C.O., Matthews, K.R., Warkup, C.C., Noble, R.C. and Hazzledine, M., 1997. The effect of dietary oil containing (n-3) fatty acids on the fatty acid, physicochemical, and organoleptic characteristics of pig meat and fat. *Journal of Animal Science,* **75,** 673-683.

Leskanich, C.O., & R.C. Noble, 1999. The comparative roles of polyunsaturated fatty acids in pig neonatal development. *British Journal of Nutrition*, **81**, 87-106.

McDonald, P., Edwards, R.A., Greenhahgh, J.F.D. and Morgan, C.A., 1995. *Fifth edition. Animal Nutrition, Longman Scientific & Technical, UK.*

Myer, J.W., Brendemuhl, J.H., Combs, G.E., Lamkey, R.O. and Walker, W.R., 1992. Performance and carcass characteristics of swine when fed diets containing canola oil and added copper to alter the unsaturated:saturated ratio of pork fat. *Journal of Animal Science,* **70**, 1417-1423.

Myer, R.O., West, R.L., Gorbet, D.W. and Brasher, C.L., 1985. Performance and carcass characteriscs of swine as affected by the consumption of peanuts remaining in the

field after harvest. *Journal of Animal Science,* **61**, 1378-1386.

National Research Council, 1998. The nutrient requirements of Swine. Nutrient Requirement Table for growing pigs. *National Research Council (US). Subcommittee on Swine Nutrition. National Academy Press. 2101 Constitution Avenue, NW. Washington, D.C. 20418, US.*

Romans, J.R., Wulf, D.M., Johnson, R.C., Libal, G.W. and Costello, W.J., 1995. Effects of ground flaxseed in swine diets on pig performance and on physical and sensory characteristics and omega-3 fatty acid content of pork. *Journal of Animal Science,* **73**, 1987-1999.

Skelley, G.C., Borgman, R.F., Handlin, D.L., Acton, J.C., McConnell, J.C., Wardlaw F.B., and Evans, E.J., 1975. Influence of diet on quality, fatty acids, and acceptability of pork. *Journal of Animal Science,* **41**, 1298 – 1304.

Smith, D.R., Knabe, D.A. and Smith, S.B., 1996. Depression of lipogenesis in swine adipose tissue by specific dietary fatty acids. *Journal of Animal Science,* **74**, 975-983.

Soler-Velasquez, M.P., Brendemuhl, J.H., McDowell, L.R., Sheppard, K.A. and Williams, J.N., 1998. Effects of supplemental vitamin E and canola oil on tissue tocopherol and liver fatty acid profile of finishing swine. *Journal of Animal Science,* **76**, 110-117.

Sumilin, I.S. and Nguyen, T.V., 1992. Feed ingredient composition in Vietnam, *Published by Agricultural Publisher. Hanoi, Vietnam.*

Warnants, N., Van Oeckel M.J. and Boucque, Ch.V., 1998. Effect of incorporation of dietary polyunsaturated fatty acids in pork backfat on the quality of salami. *Meat Science*, **49**, 435-445.

Chapter 4

Growth performance of growing pigs kept on small-holder farms in Central Vietnam and fed diets containing either ruminant or fish meal

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Abstract

We have suggested that the addition of eicosapentaenoic and docosahexaenoic acid to the diet of growing pigs, kept in small holdings in Central Vietnam, would improve growth performance. Thus, the effect was studied of dietary fish meal, as source of eicosapentaenoic and docosahexaenoic acid, on growth performance and fatty acid composition of adipose tissue in growing pigs. Fish meal was exchanged with ruminant meal so that the diets contained either 0, 10 or 20% fish meal in the dry matter. The diets were fed on 6 different small-holder farms in Central Vietnam. The farmers fed a base diet according to their personal choice, but were instructed as to the use of fish meal and ruminant meal. The diets were fed to the pigs from 70 to 126 days of age. There were three animals per treatment group per farm and biopsies of adipose tissue were analysed for their contents of linoleic, α-linolenic, eicosapentaenoic and docosahexaenoic acid. The diets without and with 20 % fish meal on average contained 0.01 and 0.03 g eicosapentaenoic acid/MJ of metabolizable energy (ME) and 0.00 and 0.25 g docosahexaenoic acid/MJ ME. The relative percentages of docosahexaenoic, α-linolenic and linoleic acid in adipose tissue were reflected by the intake of the corresponding fatty acids. Eicosapentaenoic acid was not detectable in adipose tissue. There was no impact of fish meal intake on growth performance of the growing pigs. The intake of eicosapentaenoic or docosahexaenoic acid was not related with average daily gain, and neither was the adipose tissue content of docosahexaenoic acid. Adipose tissue α-linolenic acid or linoleic acid and average daily weight gain were significantly, and directly related, the explained variance in weight gain being 44 and 69 %, respectively. The basis and implication of these relationships require further study.

Introduction

In a field study involving small holdings in Central Vietnam, we found that the content of α-linolenic acid (C18:3 n-3) in adipose tissue of growing swine was directly, and significantly, related with average daily weight gain, the explained variance of weight gain being 20 % (NGUYEN et al. 2002a). In swine, the concentration in adipose tissue of α-linolenic acid is determined by the intake of this fatty acid (NGUYEN et al. 2002b). The n-3 polyunsaturated fatty acid, α-linolenic acid, can be converted in the body into eicosapentaenoic acid (C20:5 n-3) which is the direct precursor of various eicosanoids, some of them affecting the immune system (WU and MEYDANI 1998). High intakes of eicosapentaenoic acid and docosahexaenoic acid (C22:6 n-3) in the form of fish oil may improve disease resistance in pigs (FRITSCHE et al. 1993). We have suggested that in growing pigs, kept on the small-holder farms in Central Vietnam, high intakes of α-linolenic acid were associated with high production rates of eicosapentaenoic and docosahexaenoic acid and thus enhanced disease resistance which in turn improved growth performance (NGUYEN et al. 2002a).

We wished to test our idea that the addition of eicosapentaenoic and docosahexaenoic acid to the diet of growing pigs kept in small holdings in Central Vietnam would improve growth performance. Under practical conditions, fish meal can serve as a source of eicosapentaenoic and docosahexaenoic acid, the contents being about 0.2 and 1.5 % in the product, and 3 and 20 % in the oil component, respectively. Our idea was tested by comparing the effect of exchanging fish meal and ruminant meal in the diet. The farmers participating in the study composed the base diets according to their personal choice, but were instructed as to the use of fish and ruminant meal as dietary components. The fish and ruminant meal were supplied to the farms and, under the supervision of students, the two meals were mixed with the base diets at pre-set ratios. In the pigs, growth performance and the fatty acid profile of adipose tissue were measured.

Materials and methods

Animals and experimental diets

Castrated, male weanling pigs (n=54) were purchased from a breeding farm and allocated to 6 small holdings. The piglets were of a Mong cai (female) x Large White (male) cross, were aged 70 days and had a body weight of 12.6 ± 1.12 kg (mean ± SD). Each farm received 9 animals, which were housed in pens containing three piglets each. Within each farm, the three piglets in each pen had similar average body weights and distributions. Each piglet had an ear-cut number for identification. The pigs were fed a restricted amount of dry matter according to Table 1. The farmers were individually instructed by B.Sc. students of animal science who were present on the farms. The students used a local feed table to assess the dry matter contents of the ingredients. The fish and ruminant meal were supplied to the farms by the students. The fish meal was derived from achovy and sardine. The ruminant meal consisted of lung/liver/blood in a 40/30/30 ratio on a wet-weight basis, and was sun dried prior to feeding. Table 2 shows the analysed composition of the two meals.

Table 1. Feeding schedule that was applied on each farm

	Pig body weight (kg)				
	10	20	30	40	50
	kg dry matter/day				
Base diet	0.54	0.80	1.00	1.20	1.40
Fish meal	0.13/0.07/0	0.2/0.1/0	0.25/0.13/0	0.30/0.15/0	0.35/0.18/0
Ruminant meal	0/0.07/0.13	0/0.1/0.2	0/0.13/0.25	0/0.15/0.3	0/0.18/0.35

On each farm three diets were fed, containing either 0, 10 or 20 % fish meal in the total dietary dry matter. The compositions of the base diets used on each farm are shown in Table 3.

Table 2. Analysed composition of the ruminant and fish meal

Nutrient	Ruminant meal	Fish meal
Macronutrients, g/kg		
Dry matter	901	909
Crude protein	744	596
Crude fat	97	71
Ash	43	245
Fatty acids, g methylester/100 g methylesters		
C16:0	23.7	24.8
C18:0	27.0	10.7
C18:1 n-9	20.6	8.7
C18:2 n-6	4.4	1.5
C18:3 n-3	0.9	0.7
C20:4 n-6	3.5	2.1
C20:5 n-3	0.9	2.9
C22:6 n-3	0.2	21.3

The base diets were composed by the farmers according to their own choice, but the ingredient composition was rounded off to the nearest 5 % and then kept constant. In addition, the farmers were not allowed to use fish products other than the fish meal supplied. The ruminant and fish meal concentrations in the final diets were either 0, 10 or 20% of the dietary dry matter (Table 1). On each farm, three diets were used, the ruminant/fish meal combinations being 20/0, 10/10 and 0/20. It was verified that the rations were sufficient in protein and contained at least 20 % crude protein in the dry matter as calculated on the basis of the local feed table. In our earlier study (NGUYEN et al., 2002a), we found that on various small-holder farms the supply of protein was low and limited growth of the pigs. The students recorded feed intake and ingredient composition of the rations. Table 3 shows the ingredient compositions of the diets fed on the 6 farms.

Table 3. Composition of the diets fed on each farm

Farm: Ingredient	1	2	3	4	5	6
			g/100 g dry matter			
Rice bran	30	35	30	30	25	35
Rice	20	20	20	20	20	20
Vegetables	15	15	15	15	15	15
Beer by-product	10	10	5	-	10	-
Alcohol by-product	-	-	5	10	5	10
Commercial feed	5	-	5	5	5	-
Ruminant/fish meal*	20	20	20	20	20	20

**The three ruminant/fish meal combinations on each farm were 20/0, 10/10 and 0/20.*

The feedstuffs supplied on each farm were weighed on identical balances. Any feed left overs were collected. The pigs were fed 2 times/day. Water was available ad libitum through nipples that were situated besides the trough in each pen. All pigs were weighed at the start of the experiment and every four weeks during the course of the experiment. The pigs were weighed individually while in a crate of known weight and using a calibrated balance.

Sample collection

The students took samples of the ingredients used on each farm. The samples were stored at - 20^0 C. For each farm, a composite feed sample was prepared that reflected the composition of the cumulative feed intake for the entire feeding period for each pen. Subcutaneous adipose tissue samples were taken from all piglets at the beginning of the experiment, when they were aged 70 days, and from all pigs at age of 126 days. After disinfection and induction of local anaesthesia, a 5-10 mm incision was made in the right inguinal region. About 1 g of subcutaneous fat was removed and stored at - 20^0 C. The skin would was closed. No complications occurred.

Chemical analyses

For each farm and each pen a composite feed sample was analysed. Gross energy was determined by oxygen bomb calorimetry. Dry matter, crude protein, crude fat, crude fibre and ash were measured by the Weende analysis. Total lipids in composite feed samples were extracted with chloroform:methanol 2:1 (FOLCH et al. 1957). The lipids were transesterified with 12% BF_3 in methanol. The methyl esters were extracted with

petroleum ether, taken to dryness under nitrogen, redissolved in heptane and separated and quantified by gas-liquid chromatography as described (METCALFE et al. 1966). The adipose tissue samples were saponified, methylesters of fatty acids were formed and subjected to gas-liquid chromatography.

Statistical analysis

On farm 1, 5 out of the 9 pigs died within 30 days, possibly due to samonellosis; farm 1 was excluded from data analysis. ANOVA was used to evaluate treatment effects. Linear regression analysis was done for pen mean data, which were considered to be independent. The level of statistical significance was pre-set at $P < 0.05$.

Results

Feed composition

The macronutrient composition of the diets is shown in Table 4. The diets contained at least 24 % crude protein in the dry matter, the range being 24 to 30 %. Crude fat contents of the diets were similar, the range being 7.7 to 10.4 %. Crude fibre contents of the diets were lowest on farm 4 and highest on farms 2 and 3, the difference being about 1.5 g/100 g dietary dry matter. The ash contents of the diets increased with increasing proportions of fish meal. The diets without fish meal on average contained 6.2 % of ash, whereas those with 20 % fish meal contained 10.2 % of ash in the dietary dry matter. The calculated dietary contents of metabolizable energy (ME) are given in Table 4.

Table 5 shows the fatty acid composition of the diets; the contents are expressed as g of fatty acid/ MJ ME. The levels of eicosapentaenoic and docosahexaenoic acid rose with higher inclusion levels of fish meal. Within farms, the dietary contents of α-linolenic acid were constant and this almost was the case also for linoleic (C18:2 n-6) and palmitic acid (C16:0). Between farms, the dietary concentration of linoleic acid varied on average between 1.52 and 2.15 g/MJ ME. The major fatty acids in the diets were palmitic, oleic (C18:1) and linoleic acid, these three fatty acids comprising about 75 % of the total.

Growth performance

During the feeding period of 56 days, the average daily weight gain for the pooled animals fed the diet without fish meal was 0.586 kg. For the animals fed the diets with 10 and 20 % fish meal, daily weight gain was 0.558 and 0.588 kg, respectively. ANOVA with farm as factor showed that there was no significant effect of fish meal. The standard error of the difference was 0.026 kg.

Fatty acid composition of adipose tissue

In adipose tissue, eicosapentaenoic acid was not detectable. The relative percentage of docosahexaenoic acid increased when fish meal was included in the diet (Table 6). As to docosahexaenoic acid in adipose tissue, the pigs on farm 3 showed an aberrant response to fish meal consumption. The percentage of docosahexaenoic acid on average was 0.06 % for pigs fed the diets without fish meal and 0.22 % for their counterparts given diets with 20 % fish meal. The adipose tissue contents of linoleic and α-linolenic acid were not systematically influenced by the different diets fed on each farm. The pigs on farm 3 had the highest content of linoleic acid in adipose tissue.

