Fatty acid digestion, synthesis and metabolism in broiler chickens and pigs

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Willem Smink

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Abstract

The impact of variation in the composition of dietary fat on digestion, metabolism and synthesis of fatty acids was studied in broiler chickens and in pigs. In young broiler chickens, digestion of unsaturated fatty acids was substantially higher compared with that of saturated fatty acids. Positional distribution appeared important. Particularly digestion of saturated fatty acids, e.g. palmitic acid (C16:0), esterified at the sn-1 and -3 position of the glycerol backbone was lower (51%) compared with that observed at the sn-2 position (90%). Based on these observations, an equation was developed predicting the digestion of dietary fat sources in broiler chickens and pigs, taking fatty acid composition, the positioning, and the proportion of free fatty acids into account. The deposition of fat, especially of monounsaturated fatty acids in body tissues, increased in broilers by feeding saturated fats in comparison with unsaturated fats caused by both a reduced ß-oxidation and an increased rate of *de novo* synthesis of fatty acids. In a feeding trial with pigs, starch, saturated and unsaturated fatty acid sources were compared at similar intakes of net energy. Growth performance and backfat thickness were unaffected by dietary energy source. Intramuscular fat content, however, tended to be increased in starch fed pigs when compared with pigs fed the saturated fatty acid source. To study the interactions between dietary linoleic acid (LA; C18:2 n-6) and α-linolenic acid (ALA; C18:3 n-3) and their impact on the synthesis of long-chain polyunsaturated fatty acids (LC-PUFA) in both the n-3 and n-6 chains, a trial was designed in which identical increments in the intake of ALA and LA were fed to growing pigs. Generally, dietary LA inhibited the synthesis of n-3 LC-PUFA in the liver. Dietary ALA increased the content of eicosapentaenoic acid (EPA; C20:5 n-3) but decreased that of docosahexaenoic acid (DHA; C22:6 n-3) in the liver. DHA levels in brain were hardly affected by both dietary LA and ALA. It was concluded that in addition to Δ6 desaturase, elongase 2 might be a rate-limiting enzyme in the formation of DHA. The impact of these findings on the potential of contribution of feeding LA and ALA to pigs to meet human dietary requirements for LC-PUFA by meat products was investigated by analysing the fatty acid composition of muscle and fat tissues. It appeared impossible to attain substantial improvements in tissue DHA contents by feeding different combinations of LA and ALA. However, EPA of intramuscular fat can be increased by feeding ALA, particularly when restricting LA intake. In addition, docosapentaenoic acid (DPA; C22:5 n-3) was increased in both muscle and backfat by dietary ALA. DPA might have comparable biological effects as EPA. The potential of meat products to supply DPA in food is considerable and therefore of interest.

Voorwoord

Bijna acht jaar geleden hadden Walter Gerrits en ik een aantal keer overleg over onderzoek gericht op vet bij varkens, pluimvee en vleeskalveren. Walter vroeg of ik wel eens nagedacht had over promotieonderzoek als externe PhD. Zelf was ik daar meteen enthousiast over. Na melding thuis was een tweede stap om Alex Fitié als werkgever te vragen of het akkoord was om dit te combineren met de werkzaamheden voor Feed Innovation Services (FIS). Alex ziet meestal meer voordelen dan nadelen en zei: 'Nee, sterker nog, ik heb altijd gezegd dat jij binnen FIS zou moeten promoveren'. Dat was inderdaad waar. Achteraf kan gezegd worden dat ik eigenlijk van het hele traject heb genoten. Maar dat is volgens mij ook nodig.

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Willem

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Chapter 1

General introduction

1. General introduction

Fat is an important energy source in animal diets. Due to its high energy density, inclusion of 5% of dietary fat in a pig diet, 10% in a diet for broiler chickens and 20% in a milk replacer for veal calves corresponds with approximately 15, 30 and 60% of the dietary digestible energy, respectively. Due to a lack of activity of the enzymes Δ12 and Δ15 desaturase, humans and animals are not able to synthesize linolenic and linoleic acid, also referred to as omega-3 and omega-6 fatty acids. Therefore, a dietary intake of these essential fatty acids is needed. They are needed as a precursor for longer chain polyunsaturated fatty acids. The long-chain poly-unsaturated fatty acids have important regulatory functions in immune, nervous and regulatory systems. Part of the fatty acids ingested will be incorporated into body lipids. Therefore, in animals used for meat production both the source and the amount of dietary fat will affect the fatty acid pattern of the end product, and thereby potentially the health of its consumer.

This introduction describes (1) the chemical composition of fat, (2) the digestibility of fat and (3) the synthesis, metabolism and deposition of fatty acids. The general introduction ends with the thesis outline.

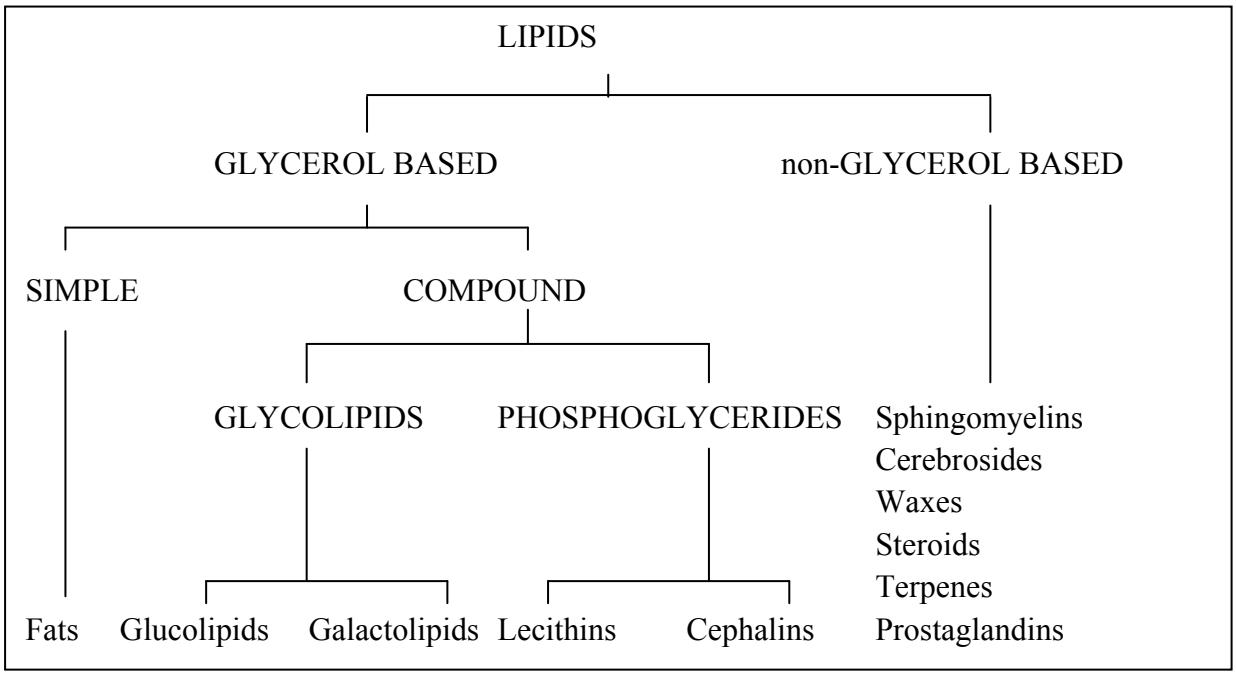

Figure 1.1. Classification of lipids (McDonald *et al.,* 1995).

Chemical composition of fats

Chemistry

The crude fat fraction as it is commonly included in the proxymate analysis of feedstuffs, is defined as the lipids that are extractable by non-polar solvents like petroleum-ether after an acid extraction. A lipid classification is provided in Figure 1.1. Simple glycerol based fats designate fats where all three residues are fatty acids. In this thesis emphasis will be on these glycerol based simple fats or triglycerides.

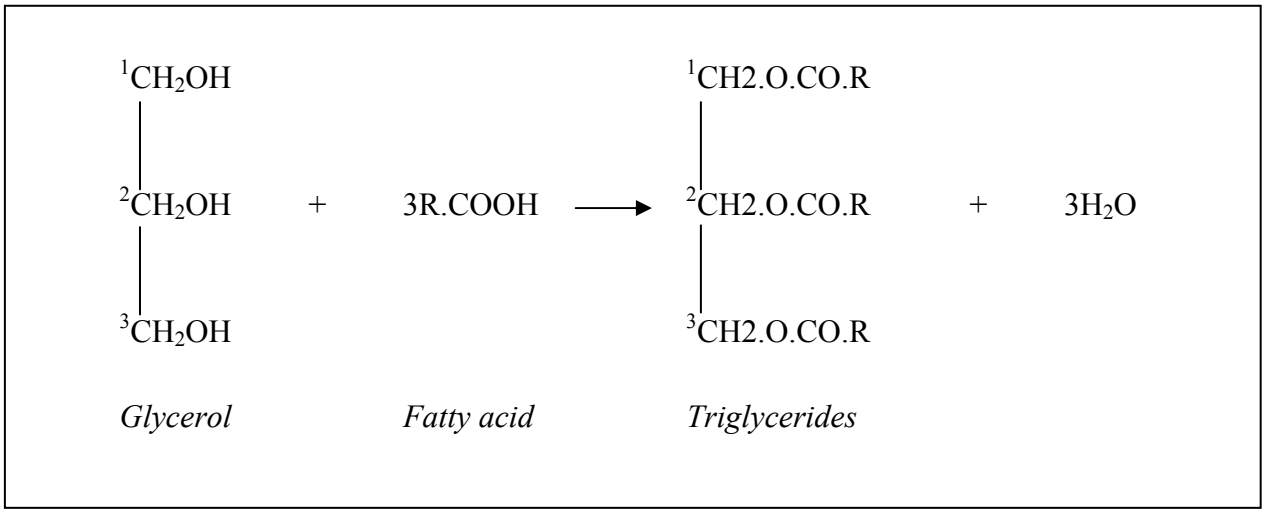

Figure 1.2. Glycerol and fatty acids from triglycerides.