Table 4. Analysed macronutrient composition of the whole diets fed on the different farms

Farm:	2			3			4			5			6		
Diet[1]:	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Dry matter (%)	92.0	92.0	92.1	92.7	92.7	92.8	92.4	92.4	92.5	92.2	92.3	92.4	92.0	92.1	92.2
Crude protein (% in dm [2])	27.3	25.8	24.3	29.1	27.6	26.1	30.4	28.9	27.4	30.1	28.6	27.1	29.1	27.6	26.1
Crude fat (% in dm)	8.8	8.6	8.3	10.7	10.4	10.2	8.6	8.4	8.1	8.7	8.5	8.2	8.2	8.0	7.7
Crude fibre (% in dm)	6.4	6.3	6.1	6.5	6.3	6.2	5.1	4.9	4.8	5.8	5.7	5.6	6.0	5.9	5.7
Ash (% in dm)	5.3	7.4	9.4	6.0	8.0	10.0	6.0	8.0	10.0	6.7	8.7	10.7	7.1	9.1	11.1
ME[3] (MJ/kg dm)	12.9	12.6	12.2	13.0	12.6	12.3	12.9	12.6	12.2	12.9	12.6	12.2	13.2	12.9	12.6

[1]Diets 1, 2 and 3 refer to the diets with 0, 10 or 20 % of fish meal, respectively.
[2]dm = dry matter.
[3]Metabolizable energy (ME) was on calculated on the basis of the measured gross energy, table values for digestibility of nutrients in the diet ingredients and by assuming that ME = 0.96 x digestible energy.

Table 5. Fatty acid composition of the whole diets fed on the farms

Farm:	2			3			4			5			6		
Diet:	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Fatty acid															
							g fatty acid/MJ ME								
C16:0	1.71	1.71	1.71	2.04	2.05	2.06	1.60	1.60	1.60	1.72	1.72	1.72	1.51	1.50	1.50
C18:0	0.52	0.39	0.25	0.55	0.42	0.28	0.53	0.39	0.25	0.53	0.39	0.25	0.51	0.38	0.24
C18:1 n-9	1.91	1.86	1.79	2.53	2.49	2.44	2.09	2.03	1.98	1.80	1.74	1.67	1.87	1.81	1.75
C18:2 n-6	1.69	1.71	1.73	2.12	2.15	2.18	1.63	1.65	1.66	1.83	1.85	1.88	1.51	1.52	1.54
C18:3 n-3	0.16	0.16	0.16	0.14	0.14	0.14	0.15	0.15	0.15	0.14	0.14	0.14	0.10	0.10	0.10
C20:4 n-6	0.05	0.04	0.02	0.05	0.04	0.02	0.05	0.04	0.02	0.05	0.04	0.02	0.05	0.04	0.02
C20:5 n-3	0.01	0.02	0.03	0.01	0.02	0.03	0.01	0.02	0.03	0.01	0.02	0.03	0.01	0.02	0.03
C22:6 n-3	0.00	0.12	0.25	0.00	0.12	0.25	0.00	0.12	0.25	0.00	0.12	0.25	0.00	0.12	0.24

Table 6. Fatty acid composition of adipose tissue from pigs fed the different diets

Farm:	2			3			4			5			6		
Diet:	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Fatty acid	g fatty acid methylester/100 g methylesters														
C18:2 n-6	6.03	6.02	7.78	14.60	12.80	15.41	11.21	10.85	11.35	11.13	12.06	11.37	8.91	10.37	9.32
C18:3 n-3	0.60	0.78	0.89	1.00	0.90	1.03	0.82	0.86	0.84	0.82	1.17	1.10	0.76	0.87	0.65
C22:6 n-3	0.00	0.14	0.12	0.22	0.23	0.17	0.00	0.24	0.26	0.07	0.10	0.25	0.00	0.09	0.29

Means for 3 pigs per diet on each farm are given

Correlations between fatty acids in diets and in adipose tissue

For all pen mean data combined, linear correlation coefficients were calculated between the dietary contents of docosahexaenoic, α-linolenic and linoleic acid and those of adipose tissue. Dietary fatty acids were expressed as g/MJ ME. Adipose tissue fatty acids were expressed as % of total fatty acids. The linear correlation coefficients (r) were found to be 0.67, 0.13 and 0.69 for docosahexaenoic, α-linolenic and linoleic acid, respectively. The high correlation coefficient for docosahexaenoic acid was due to clustering of the data according to the three treatments that were installed. Fig. 1 shows the relationship for linoleic acid; the correlation was based on clustering of the data for each of the five farms.

Correlations between dietary fatty acids and growth

There were no significant correlations between the intakes of docosahexaenoic or α-linolenic acid and growth, the correlation coefficients (r) were 0.02 and –0.04, respectively. However, the intake of linoleic acid and growth were significantly associated (Fig. 2), the linear correlation coefficient being 0.50.

Correlations between adipose tissue fatty acids and weight gain

For the pen means, the linear correlations were calculated between the contents of docosahexaenoic or α-linolenic acid in adipose tissue and growth. The correlations were found to be 0.22, and 0.66, respectively. Fig. 3 shows the relationship between α-linolenic acid in adipose tissue and average daily weight gain.

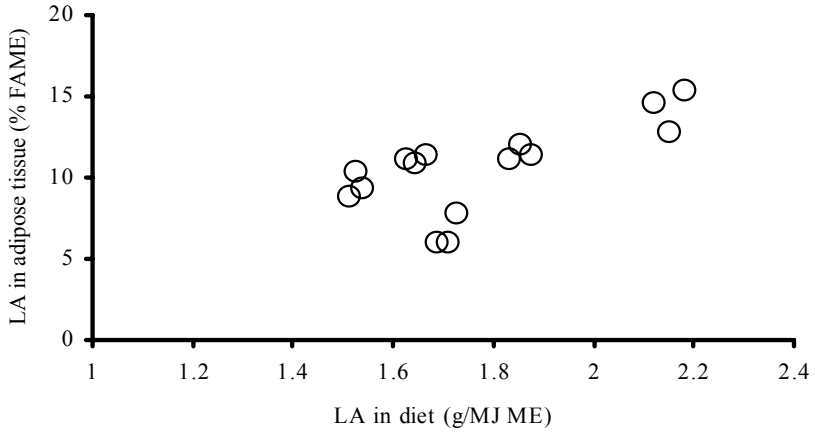

Fig. 1. Relationship between dietary and adipose tissue linoleic acid (LA) for pen means. The linear correlation coefficient was 0.69 (P < 0.05) and the regression equation is y = -4.21 + 8.34x.

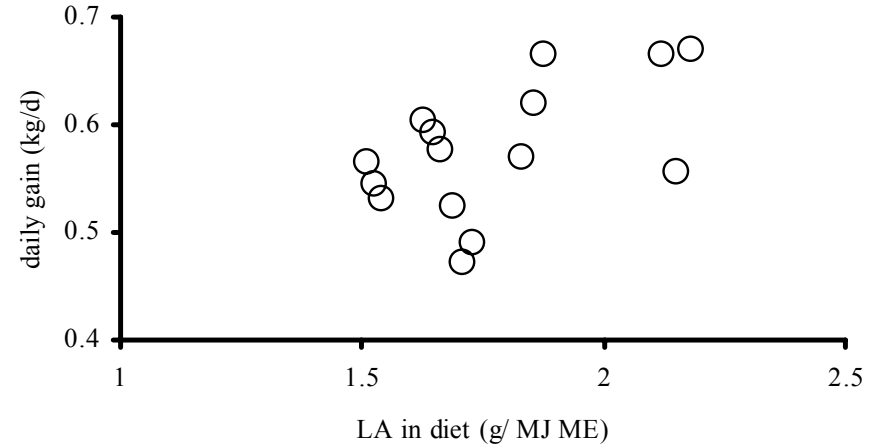

Fig. 2. Relationship between dietary linoleic acid (LA) and growth for pen means. The linear correlation coefficient was 0.50 (P < 0.05) and the regression equation is y = 0.31 + 0.15x.

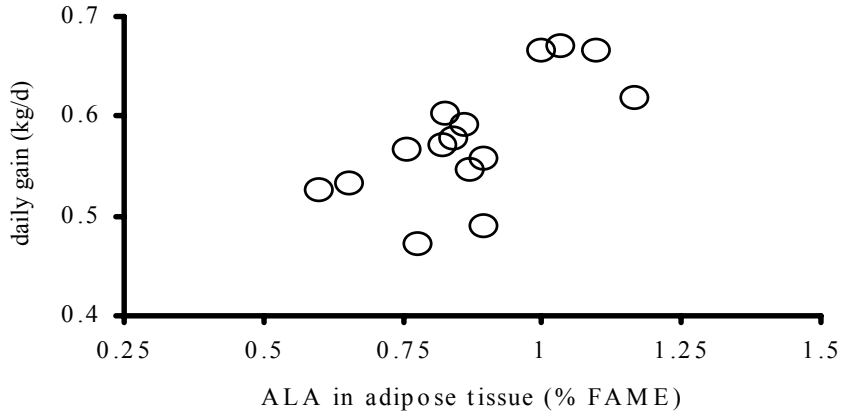

Fig. 3. Relationship between adipose tissue α-linolenic acid (ALA) and growth for pen means. The linear correlation coefficient was 0.66 (P < 0.05) and the regression equation is y = 0.39 + 0.27x.

The relationship for linoleic acid is illustrated in Fig. 4; the linear correlation coefficient is 0.83.

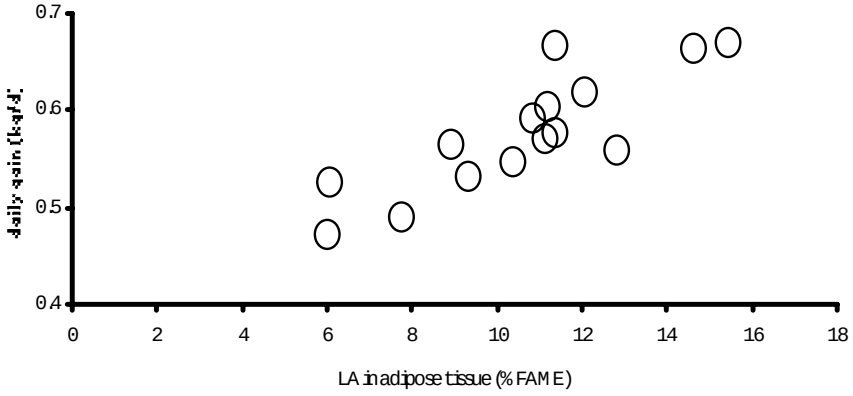

Fig. 4. Relationship between adipose tissue linoleic acid (LA) and growth for pen means. The linear correlation coefficient was 0.83 ($P < 0.05$) and the regression equation is $y = 0.38 + 0.02$

Discussion

This study confirms earlier work (NGUYEN et al. 2002b) in that the fatty acid composition of the diet is related with the fatty acid composition of adipose tissue in growing swine. The incorporation of fish meal into the diet raised the intake of eicosapentaenoic and docosahexaenoic acid. In the pigs fed fish meal, the adipose tissue generally was enriched with docosahexaenoic acid, but eicosapentaenoic acid remained undetectable. On the basis of literature data we have shown that dietary eicosapentaenoic acid is incorporated into adipose tissue less efficiently than is docosahexaenoic acid (NGUYEN et al. 2002b). When the diets contained 20 % fish meal, the average dietary eicosapentaenoic and docosahexaenoic contents were 0.03 and 0.25 g/MJ ME, respectively. According to previously established regression equations (NGUYEN et al. 2002b), the expected relative percentages of eicosapentaenoic and docosahexaenoic acid in adipose tissue would be 0.19 and 1.36 %. As mentioned above, eicosapentaenoic acid in adipose tissue was undetectable and the group mean percentage of docosahexaenoic acid in the adipose tissue of the pigs fed the diet with 20 % fish meal was 0.22 %. Thus, the observed concentrations of eicosapentaenoic and docosahexaenoic acid in adipose tissue were much lower than those expected. Possibly, the discrepancy relates to a high rate of de novo fatty acid synthesis in the Vietnamese swine, leading to extra dilution of the flux of dietary fatty acids into adipose tissue.

Because the farmers were allowed to compose the base diet according to their own preferences, there were between-farm differences in the composition, including the content

of linoleic acid. There was a direct relationship between linoleic acid intake and its percentage in adipose tissue (Fig. 1). As was the case for eicosapentaenoic and docosahexaenoic acid, the percentage of linoleic acid in adipose tissue in relation to linoleic acid intake was lower than that expected on the basis of previously established regression formulas (NGUYEN et al., 2002b).

The hypothesis tested in this study was that the feeding of extra eicosapentaenoic and docosahexaenoic acid in the form of fish meal would enhance growth. In order to test the hypothesis, the diets were formulated so that protein supply was abundant and would not limit growth. There was no effect of fish meal on growth and no relationship between the intake of either eicosapentaenoic or docosahexaenoic acid and daily gain. Thus, the hypothesis would be rejected. It could be argued that the intake of eicosapentaenoic acid was not sufficiently raised or did not induce differences between the treatments because it remained undetectable in adipose tissue. We did observe an increase in the percentage of docosahexaenoic acid in adipose tissue after fish meal feeding. However, there was no relation between docosahexaenoic acid in adipose tissue and growth. Thus, again the hypothesis would be rejected. There was a statistically significant, direct relationship between the percentage of α-linolenic acid in adipose tissue and growth. The explained variance of average daily weight gain was 44 %. This observation supports our field study in which we compared twelve small holdings in Central Vietnam (NGUYEN et al., 2002b). The observed positive relationship between α-linolenic acid in adipose tissue and average daily gain might indicate that α-linolenic acid intake was too low to sustain maximum growth.

A surprising finding emerged from this study. There was a direct relationship between linoleic acid either in the diet or in adipose tissue and growth. The requirement of linoleic acid in growing pigs is about 0.07 g/MJ ME (NATIONAL RESEARCH COUNCIL, 1998) which is equivalent to 7.8 % linoleic acid in adipose tissue (NGUYEN et al. 2002b). The lowest adipose tissue level of linoleic acid in this study was 6 %. Thus, linoleic acid supply may not have been limiting for growth. It would appear that linoleic acid intake, and thus also adipose tissue linoleic acid, in this study acted a surrogate variable for a powerful determinant of growth. The identity of the determinant is unknown.

In conclusion, the intake of fish meal at the expense of ruminant meal did not affect growth of swine kept in small holdings in Central Vietnam. It would thus appear that extra intake of eicosapentaenoic and docosahexaenoic acid is not required for an increase in average daily weight gain. There were significant, and direct relationships between the intakes of either α-linolenic acid or linoleic acid and weight gain. The basis and implication of these relationships require further study.

Acknowledgement

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References

FOLCH, J.; LEES, M.; SLOANE-STANLEY, G.H., 1957: J. Biol. Chem. 226, 497-509.

FRITSCHE, K.L.; HUANG, S.-C.; CASSITY, N.A., 1993: J. Anim. Sci. 71, 1841-1847.

NATIONAL RESEARCH COUNCIL, 1998: Nutrient Requirements of Swine, National Academy Press, Washington, D.C.

NGUYEN, L.Q.; EVERTS, H.; BEYNEN, A.C., 2002a: Trop. Anim. Health Prod. (in press)

NGUYEN, L.Q.; NUIJENS, M.C.G.A.; EVERTS, H.; SALDEN, N.; BEYNEN, A.C., 2002b: Meat Sci. (in press)

METCALFE, L.D.; SCHMITZ, A.A.; PELKA, J.R., 1966: Anal. Chem. 18, 514-516.