Numbers in superscript as shown left to the CH_2 in Figure 1.2 indicate the position of the C-atoms of the glycerol. Number 1, 2 and 3 is the so called sn-1, sn-2 and sn-3 position at the glycerol molecule, respectively. Lipids of fat sources are in general triglycerides, i.e. three fatty acids are esterified to one glycerol molecule. The fatty acids can be divided into saturated fatty acids (fatty acids with no double bond between the C-atoms e.g. stearic, palmitic, myristic and lauric acid with respectively 18, 16, 14 and 12 C-atoms) and unsaturated fatty acids. The unsaturated ones can be further divided into mono- and polyunsaturated fatty acids. Polyunsaturated fatty acids are divided into families dependent on the position of the first double bond in the carbon chain counted from the end of the fatty acid. When the first double bond is then at the third C-atom, the chain is called omega-3 (or n-3). When the first double bond occurs at the sixth C-atom, the fatty acid belongs to the omega-6 (or n-6) family. Oleic acid contains one double bond at the ninth C-atom (n-9). Names and formulas of common fatty acids are presented in Table 1.1.

Chemical composition of fat sources

The fatty acid composition of fat differs between sources. Most vegetable fats consist mainly of unsaturated fatty acids, with proportions of linoleic or sometimes linolenic acid up to 60%. Animal fats contain both saturated and monounsaturated fatty acids. Long-chain polyunsaturated fatty acids (LC-PUFA; C20 or higher) occurs mainly in fish oils. Apart from the composition of fatty acids, the positional distribution of fatty acids on the glycerol

molecule can differ between fat sources. These positions of fatty acids have physiological and technological aspects. This may have considerable impact for its digestion in animals, but is not included in feed composition databases (e.g. INRA, 2004; CVB, 2011), while Doppenberg and Van der Aar (2010) used an extra energy value via a 5% higher digestibility coefficient in broilers for the fat sources lard and poultry fat, due to their position of fatty acids. The positional distribution can be measured via an enzymatic method described by Breckenridge (1978). The fatty acid composition and its positional distribution are given in Table 1.2. The proportion (in % of the fatty acid present in the fat) that is situated at the sn-2 position is presented in italics. When the distribution is random, 33% is present at each position, so also at the sn-2 position. A percentage of for example 54% for C12:0 in coconut oil means that 54% of all C12:0 in coconut oil is present at the sn-2 position. This implies that 46% is esterified at either the sn-1 or sn-3 position. Generally, C18:2 from vegetable oils are predominantly positioned at the sn-2 position, while the proportion of C16:0 at the sn-2 position is rather low. Due to the high content of C16:0 in palm oil, a rather high absolute amount of C16:0 is at the sn-1,3 positions. Animal fats are different. Palmitic acid in tallow is rather evenly distributed, while in lard the majority of this fatty acid is esterified at the sn-2 position.

Table 1.1 Name and chemical notation of common fatty acids in fat sources.

Fatty acid	Notation
Butyric acid	C4:0
Caproic acid	C6:0
Caprylic acid	C8:0
Capric acid	C10:0
Lauric acid	C12:0
Myristic acid	C14:0
Palmitic acid	C16:0
Palmitoleic acid	C16:1
Stearic acid	C18:0
Oleic acid	C18:1 n-9
Linoleic acid (LA)	C18:2 n-6
α-Linolenic acid (ALA)	C18:3 n-3
γ-Linolenic acid	C18:3 n-6
Gadoleic acid	C20:1 n-9
Arachidic acid	C20:0
Dihomo-γ-linolenic acid	C20:3 n-6
Arachidonic acid (ARA)	C20:4 n-6
Eicosapentaenoic acid (EPA)	C20:5 n-3
Docosatetraenoic acid (DTA)	C22:4 n-6
Docosapentaenoic acid (DPA)	C22:5 n-3
Docosahexaenoic acid (DHA)	C22:6 n-3

Table 1.2 Fatty acid composition of fat sources in % of total fatty acids of the main fatty acids (INRA, 2004; Gunstone and Harwood, 2007; Mitchoathai, 2007; CVB, 2011) and the relative amount (presented *italic*) of those fatty acids at the sn-2 position of the glycerol molecule (Breckenridge, 1978; Gunstone and Harwood, 2007).

Fat source	Fatty acid													
	C10:0	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2 n-6	C18:3 n-3	C20:1	C22:1	C20:5 n-3	C22:5 n-3	C22:6 n-3
Vegetable oil														
Coconut oil	6.4	48	18	9.0	-	2.5	5.7	1.4	-	-	-	-	-	-
	6	*54*	*19*	*10*	*-*	*0*	*35*	*50*	-	-	-	-	-	-
Linseed oil	-	-	-	4.8	-	3.6	17	16	53	-	-	-	-	-
	-	-	-	*11*	*-*	*9*	*37*	*45*	*35*	-	-	-	-	-
Maize oil	-	-	-	12	0.1	2.5	32	52	1.0	-	-	-	-	-
	-	-	-	*6*	*-*	*2*	*31*	*41*	-	-	-	-	-	-
Olive oil	-	-	-	11	0.7	2.4	69	12	0.8	-	-	-	-	-
	-	-	-	*9*	*-*	*12*	*37*	*36*	-	-	-	-	-	-
Palm oil	-	0.2	0.9	43	0.3	4.8	38	11	0.3	-	-	-	-	-
	-	-	-	*18*	*-*	*18*	*52*	*57*	-	-	-	-	-	-
Palm kernel oil	7.0	47	15	9.0	-	2.5	16	2.5	0.5	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rapeseed oil	-	-	0.5	5.0	0.4	2.1	57	22	10	-	-	-	-	-
	-	-	-	*11*	*-*	*11*	*30*	*47*	*44*	-	-	-	-	-
Soybean oil	-	-	0.2	11	0.2	4.0	22	54	8.0	-	-	-	-	-
	-	-	-	*6*	*-*	*3*	*31*	*43*	*29*	-	-	-	-	-
Sunflower oil	-	0.2	0.3	7.1	0.3	4.0	22	65	0.4	-	-	-	-	-
	-	-	-	*20*	*-*	*21*	*33*	*35*	-	-	-	-	-	-

Fat source	Fatty acid													
	C10:0	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2 n-6	C18:3 n-3	C20:1	C22:1	C20:5 n-3	C22:5 n-3	C22:6 n-3
<u>Animal fat</u>														
Poultry fat	-	0.1	0.1	15	2.4	5.4	31	37	4.4	-	-	-	-	-
	-	-	-	*14*	*26*	*35*	*41*	*60*	-	-	-	-	-	-
Tallow	-	-	2.1	27	2.5	19	35	4.9	4.3	-	-	-	-	-
	-	-	*15*	*28*	*14*	*14*	*52*	*85*	-	-	-	-	-	-
Lard	-	0.2	1.8	27	2.3	17	39	11	1.0	-	-	-	-	-
	-	-	*72*	*81*	*49*	*7*	*11*	*12*	-	-	-	-	-	-
Milk cow	3.7	3.9	10	23	2.6	7	24	2.5	1.2	-	-	-	-	-
	27	*53*	*57*	*44*	*46*	*45*	*26*	*40*	-	-	-	-	-	-
Milk sow	-	-	4	33	11	1.8	35	12	0.3	-	-	-	-	-
	-	-	*71*	*60*	-	*10*	*20*	*26*	-	-	-	-	-	-
<u>Fish oil</u>														
Anchovy oil	-	-	7.2	17	11	3.9	12	1.1	0.8	1.9	1.4	18	1.5	9.0
	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cod liver oil	-	-	6.2	13	7.4	1.6	20	0.9	0.5	11	4.6	11	1.4	11
	-	-	*44*	*42*	*30*	*13*	*15*	*33*	-	*19*	*28*	*44*	*60*	*74*
Menhaden oil	-	-	9.9	21	12	3.3	14	1.1	0.8	1.9	0.7	13	1.7	7.9
	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Herring	-	-	6.2	13	7.5	1.1	13	1.1	0.7	15	22	6.8	0.8	5.8
	-	-	*50*	*58*	*30*	*33*	*29*	*42*	-	*11*	*7*	*72*	*60*	*86*
Salmon oil (wild)	-	-	5.3	16	9.3	3.3	15	3.4	1	8.4	5.5	17	2.5	13
	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sardine oil	-	-	6.7	19	8.8	3.4	14	1.1	0.1	3.2	3.8	17	2.5	16
	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Digestibility of fats

Hydrolysis, micelle formation and absorption
Digestion of an ingested triglyceride occurs in three steps:
1. Hydrolysis of the bonds between glycerol and the two fatty acids at the sn-1 and sn-3 position by lipase, leaving two free fatty acids and a monoglyceride.
2. Micelle formation of fatty acids and monoglycerides in the small intestine occurs, facilitated by bile salts.
3. The micelles move towards the intestinal wall, mostly in the jejunum, at which an exchange occurs and fatty acids and monoglycerides are absorbed. Re-esterification occurs in the enterocyte, chylomicrons are formed and drained from the intestinal wall mainly into lymphatic vessels.

Lipase, phospholipase and cholesterolesterase are involved in hydrolysis of lipids. Lipase is splitting the fatty acids from their first and third position of the glycerol molecule, resulting in two free fatty acids and one monoglyceride. Lipase is mainly produced by the pancreas. In mammals, however, already some lipase activity consist in the mouth and stomach (Nelson *et al.*, 1977). These lipases have a high affinity for short and medium-chain fatty acids. The activity of lipase is positively affected by colipase and bile salts. Phospholipase is splitting the second position of the glycerol molecule whereby lysophospholipids are created.

The end products of fat hydrolysis are bile acid micelles. These micelles develop by an interaction with bile salt and amphipatic products (fatty acids with a hydrophobic and a hydrophilic part) such as monoglycerides, medium-chain fatty acids, unsaturated fatty acids and lecithin. These products lead to swelling of micelles and therefore create space for hydrophobic products in the micelles like diglycerides, long-chain saturated fatty acids and fat soluble vitamins. The bile required is produced in the liver and stored in the bile bladder. The concentration of bile salts in the intestinal contents should exceed 2 mmol bile/L (Argenzio, 1984), as below this concentration micelle formation is hampered. This concentration is referred to as critical micelle concentration. Higher concentrations of monoglycerides decrease the critical micelle concentration (Freeman, 1984). Formed micelle's have an estimated particle size of 30-40Å, small enough to pass between the microvilli of mucosal cells. Absorption of fat occurs in the region of the end of the duodenum to the end of the ileum in monogastric animals. Bile salt in the micelles will be absorbed via an active and passive transport mechanism. Approximately 95% will be re-used. The re-use of bile acids is important for the digestibility. The main site of absorption of lipids is the proximal part of the jejunum. In the mucosal cells, the fatty acids and monoglycerides are re-synthesized into triglycerides, coated with protein (chylomicrons) and transported into the portal vein or via the lymph. Chickens absorb the fat directly into the portal vein (Krogdahl, 1985). The transport of fat in mammals is only for short and medium-chain fatty acids via the portal vein. The long-chain fatty acids are transported via the lymph. The process is presented in Figure 1.3.

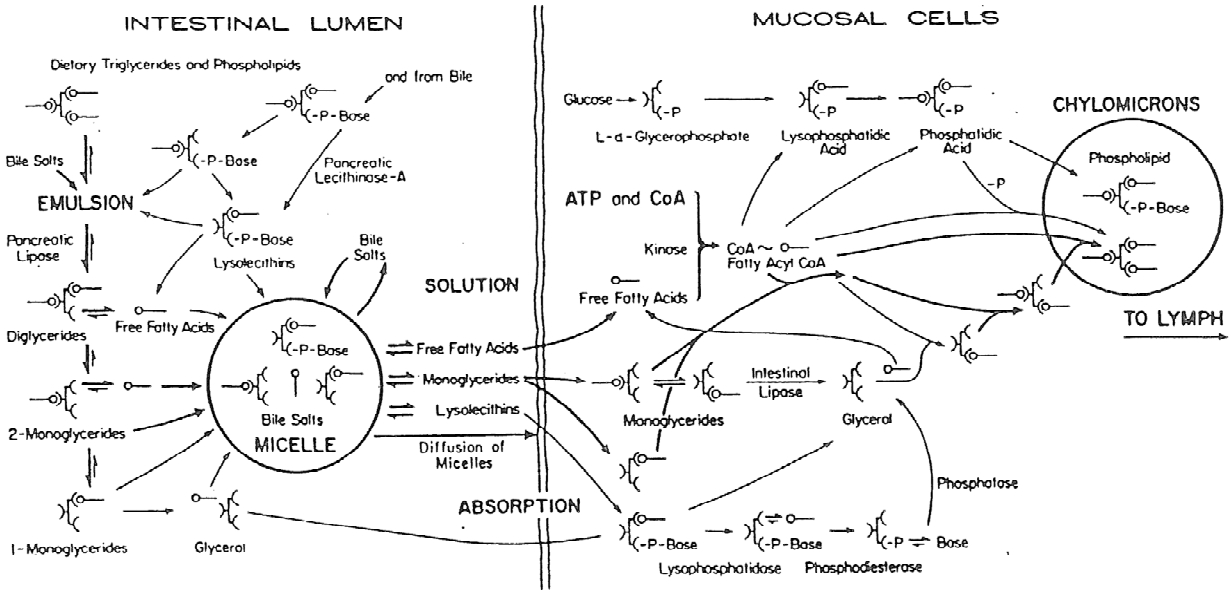

Figure 1.3. General hydrolysis and resynthesis of lipid (Davenport, 1982).

Digestibility of dietary fat sources

The major part of the variation in energy value of dietary fat sources is related to variation in digestibility. Fat digestion is influenced by both dietary and animal factors, as outlined below.

Animal factors and fatty acid composition

Current protocols for fat digestion by CVB (CVB 2011) require measurements at an age of approximately 4 weeks for broiler chickens and a body weight range of 40-100 kg in pigs. The calculated energy value of fat for diets of broiler chickens and pigs in the Netherlands are based on such measurement standards. Fat digestibility differs between sources but also between types of animals. For example, saturated fat sources are poorly digested in broilers when compared with laying hens, pigs and veal calves. In addition to the differences between types of animal, the digestibility is lower in younger animals. Results of a study in broilers fed with two different fats at an age between 2 and 8 weeks are presented in Table 1.3.

Table 1.3. Faecal digestibility (%) of tallow and soybean oil in broiler chickens at different ages (Ketels, 1994).

Age (weeks)	Soybean oil	Tallow
2	75	42
3	87	53
4	92	63
8	91	67

The digestibility of saturated fat sources like tallow is rather low in broilers. A limited number of studies were carried out to study the digestion of individual fatty acids in broilers. Some results are summarized in Table 1.4.

Table 1.4. Digestibility (%) of fatty acids in broiler chickens.

Fatty acid	Animal fat[1]	Soybean oil 8% / tallow 2%[2]	Soybean oil 2% / tallow 8%[2]
C14:0	86	-	-
C16:0	73	65	53
C18:0	67	49	41
C18:1	91	83	80
C18:2	90	88	81

[1]: Kussaibati *et al.*, 1982; [2:] Dänicke *et al.*, 2000.

The ability of the young chicken to digest long-chain saturated fatty acids, especially C16:0 and C18:0 is rather low. It is expected that bile salts is a limiting factor for the digestibility of saturated fat sources (Ketels, 1994). Addition of bile salts increased the digestibility of C16:0 and C18:0 by 2 % points (Kussaibati *et al.*, 1982). There were no effects of the bile salt on the digestibility of unsaturated fatty acids C18:1 and C18:2. Increasing the intake of saturated fatty acid sources decreases their digestibility (Ketels, 1994), indicating that the capacity for digestion of fat can easily be exceeded in young birds.

Growing/fattening pigs appear to be able to digest much higher fat levels than commonly used in commercial diets. In experiments with growing pigs, Powles *et al.* (1994), did not find a clear relation between the inclusion level of fat and the digestibility coefficient in the range of 4 to 12% dietary fat. Even higher fat levels of a long-chain saturated fat source did not have a negative effect on its digestibility (Jørgensen and Fernandez, 2000). Low inclusion levels of fat increases the contribution of endogenous fat to faecal fat excretion, thereby decreasing the apparent digestibility coefficient.

Free fatty acids

An important part of the fat sources used in animal nutrition are by-products from the food and cosmetic industry and contain a large proportion of free fatty acids. A good estimate of the digestibility of free fatty acids is therefore important. Several studies showed that the digestibility of free fatty acids in comparison with triglycerides is lower in pigs (e.g. Powles *et al.*, 1993; 1994), calves (Jenkins *et al.*, 1985) and broiler chickens (e.g. Wiseman and Salvador, 1991; Ketels, 1994). The magnitude of the effect of free fatty acids on the digestibility is largest for younger animals, as illustrated in Table 1.5.

Table 1.5. Digestibility of tallow, soybean oil and palm oil with a different proportion being present in the form of free fatty acids (FFA) in broilers at an age of 10 and 50 days (Wiseman and Salvador, 1991).

	Age	Free fatty acids (%)				
	(days)	10	30	50	70	90
Tallow	10	74	66	61	56	41
	50	82	80	78	74	69
Palm oil	10	79	77	66	62	53
	50	84	83	77	74	71
Soybean oil	10	95[1]	90	88	84	-
	50	95[1]	93	91	88	-

[1]: FFA is 1.4%.

In diets with large amounts of free fatty acids, micelle formation is likely impaired due to a reduced concentration of monoglycerides in the intestinal lumen. Monoglycerides are important in mixed micelles because they will give a swelling ability of the micelles whereby the fatty acids with a hydrophobic character can be absorbed (Freeman, 1984). Based on a study with pigs, Lynn *et al.* (1976) concluded that the lower digestibility is not due to an absence of monoglycerides. The authors suggest an endogenous production of monoglycerides by the pigs fed the free fatty acids. Other explanations for a lowered digestibility are for example that there might be more soap formation with calcium and magnesium, especially when saturated free fatty acids were used.