WU, D.; MEYDANI, S.N., 1998: Proc. Nutr. Soc. 57, 503-509.

Chapter 5

Shrimp byproduct feeding and growth performance of growing pigs kept on small holdings in Central Vietnam

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Abstract

The effect studied was that of the feeding of shrimp byproduct meal, as source of eicosapentaenoic and docosahexaenoic acid, on growth performance and fatty acid composition of adipose tissue in growing pigs kept on small holdings in Central Vietnam. Shrimp meal was exchanged with ruminant meal so that the diets contained either 0, 10 or 20 % shrimp meal in the dry matter. The diets were fed on 6 different small-holder farms. The farmers fed a base diet according to their personal choice, but were instructed as to the use of shrimp and ruminant meal. The diets were fed to the pigs from 70 to 126 days of age. There were three animals per treatment group per farm. The diets without and with 20 % shrimp meal on average contained 0.01 and 0.13 g docosahexaenoic acid/MJ of metabolizable energy (ME). Due to the higher contents of ash and crude fiber, the shrimp containing diets had lower energy densities than the control diets. The relative percentage of α-linolenic acid in adipose tissue was directly related with the intake of this fatty acid. Eicosapentaenoic acid was not detectable in adipose tissue; the content of docosahexaenoic acid was generally increased after consumption of shrimp byproduct meal. In spite of the concurrent high intakes of ash and crude fiber, the feeding of shrimp byproduct meal had a general stimulatory effect on growth performance of the growing pigs. The intake of docosahexaenoic acid or its content in adipose tissue was not related with average daily gain. It is suggested that shrimp byproduct meal may contain an unknown growth enhancing factor.

Introduction

High intakes of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the form of fish oil may improve disease resistance in pigs (Fritsche et al., 1993; Calder, 2001). In a field study involving small holdings in Central Vietnam, we found that the percentage of α-linolenic acid (ALA) in adipose tissue was positively related with growth (Nguyen et al., 2002a). The content of ALA in adipose tissue of growing pigs reflects that in the diet (Nguyen et al. 2002b). In the body of the pig, ALA is converted into EPA and DHA (Innis, 1991). Possibly, the observed positive relationship between ALA in adipose tissue and growth was related to suboptimal production of EPA and DHA. Shrimp byproducts may contain 7 % fat of which 6 and 10 % may be EPA and DHA, respectively. Thus, shrimp byproducts can be used as a source of EPA and DHA so that its addition to the diet of growing-finishing pigs in small holdings in Central Vietnam could improve growth performance. This idea was tested by comparing the effect of exchanging shrimp byproducts for ruminant meal in the diet. The farmers composed the base diets according to their personal choice, but were instructed to use shrimp byproduct or ruminant meal as components. The dietary variables were supplied to the pig farms and under the supervision of students the two components were mixed with the base diets at pre-set ratios.

Material and methods

Animals and experimental diets

Castrated, male weanling pigs (n = 54) were purchased and allocated to 6 small-holdings. The piglets were of a Mong cai (female) x Large White (male) cross, were aged about 70 days and had a body weight of 13.2 ± 0.54 kg (mean ± SD). Each farm received 9 animals, which were housed in pens containing three piglets each. Within each farm, the three piglets in each pen had similar average body weights and distributions. Each piglet had an ear-cut number for identification. The pigs were fed a restricted amount of dry matter according to Table 1.

Table 1. Feeding schedule that was applied on each farm

	Pig body weight (kg)				
	10	*20*	*30*	*40*	*50*
	kg dry matter/day				
Base diet	*0.54*	*0.80*	*1.00*	*1.20*	*1.40*
Shrimp byproduct	*0/0.07/0.13*	*0/0.1/0.2*	*0/0.13/0.25*	*0/0.15/0.30*	*0/0.18/0.35*
Ruminant meal	*0.13/0.07/0*	*0.2/0.1/0*	*0.25/0.13/0*	*0.30/0.15/0*	*0.35/0.18/0*

On each farm three diets were fed, containing either 0, 10, 20 % shrimp by-product meal in total dietary matter. The compositions of the base diets used on each farm are shown in Table 3.

The farmers were individually instructed by B.Sc students of animal science who were present on the farms. The students used a local feed table to check the dry matter contents of the ingredients. The shrimp byproduct and ruminant meal were supplied by the students. The shrimp byproduct was obtained from a local company and consisted of heads mainly. The ruminant meal was sun dried and consisted of lung/liver/blood in a 40/30/30 ratio on a wet-weight basis. Table 2 shows the analysed composition of the two dietary variables.

Table 2. Analysed composition of the ruminant and shrimp byproduct meal

Nutrient	*Ruminant meal*	*Shrimp meal*
Macronutrients, g/kg		
Dry matter	*901*	*930*
Crude protein	*744*	*482*
Crude fat	*97*	*75*
Crude fibre	*21*	*150*
Ash	*43*	*249*
Fatty acids (g methylester/100 g methylester)		
C16:0	*23.7*	*18.4*
C18:0	*27.0*	*8.3*
C18:1 n-9	*20.6*	*19.2*
C18:2 n-6	*4.4*	*10.4*
C18:3 n-3	*0.9*	*0.6*
C20:4 n-6	*3.5*	*5.2*
C20:5 n-3	*0.9*	*5.9*
C22:6 n-3	*0.2*	*10.5*

The base diets were composed by the farmers according to their own choice, but the ingredient composition was rounded off to the nearest 5 % and then kept constant. In addition, the farmers were not allowed to use fish products other than the shrimp byproduct meal supplied. The ruminant and shrimp byproduct meal concentrations in the final diets were 0, 10 or 20 % of the dietary dry matter (Table 1). On each farm three diets were used, the ruminant/shrimp byproduct meal combination being 20/0, 10/10 and 0/20. It was verified that the rations contained at least 20 % crude protein in the dry matter as calculated on the basis of the feed table. In our earlier study (Nguyen et al., 2002a), we found that on various small-holder farms the supply of protein was low and limited growth of the pigs. The students recorded feed intake and the ingredient composition of the rations. Table 3 shows the ingredient composition of the diets fed on the 6 farms.

Table 3. Composition of the diets fed on each farm

Farm	*1*	*2*	*3*	*4*	*5*	*6*

Ingredient						
Rice bran	*25*	*25*	*25*	*25*	*25*	*20*
Rice	*20*	*20*	*20*	*20*	*20*	*25*
Pea byproduct	*10*	*-*	*5*	*-*	*-*	*-*
Beer byproduct	*-*	*10*	*5*	*10*	*10*	*10*
Commercial feed	*10*	*10*	*10*	*10*	*10*	*10*
Vegetables	*15*	*15*	*15*	*15*	*15*	*15*
Variable meal[1]	*-*	*-*	*-*	*-*	*-*	*-*

[1]The three ruminant/shrimp byproduct meal combinations on each farm were 20/0, 10/10 and 0/20.

The feedstuffs supplied were weighed on a calibrated balance. The pigs were fed 2 times/day. Water was available ad libitum through nipples that were situated besides the trough in each pen. All pigs were weighed at the start and end of the experiment. The pigs were weighed individually while in a crate of known weight and using a calibrated balance.

Sample collection

The students took samples of the ingredients used on each farm. Each new batch of feedstuff was sampled. The samples were stored at – 20 ^{o} C. For each farm, a composite feed sample was prepared that reflected the composition of the cummulative feed intake for the entire feeding period for each pen. Subcutaneous adipose tissue samples were taken from the pigs at the end of the experiment, when they were aged about 126 days. After disinfection and induction of local anaesthesia, a 5-10 mm incision was made in the right inguinal region. About 1 g of subcutaneous fat was removed and stored at – 20 ^{o} C. The skin would was closed. No complications occurred.

Chemical analyses

For each farm and each pen a composite feed sample was analysed. Gross energy (GE) was determined by oxygen bomb calorimetry. Dry matter (DM), crude protein, crude fat, crude fibre and ash were measured by the Weende analysis. Total lipids in composite feed samples were extracted with chloroform:methanol 2:1 (Folch at al. 1957). The lipids were transesterified with 12% BF_3 in methanol at 80^{0}C. The methyl esters were extracted with water and petroleum ether, taken to dryness under nitrogen, redissolved in heptane and separated and quantified by gas liquid chromatography (GC) as described (Metcalfe et al. 1966). The fat in adipose tissue or experimental fats was saponified, methylesters of fatty acids were formed and subjected to GC.

Statistical analysis

ANOVA was used to evaluate treatment effects. Linear regression analysis was done for pen mean data. The level of statistical significance was pre-set at $P < 0.05$.

Results

Feed composition

The macronutrient composition of the diets is shown in Table 4. The diets contained at least 24 % crude protein in the dry matter, the range being 24.2 to 30.2 %.

Crude fat contents of the diets were lower in diet 3 than in diet 1. Crude fibre contents of diets 1 and 3 were higher than of diet 1. The ash contents of the diets increased with increasing proportions of shrimp byproduct meal. The diets without shrimp byproduct meal on average contained 7.4 % of ash, whereas those with 20 % shrimp byproduct meal contained 11.7 % of ash in the dietary dry matter. The calculated dietary contents of metabolizable energy (ME) are given in Table 4.

Table 5 shows the fatty acid composition of the diets; the contents are expressed as g of fatty acid/ MJ ME. The levels of EPA (C20:5 n-3, not shown) and DHA (C22:6 n-3) rose with higher inclusion levels of shrimp product meal. Within farms, the dietary contents of ALA were constant and this almost was the case also for linoleic acid (C18:2 n-6). Between farms, the dietary concentration of linoleic acid and ALA varied on average between 1.41 and 1.82 and 0.19 and 0.23 g/MJ ME, respectively.

Growth performance

Table 6 shows the average initial and final body weight and weight gain for each pen. On farms 2, 4, 5 and 6, the feeding of shrimp byproduct instead of ruminant meal produced an increase in weight gain. On farm 1, 10 % shrimp byproduct in the ration raised weight gain, but an inclusion level of 20 % lowered it. On farm 3, no influence of shrimp byproduct was seen. During the feeding period of 56 days, the average daily weight gain for the pooled animals fed the diet without shrimp byproduct meal was 0.480 kg. For the animals fed the diets with 10 and 20 % shrimp byproduct meal, daily weight gain was 0.505 and 0.511 kg, respectively. ANOVA with farm as factor showed that there was no significant effect of shrimp byproduct meal (P = 0.15). The standard error of the difference was 0.017 kg. When the two shrimp treatments were combined, the difference between shrimp byproduct feeding and the control treatment almost reached statistical significance (P = 0.056).

Fatty acid composition of adipose tissue

In adipose tissue, EPA was not detectable. The relative percentage of DHA increased when shrimp byproduct meal was included in the diet (Table 5). As to DHA in adipose tissue, the pigs on farm 1 showed an aberrant response to shrimp byproduct meal consumption. The percentage of DHA on average was 0.06 % for pigs fed the diets without shrimp byproduct meal and 0.19 % for their counterparts given diets with 20 % shrimp byproduct meal. The adipose tissue contents of linoleic acid and ALA were influenced by the different diets fed on each farm, the range being 7.19 to 12.69 % and 0.79 to 1.23 % of total fatty acids for farms without shrimp byproduct meal, 8.29 to 12.44 % and 0.77 to 1.19 for diets with 10 % of shrimp byproduct meal, and 8.71 to 13.18 % and 0.81 to 1.38 % for diets with 20 % of shrimp byproduct meal, respectively.

Table 4. Analysed macronutrient composition of the whole diets fed on the different farms

Farm	*1*			*2*			*3*			*4*			*5*			*6*		
Diet[1]	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*
Dry matter (%)	*94.3*	*94.6*	*94.9*	*94.3*	*94.6*	*94.9*	*94.3*	*94.5*	*94.9*	*94.3*	*94.6*	*94.9*	*94.3*	*94.6*	*94.9*	*94.2*	*94.5*	*94.8*
Crude protein (% in dm)	*29.5*	*26.9*	*24.2*	*30.2*	*27.6*	*24.9*	*29.8*	*27.2*	*24.6*	*30.2*	*27.6*	*24.9*	*30.2*	*27.6*	*24.9*	*29.8*	*27.2*	*24.6*
Crude fat (% in dm)	*8.4*	*8.2*	*7.9*	*9.3*	*9.1*	*8.9*	*8.8*	*8.6*	*8.4*	*9.3*	*9.1*	*8.9*	*9.3*	*9.1*	*8.9*	*8.5*	*8.3*	*8.1*
Crude fibre (% in dm)	*7.6*	*8.9*	*10.2*	*7.3*	*8.6*	*9.9*	*7.4*	*8.7*	*10.0*	*7.3*	*8.6*	*9.9*	*7.3*	*8.6*	*9.9*	*6.9*	*8.2*	*9.5*
Ash (% in dm)	*7.6*	*9.3*	*11.4*	*7.6*	*9.3*	*11.4*	*6.8*	*9.3*	*11.4*	*7.6*	*9.3*	*11.4*	*7.6*	*9.3*	*11.4*	*7.2*	*8.9*	*11.0*
ME[3] *(MJ/kg dm)*	*12.7*	*12.2*	*11.8*	*12.4*	*12.0*	*11.6*	*12.6*	*12.1*	*11.7*	*12.4*	*12.0*	*11.6*	*12.4*	*12.0*	*11.6*	*12.5*	*12.0*	*11.6*

[1] Diets 1, 2 and 3 refer to the diets with 0, 10 or 20 % of shrimp byproduct meal, respectively.

[2] dm = dry matter.

[3] Metabolizable energy (ME) was on calculated on the basis of the measured gross energy, table values for digestibility of nutrients in the diet ingredients and by assuming that ME = 0.96 x digestible energy.

Table 5. Fatty acid composition of the whole diets fed on the farms and in adipose tissue of the pigs

Farm	*1*			*2*			*3*			*4*			*5*			*6*		
Diet[1]	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*
Fatty acid	*g fatty acid/MJ ME*																	
C18: 2n-6	*1.52*	*1.60*	*1.69*	*1.92*	*2.02*	*2.11*	*1.72*	*1.81*	*1.93*	*1.92*	*2.02*	*2.13*	*1.92*	*2.02*	*2.13*	*1.71*	*1.80*	*1.90*
C18: 3n-3	*0.22*	*0.23*	*0.23*	*0.25*	*0.26*	*0.23*	*0.24*	*0.25*	*0.25*	*0.25*	*0.25*	*0.26*	*0.25*	*0.25*	*0.26*	*0.24*	*0.24*	*0.25*
C20:5 n-3	*0.02*	*0.05*	*0.08*	*0.02*	*0.05*	*0.08*	*0.02*	*0.05*	*0.08*	*0.02*	*0.05*	*0.08*	*0.02*	*0.05*	*0.08*	*0.02*	*0.05*	*0.08*
C22: 6n-3	*0.01*	*0.07*	*0.14*	*0.01*	*0.08*	*0.15*	*0.01*	*0.08*	*0.15*	*0.01*	*0.08*	*0.15*	*0.01*	*0.08*	*0.15*	*0.01*	*0.08*	*0.15*
C18: 2n-6	*11.42*	*11.53*	*12.05*	*10.14*	*12.23*	*11.18*	*12.69*	*12.39*	*12.16*	*7.19*	*8.29*	*8.71*	*13.18*	*12.44*	*11.35*	*8.84*	*9.61*	*9.89*
C18: 3n-3	*0.79*	*0.77*	*0.81*	*1.04*	*1.19*	*1.08*	*1.07*	*0.97*	*0.96*	*1.16*	*1.19*	*1.38*	*1.23*	*1.02*	*0.96*	*1.14*	*1.09*	*1.26*
C22: 6n-3	*0*	*0*	*0*	*0.07*	*0.05*	*0.15*	*0*	*0*	*0.04*	*0.17*	*0.21*	*0.28*	*0.05*	*0.09*	*0.20*	*0.09*	*0.13*	*0.27*

[1]Diets 1, 2 and 3 refer to the diets with 0, 10 or 20 % of shrimp byproduct meal, respectively.

Table 6. Average initial and final body weight for each pen

Farm	*1*			*2*			*3*			*4*			*5*			*6*		
Diet[1]	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*
Body weight																		
Initial	*13.5*	*13.7*	*12.5*	*13.0*	*13.2*	*13.1*	*13.5*	*13.7*	*13.5*	*13.0*	*13.1*	*12.9*	*13.0*	*13.1*	*13.2*	*13.7*	*12.8*	*12.8*
Final	*43.0*	*45.0*	*41.0*	*38.3*	*42.0*	*42.7*	*41.0*	*41.3*	*41.3*	*38.7*	*39.0*	*40.7*	*38.7*	*40.3*	*40.5*	*41.2*	*41.5*	*43.3*
Weight gain (kg/day)	*0.527*	*0.560*	*0.509*	*0.452*	*0.514*	*0.528*	*0.491*	*0.494*	*0.497*	*0.458*	*0.463*	*0.495*	*0.458*	*0.487*	*0.491*	*0.491*	*0.512*	*0.545*

[1]Diets 1, 2 and 3 refer to the diets with 0, 10 or 20 % of shrimp byproduct meal, respectively.

Correlations on a pen basis

For all pen mean data combined, linear correlation coefficients were calculated between the dietary contents of DHA, ALA and linoleic acid and those of adipose tissue. Dietary fatty acids were expressed as g/MJ ME. Adipose tissue fatty acids were expressed as % of total fatty acids. The linear correlation coefficients (r) were found to be 0.43, 0.54 and 0.29 for DHA, ALA and linoleic acid, respectively. Fig. 1 shows the relationship for ALA.

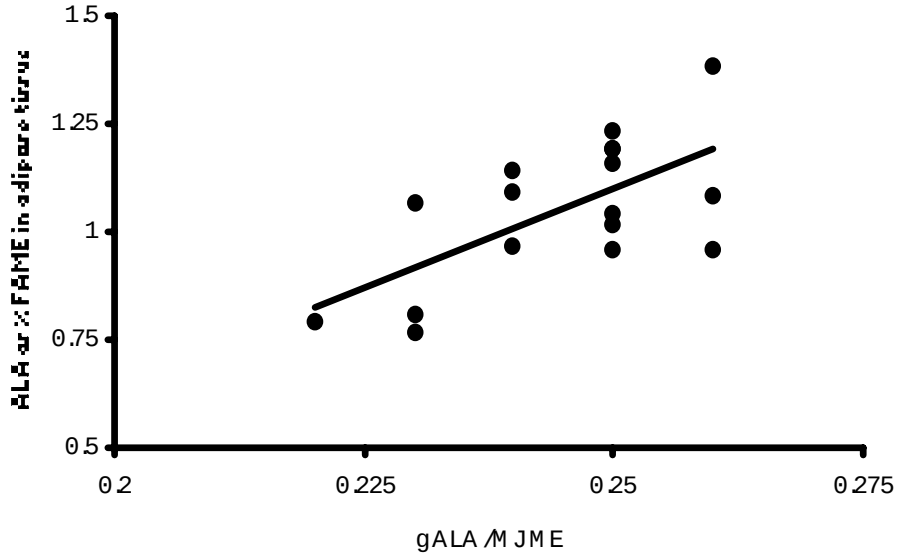

Figure 1. Relationship between dietary and adipose tissue α-linolenic acid (ALA) for pen means. The linear correlation coefficient was 0.65 (P < 0.01) and the regression equation is y = 8.99x –1.15.

There were no significant correlations between the intakes of DHA, ALA or linoleic acid and growth, the correlation coefficients (r) were -0.34, - 0.17 and –0.20, respectively. For the pen means, the linear correlations also were calculated between the contents of DHA, ALA or linoleic acid in adipose tissue and growth; there were no significant correlations.

Discussion

This study confirms earlier work (Nguyen et al. 2002b) in that the fatty acid composition of the diet affects the fatty acid composition of adipose tissue in growing swine. Because the farmers were allowed to compose the base diet according to their own preferences, there were between-farm differences in diet composition, including the content of ALA. There was a direct relationship between ALA intake and its percentage in adipose tissue (Fig. 1). The incorporation of shrimp byproduct meal into the diet raised the intake

of EPA and DHA. When the diets contained 20 % shrimp byproduct meal, the average dietary DHA content was 0.12 g/MJ ME. In the pigs fed shrimp byproduct meal, the adipose tissue generally was enriched with DHA, but EPA remained undetectable.

The question addressed in this study was whether the feeding of extra EPA and DHA in the form of shrimp byproduct meal would enhance growth. The diets were formulated so that protein supply was abundant and would not limit growth. However, the shrimp byproduct contained 6- and 7-fold more ash and crude fiber than did the ruminant meal. As a result, the shrimp-meal containing diets contained more ash and fiber and had lower energy densities than the control diet. These characteristics of the shrimp diets by itself would depress body-weight gain. Nevertheless, there was a general stimulatory effect of shrimp byproduct meal on growth, but there was no relationship between the intake of DHA or DHA in adipose tissue and daily gain. It follows that the intake of DHA was not limiting growth, but that another component of the shrimp byproduct acted as a powerful determinant of growth. The identity of the component is unknown.

In conclusion, the intake of shrimp byproduct meal at the expense of ruminant meal stimulated growth of swine kept in small holdings in Central Vietnam. The data indicate that the extra intake of EPA and DHA associated with the shrimp byproduct did not cause the increase in average daily weight gain.

Acknowledgements

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References

Calder, P.C. (2001). Polyunsaturated fatty acids, inflammation, and immunity. Lipids, 36: 1007-1024.

Fritsche, K. L., D.W. Alexander, N.A. Cassity and Shu-cai Huang (1993). Maternally supplied fish oil alters piglet immune cell fatty acid profile and eicosanoid production. Lipids, 28: 677-682.

Folch, J., Lees, M., & Sloane Stanley, G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry, 226, 497-509.

Innis, S.M. (1991). Essential fatty acids in growth and development. Progress in Lipid Research, 30: 39-103.

Metcalfe, L.D., Schmitz A.A., & Pekka J.R. (1966). Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Analytical Chemistry, 18, 514-516.

Nguyen, L.Q., Everts, H., & Beynen, A.C. (2002a). Intake of essential fatty acids by growing-finishing pigs kept on small holdings in Central Vietnam. Tropical Animal Health and Production (in press).

Nguyen, L.Q.; Nuijens, M.C.G.A.; Everts, H.; Salden, N.; Beynen, A.C. (2002b). Mathematical relationships between the intake of n-6 and n-3 polyunsaturated fatty acids and their contents in adipose tissue of growing pigs. Meat Science (in press)

Chapter 6

Dietary linseed oil versus either fish or coconut oil enhances growth performance of growing pigs kept on small holdings in Central Vietnam

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Abstract

We have investigated the effect on growth performance of the addition of 5 % of either linseed, fish or coconut oil to the diet of growing pigs kept on small holdings in Central Vietnam. The diets were fed on 6 different farms; there were three animals per treatment per farm. The farmers fed a base diet according to personal choice, but were instructed as to the use of linseed, fish and coconut oil. The diets were fed to the pigs from 70 to 130 days of age. The diets with 5% linseed oil on average contained 2.4 g α-linolenic acid /MJ of metabolizable energy (ME) and the diets with 5 % fish oil on average contained 0.06 and 0.75 g eicosapentaenoic and docosahexaenoic acid/MJ ME, respectively. The relative percentages of docosahexaenoic, eicosapentaenoic, α-linolenic and linoleic acid in adipose tissue were determined by the intake of the corresponding fatty acids. Dietary linseed oil versus coconut oil significantly enhanced daily weight gain and versus fish oil it also stimulated growth, but this effect just failed to reach statistical significance. It is concluded that extra intake of α-linolenic acid may stimulate growth in growing-finishing pigs, this effect being independent of the conversion of α-linolenic acid into eicosapentaenoic and docosahexaenoic acid.

Introduction

In a field study involving small holdings in Central Vietnam, we have found a weak, but statistically significant, positive relation between the content of α-linolenic acid (ALA) in subcutaneous adipose tissue and average daily gain (ADG) of growing-finishing pigs (Nguyen et al. 2002a). The amount of ALA in swine adipose tissue is a valid index of ALA intake (Nguyen et al. 2002b). We suggested that on the small holdings in Central Vietnam the dietary provision with the essential fatty acid ALA might have been too low to sustain maximum growth. There is no formal recommendation as to the requirement of ALA by growing-finishing pigs. In the body, ALA is converted into eicosapentaenoic acid (EPA) (Innis, 1991) which is the direct precursor of various eicosanoids, some of them affecting the immune system (Wu and Meydani, 1998). High intakes of EPA in the form of fish oil may improve disease resistance in pigs (Fritsche, et al., 1993; Calder, 1996, 1997; Turek, et al., 1994, 1996; Thies, et al., 1999). There is also evidence that ingestion of extra docosahexaenoic acid (DHA), which can be synthesized from EPA, stimulates the immune system (Wu and Meydani, 1998).

We hypothesized that supplementation of the swine rations in Central Vietnam with either ALA, EPA and/or DHA would enhance growth performance either due to meeting the nutritional requirement or due to improved disease resistance. Our hypothesis was tested by enrichment of the rations with either ALA in the form of linseed oil or EPA plus DHA in the form of fish oil. A ration mixed with coconut fat served as control treatment. The farmers composed the base diets according to their personal choice, but were instructed not to use fish products and soybean byproducts as ingredients. The experimental fats were supplied to the farmers and, under the supervision of students, the oils were mixed with the base diets at a pre-set ratio.

Materials and methods

Animals and experimental diets

Castrated, male weanling pigs, (n = 54) were purchased and allocated to 6 small holdings. The piglets were of a Mong cai (female) x Large White (male) cross, were aged about 70 days and had a body weight of 11.8 ± 0.96 kg (mean ± SD). Each farm received 9 animals, which were housed in pens containing three piglets each. Within each farm, the three piglets in each pen had similar average body weights and distributions. Each piglet had an ear-cut number for identification. The pigs were fed a restricted amount of dry matter according to Table 1. The farmers were individually instructed by B.Sc students of animal science who were present on the farms. The students used a local feed table to assess the dry matter contents of the ingredients. The experimental fats (coconut fat, linseed oil, fish oil) were supplied by the students. The experimental fats were stored at –20 ^{0}C and taken to the farms in cans while kept under a nitrogen atmosphere. The cans contained an amount of oil sufficient for feeding for a period of at least 5 days. Table 2 shows the analysed fatty acid composition of the experimental fats. The base diets were composed by the farmers according to their own choice, but they were not allowed to use fish products or soybean byproducts in the diets. Sudents verified that the rations contained at least 15 % crude protein in the dry matter as calculated on the basis of the feed table. All pigs were weighed at the start of the experiment and again at the end of the experiment when they were aged

about 130 days. The pigs were weighed while in a crate of known weight and using a calibrated balance.

Table 1. Feeding schedule that was applied on each farm

	Pig body weight (kg)				
	10	20	30	40	50
	kg DM[1]				
Base diet	0.636	0.95	1.187	1.425	1.66
Variable oil	0.034	0.05	0.063	0.075	0.09

On each farm three diets were fed, containing either 5 % of coconut, linseed or fish oil in the total dietary matter. The compositions of the base diets used on each farm are shown in Table 3.
[1]DM = dry matter.

Table 2. Analysed fatty acid composition of the experimental oils

	Coconut oil	Fish oil	Linseed oil
	g methylester/100 g methylesters		
C8:0	8.2	0.0	0.0
C10:0	5.9	0.0	0.0
C12:0	46.7	0.1	0.1
C14:0	19.0	2.8	0.0
C16:0	9.2	21.0	5.4
C18:0	3.1	5.7	3.3
C18:1 n-9	6.2	17.8	17.8
C18:2 n-6	1.5	1.3	15.1
C18:3 n-3	0.0	0.4	56.9
C20:4 n-6	0.0	1.7	0.0
C20:5 n-3	0.0	1.5	0.0
C22:6 n-3	0.0	19.8	0.0

Sample collection

The students took samples of the ingredients used on each farm. Each new batch of feedstuff was sampled. The samples were stored at – 20 ^{o} C. For each farm, a composite feed sample was prepared that reflected the composition of the cumulative feed intake for the entire feeding period for each pen.

Subcutaneous adipose tissue samples were taken from six piglets at the beginning of the experiment, when they were aged about 70 days, and from all pigs at the age of about 130 days. After disinfection and induction of local anaesthesia, a 5-10 mm incision was made in the right inguinal region. About 1 g of subcutaneous fat was removed and stored at

-20 ^{o} C. The skin wound was closed. No complications occurred.

Chemical analyses

The composite feed samples were analysed. Gross energy (GE) was determined by oxygen bomb calorimetry. Dry matter (DM), crude protein, crude fat, crude fibre and ash was measured by the Weende method.

Total lipids in feedstuffs were extracted with chloroform:methanol 2:1 (Folch et al. 1957). The lipids were transesterified with 12% BF_3 in methanol at 80 ^{0}C. The methyl esters were then extracted with petroleum ether, taken to dryness under nitrogen, redissolved in heptane and separated and quantified by gas liquid chromatography (GC) as described (Metcalfe et al. 1966). The fat in adipose tissue was saponified, methylesters of fatty acids were formed and subjected to GC.