Position of fatty acids at the glycerol molecule

Considering the strong specificity of endogenous lipases for cleaving at specific sites at the glycerol backbone (Nelson *et al.*, 1977) it can be postulated that the fatty acid distribution over the glycerol backbone and the subsequent hydrolysis by endogenous enzymes plays a role in the digestion of the lipids. Especially the fatty acids, esterified at sn- 2 of the glycerol backbone are thought to be absorbed at a higher rate than the free fatty acids due to the more hydrophilic character of the glycerol + fatty acids (monoglycerides) than the free fatty acid alone. Studies with rats showed that the digestibility of long-chain saturated fatty acids at the sn-2 position is higher compared with those bound at the sn-1,3 position (Brink *et al.*, 1995; Renaud *et al.*, 1995)

Other factors and aspects of fat

When digestibility measurements are performed to determine the digestibility of a fat source, usually a basal diet is composed, delivering nutrients other than fat to the animal. There is evidence that the composition of such basal diet affects the digestibility of fats. Examples are protein sources such as a replacement of skimmed milk powder by vegetable protein sources for veal calves. The reason for this effect is an increased binding of minerals with bile salts and increasing bile excretion with faeces (Xu, 1998). In broilers, the use of wheat, barley and

cereal by-products as a replacement of maize has its impact on fat digestibility. It is known that the carbohydrate sources with specific non starch polysaccharides (NSP) in rye, barley and wheat will give an increased viscosity in the gut. As a result, nutrient digestibility is decreased. In this respect, especially saturated fat is sensitive to impairment of digestion. Several studies have indicated that by including viscous water-soluble NSP in diet, the microbial activity in the small intestine is increased markedly. As a result of degradation of bile acids may occur, making fat emulsification less effective. Langhout (1998) showed that an increased microbial activity in the small intestine will increase the deconjugation and excretion of bile acids with the droppings. Enzymes for the breakdown of water soluble polysaccharides in wheat, barley and rye will clearly improve the digestibility of fat (e.g. Langhout, 1998; Dänicke *et al.*, 2000). This effect is more pronounced in tallow in comparison with soybean oil.

As presented in Table 1.2, most fatty acids consist of long-chain fatty acids (>C12). The fatty acids of palmkernel oil and coconut oil, however, are mainly medium-chain fatty acids. These fatty acids with a chain length of 8, 10 or 12 C-atoms have specific antibacterial properties (Petshow *et al.*, 1998; Decuypere and Dierick, 2003) and therefore potentially health promoting. In addition, a depression of fat digestibility after an *Eimeria acervulina* infection in broilers was less severe with coconut oil in comparison with animal fat or soybean oil (Adams *et al.*, 1996).

State of art

The major part of the energy from dietary fat originates from sources with long-chain saturated and long-chain unsaturated triglycerides and fatty acids. Several studies were carried out to compare long-chain saturated with long-chain unsaturated fat sources. Data to compare the digestion of C16:0 and C18:0 is largely lacking, but is expected to be relevant. In addition, there is hardly any information about the effect of the position of fatty acids on the glycerol molecule on the digestibility of fatty acids in pigs and poultry. Because important fats like palm and lard have a very specific distribution of fatty acids on the glycerol molecule, this information would improve current estimates of digestion of these fat sources.

Synthesis, metabolism and deposition of fatty acids

After absorption, fatty acids can be oxidized to yield ATP, deposited or serve as a precursor for other bioactive molecules. In addition, a substantial amount of fatty acids will be synthesized *de novo* using acetyl co-enzyme A units originating from other metabolic processes as precursor.

ß-oxidation

Fats and oils are highly concentrated stores of energy. The complete oxidation of fatty acids yields approximately 39 kJ/g, whereas carbohydrates and proteins yield approximately 17 and 24 kJ/g, respectively. The process of production of ATP from fatty acids is generally referred to as the β-oxidation pathway. Briefly, it consists of the sequential breakdown of two carbon

units during every round of the cycle. Thus a C18:0 becomes after one cycle a C16:0, which becomes a C14:0 etc. During this enzymatic breakdown energy-rich molecules are formed (NADH, $FADH_2$) which eventually are converted into ATP, what is used for energy-consuming processes in the body. The degradation of fatty acids to acetyl CoA in the β-oxidation pathway occurs mainly in the mitochondria. The oxidation rate of medium-chain fatty acids is faster than long-chain fatty acids. In addition the degree of unsaturation increases the oxidation of fatty acids (Bach *et al.*, 1996; DeLany *et al.*, 2000).

De novo fatty acid synthesis

Biosynthesis of long-chain saturated fatty acids occurs in the cytoplasm of cells. Although it occurs by sequential addition of acetyl groups and involves similar chemical reactions, fatty acid biosynthesis is not ß-oxidation in reverse.

Two enzyme complexes involved in fatty acid biosynthesis are acetyl-CoA carboxylase and fatty acid synthase. Acetyl CoA carboxylase adds carbon dioxide to acetyl CoA. The major end product of the fatty acid synthesis is C16:0, which in turn can be elongated to C18:0. Cell membranes require unsaturated fatty acids to maintain their structure, fluidity and function (Enser, 1984). The introduction of a single double bond between carbon atoms 9 and 10 is catalysed by the enzyme Δ9-desaturase, which is universally present in both plants and animals. This enzyme results in the conversion of C18:0 to C18:1 n-9.

The length of the fatty acid synthesized varies with the tissue in which it is synthesized. In the liver and adipose tissue, palmitic acid is the major product. In the mammary gland, shorter chain fatty acids are produced. The addition of two carbon units to a two-carbon acetyl primer results in the formation of the common even-chain fatty acids. The synthesis of fatty acids occurs in different tissues. The *de novo* fat synthesis in pigs occurs predominantly in adipose tissue (O'Hea and Leveille, 1968), while the *de novo* fat synthesis in broilers occurs mainly in the liver (Leveille *et al.*, 1975).

Elongation and desaturation

Plants, unlike animals, can insert additional double bonds into oleic acid; a Δ12-desaturase converting oleic acid into linoleic acid (18:2 n-6; LA) whiles a Δl5-desaturase converts linoleic acid into α-linolenic acid (18:3 n-3; ALA). As animal tissues are unable to synthesize linoleic and α-linolenic acids, these fatty acids must be consumed via the diet and so are termed essential fatty acids. Using the pathway outlined in Figure 1.4, animal cells can convert dietary α-linolenic acid into eicosapentaenoic acid (EPA; C20:5 n-3), docosapentaenoic acid (DPA; C22:5 n-3) and docosahexaenoic acid (DHA; C22:6 n-3). By a similar series of reactions, dietary linoleic acid is converted via γ-linolenic (C18:3 n-6) and dihomo-γ-linolenic (C20:3 n-6) acids to arachidonic acid (ARA; C20:4 n-6). The n-6 and n-3 families of PUFA are not metabolically interconvertible in mammals. Many marine plants, especially the unicellular algae in phytoplankton, also carry out chain elongation and further desaturation of α-linolenic acid to yield the n-3 long-chain PUFA (LC-PUFA). The formation of these n-3 LC-PUFA by marine algae and their transfer through the food chain to fish is accountable for the abundance of the n-3 PUFA in some marine fish oils (Calder, 1998).

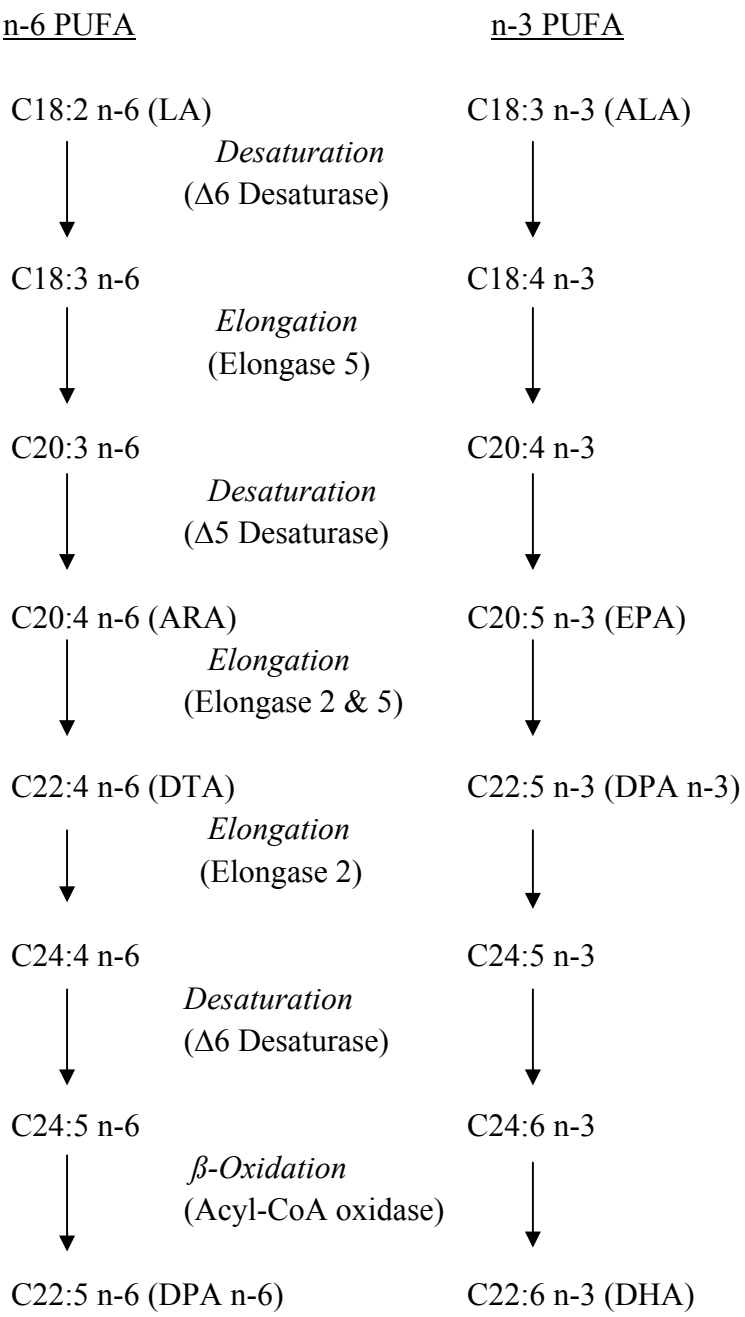

Figure 1.4. General metabolic pathway of omega 6 and -3 fatty acids (adapted from Igarashi *et al.*, 2007). LA, linoleic acid; ALA, α-linolenic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DTA, docosatetraenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.