Statistical analysis

ANOVA was used to evaluate treatment effects. Linear regression analysis was done for pen mean data. The level of statistical significance was pre-set at $P < 0.05$.

Results

Feed composition

Table 3 illustrates the ingredient composition of the diets. The analysed macronutrient composition is shown in Table 4.

Table 3. Composition of the diets fed on each farm

Farm	1	2	3	4	5	6
Ingredient						
			g/100 g DM[1]			
Rice bran	30	30	36	30	30	36
Rice	24	24	24	24	24	27
Vegetables	17	17	17	17	17	17
Pea byproduct	12	-	-	-	12	12
Maize	-	12	-	-	-	-
Soybean byproduct	6	6	-	-	6	-
Commercial feed	6	6	-	-	6	-
Fresh fish	-	-	-	2	-	3
Beer byproduct	-	-	12	-	-	-
Alcohol byproduct	-	-	6	10	-	-
Variable oils	5	5	5	5	5	5

[1]DM = dry matter.

Table 4. Analysed macronutrient composition of the whole diets fed on the different farms

Farm	1	2	3	4	5	6
Dry matter (%)	94.5	94.1	94.7	94.7	94.1	94
Crude protein (% in DM)[1]	19.2	16.2	17.8	16.7	19.2	14.9
Crude fat (% in DM)	13.66	13.92	15.64	14.43	13.97	13.08
Crude fibre (% in DM)	8.01	6.55	8.73	6.70	7.45	9.69
Ash (% in DM)	6.02	6.08	5.54	6.11	6.32	5.79
ME[2] (MJ/kg DM)	13.78	13.80	13.22	13.20	13.78	13.50

[1]DM = dry matter

[2] Metabolizable energy (ME) was calculated on the basis of the measured gross energy, table values for digestibility of nutrients in the diet ingredients and by assuming that ME = 0.96 x digestible energy.

The data for the three diets on each farm were pooled because the differences between diets within farms were negligible. The diets contained at least 15 % crude protein in the dry matter, the range being 14.9 to 19.2 %. Crude fat contents of the diets were similar, the range being 13.1 to 15.6 %, including the 5 % of added oils. Crude fibre contents of the diets were lowest on farm 2 and highest on farm 6, the difference being about 3.1g/100 g dietary dry matter. The ash contents of the diets were similar, the range being 5.54 to 6.32 %. The calculated dietary contents of metabolizable energy (ME) differed slightly between the diets (Table 4).

Table 5 shows the fatty acid composition of the diets; the contents are expressed as g of fatty acid/ MJ ME. The fatty acid composition of the whole diets reflected that of the added oils. The levels of EPA (C20:5 n-3) and DHA (C22:6 n-3) were raised by the inclusion of fish oil in diet 2 of each farm. The content of ALA (C18:3 n-3) was highest for diet 3, containing linseed oil. Within farms, the diets containing linseed oil had higher levels of linoleic acid (C18:2 n-6) than did the diets with either coconut fat or fish oil. Between farms, the dietary concentration of linoleic acid varied between 1.64 and 2.90 g/MJ ME.

Growth performance

Table 6 shows the average initial and final body weight and weight gain for each pen. During the feeding period of 56 days, the average daily weight gain for the pooled animals fed the diet with linseed oil was 0.518 kg. For the animals fed the diets with fish oil daily weight gain was 0.486 kg and for animals fed the diets with coconut oil it was 0.471 kg. ANOVA with farm as factor showed that the effect of different oils just failed to reach statistical significance (P = 0.077). The standard error of the difference was 0.021 kg. When the oil effects within farms were compared, it was found that linseed versus coconut oil significantly raised weight gain (P = 0.038; paired Student's t test) and that the stimulatory effect of linseed versus fish oil almost reached statistical significance (P=0.078).

Table 5. Fatty acid composition of the whole diets fed on the farms

Farm	*1*			*2*			*3*			*4*			*5*			*6*		
Diet[1]	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*
								Fatty acid (g fatty acid/MJ ME)										
C8:0	*0.32*	*0.02*	*0.02*	*0.30*	*0.00*	*0.00*	*0.33*	*0.02*	*0.02*	*0.33*	*0.02*	*0.02*	*0.32*	*0.02*	*0.02*	*0.32*	*0.02*	*0.02*
C10:0	*0.21*	*0.00*	*0.00*	*0.21*	*0.00*	*0.00*	*0.22*	*0.00*	*0.00*	*0.22*	*0.00*	*0.00*	*0.22*	*0.00*	*0.00*	*0.22*	*0.00*	*0.00*
C12:0	*1.69*	*0.00*	*0.00*	*1.69*	*0.00*	*0.00*	*1.76*	*0.00*	*0.00*	*1.77*	*0.00*	*0.00*	*1.69*	*0.00*	*0.00*	*1.73*	*0.00*	*0.00*
C14:0	*0.72*	*0.13*	*0.03*	*0.72*	*0.13*	*0.03*	*0.75*	*0.13*	*0.03*	*0.75*	*0.14*	*0.03*	*0.72*	*0.13*	*0.03*	*0.73*	*0.13*	*0.03*
C16: 0	*1.69*	*2.11*	*1.55*	*1.76*	*2.19*	*1.62*	*2.07*	*2.51*	*1.92*	*1.78*	*2.22*	*1.63*	*1.78*	*2.20*	*1.64*	*1.63*	*2.07*	*1.49*
C18: 0	*0.27*	*0.36*	*0.28*	*0.27*	*0.36*	*0.28*	*0.29*	*0.39*	*0.30*	*0.30*	*0.39*	*0.3*	*0.28*	*0.37*	*0.28*	*0.27*	*0.36*	*0.27*
C18: 1n-9	*2.14*	*2.56*	*2.56*	*2.22*	*2.64*	*2.64*	*2.64*	*3.08*	*3.08*	*2.51*	*2.94*	*2.94*	*2.13*	*2.55*	*1.96*	*1.96*	*2.39*	*2.39*
C18: 2n-6	*1.82*	*1.81*	*2.31*	*1.87*	*1.86*	*2.37*	*2.39*	*2.38*	*2.90*	*2.08*	*2.07*	*2.60*	*1.81*	*1.80*	*2.31*	*1.65*	*1.64*	*2.15*
C18: 3n-3	*0.26*	*0.28*	*2.33*	*0.17*	*0.19*	*2.23*	*0.29*	*0.31*	*2.44*	*0.29*	*0.30*	*2.44*	*0.28*	*0.29*	*2.34*	*0.31*	*0.32*	*2.41*
C20: 5n-3	*0*	*0.06*	*0*	*0*	*0.06*	*0*	*0*	*0.06*	*0*	*0.00*	*0.06*	*0.00*	*0*	*0.06*	*0*	*0.00*	*0.06*	*0.00*
C22: 6n-3	*0*	*0.72*	*0*	*0.02*	*0.73*	*0.01*	*0.01*	*0.76*	*0.01*	*0.03*	*0.78*	*0.03*	*0*	*0.72*	*0.03*	*0.03*	*0.76*	*0.03*

[1]Diet 1 contained coconut fat, diet 2 contained fish oil and diet 3 contained linseed oil.

Table 6. Average initial and final body weight for each pen (= diet) on the six small holdings

Farm	*1*			*2*			*3*			*4*			*5*			*6*		
Diet[1]	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*
Body weight (kg)																		
Initial	*12.0*	*12.7*	*12.3*	*11.5*	*10.4*	*10.5*	*11.2*	*12.3*	*12.1*	*12.2*	*12.2*	*12.3*	*11.8*	*11.7*	*11.8*	*11.8*	*12.1*	*12.2*
Final	*33.3*	*36.2*	*40.2*	*34.5*	*35.3*	*35.8*	*33.9*	*32.2*	*35.9*	*40.5*	*40.0*	*39.8*	*45.5*	*45.0*	*46.3*	*41.2*	*45.8*	*47.8*
Body-weight gain (kg/day)	*0.381*	*0.420*	*0.497*	*0.411*	*0.445*	*0.452*	*0.405*	*0.355*	*0.425*	*0.506*	*0.497*	*0.492*	*0.601*	*0.595*	*0.616*	*0.524*	*0.602*	*0.634*

[1]*Diet 1 contained coconut fat, diet 2 contained fish oil and diet 3 contained linseed oil.*

Fatty acid composition of adipose tissue

The relative percentage of EPA and DHA had increased when fish oil was included in the diet (Table 7). EPA was not detectable in pigs fed diets with coconut oil. The percentages of EPA and DHA on average were 0.0 and 0.21 % for pigs fed the diets with coconut oil, 0.05 and 0.26 % for pigs fed the diets with linseed oil, and 0.15 and 1.13 % for pigs fed the diets with fish oil. Linseed oil feeding induced high adipose tissue contents of ALA, the range being 7.63 to 13.19 %. The adipose tissue content of linoleic acid was not systematically influenced by the different diets fed on each farm. The pigs on farms 1 and 3 had the highest content of linoleic acid in adipose tissue and the lowest values were seen in the pigs on farm 6.

Correlations on a pen basis

For all pen mean data combined, linear correlation coefficients were calculated between the dietary contents of linoleic acid, ALA, EPA or DHA and those of adipose tissue. Dietary fatty acids were expressed as g/MJ ME and adipose tissue fatty acids as % of total fatty acids. There was a significant relationship between linoleic acid in diets and adipose tissue, the linear correlation coefficient (r) being 0.734. Fig. 1 shows the relationship for linoleic acid.

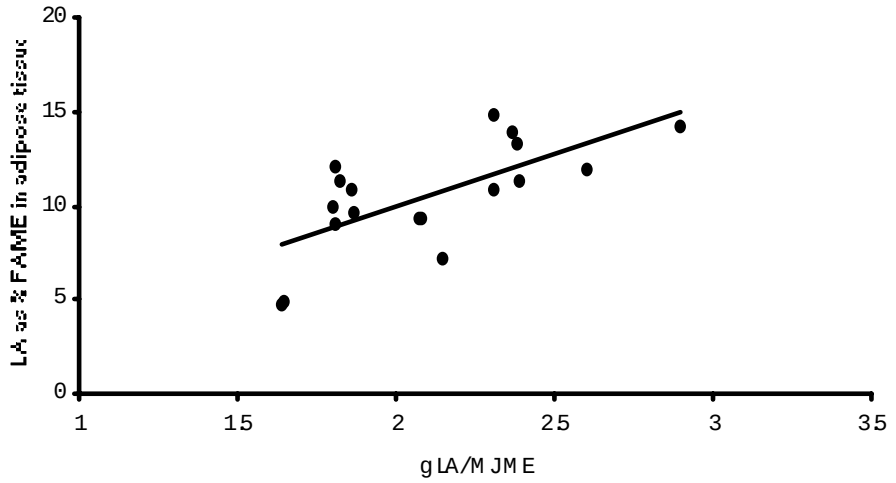

Fig. 1. Relationship between dietary and adipose tissue linoleic acid (LA) for pen means . The linear correlation coefficient was 0.73 and the regression equation is Y = -3.08 + 6.12x.

The relationships for ALA, EPA and DHA were based on clustering of the data on two sides of the scale. There were no significant correlations between the contents of one of the four polyunsaturated fatty acids in either the diet or adipose tissue on the one hand and growth on the other hand.

Table 7. Fatty acid composition of adipose tissue from pigs fed the different diets

Farm	*1*			*2*			*3*			*4*			*5*			*6*		
Diet[1]	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*
	Fatty acid (g fatty acid methylester/100 g methylesters)																	
C12:0	*2.69*	*0.00*	*0.05*	*2.20*	*0.14*	*0.09*	*2.68*	*0.41*	*0.33*	*1.69*	*0.39*	*0.34*	*1.57*	*0.06*	*0.07*	*1.14*	*0.05*	*0.04*
C14:0	*6.26*	*1.54*	*1.23*	*5.69*	*1.53*	*1.06*	*5.79*	*2.08*	*1.64*	*4.53*	*1.94*	*1.74*	*4.55*	*1.38*	*1.36*	*4.13*	*1.52*	*1.18*
C16:0	*25.3*	*23.34*	*19.9*	*25.65*	*24.2*	*18.9*	*25.34*	*24.32*	*20.55*	*26.10*	*24.54*	*21.85*	*25.76*	*23.85*	*22.96*	*27.20*	*25.40*	*21.78*
C18:1 n-9	*34.65*	*39.78*	*34.58*	*36.77*	*38.72*	*36.56*	*34.67*	*35.04*	*34.29*	*35.13*	*37.72*	*36.27*	*38.41*	*40.57*	*38.49*	*38.79*	*41.69*	*37.12*
C18: 2n-6	*11.27*	*12.11*	*14.81*	*9.68*	*10.76*	*13.95*	*11.27*	*13.33*	*14.22*	*9.28*	*9.38*	*11.93*	*9.08*	*9.92*	*10.84*	*4.88*	*4.78*	*7.24*
C18: 3n-3	*1.53*	*1.92*	*13.08*	*0.95*	*1.64*	*13.19*	*1.57*	*3.88*	*12.03*	*2.07*	*2.37*	*8.75*	*0.96*	*1.36*	*7.63*	*1.08*	*1.28*	*11.59*
C20:4n-6	*0.17*	*0.41*	*0.05*	*0.14*	*0.37*	*0.03*	*0.21*	*0.42*	*0.11*	*0.19*	*0.34*	*0.18*	*0.13*	*0.32*	*0.15*	*0.06*	*0.29*	*0.14*
C20:5n-3	*0*	*0.08*	*0.04*	*0*	*0.19*	*0.04*	*0.03*	*0.16*	*0.05*	*0*	*0.19*	*0.05*	*0*	*0.11*	*0.04*	*0*	*0.17*	*0.14*
C22: 6n-3	*0.23*	*1.31*	*0.23*	*0.05*	*0.82*	*0.1*	*0.30*	*1.34*	*0.34*	*0.5*	*1.24*	*0.45*	*0.11*	*1.07*	*0.12*	*0.34*	*1.0*	*0.36*

[1]*Diet 1 contained coconut fat, diet 2 contained fish oil and diet 3 contained linseed oil.*

Discussion

In agreement with earlier work (Nguyen et al. 2002a), the fatty acid composition of the diet was found to be related with the fatty acid composition of adipose tissue in growing swine. In the pigs fed fish oil, the adipose tissue generally was enriched with DHA. On the basis of literature data (Nguyen et al. 2002b), the expected relative percentage of DHA in adipose tissue of the pigs fed fish oil would be 1.36 %, whereas the observed value was 1.13 %. When the diets contained 5% linseed oil, the dietary ALA content was 2.4 g/MJ ME. The expected relative percentage of ALA in adipose tissue would be 10.0 %. The observed, average percentage of ALA in adipose tissue was 10.4 %. Thus, the observed concentrations of DHA and ALA in adipose tissue agreed with those expected. There was a direct relationship between linoleic acid intake and its percentage in adipose tissue (Fig. 1). The slope of the regression equation was similar to that calculated earlier on the basis of literature data (Nguyen et al. 2002b), but the intercept was negative and much lower.