The pathway presented in Figure 1.4 occurs in various tissues. The magnitude of this elongation and desaturation steps differs among tissues, genetics, physiological and dietary factors (Bézard *et al.*, 1994; Burdge and Calder, 2005). Fatty acids compete for the same enzymes in tissues. For example, the same desaturases and elongases are used for desaturation and elongation steps of both ALA and LA. In addition, fatty acids may have a direct effect on enzymes involved in the conversion process. LA depresses the concentration of EPA in human phospholipids in blood (Goyens *et al.*, 2006). Also, other dietary fatty acids such as C14:0 and C18:1 can alter one of the enzymes involved in the conversion steps of the n-3 and n-6 chain (Jan *et al.*, 2004; Portolesie *et al.*, 2008; Rioux *et al.*, 2008). Vegetable oils contain very little n-3 fatty acids other than ALA. The average human intake of EPA and DHA are insufficient in many Western countries and consequently, additional intake of these fatty acids is needed to achieve the recommended value of EFSA (2010). It is important to know the animals' potential to convert ALA into EPA and DHA and the way these conversions may be impaired by other dietary fatty acids.

Fat deposition

The liver and adipose tissue are the two major sites of biosynthesis of fatty acids and triglycerides in broiler chickens and pigs, respectively. Fat deposition in body tissues is the net result of absorption, *de novo* synthesis and ß-oxidation.

Broilers

De novo synthesis of non-essential fatty acids, mainly monounsaturated, is high in broilers fed with low fat diets (Villaverde *et al.*, 2006). Several studies with broilers were carried out to determine the effect of fat source on abdominal fat accumulation. The results of several studies with a replacement of tallow for vegetable oil are summarized in Table 1.6.

From this table it is quite clear that replacement of tallow by vegetable sources decreases the abdominal fat deposition in broilers. Attention has focused mainly on abdominal fat, which is not the major part of body fat in broilers, and it is not clear to what extent the effect on abdominal fat originates from a re-distribution of body fats, from an increase in fatty acid oxidation, or from a reduced rate of *de novo* fatty acid synthesis. Research by Ferrini *et al.* (2008) suggests that the abdominal fat was not the only fat depot that is affected, as they observed a significant decrease of skin fat when replacing tallow by sunflower oil. There is, however, no agreement in literature on the effects of replacing animal fats by vegetable oil on fatty acid synthesis and –oxidation. Sanz *et al.* (2000) concluded that a reduced rate of fatty acid oxidation and a simultaneous increased in *de novo* fatty acid synthesis are responsible for the increase of abdominal fat. Crespo and Esteve-Garcia (2002) found that both pathways were increased when tallow was replaced by linseed oil.

Table 1.6. Abdominal fat (g/bird) of broilers fed either tallow or vegetable oils at the same dietary concentrations and a comparable intake.

Vegetable fat	Abdominal fat of dietary fat group (g/bird)		Dietary fat (%)	Reference
	Tallow	Vegetable fat		
Sunflower oil	115	95	8	1
Sunflower oil	82	70	8	2
Sunflower oil	47	41	10	3
Linseed oil	47	36	10	3
Olive oil	47	43	10	3
Sunflower oil	38	27	10	4
High oleic acid sunflower oil	38	32	10	4
Linseed oil	38	26	10	4
Soybean oil	55	41	8	5
Sunflower oil	107	42	8	6

1=Sanz *et al.*, 1999; 2 = Sanz *et al*., 2000; 3 = Crespo and Esteve-Garcia, 2002; 4 = Ferrini *et al.*, 2008; 5 = Wongsuthavas *et al.*, 2008; 6 = Newman *et al.*, 2002.

Pigs

In an experiment with pigs of 20 – 45 kg body weight, Bikker *et al.* (1996) found, feeding low-fat diets to growing pigs, that when digestible fat intake (from a constant diet, i.e. by increasing feed intake) increased from 40 to 100 g/d, total body fat deposition increased from 30 to 190 g/d. Similarly, with pigs from 45 to 85 kg BW, body fat retention increased from 45 to 390 g/d when digestible fat intake increased from 65 to 160 g/d. These results are presented in Figure 1.5. Also in studies of Mitchaothai (2007) with relative high dietary fat contents (7-8%), the deposition of fat was 1.5 to 2 times higher than their intake.

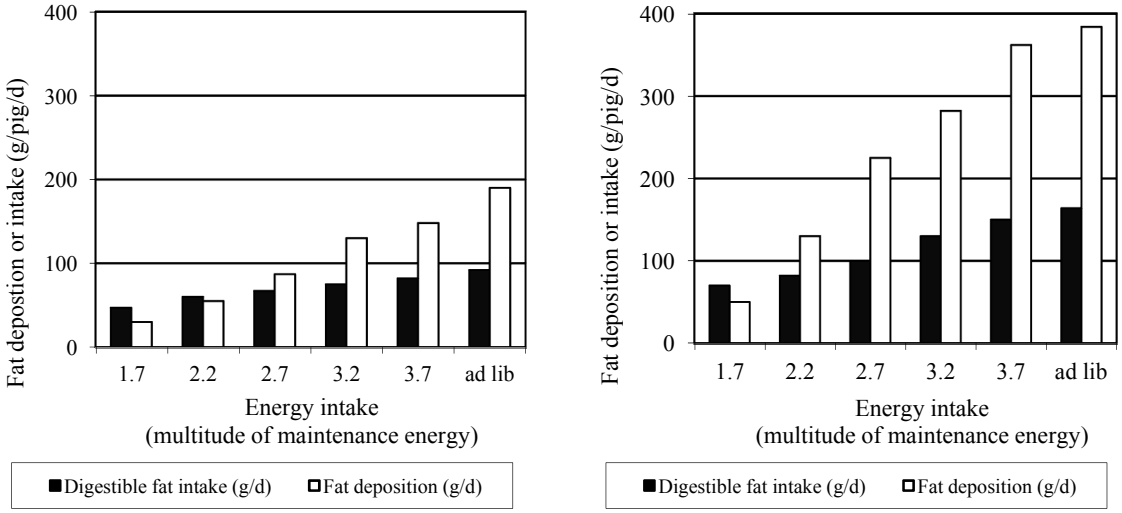

Figure 1.5. Digestible fat intake and fat deposition of pigs in the weight range 20-45 kg (left) and in the weight range 45-85 kg (right) fed in a range of 1.7 to 3.7 x energy intake required for maintenance or ad lib (Bikker *et al.*, 1996).

From these data it is clear that only at extremely low feed (and fat) intake, dietary fat intake exceeds body fat deposition in the pig diets. With increasing feed intake, fat deposition greatly exceeded digestible fat intake. At intakes near ad libitum of pigs from 20 – 45 kg BW, at least 50 % of the deposited body fat originated from *de novo* fatty acid synthesis, while this was at least 60% in pigs from 45 – 85 kg BW. These minimum amounts are calculated assuming that 100% of the dietary fat is deposited as body fat. In most feed evaluation systems for pigs that are based on net energy, it is assumed the efficiency of digested dietary fat into fat retention is 90% (CVB, 2000). This is close to the estimate by Van Milgen *et al.* (2001). There is, however, considerable variation in this estimate, as is for example Chwalibog *et al.* (1992) could not find any heat production from added dietary fat (i.e. dietary fat is retained with an efficiency close to 100%). On the other hand, Halas *et al.* (2010) only recovered between 45 and 55% of added digestible fat in body tissues. So when feeding low fat diets, the majority of the fatty acids deposited are synthesized *de novo*. Nonetheless, the incorporation of dietary fatty acids into body tissues can be very efficient both in pigs and in poultry, as illustrated by the high NE coefficients used in feed evaluation systems (CVB, 2000; Van Milgen *et al.*, 2001). Consequently, the fatty acid pattern of body lipids can largely reflect those of the dietary sources fed, particularly in high-fat diets.

The majority of fat deposited in pigs is originated from *de novo* synthesis. Nevertheless the fatty acid pattern of the diet clearly affects the body fatty acid composition. In general, the proportion of essential fatty acid in tissues clearly increases with the fatty composition of the pig diet (Nguyen *et al.*, 2003; Wood *et al.*, 2008) and broiler chickens (Bavelaar and Beynen, 2003). Essential fatty acids can be used for both energy (oxidation) and deposition. There is not much information available about the efficiency of deposition of essential fatty acids. Studies of Mitchaothai (2007) indicated efficiencies of 25-80% for LA and ALA in different pig breeds and diets. The relation between dietary essential fatty acids and fatty acid composition in backfat of pigs in the study of Nguyen *et al.* (2003) is presented in Figure 1.6.