The hypothesis tested in this study was that the feeding of extra ALA in the form of linseed oil, or EPA and DHA in the form of fish oil, would enhance growth. There was a stimulatory effect of linseed oil on growth when compared with either coconut fat or fish oil. However, there was no effect of fish oil when compared with coconut oil. It could be argued that the intakes of EPA and DHA were not sufficiently raised to induce differences in growth performance between the treatments.Nevertheless, we did observe an increase in the percentages of EPA and DHA in adipose tissue after fish oil feeding. The stimulatory effect of linseed oil on growth may be unrelated to enhanced conversion of ALA into EPA and DHA, but points at the contents of ALA in the diets with either coconut fat or fish oil being insufficient to sustain maximum growth. Possibly, ALA has a specific and direct action on growth, the underlying mechanism being obscure. Alternatively, linseed oil may contain an unknown factor that stimulates growth. In any event, the present observation supports our field study in which we compared twelve small holdings in Central Vietnam and found a positive relationship between ALA in adipose tissue and average daily gain (Nguyen et al., 2002a). As linseed oil was not fed on those farms, it would follow that ALA rather than another component of linseed oil is responsible for the growth-enhancing effect seen in this study.

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References

Calder, P.C. (1996). Immunomodulatory and anti-inflammatory effects of n-3 polyunsaturated fatty acids. Proc. Nutr. Soc., 55: 737-774.

Calder, P.C. (1997). N-3 polyunsaturated fatty acids and cytokine production in health and disease. Ann. Nutr. Metab, 41: 203-234.

Folch, J., Lees, M., & Sloane Stanley, G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry, 226, 497-509.

Fritsche, K. L., D.W. Alexander, N.A. Cassity and S.C. Huang, (1993). Maternally supplied fish oil alters piglet immune cell fatty acid profile and eicosanoid production. Lipids, 28: 677-682.

Fritsche K.L., S.C. Huang, N.A. Cassity (1993). Enrichment of omega-3 fatty acids in suckling pigs by maternal dietary fish oil supplementation. J. Anim. Sci. 71:1841-1847.

Innis, S.M. (1991). Essential fatty acids in growth and development. Progress in Lipid Research, 30: 39-103.

Metcalfe, L.D., A.A. Schmitz and J.R. Pekka (1966). Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Analytical Chemistry, 18: 514-516.

Nguyen, L.Q., Everts, H., & Beynen, A.C. (2002a). Intake of essential fatty acids by growing-finishing pigs kept on small holdings in Central Vietnam. Tropical Animal Health and Production (in press).

Nguyen, L.Q., M.C.G.A. Nuijens, H. Everts, N. Salden, and A.C. Beynen (2002b). Mathematical relationships between the intake of n-6 and n-3 polyunsaturated fatty acids and their contents in adipose tissue of growing pigs. Meat Science (in press).

Thies, F., L.D. Peterson, J.R. Powell, G. Nebe-von-Caron, T.L. Hurst, K.R. Matthews, E.A. Newsholme, and P.C. Calder, (1999). Manipulation of the type of fat consumed by growing pigs affects plasma and mononuclear cell fatty acid compositions and lymphocyte and phagocyte functions. J. Anim. Sci. 77: 137-147.

Turek, J.J., I.A. Schoenlein, B.A.Watkins, W.G. van Alstine, (1994). Dietary polyunsaturated fatty acid effects on immune cells of porcine lung. J. Leukc. Biol. 56: 599-604.

Turek, J.J., I.A. Schoenlein, B.A.Watkins, W.G. van Alstine, L.K. Clark and K. Knox, (1996). Dietary polyunsaturated fatty acids modulate responses of pigs to mycoplasma hyopneumoniae infection. J. Nutr. 126: 1541-1548.

Wu, D., and N. Meydani (1998). n-3 Polyunsaturated fatty acids and immune function. Proceedings of the Nutrition Society, 57: 503-509.

Chapter 7

Feeding of spinach or sweet-potato leaves and growth performance of growing pigs kept on small-holder farms in Central Vietnam

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Abstract

We have investigated whether the addition of either spinach or sweet-potato leaves to the diet of growing pigs, kept in small holdings in Central Vietnam, would improve growth performance. A control diet was formulated and mixed with each of the vegetables to a final concentration of 15 % in the total dietary dry matter. The diets were fed to the pigs from 70 to 100 days of age on 6 different small-holder farms in Central Vietnam. There were three animals per treatment group per farm and biopsies of adipose tissue were analysed for their contents of α-linolenic, eicosapentaenoic and docosahexaenoic acid. The diets without and with vegetables on average contained 0.14 and 0.32 g α-linolenic acid/MJ of metabolizable energy (ME). The relative percentage of α-linolenic acid in adipose tissue was raised by the intake of the vegetables. Eicosapentaenoic acid was not detectable in adipose tissue and the level of docosahexaenoic acid was unchanged. There was a significant stimulatory impact of the intake of either spinach or sweet-potato leaves on growth performance of the growing pigs. It is suggested that an adipose tissue content of α-linolenic acid less than 1 % of total fatty acids does not allow maximum growth in growing pigs.

Introduction

In a field study involving small holdings in Central Vietnam, we found that the content of α-linolenic acid ALA, (C18:3 n-3) in adipose tissue of growing swine was directly, and significantly, related with average daily weight gain, the explained variance of weight gain being 20 % (NGUYEN et al. 2002a). In swine, the concentration in adipose tissue of ALA is determined by the intake of this fatty acid (NGUYEN et al. 2002b). The n-3 polyunsaturated fatty acid, ALA, can be converted in the body into eicosapentaenoic acid (C20:5 n-3) which is the direct precursor of various eicosanoids, some of them affecting the immune system (WU and MEYDANI 1998). High intakes of eicosapentaenoic acid and docosahexaenoic acid (C22:6 n-3) in the form of fish oil may improve disease resistance in pigs (FRITSCHE et al. 1993). We have suggested that in growing pigs, kept on the small-holder farms in Central Vietnam, high intakes of ALA were associated with high production rates of eicosapentaenoic and docosahexaenoic acid and thus enhanced disease resistance which in turn improved growth performance (NGUYEN et al. 2002a).

We wished to test our idea that the addition of ALA to the diet of growing pigs kept in small holdings in Central Vietnam would improve growth performance. Under practical conditions, spinach or sweet-potato leaves can serve as a source of ALA, the contents being about 35 and 40 % in the oil component, respectively. Our idea was tested by mixing fresh spinach or sweet-potato leaves with a base diet to a final concentration of 15 % in the total dietary dry matter. The farmers participating in the study composed the base diets according to our instructions and added the dietary variables. The spinach and sweet-potato leaves were supplied to the farms and, under the supervision of students, the two vegetables were mixed with the base diets at the pre-set ratios. In the pigs, growth performance and the fatty acid profile of adipose tissue were measured.

Materials and methods

Animals and experimental diets

Castrated, male weanling pigs (n=54) were purchased from a breeding farm and allocated to 6 small holdings. The piglets were of a Mong cai (female) x Large White (male) cross, were aged 70 days and had a body weight of 10.7 ± 1.06 kg (mean ± SD). Each farm received 9 animals, which were housed in pens containing three piglets each. Within each farm, the three piglets in each pen had similar average body weights and distributions. Each piglet had an ear-cut number for identification. The pigs were fed a restricted amount of dry matter according to body weight. The feeding schedule was as follows. On arrival the pigs were given 0.54 kg dry matter/day, this amount being gradually raised to 0.85 kg/day by the end of the experiment.

The farmers were individually instructed by B.Sc. students of animal science who were present on the farms. The students used a local feed table to assess the dry matter contents of the ingredients. The spinach and sweet-potato leaves were supplied to the farms by the students. The base diets were composed by the farmers according to our instructions and consited of the following (g/kg as fed): rice bran, 320; maize, 220; cassava meal, 180; beer byproduct, 120; groundnut meal, 150; bone meal, 10. The calculated dry matter content of the base diet was 806 g/kg. To 1 kg of base diet, which also served as control diet, either 1700 g of fresh spinach or 1070 g of sweet-potato leaves were added. The added amounts of spinach and potato leaves were equivalent to 150 g of dry matter. The

calculated dry matter contents of the diets containing either spinach or potato leaves were 313 and 405 g/kg, respectively. On each farm, the three diets were used. It was verified that the rations contained at least 17 % crude protein in the dry matter as calculated on the basis of the local feed table. The feedstuffs supplied on each farm were weighed on identical balances. The pigs were fed 2 times/day. Water was available ad libitum through nipples that were situated besides the trough in each pen. All pigs were weighed at the start and end of the experiment. The pigs were weighed individually while in a crate of known weight and using a calibrated balance.

Sample collection

For each diet, a composite sample was prepared. Subcutaneous adipose tissue samples were taken from all piglets at the end of the experiment, when they were aged about 100 days. After disinfection and induction of local anaesthesia, a 5-10 mm incision was made in the right inguinal region. About 1 g of subcutaneous fat was removed and stored at - 20 ^{0} C. The skin wound was closed. No complications occurred.

Chemical analyses

Gross energy in the feed samples was determined by oxygen bomb calorimetry. Dry matter, crude protein, crude fat, crude fibre and ash were measured by the Weende analysis. Total lipids in the feed samples were extracted with chloroform:methanol, 2:1 (FOLCH et al. 1957). The lipids were transesterified with 12% BF_3 in methanol. The methyl esters were extracted with petroleum ether, taken to dryness under nitrogen, redissolved in heptane and separated and quantified by gas-liquid chromatography as described (METCALFE et al. 1966). The adipose tissue samples were saponified, methylesters of fatty acids were formed and subjected to gas-liquid chromatography.

Statistical analysis

ANOVA and the paired Student's t test were used to evaluate treatment effects. The level of statistical significance was pre-set at $P < 0.05$.

Results

Feed composition

The analysed composition of the spinach and sweet-potato leaves is shown in Table 1. The vegetables contained about 6 % crude fat in the dry matter of which 35 or 41 % was ALA. The macronutrient composition of the diets is shown in Table 2. The diets contained 17.2 – 18.3 % crude protein in the dry matter. Crude fat contents of the diets had a range of 11.1 to 11.5 %. Crude fibre and ash contents of the diets were higher for the diets with added vegetables than for the control diet. The calculated dietary contents of metabolizable energy (ME) are given in Table 2.

Table 1. Analysed composition of the spinach and sweet-potato leaves

Nutrient	Spinach	Sweet-potato leaves
Macronutrients, g/kg DM[1]		
Crude protein	209	253
Crude fat	62	64
Crude fiber	137	127
Ash	102	118
Fatty acids, g methylester/100 g methylesters		
C16:0	21.1	16.5
C18:0	3.8	3.5
C18:1 n-9	3.3	3.4
C18:2 n-6	17.1	17.7
C18:3 n-3	35.0	40.9

[1]*DM = dry matter.*

Table 2. Analysed macronutrient composition of the experimental diets fed on the different farms

	Diet		
	Control	Spinach	Sweet-potato leaves
Crude protein (% in dm [1])	17.2	18.3	18.2
Crude fat (% in dm)	11.3	11.1	11.5
Crude fibre (% in dm)	6.7	8.0	7.5
Ash (% in dm)	5.5	6.7	6.9
ME[2] (MJ/kg dm)	13.13	12.74	12.55

[1]*dm = dry matter.*
[2]*Metabolizable energy (ME) was on calculated on the basis of the measured gross energy, table values for digestibility of nutrients in the diet ingredients and by assuming that ME = 0.96 x digestible energy.*

Table 3 shows the fatty acid composition of the diets; the contents are expressed as g of fatty acid/ MJ ME. The levels of ALA were about two-fold higher for the diets with vegetables than for the control diet. The linoleic acid content of the control diet was somewhat higher.

Table 3. Fatty acid composition of the experimental diets fed on the farms

	Diet		
	Control	Spinach	Sweet-potato leaves
	g fatty acid/MJ ME		
C16:0	1.60	1.82	1.91
C18:0	0.25	0.27	0.29
C18:1 n-9	2.98	2.66	2.81
C18:2 n-6	3.07	2.65	2.75
C18:3 n-3	0.14	0.32	0.32

Growth performance

Table 4 shows the average initial and final body weight gain for each pen. During the feeding period of 28 days, the average daily weight gain for the pooled animals fed the control diet was 0.367 kg. For the animals fed the diets with spinach or potato leaves, daily weight gain was 0.474 and 0.432 kg, respectively. ANOVA with farm as factor showed that there was a significant effect of diet (P = 0.027). The standard error of the difference was 0.038 kg. When compared with the control diet, both the diet containing spinach and that containing sweet-potato leaves significantly enhanced growth (paired Student's t-test).

Fatty acid composition of adipose tissue

Feeding either spinach or sweet-potato leaves significantly raised the percentage ALA in adipose tissue (Table 5). In adipose tissue, eicosapentaenoic acid was not detectable. The relative percentage of docosahexaenoic acid was not altered when the vegetables were included in the diet (Table 5).

Table 5. Fatty acid composition of adipose tissue from pigs pooled per diet

	Diet				
Fatty acid	Control	Spinach	Sweet-potato leaves	Pvalue[1]	SED[2]
	g methylester/100 g methylesters				
C18:	10.4	9.8	10.5	0.258	0.40
C18:1 n-9	40.3	39.7	39.5	0.416	0.56
C18:2 n-6	13.6	14.4	14.3	0.483	0.71
C18:3 n-3	0.96	1.33	1.17	0.006	0.09
C20:4 n-6	0.29	0.28	0.28	0.977	0.03
C20:5 n-3	0	0	0	-	-
C22:6 n-3	0.22	0.22	0.17	0.370	0.04

Results presented as means for 18 pigs per diet
[1]P value based on ANOVA with farm as factor.
[2]SED = standard error of difference.