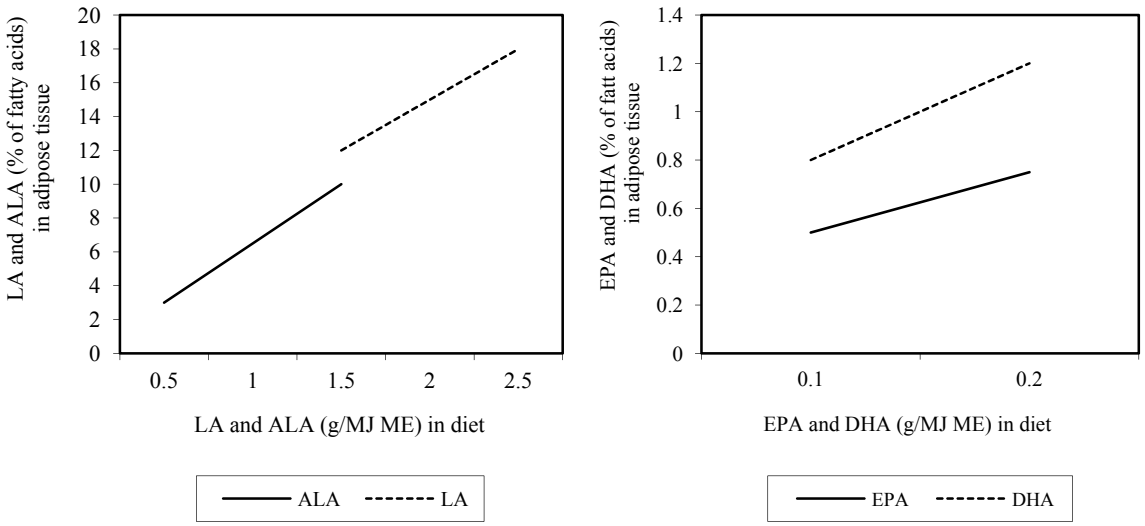

Figure 1.6. Relation between linoleic (LA), α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the diet and those fatty acid content in adipose tissue of pigs (modified from Nguyen, *et al.*, 2003).

Recommended daily allowance of EPA+DHA for humans by EFSA (2010) is 250 mg/d, in addition to an intake of ALA. This EPA+DHA can be achieved by consumption of fish (or fish oil capsules). Figure 1.6 shows that, when feeding DHA or EPA, enrichment of backfat with these LC-PUFA occurs. However, such deposition in backfat acquires intake fish oil or EPA+DHA rich algae by the pigs. Rather than feeding these LC-PUFA, it is interesting to investigate to what extent enrichment of meat and backfat of pigs and poultry can be realized by feeding only the precursor (ALA) and minimizing interference from other dietary fatty acids.

State of art
There is conflicting information to what extent the post-absorptive processes are affected by the properties of fatty acids. In poultry, abdominal fat is reduced when replacing dietary saturated with unsaturated fatty acids. It is however, not clear to what extent total body fat deposition is affected, and whether this is related to alterations in *de novo* fatty acid synthesis or fatty acid oxidation. In pigs, a difference in efficiency in the use of digestible nutrients for energy deposition is accounted for in most feed evaluation systems. It is not clear to what extent the location of *de novo* fatty acid synthesis is affected by dietary energy source. This may have an important effect on meat quality characteristics.

It is obvious that fatty acid profiles in tissues of broilers and pigs largely reflect the dietary profile. The n-3 LC-PUFA in the diet are beneficial for human health. The main sources of n-3 LC-PUFA are fish oils and also some algae species. ALA is the precursor of n-3 LC-PUFA. Information about efficiency of ALA into n-3 LC-PUFA in blood and tissues in pigs and the effects of the antagonist LA will be studied. There is hardly any information available which enzymes are limiting.

Thesis outline

The ratio of unsaturated to saturated fatty acids, fatty acid chain length and the proportion of free fatty acids in the diet are known to affect the digestibility of fat. It is expected that the positioning of fatty acids on the glycerol molecule influences their digestibility, particularly in young birds. This hypothesis has been investigated in an experiment with broiler chickens (**Chapter 2**).
Replacing animal fats by vegetable fats generally results in a decrease in abdominal fat deposition in broiler chickens. It is, however, not known to what extent this effect is caused by an increase in fatty acid oxidation, a reduction in *de novo* fatty acid synthesis, or a combination of the two. In **Chapter 3**, a study is described in which this is tested.
In pig nutrition, differences in the energy value of dietary fat sources are mainly based on their difference in digestibility. Differences in the energy values of starch and fat are based on their potential conversion into body fat. As these differences are based on whole-body measurements, differences in the location of fat deposition, with relevant consequences for meat quality may still occur. In **Chapter 4**, a comparison of three energy sources; starch,

saturated fat and unsaturated fat in relation to performance and carcass quality in fattening pigs is presented.
The essential fatty acids LA and ALA are precursors for n-6 and n-3 LC-PUFA. It is expected that LA interferes in the production of n-3 LC-PUFA from ALA. It is known that ALA interferes with the synthesis from LA into n-6 LC-PUFA. This is information available from studies in which LA or ALA is replaced by non-essential fatty acids (mostly oleic acid). It is not known to what extent the desirable LC-PUFA (ARA, DHA, EPA) can be synthesized (and deposited) by varying the intake, rather than the ratio, of both LA and ALA. In addition, the rate limiting step in these conversions is unknown. The study presented in **Chapter 5** is carried out with equal increments of LA and ALA intake between treatments. The effect on fatty acids, elongases and desaturases in liver and brain was determined.
The n-3 LC-PUFA are desirable for human health. The intake of fish in Western countries is too low to achieve a sufficient intake of EPA and DHA. Therefore it is of interest to increase the content of n-3 LCPUFA in pig meat. This is possible via a deposition of n-3 LC-PUFA from fish oil or algae in the pig diet. It is of interest if a pig could synthesize relevant amounts of EPA and DHA from dietary ALA (**Chapter 6**).
In the general discussion (**Chapter 7**), a calculation model is proposed that facilitates the prediction of digestibility of dietary fat sources in broiler chickens and growing pigs. In addition to variation in degree of saturation, the calculation model considers differences in digestion between C16:0 and C18:0 into account, differences in digestion of FFA, and differences in positional distribution of fatty acids on the glycerol molecule. Secondly, in the general discussion some results regarding the correlation of LC-PUFA and behaviour of pigs will be summarized. Thirdly, a discussion is presented about the extent to which changes in dietary ALA and LA intake can, via the consumption of pig meat products, contribute to meeting human recommended daily intakes of ALA, EPA and DHA.

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Chapter 2

Fatty acid digestion and deposition in broiler chickens fed diets containing either native or randomized palm oil

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2. Fatty acid digestion and deposition in broiler chickens fed diets containing either native or randomized palm oil

Abstract

The hypothesis tested was that randomization of palm oil would increase its digestibility, especially that of its palmitic acid (C16:0) component, with subsequent changes in the fatty acid composition in body tissues. Broiler chickens were fed diets containing either native or randomized palm oil. Diets with either native or a 50/50 mix of native and hydrogenated sunflower oil were also fed. Randomization of palm oil raised the fraction of C16:0 at the sn-2 position of the glycerol molecule from 14 to 32%. Hydrogenation of sunflower oil reduced fat and total saturated fatty acid digestibility, while no change in digestibility of total unsaturated fatty acids was found. Randomization of palm oil raised the group mean apparent digestibility of C16:0 by 2.6 and 5.8% units during the starter and grower-finisher phase, respectively. On the basis of the observed digestibilities in the grower-finisher period, it was calculated that the digestibility for C16:0 at the sn-2 and sn-1,3 position was 90 and 51%, respectively. The feeding of randomized instead of native palm oil significantly raised the palmitic content of breast meat and abdominal fat and lowered the ratio of unsaturated to saturated fatty acids. It is concluded that randomized palm oil may be used as vegetable oil in broiler nutrition with positive effect on saturated fatty acid digestibility when compared with native palm oil and positive effect on firmness of meat when compared with vegetable oils rich in unsaturated fatty acids.

Keywords: chicken, palm oil, fatty acid, digestibility

Introduction

There is increasing interest to replace animal fats by vegetable fat sources in the diet for broiler chickens. Animal fats such as tallow and lard are rich in long-chain saturated fatty acids. Most vegetable fat sources have a high content of unsaturated fatty acids. The use of unsaturated dietary fats decreases the melting point of the fat in the broiler carcass (Bavelaar and Beynen, 2003), diminishing the firmness or consistency of the fat (Gläser *et al.*, 2004).

Palm oil is of vegetable origin, but is rich in the saturated fatty acid palmitic acid (C16:0), the content is about 45% of the total fatty acids. The use of palm oil in broiler diets is attractive because it is a saturated source that may be associated with a positive influence on meat firmness. However, saturated fats rich in long-chain fatty acids (> 14 C-atoms) are less digestible than fats high in medium-chain fatty acids or unsaturated fatty acids (Renner and Hill, 1961; Young, 1961; Garrett and Young, 1975; Vila and Esteve-Garcia, 1996). In addition, a high fraction of C16:0 in palm oil is bound at the sn-1 or sn-3 position of the glycerol molecule (Breckenridge, 1978; Mu and Høy, 2004). Long-chain saturated fatty acids on the sn-1 and sn-3 positions are thought to be absorbed less efficiently than those bound on

the sn-2 position. This is because of the more hydrophilic character of the monoglyceride in comparison with, by lipase hydrolyzed, fatty acids from the sn-1 or sn-3 position of the glycerol backbone.
The position of fatty acids in triacylglycerols can be manipulated by hydrolysis and chemical re-esterification (Mukherjee and Warwel, 1997). Randomization is a process of non-specific random esterification of fatty acids at the three positions of the glycerol molecule. In lard the C16:0 is mainly bound at the sn-2 position of the glycerol molecule (Breckenridge, 1978; Mu and Høy, 2004). Randomization of lard does decrease the digestibility of C16:0 in broilers (Renner and Hill, 1961). A higher digestibility of long-chain saturated fatty acids at the sn-2 position is probably also responsible for a higher deposition rate of these fatty acids in broilers (Scheeder *et al.*, 2003). As far as known, the effect of randomization of palm oil on C16:0 digestibility and deposition in broiler chickens has not yet been quantified. We hypothesized that randomization of palm oil would increase its digestibility, in particular that of its C16:0 component. In this study, our hypothesis was put to the test. Broiler chickens were fed diets containing either native or randomized palm oil and the digestibility and deposition of fatty acids were measured.
In addition to studying the effect of randomization of palm oil, we also assessed the impact on digestibility of the degree of fatty acid saturation and chain length of fatty acids. Mathematical models used to calculate the metabolizable energy of fat sources in broiler nutrition are generally based on the contents of long-chain unsaturated and saturated fatty acids without taking into account chain length differences between C16:0 and C18:0 (Ketels, 1994; Wiseman *et al.*, 1998). It is well known that saturation of unsaturated fatty acids will decrease their digestibility and that of the oils they are components of (Ketels, 1994; Wiseman *et al.*, 1998). As far as we know, it is not known to what extent the total digestibility of oils varies when their saturated fatty acid constituents have different chain length. As a positive control in this study we determined the effect of saturation by comparing sunflower oil and a 50/50 mix of fully hydrogenated sunflower oil and sunflower oil. To assess effect of chain length in the form of C16:0 versus C18:0, we used the comparison of randomized palm oil versus the mixture of hydrogenated and native sunflower oil.

Materials and methods

Birds and housing
One-day old female broilers (Ross 308) were purchased from a local hatchery. On arrival, they were wing-banded, weighed and housed in wire-floor, suspended cages. Each of the four experimental groups contained 12 replicates (cages). The experiment started with 6 birds per cage during the starter period of 2 weeks. Then up to two randomly chosen birds in each cage were removed, so there were 4 birds for the grower-finisher period. Continuous lighting was provided throughout the experiment. The temperature in the cage at arrival was 32°C and was decreased gradually to ambient temperatures during the course of the experiment.

Diets

The birds received a starter feed until day 14 and a grower-finisher feed between days 15 to 35. The composition of the diets is presented in Table 2.1. Four different fat sources were used in the starter and grower-finisher diets. The fat sources were delivered by Cargill (Rotterdam-Botlek, The Netherlands). The diets were in pelleted form (2.5 mm). The diets were fed on an *ad libitum* basis and the birds had free access to water. The inclusion level of experimental fat was 4 and 8% (w/w) in the starter and grower-finisher period, respectively (Table 2.1). The inclusion levels are in agreement with European high energy and fat diets for broiler chickens. The four experimental fats consisted of sunflower oil (SO), a 50/50 mix of fully hydrogenated sunflower oil and sunflower oil (HSO+SO), palm oil (PO) and chemical randomized palm oil (RPO). The analyzed fatty acid composition of the experimental fats is presented in Table 2.2. The macronutrient and fatty acid composition of the diets are presented in Table 2.3. The experimental fats allow assessment of the effect of saturation (HSO+SO vs SO or C18:0 vs C18:1+C18:2), the effect of the chain length (HSO+SO vs RPO or C18:0 vs C16:0), and the effect of the position of long-chain saturated fatty acids on the glycerol molecule (PO vs RPO or 20 vs 45 % of C16:0 at the sn-2 position) in a situation with a high fat intake.

Sampling and analysis

Excreta were collected in the starter period from days 10 to 14 and in the growing-finishing period from days 31 to 33. It is known that fat digestibility in broiler chickens is lower during the starter than grower-finisher period (Katangole and March, 1980; Ketels, 1994). Thus, we collected excreta during the two periods in order to determine apparent fecal digestibility of total fat and individual fatty acids. Excreta were collected quantitatively per cage, dried at 60°C, weight and ground. On day 35, two broilers per pen were used to determine the fatty acid composition of breast meat and abdominal fat. Crude fat determination of diets and excreta were determined with the acid hydrolysis method (AOAC, 1975). To determine the fatty acid composition of the diets, breast meat and faeces, a 10-g sample was extracted with a choloroform:methanol (2:1, v/v) mixture according to the method of Folch *et al.* (1957). Then, 20-25 mg of the extracted fat was saponified with 0.5 M methanolic sodium hydroxide and methylated with boronitrifluoride in methanol according to the method of Metcalfe *et al.* (1966). The fatty acid methylesters obtained were separated and analyzed by gas chromatography. The fat of abdominal fat was directly saponified and methylated and the fatty acid composition determined with gas chromatography. The concentration of fatty acids at the sn-2 position of PO and RPO was determined by gas liquid chromatography after hydrolysis with pancreas lipase. The diets were analyzed according to the Dutch Normalization Institute for dry matter (NEN 3332), ash (NEN 3329), crude fibre (NEN 3326) and nitrogen (N). N in the diets was analyzed with the Kjeldahl method (NEN 3145). Crude protein (g) was calculated as 6.25 x N (g).

Table 2.1. Ingredient composition of the diets (g/kg).

Ingredients	Starter diet	Grower-finisher diet
Wheat (+ xylanase)	300.0	490.5
Maize	143.2	-
Soybean meal (467 CP)	170.0	160.0
Peas	100.0	100.0
Sunflower meal (320 CP)	50.0	50.0
Rapeseed meal (340 CP)	50.0	50.0
Potato protein	15.0	10.0
Maize gluten meal (580 CP)	35.0	20.0
Maize starch	50.0	-
Soybean oil	10.0	10.0
Experimental oils[1]	40.0	80.0
Mineral-vitamin mix[2]	5.0	5.0
Limestone	15.0	12.0
Mono calcium phosphate	8.0	3.5
Natuphos 5000G phytase	0.1	0.1
Salt	2.5	2.0
Na-bicarbonate	1.5	1.7
L-lysine HCl	2.5	2.6
DL-methionine	1.8	1.9
L-threonine	0.4	0.7

[1]: Experimental oils are hydrogenated sunflower oil mixed (50/50) with sunflower oil (HSO+SO), sunflower oil (SO), palm oil (PO) and randomized palm oil (RPO)

[2:] Supplied per kg of diet: retinyl acetate, 12,000 IU; cholecalciferol, 2,400 IU; DL-α-tocopherol, 30 mg; menadione, 1.5 mg; thiamin, 2.0 mg; riboflavine 7.5 mg; pyridoxine-HCl, 3.5 mg; cyanocobalamin, 20 µg; niacin, 35 mg; D-pantothenic acid, 10 mg; choline chloride, 460 mg; folic acid, 1.0 mg; biotin, 0.2 mg; $FeSO_4.H_2O$, 267 mg; $CuSO_4.5H_2O$, 48 mg; MnO, 135 mg; $ZnSO_4.H_2O$, 165 mg; $CoSO_4.7H_2O$, 2 mg; Na_2SeO_3, 0.22 mg; KI, 1 mg; limestone, 540 mg; anti-oxidant, 125 mg.

Table 2.2. Fatty acid composition (% of methyl esters) of the total fatty acids in the experimental fats[1] and the composition of fatty acids at the sn-2 position of the glycerol molecule of the palm oil and the randomized palm oil.

	HSO+SO	SO	PO		RPO	
	Total	Total	Total	Sn-2	Total	Sn-2
C14:0	0.1	0.1	1.1	0.6	1.1	1.1
C16:0	6.4	5.9	46.8	20.1	46.9	45.1
C18:0	45.7	3.9	4.6	1.5	4.8	4.8
C18:1 *t*	0.7	0.0	0.1	0.2	0.2	0.2
C18:1	12.8	24.5	37.3	60.8	36.8	38.6
C18:2 *t*	0.1	0.4	0.7	1.2	0.8	0.8
C18:2	32.8	63.9	8.4	15.6	8.1	8.6

[1]: Experimental oils are hydrogenated sunflower oil mixed (50/50) with sunflower oil (HSO+SO), sunflower oil (SO), palm oil (PO) and randomized palm oil (RPO)

Statistical analysis

Cage served as experimental unit so that there were 12 units per diet. The effect of diet on digestibility of fat and fatty acids, profiles of breast meat and adipose tissue were statistically analyzed by one-way ANOVA with diet as factor. In case of a significant diet-effect, the effects of dietary fatty acid saturation (HSO+SO vs SO), chain length (HSO+SO vs RPO) and position on the glycerol molecule (RPO vs PO) were analyzed via a Least Square Means (LSM) contrast test. The level of statistical significance was pre-set at $P<0.05$. Results are presented as LSM and a pooled standard error of the mean (pooled SEM). Statistical analysis was done with the SAS program (SAS JMP, 2000).

Table 2.3. Analyzed macronutrient content (%) and fatty acid profile (% of methyl esters) of the experimental diets[1] in the starter and grower-finisher period.