Table 4. Average initial and final body weight for each pen (= diet) on the six small holdings

Farm	1			2			3			4			5			6		
Diet[1]	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Body weight (kg)																		
Initial	11.2	11.5	11.3	10.8	9.2	9.3	11.7	11.4	11.8	9.8	9.7	9.8	11.0	10.8	10.8	1.7	10.5	10.5
Final	26.1	28.3	26.8	20.8	22.7	21.3	20.0	25.0	24.5	17.9	22.3	20.6	23.0	23.8	23.7	19.0	20.6	19.3
Weight gain (kg/day)	0.531	0.600	0.554	0.356	0.483	0.426	0.296	0.485	0.454	0.289	0.451	0.385	0.430	0.462	0.461	0.299	0.361	0.315

[1]*Diet 1 was the control diet, diet 2 contained spinach and diet 3 contained sweet-potato leaves.*

Discussion

The incorporation of either spinach or sweet-potato leaves into the diet raised the intake of ALA. In the pigs fed the vegetables, the adipose tissue contained slightly, but significantly more ALA, whereas eicosapentaenoic acid remained undetectable and docosahexaenoic acid was unaltered. When the diets contained the vegetables, the average dietary ALA content was 0.32 g/MJ ME. According to previously established regression equations (NGUYEN et al. 2002b), the expected relative percentage of ALA in adipose tissue would be 2.57 %. The mean percentage of ALA in the adipose tissue of the pigs fed the diets with the vegetables was 1.23 %. Thus, the observed concentration of ALA in adipose tissue was much lower than that expected. Possibly, the discrepancy relates to a high rate of de novo fatty acid synthesis in the Vietnamese swine, leading to extra dilution of the flux of dietary fatty acids into adipose tissue.

The question addressed in this study was whether the feeding of extra ALA in the form of either spinach or sweet-potato leaves would enhance growth. The diets were formulated so that protein supply would not limit growth. There was a significant effect of the two vegetables on growth. Thus, the data indicate that extra intake of ALA stimulated growth. In another feeding trial with ruminant and fish meal as dietary variables (NGUYEN et al. 2002c), we found a statistically significant, direct relationship between the percentage of ALA in adipose tissue and growth, the explained variance of average daily weight gain being 44 %. That observation and the present one support our field study in which we compared twelve small holdings in Central Vietnam (NGUYEN et al., 2002b). The observed positive relationship between ALA in adipose tissue and average daily gain might indicate that ALA intake with the control diet was too low to sustain maximum growth.

The growth-enhancing effect of the feeding of either spinach or sweet-potato leaves was associated with an increase of the ALA concentration in adipose tissue, but eicosapentaenoic acid remained undetectable and docosahexaenoic acid was unchanged. The putative effect of ALA on growth may be independent of the formation of eicosapentaenoic and docosahexaenoic acid. The requirement of ALA for growing pigs is not known. This study and our previous studies (NGUYEN et al., 2002a,c) indicate that an ALA content less than 1 % of total fatty acids in adipose tissue may be associated with low growth rates. On the basis of literature data it follows that 1 % of ALA in adipose tissue is equivalent with about 0.11 g ALA/MJ ME of diet (NGUYEN et al. 2002b). The control diet in this study contained 0.14 g ALA/MJ ME.

In conclusion, the intake of additional spinach or sweet-potato leaves stimulated growth of swine kept in small holdings in Central Vietnam. It would thus appear that extra intake of ALA is required for an increase in average daily weight gain. The basis and implication of this effect of ALA requires further study.

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References

FOLCH, J.; LEES, M.; SLOANE-STANLEY, G.H., 1957: J. Biol. Chem. 226, 497-509.

FRITSCHE, K.L.; HUANG, S.-C.; CASSITY, N.A., 1993: J. Anim. Sci. 71, 1841-1847.

NATIONAL RESEARCH COUNCIL, 1998: Nutrient Requirements of Swine, National Academy Press, Washington, D.C.

NGUYEN, L.Q.; EVERTS, H.; BEYNEN, A.C., 2002a: Trop. Anim. Health Prod. (in press)

NGUYEN, L.Q.; NUIJENS, M.C.G.A.; EVERTS, H.; SALDEN, N.; BEYNEN, A.C., 2002b: Meat Sci. (in press)

NGUYEN, L.Q.; EVERTS, H.; DUONG, N..; TANH, P.V.; BEYNEN, A.C., 2002c: J. Anim. Physiol. a. Anim. Nutr. (in press).

METCALFE, L.D.; SCHMITZ, A.A.; PELKA, J.R., 1966: Anal. Chem. 18, 514-516.

WU, D.; MEYDANI, S.N., 1998: Proc. Nutr. Soc. 57, 503-509.

General conclusions

This thesis has focussed on the dietary provision of linoleic acid (LA), α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) to growing pigs in relation to growth performance. In particular, this study aimed at obtaining information so as to optimise the level of polyunsaturated fatty acids (PUFAs) in rations used on small-scale farms in Central Vietnam. The major results and conclusions of the various chapters above may be summarized as follows.

The intakes of LA, ALA, EPA or DHA are directly correlated with their concentrations in adipose tissue of growing pigs, and the correlations can be expressed in the form of equations with high degree of predictability

Chapter 2 presents mathematical relationships between the intakes of individual PUFAs and their concentrations in adipose tissue under controlled conditions. The intake of fatty acids is expressed as g/MJ of metabolizable energy (ME) and the level in adipose tissue as percentage of total fatty acids. The observed linear correlation coefficients are as high as 0.83. Under less controlled conditions at the small-holder farms, direct relationships between dietary and adipose tissue LA (Chapters 2, 3, 4, 5, 6), ALA (Chapters 2, 3, 4, 5) or DHA (Chapters 4, 5) were also observed, but the correlations were less strong.

The current recommended intake of LA for growing pigs, i.e. 0.08 g/MJ of metabolizable energy, may be too low to support maximum growth in growing pigs.

A meta-analysis based on literature data indicates that for rapidly growing pigs (average daily gain, ADG > 750 g/day) an increase in LA intake by 1.0 g/MJ is associated with an increase in weight gain by 50 g/day (Chapter 1). In a field study involving 12 small holdings in Central Vietnam, there was a significant correlation between the LA content of adipose tissue and ADG, the explained variation of ADG being 14 % (Chapter 3). Adipose tissue LA contents were above 8 % of total fatty acids, which is equivalent to LA intakes above 0.1 g/MJ (Chapter 2). In another study in which farmers added either ruminant or fish meal to the ration of their own choice, there also was a direct relationship between LA in the diet and ADG (Chapter 4). Intakes above 1.5 g LA/MJ ME were associated with enhanced growth.

ALA intakes above 0.05 g/MJ may not stimulate growth in growing pigs with ADG above 700 g/MJ, but up to an intake level of 0.3 g/MJ, the ingestion of extra ALA may increase ADG in pigs kept on the Vietnamese farms.

Based on literature data it is concluded that maximum growth in relation to ALA intake may be achieved at intake levels of about 0.05 g/MJ (Chapter 1). Various studies carried out on small-holder farms in Central Vietnam indicate that ALA intake is directly associated with ADG (Chapters 3, 4, 6 and 7). In the field study (Chapter 3), ALA in adipose tissue and ADG were significantly correlated (r = 0.45) and this was also found (r = 0.66) in another study (Chapter 4) in which three different diets were fed on each of the six farms. The incorporation of linseed oil into the ration, instead of coconut fat, was found to significantly raise ADG (Chapter 6). The consumption of linseed oil caused a marked

increase in ALA intake and consequently raised the ALA concentration in subcutaneous adipose tissue. When the diet was mixed with either spinach or sweet-potato leaves there was a significant increase in ADG associated with an increase in the ALA content of adipose tissue (Chapter 7). An ALA content in adipose tissue less than 1 % of total fatty acids may be associated with low growth rates, which is equivalent with a dietary ALA concentration less than 0.11 g/MJ. The addition of either spinach or sweet-potato leaves to the diet raised the ALA level from 0.14 to 0.32 g/MJ ME. In the trial with linseed oil an increase in ALA intake from 0.26 to 2.36 g/MJ was associated with higher ADG. It is likely that maximum growth was reached at an ALA intake much lower than 2.36 g/MJ ME. It is suggested that a dietary ALA level of at least 0.3 g/MJ ME is required for maximum growth.

The stimulatory effect of extra ALA intake on ADG in low-performing pigs may not relate to improved disease resistance.

There is evidence from rodent studies, that extra intake of EPA and DHA in the form of fish oil improves resistance against infectious disease. However, on the basis of literature data it is concluded that there is no evidence that under conditions of infectious pressure in practice, growth of pigs may be enhanced by the intake of extra n-3 PUFAs. In the body ALA is converted into EPA and DHA, but the positive effects of ALA on ADG may not relate to this conversion. Fish oil, which is rich in DHA, was found to stimulate growth when compared with coconut fat, but not when compared with linseed oil, which is rich in ALA (Chapter 6). The observed effects of ALA on ADG were associated with an increase in the ALA content of adipose tissue, but not with an increase of EPA and DHA in adipose tissue (Chapters 6 and 7). The lack of increase of EPA and DHA in adipose tissue points at ALA conversion in tissues other than adipose tissue. The observed lack of effect of fish-meal versus ruminant-feeding on ADG may be explained by either lack of EPA effect on growth or the intake of EPA not being sufficiently raised (Chapter 4). Like the fat fraction of fish meal, that of shrimp byproduct meal also is rich in EPA. Shrimp byproduct meal versus ruminant meal was found to raise ADG, but this effect probably was unrelated to the extra intake of EPA (Chapter 5). Thus, the data indicate that for maximum growth dietary ALA is more crucial than are EPA and DHA.

For optimum growth as function of fatty acid intake the swine rations on small holdings in Central Vietnam should contain at least 2 g LA/MJ and 0.3 g ALA/MJ ME.

The studies described have yielded knowledge on the relationship between PUFA intake and ADG in growing-finishing pigs kept on small-scale farms in Central Vietnam. It is suggested that supplementary intakes of LA and ALA, up to dietary concentrations of 2 and 0.3 g/MJ ME, respectively, may increase ADG. Such intakes are high when compared to the optimum intakes that were derived from the literature. However, the pigs in the present studies had relatively low weight gain and high body fat. Furthermore, in these studies LA and ALA were not the only dietary variables so that unknown, associated factors may have been responsible, either fully or in part, for the observed direct correlations between the intakes of either LA or ALA and growth.

SUMMARY

This thesis concerns the influence of essential dietary fatty acids on the fatty acid composition of adipose tissue and growth performance of growing pigs. Essential fatty acids cannot be synthesized by the body and have to be ingested with the feed. There are two families of essential polyunsaturated fatty acids (PUFAs) that cannot be metabolically interconverted. Linoleic acid (LA; C18:2 n-6) is the parent compound of the n-6 PUFAs and α-linolenic acid (ALA; C18:3 n-3) that of the n-3 PUFAs. In the body, LA can be converted into the n-6 PUFA arachidonic acid (AA; C20:4 n-6) and ALA can be elongated and desaturated to eicosapentaenoic acid (EPA; C20:5 n-3) and docosahexaenoic acid (DHA; C22:6 n-3). The n-6 and n-3 PUFAs are essential structural components of the cell membranes and are precursors of eicosanoids, compounds affecting functions such as immunity, platelet aggregation and vasoconstriction. It is well-known that the quantitative intakes of n-6 and n-3 PUFAs are reflected in the adipose tissue of non-ruminants. This implies not only that the fatty acid composition of adipose tissue can be modulated by diet, but also that the adipose tissue composition can be used as an index of the qualitative PUFA intake.

The LA requirement of growing pigs has been set at 0.08 g/MJ metabolizable energy (ME), but the requirement of ALA is not known. On the basis of literature data, Chapter 1 indicates that the current recommended intake of LA is too low to sustain maximum growth. Intakes of ALA higher than 0.05 g/MJ may not further enhance growth. Review of the literature did not provide evidence for a positive effect of the intake of extra n-3 PUFAs on disease resistance, at least not under realistic, practical conditions. In one published study, a high intake of EPA and DHA reduced the severity of lung laesions as induced by *Mycoplasma hyopneumoniae* infection, but the EPA and DHA intakes were unrealistically high and the diet used had a LA level below the requirement for maximum growth.

Chapter 2 describes the mathematical relationships between the fatty acid composition of adipose tissue and PUFA intake by growing pigs. For LA, ALA, EPA and DHA there were strong correlations. The slopes of the regression equations for the relationships between dietary and adipose tissue PUFAs can be considered a measure of the efficiency of incorporation of the dietary fatty acids into adipose tissue. The slope was steepest for LA and most shallow for EPA. The mathematical relationships were used to verify PUFA intake in subsequent studies carried out on small-holder farms in Central Vietnam. On those farms, mixtures of locally available feedstuffs with varying quality are fed which makes it difficult to assess fatty acid intake by the pigs.

An inventory of fatty acid intake and average daily gain (ADG) of pigs was made on 12 small-scale farms on which the pigs were fed according to the farmers' choice of feedstuff mixtures. The feeding of higher amounts of LA and ALA was associated with higher percentages of these fatty acids in adipose tissue of growing pigs (Chapter 3). There were weak, but statistically significant, positive correlations between either adipose tissue content of LA or that of ALA and ADG.

In an attempt to raise EPA and DHA intake, the farmers were instructed to use either fish meal or specially prepared ruminant meal as constituent of the ration (Chapter 4). The diets without and with 20% (% in total dietary dry matter) fish meal on average contained

0.01 and 0.03 g EPA/MJ ME and 0.00 and 0.25 g DHA/MJ ME. The relative percentages of DHA, ALA and LA in adipose tissue were reflected by the intake of the corresponding fatty acids. EPA was not detectable in adipose tissue. There was no impact of fish meal intake on growth performance of the growing pigs. The intake of EPA or DHA was not related with ADG, and neither was the adipose tissue content of DHA. Adipose tissue content of ALA or that of LA were significantly correlated with ADG. Thus, it was concluded that on the farms ALA and LA intake were limiting growth.

In a further study (Chapter 5) EPA and DHA were fed in the form of shrimp by-product meal, essentially consisting of shrimp heads. The diets without and with 20% shrimp meal on average contained 0.01 and 0.13 g DHA/MJ ME. The relative percentage of ALA in adipose tissue was directly related with the intake of this fatty acid. EPA was not detectable in adipose tissue, but the content of DHA was increased after consumption of shrimp by-product meal. The intake of DHA or its content in adipose tissue was not related with ADG. Inclusion of shrimp by-product meal caused an increase in the ash and crude-fibre content of the ration. Nevertheless, there was a general stimulatory effect of shrimp by-product meal on growth.

The hypothesis tested in Chapter 6 was that feeding of either linseed or fish oil, instead of coconut oil, would enhance growth of growing pigs kept on small holdings. The linseed oil used contained 57 % ALA and the fish oil contained 1.5 % EPA and 20 % DHA. The diet with 5% linseed oil on average contained 2.4 g ALA/MJ and the diet with 5% fish oil on average contained 0.06 and 0.75 g EPA and DHA/MJ ME, respectively. The relative percentages of DHA, EPA, ALA and LA in adipose tissue were found to be determined by the intake of the corresponding fatty acids. Dietary linseed oil versus coconut oil significantly enhanced ADG, and when compared with fish oil, it also stimulated growth, but this effect just failed to reach statistical significance. It was concluded that extra intake of ALA may stimulate growth, this effect being independent of conversion of ALA into EPA and DHA.

In Chapter 7, the question addressed was whether the addition of either spinach or sweet-potato leaves to the diet of growing pigs, kept in small holdings in Central Vietnam, would improve growth performance. The diets contained 15 % of each of the vegetables in the total dietary dry matter. The spinach leaves contained 35 % ALA in its fat component and for the sweet-potato forage the value was 41 %. The diets without and with vegetables on average contained 0.14 and 0.32 g of ALA/MJ ME. The relative percentage of ALA in adipose tissue was raised by the intake of the vegetables. There was a significant, stimulatory impact of the intake of either spinach or sweet-potato leaves on growth performance of the growing pigs.

The studies described have yielded knowledge on the relationship between PUFA intake and ADG in growing-finishing pigs kept on small-scale farms in Central Vietnam. It is suggested that supplementary intakes of LA and ALA, up to dietary concentrations of 2 and 0.3 g/MJ ME, respectively, may increase ADG. Such intakes are high when compared to the optimum intakes that were derived from the literature. However, the pigs in the present studies had relatively low weight gain and high body fat. Furthermore, in these studies LA and ALA were not the only dietary variables so that unknown, associated factors may have been responsible, either fully or in part, for the observed direct correlations between the intakes of either LA or ALA and growth.

SAMENVATTING

Dit proefschrift beschrijft de invloed van essentiële vetzuren in de voeding op de vetzuursamenstelling van onderhuids vetweefsel en de groei van vleesvarkens. Essentiële vetzuren kunnen niet in het lichaam worden gesynthetiseerd en moeten met de voeding worden opgenomen. Er zijn twee families van essentiële, meervoudig onverzadigde vetzuren (polyunsaturated fatty acids, PUFAs) die niet in het lichaam in elkaar kunnen worden omgezet. Linolzuur (linoleic acid, LA; C18:2 n-6) is het stamvetzuur van de n-6 PUFAs en α-linoleenzuur (α-linolenic acid, ALA; C18:3 n-3) is het stamvetzuur van de n-3 PUFAs. In het lichaam kan LA worden omgezet in arachidonzuur (arachidonic acid, AA; C20:4 n-6) en ALA kan worden verlengd en gedesatureerd tot eicosapentaeenzuur (eicosapentaenoic acid, EPA; C20:5 n-3) en docosahexaeenzuur (docosahexaenoic acid, DHA; C22:6 n-3). De n-6 en n-3 PUFAs zijn essentiële, structurele componenten van membranen en fungeren als bouwstenen voor de eicosanoiden, een groep van hormoonachtige stoffen die functies beïnvloeden zoals immuniteit, aggregatie van bloedplaatjes en vasoconstrictie. Het is bekend dat de kwantitatieve opname van n-6 en n-3 PUFAs wordt weerspiegeld in het vetweefsel van niet-herkauwers. Dit betekent niet alleen dat de vetzuursamenstelling van het vetweefsel beïnvloed wordt door de voeding maar ook dat de vetweefselsamenstelling gebruikt kan worden als indicator van de kwalitatieve PUFA-opname.

De LA-behoefte van vleesvarkens is gesteld op 0,08 g/MJ metaboliseerbare energie (ME), maar de ALA-behoefte is onbekend. Op basis van literatuurgegevens geeft Hoofdstuk 1 aan dat de huidige LA-aanbeveling te laag is om maximale groei te realiseren. Opnames van ALA hoger dan 0,05 g/MJ leidden niet tot extra groei. Literatuuronderzoek gaf geen onderbouwing voor een positief effect van extra opname van n-3 PUFAs op ziekteweerstand, tenminste niet onder realistische, praktische condities. In één onderzoek reduceerde een hoge opname van EPA en DHA de ernst en omvang van longlaesies na infectie met *Mycoplasma hyopneumoniae*, maar de opnameniveaus van EPA en DHA waren onrealistisch hoog terwijl het gebruikte rantsoen een LA-gehalte had dat lager was dan de aanbevolen hoeveelheid voor maximale groei.

Hoofdstuk 2 beschrijft de mathematische verbanden tussen de vetzuursamenstelling van het vetweefsel en de PUFA-opname bij vleesvarkens. Voor zowel LA, ALA, EPA als DHA werden sterke correlaties gevonden. De helling van de regressielijnen voor de relatie tussen voedings- en vetweefsel-PUFAs kan worden beschouwd als maat voor de efficiëntie van de vetzuurinbouw in het vetweefsel. De helling was het steilst voor LA en het geringst voor EPA. De mathematische verbanden werden gebruikt om de PUFA-opname te verifiëren in vervolgonderzoek bij varkens van keuterboeren in Centraal Vietnam. Op de bedrijfjes worden mengsels van lokaal verkrijgbare voedermiddelen met wisselende kwaliteit gevoerd hetgeen het moeilijk maakt om een betrouwbare indruk van de vetzuuropname door de varkens te krijgen.

Een inventarisatie is gemaakt van de vetzuuropname en de gemiddelde dagelijkse gewichtstoename (average daily gain, ADG) op 12 bedrijfjes alwaar de varkens werden gevoerd op basis van de voedermiddelenkeuze van de keuterboeren. Verstrekking van grotere hoeveelheden LA en ALA was geassocieerd met hogere gehalten van deze vetzuren in het vetweefsel van de vleesvarkens (Hoofdstuk 3). Er waren zwakke, maar statistisch

significante, positieve correlaties tussen zowel het LA- als ALA-gehalte van het vetweefsel enerzijds en de ADG anderzijds.

In een poging om de EPA- en DHA-opname te verhogen, werden de varkenshouders geïnstrueerd om vismeel of speciaal bereid herkauwermeel als onderdeel van het rantsoen te gebruiken. De rantsoenen zonder en met 20% (% in de totale droge stof van de voeding) vismeel bevatten gemiddeld 0,01 en 0,03 g EPA/MJ en 0,00 en 0,25 g DHA/MJ ME. De percentages DHA, ALA en LA in vetweefselvet werden gereflecteerd door de opname van de corresponderende vetzuren. EPA was niet detecteerbaar in het vetweefsel. Vismeel in het rantsoen had geen invloed op de groei van de varkens. De opname van EPA of DHA was niet gerelateerd aan de ADG en dit gold ook voor het DHA-gehalte in het vetweefsel. Zowel het ALA- als het LA-gehalte van het vetweefsel was significant gecorreleerd met de ADG. Het werd geconcludeerd dat op de bedrijven de ALA- en LA-opname beperkend waren voor de groei van de varkens.

In verder onderzoek op de bedrijfjes (Hoofdstuk 5) werden EPA en DHA verstrekt in de vorm van meel van een garnalenbijproduct, met name bestaande uit garnalenkoppen. De rantsoenen met 20% garnalenmeel bevatten gemiddeld 0,01 en 0,13 g DHA/MJ ME. Het percentage ALA in vetweefselvet was direct gerelateerd aan de opname van dit vetzuur. EPA was niet aantoonbaar in vetweefsel, maar het DHA-gehalte was verhoogd na consumptie van het garnalenbijproduct. Noch de DHA-opname, noch het DHA-gehalte van het vetweefsel, was gecorreleerd met de ADG. Toevoeging van garnalenmeel aan het rantsoen veroorzaakte een hoger as- en ruwe celstofgehalte. Desalniettemin was er een stimulerend effect van het garnalenbijproduct op de groei.

De te toetsen hypothese in Hoofdstuk 6 was dat de verstrekking van lijnzaadolie of visolie, in plaats van kokosvet, de groei zou stimuleren van vleesvarkens op Vietnamese bedrijfjes. De gebruikte lijnzaadolie bevatte 57 % ALA en de visolie bevatte 1,5 % EPA en 20 % DHA. Het rantsoen met 5% lijnzaadolie bevatte gemiddeld 2,4 g ALA/MJ en het rantsoen met 5% visolie bevatte gemiddeld respectievelijk 0,06 en 0,75 g EPA en DHA/MJ ME. De percentages van LA, ALA, EPA en DHA in vetweefselvet werden bepaald door de opname van de respectievelijke vetzuren. Lijnzaadolie in de voeding, vergeleken met kokosvet, verhoogde de ADG significant en vergeleken met visolie was de ADG ook toegenomen, maar net niet significant. De conclusie luidde dat extra opname van ALA de groei verhoogt en dat dit effect onafhankelijk is van de omzetting van ALA in EPA en DHA.

In Hoofdstuk 7 werd de vraag gesteld of dat toevoeging van het blad van spinazie of dat van de zoete aardappel aan het rantsoen de groei van varkens op Vietnamese bedrijfjes zou bevorderen. De rantsoenen bevatten een van de bladgroenten in een concentratie van 15% in de totale droge stof. Spinazieblad heeft een ALA-gehalte van 35 % in de vetcomponent en voor het blad van de zoete aardappel was de waarde 41 %. De rantsoenen zonder en met bladgroenten bevatten gemiddeld 0,14 en 0,32 g ALA/MJ ME. De opname van de bladgroenten resulteerde in een stijging van het ALA-gehalte in het vetweefselvet. Er was een significante toename van de groei door toevoeging van spinazie of aardappelblad aan het rantsoen.

Het beschreven onderzoek heeft extra kennis gegenereerd inzake de relatie tussen de opname van PUFAs en de ADG van vleesvarkens op Vietnamese bedrijfjes van keuterboeren. Er wordt gesteld dat extra opname van LA en ALA, tot gehalten in de voeding van respectievelijk 2 en 0,3 g/MJ ME, de ADG zal verhogen. Dergelijke

opnameniveaus zijn hoog, vergeleken met de optimale niveaus gebaseerd op het literatuuronderzoek. Echter, de varkens in het huidige onderzoek hadden een relatief lage groeisnelheid en een hoog gehalte lichaamsvet. Bovendien waren in dit onderzoek LA en ALA niet de enige variabelen zodat onbekende, verstrengelde factoren volledig of deels verantwoordelijk kunnen zijn voor de toegenomen verbanden tussen de opname van LA of ALA en de groei.

CURRICULUM VITAE

NGUYEN QUANG LINH was born on November 24, 1961 in Ha Tinh province, Vietnam. He passed the national examination to enter university in 1979 and in the same year he started his study of Animal Husbandry and Veterinary Medicine at Habac Agricultural University. He graduated in 1985.

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1998: Birmingham, UK. 15th IPVS Congress.

1998: Utrecht, The Netherlands. Symposium on Tropical Animal Diseases.

2001: Beijing, China. Workshop on Sustainable Development in Agriculture.

2001: Canberra, Australia. Sixth Conference on Asian Women.

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List of publications other than those in this thesis:

1- Linh Q. Nguyen, Vu B. Hoa, (1995). Effect of Bomogalactogen and Galactogil on milk yield and resistance to diseases in sows. *J. Sci. Technol. Econ. Manag. Ministry of Agriculture and Food Industry, Vietnam,* 10:42-48.

2- Linh Q. Nguyen & H. D. Dinh, (1988). Effect of protein and energy levels on disease resistance of growing-finishing pigs in Central Vietnam. *Magazine of INVR,* 10:67-72.

3- Linh Q. Nguyen, (1994). Small scale pig fattening in areas of Vietnam. *N- C. T.-.R Netherlands' Centres for Training on Animal Resources Management 6.2, 1994, pp. 25-29.*

4- Linh Q. Nguyen., N.V. Hanh, & T.L. To, (1996). First research results of pathogenic bacteria of contagious diarrhea in pigs in Central Vietnam. *Proceedings of the 14th IPVS' Congress, Bologna, Italy. P.346.*

5- Linh Q. Nguyen, (1996). Research on the effect of protein diet and fosterage ways to the productivity and meat quality of F1 hybrid pigs (Large White x MC-Local Breed). *J. Sci. Technol. Econ. Manag. Ministry of Agriculture and Food Industry, Vietnam.* 10:94-105.

6- Linh Q. Nguyen, (1997). Research on the effect of protein diet and fosterage ways to the productivity and meat quality of F1 hybrid pigs (Large White x MC-Local Breed) and (Landrace x MC-local breed) in Central Vietnam. *N.-. C. T.-.R Netherlands' Centres for Training on Animal Resources Management. Vol.8.1, 1997, pp. 27-31.*

7- Linh Q. Nguyen, (1997). Research on the effect of Peterhand and Kemzyme to productivity and meat quality of F1 pigs (Large White x Mong Cai). *Proceedings of*

Research Results in Sci., Tech. and Econ. Manag., Hue Uni. of Agri. And Forestry. 1997, pp. 175-179.

8- Linh Q. Nguyen, (1997). Research on the effect of supplementary to growing-finishing pigs in central Vietnam. *J. Sci. Technol. Econ. Manag. Ministry of Agriculture and Food Industry, Vietnam.*11:32-39.

9- Linh Q. Nguyen, (1998). Research on the relations between animal income, farm income and householder characteristics in Nghe An province. *J. Sci. Technol. Econ. Manag. Ministry of Agriculture and Rural Development, Vietnam, 10/1998, pp. 415-419*

10- Linh Q. Nguyen & L.V. Lien, (1998). Research on the effect of fermented feed on growing-finishing pigs in Central Vietnam. *J. Sci. Technol. Econ. Manag. Ministry of Agriculture and Food Industry, Vietnam.*11:48-52.

11- Linh Q. Nguyen & D.M. Nhat, (1998). Effect of dietary active dry yeast supplement on growing-finishing pigs in Central Vietnam. *Proceedings of the 15th IPVS' Congress, Birmingham, England. P.43*

12. Linh Q. Nguyen, (1999). The use of Large White, Mong Cai pigs and their crossbreeds in different farming systems in Central Vietnam. *Proceedings of National Conference in Animal Science, Hue City, July 1999.*

13. Linh Q. Nguyen, (1999). The use of pig Large white, Mong cai and their crossbreeds in different farming systems in Central Vietnam. MSc. Thesis, Wageningen Agricultural University, The Netherlands.

14. Linh Q. Nguyen & L. C. Tu, (1999). The use of mathematical models in analysis of livestock systems. *Proceedings of Hue University Conference in Animal Husbandry and Veterinary Medicine, December.* P.217.

15. Linh Q. Nguyen, Duong H. Duong & Thanh V. Tran, (2002). The influence of supplemental (n-3) fatty acids on growth performance and resistance to disease of growing pigs. *Proceedings of the 17th IPVS Congress, Ames, Iowa.*P.114.

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As from 1985, Nguyen Quang Linh is a member of the teaching staff of Hue University of Agriculture. Lectures given are on pig husbandry and animal production systems. He has had under his guidance 45 students to fulfil their theses within the framework of graduate courses in Animal Husbandry and Veterinary Medicine. He is a member of the International Pig Veterinary Society since 1996. He is vice-chairman of the scientific committee of Animal Sciences, Hue University of Agriculture and Forestry, Vietnam. Since 2001, he is vice-dean for Scientific Research and International Relations, Faculty of Animal Sciences, Hue University of Agriculture and Forestry, Vietnam.