	Starter period				Grower-finisher period			
	HSO+SO	SO	PO	RPO	HSO+SO	SO	PO	RPO
Ash	4.9	4.9	5.3	5.2	4.5	4.5	5.3	5.2
Crude protein	21.6	21.4	21.7	21.3	20.4	20.1	20.3	19.7
Crude fiber	4.3	4.2	4.3	4.2	4.4	4.5	4.4	4.3
Crude fat	9.5	9.7	9.3	9.5	13.0	13.1	12.7	12.5
Fatty acid								
C12:0	0.21	0.20	0.27	0.37	0.16	0.15	0.25	0.39
C14:0	0.17	0.15	0.65	0.70	0.15	0.07	0.83	0.89
C16:0	11.1	10.4	30.9	31.1	9.61	9.14	38.0	37.6
C16:1	0.00	0.14	0.17	0.17	0.00	0.00	0.16	0.17
C18:0	25.6	3.41	3.76	3.80	34.1	3.63	4.06	4.22
C18:1 n-9	17.3	22.7	28.7	29.0	14.7	22.6	30.6	30.5
C18:1 n-7	1.22	1.25	1.31	1.31	1.01	1.08	1.11	1.14
C18:2 n-6	38.9	55.6	29.1	28.5	36.3	58.8	21.0	20.6
C18:3 n-3	2.22	2.27	2.32	2.33	1.53	1.58	1.59	1.61
C20:0	0.40	0.33	0.40	0.40	0.37	0.29	0.38	0.38
C20:1 n-9	0.34	0.41	0.36	0.36	0.26	0.37	0.30	0.31
C20:5 n-3	0.39	0.38	0.40	0.41	0.21	0.23	0.30	0.32
C22:0	0.74	0.75	0.38	0.37	0.75	0.76	0.27	0.29
C24:0	0.27	0.26	0.19	0.18	0.25	0.25	0.14	0.14
Saturated (S)	38.5	15.5	36.6	36.9	45.4	14.4	43.9	43.9
Unsaturated (U)	60.4	82.8	62.4	62.1	54.0	84.7	55.1	54.7
U/S ratio	1.57	5.34	1.70	1.68	1.19	5.88	1.26	1.25
C16:0 (% at sn-2*)			21	33			18	33
C18:0 (% at sn-2*)			22	33			17	33

[1]: Diets with hydrogenated sunflower oil mixed (50/50) with sunflower oil (HSO+SO), sunflower oil (SO), palm oil (PO) and randomized palm oil (RPO)

*: calculated as based on the assumption that C16:0 and C18:0 are randomly distributed at the glycerol molecule in all raw materials, except for PO.

Results

The effect of dietary fat source on body weight gain, feed intake and feed conversion is presented in Table 2.4. The weight gain was not affected by the diet. The feed to gain ratio was significant lower in the SO group in comparison with the HSO+SO group in both starter and grower-finisher period.

Table 2.4. Effect of dietary fat characteristics on feed intake, daily weight gain and feed conversion during the starter (days 1-14) and grower-finisher (days 15-33) period.

	Diet[1]				Pooled SEM	P-values of contrasts[2]		
	HSO+SO	SO	PO	RPO		HSO+SO vs SO	HSO+SO vs RPO	PO vs RPO
Day 1-14								
Feed intake (g/bird/d)	34.5	33.4	34.2	35.2	0.63	-	-	-
Weight gain (g/bird/d)	26.3	26.5	26.5	27.0	0.43	-	-	-
Feed/gain (g/g)	1.31	1.26	1.29	1.30	0.014	0.012	0.74	0.54
Day 15-33								
Feed intake (g/bird/d)	130.3	119.1	129.0	127.7	1.61	<0.001	0.26	0.57
Weight gain (g/bird/d)	70.1	69.6	70.8	69.6	0.81	-	-	-
Feed/gain (g/g)	1.86	1.71	1.82	1.84	0.021	<0.001	0.45	0.63

[1]: Diets with hydrogenated sunflower oil mixed (50/50) with sunflower oil (HSO+SO), sunflower oil (SO), palm oil (PO) and randomized palm oil (RPO)
[2]: Only calculated when the P-value of the dietary effect was <0.05

The effects of treatment on the apparent faecal digestibility of total fat and individual fatty acids are presented in Tables 2.5 and 2.6. The digestibility of crude fat in broilers fed the HSO+SO was significantly (P<0.001) lower than in those fed the SO in both the starter and grower-finisher period. The digestibilities of C18:0, C16:0 and total saturated fatty acids (S) was highest in the SO group, irrespective of the age of the broilers. There was no significant difference in the digestibility of total unsaturated fatty acids (U) between the HSO+SO and SO or RPO groups. During the age period of 31-33 days, almost all differences between digestibility of saturated fatty acids in HSO+SO and RPO were significant. During the age period of 10-14 days, the significant higher digestibility of total S was mainly based on a higher digestibility of C18:0 (Table 2.5). The difference for the digestibility of total U was not significant. The randomization of palm oil (RPO versus PO) did not significantly affect fat and fatty acid digestibility. However, a numerical increase (P<0.1) of the digestibility of

C16:0, C18:0 and total S for the growing-finishing birds fed with RPO in comparison with the PO group was found.
The effect of the dietary fat source on the fatty acid profile of breast meat and abdominal fat is presented in Table 2.7 and 2.8. The differences in fatty acid composition for the animals fed the HSO+SO and SO diets were nearly all significant. Broilers fed the SO diet had higher concentrations of unsaturated fatty acids in breast meat and abdominal fat. The calculated U/S ratio was highest in the SO group. The differences in fatty acid profile for broilers fed either the RPO diet or HSO+SO diet were significant for most fatty acids. The concentrations of C16:0 and C18:1 were higher and those of C18:0 and C18:2 were lower in the RPO group. The U/S ratio of breast meat and abdominal fat was lowest in broiler chickens fed the RPO diet. Feeding of RPO instead of PO resulted in significantly increased C16:0 in abdominal fat and breast meat. The U/S ratio of both breast meat and abdominal fat was significantly lower in the RPO group when compared with the PO group.

Table 2.5. Effect of dietary fat characteristics on the apparent faecal digestibility (%) of dry matter, crude fat and fatty acids in the starter period (days 10-14).

	Diet[1]				Pooled	P-values of contrasts[2]		
Component for digestibility	HSO + SO	SO	PO	RPO	SEM	HSO+SO vs SO	HSO+SO vs RPO	PO vs RPO
Dry matter	67.1	68.3	68.2	67.9	0.79	-	-	-
Crude fat	45.4	69.1	56.5	56.3	2.14	<0.001	<0.001	0.94
Fatty acids								
C14:0	23.9	16.2	51.9	54.0	3.20	0.10	<0.001	0.64
C16:0	35.6	54.9	37.9	40.5	2.74	<0.001	0.21	0.50
C18:0	-5.7	17.2	9.2	11.3	3.32	<0.001	<0.001	0.66
C18:1 n-7	28.9	26.1	38.5	36.7	4.27	-	-	-
C18:1 n-9	67.2	71.6	69.5	68.1	2.04	-	-	-
C18:2 n-6	73.8	79.2	74.1	73.0	1.87	-	-	-
C18:3 n-3	74.2	78.7	75.6	73.2	1.88	-	-	-
C20:0	9.4	17.5	12.6	15.3	3.23	-	-	-
C20:1 n-9	36.3	33.0	33.0	35.4	3.60	-	-	-
C20:5 n-3	67.5	45.1	48.7	49.0	2.31	<0.001	<0.001	0.94
C22:0	25.3	26.4	29.4	30.9	2.44	-	-	-
C24:0	5.1	-0.9	2.5	-0.6	2.81	-	-	-
Total unsaturated	64.9	68.9	67.9	66.5	2.06	-	-	-
Total saturated	7.5	42.9	34.6	37.4	2.70	<0.001	<0.001	0.47

[1]: Diets with hydrogenated sunflower oil mixed (50/50) with sunflower oil (HSO+SO), sunflower oil (SO), palm oil (PO) and randomized palm oil (RPO)
[2]: Only calculated when the P-value of the dietary effect was <0.05

Table 2.6. Effect of dietary fat characteristics on the apparent faecal digestibility (%) of dry matter, crude fat and fatty acids in the grower-finisher period (days 31-33).

Component for digestibility	Diet[1]				Pooled SEM	P-values of contrasts[2]		
	HSO+SO	SO	PO	RPO		HSO+SO vs SO	HSO+SO vs RPO	PO vs RPO
Dry matter	66.3	67.1	66.9	66.4	1.69	0.62	0.95	0.76
Crude fat	56.7	77.5	70.2	72.1	1.58	<0.001	<0.001	0.40
Fatty acids								
C14:0	41.5	-47.5	68.8	71.1	4.90	<0.001	<0.001	0.74
C16:0	50.1	63.0	57.9	63.7	2.38	<0.001	<0.001	0.09
C18:0	15.4	52.1	45.2	52.7	3.06	<0.001	<0.001	0.09
C18:1 n-7	64.1	52.4	63.4	61.5	1.73	<0.001	0.29	0.45
C18:1 n-9	83.0	79.6	83.0	81.7	1.48	-	-	-
C18:2 n-6	85.7	83.1	79.8	78.9	1.43	0.19	0.002	0.65
C18:3 n-3	82.1	76.0	78.9	77.9	1.27	0.001	0.022	0.55
C20:0	24.0	48.0	42.7	48.6	2.33	<0.001	<0.001	0.08
C20:1 n-9	69.8	67.1	67.1	66.7	2.34	-	-	-
C20:5 n-3	93.7	62.3	89.5	76.6	6.24	<0.001	0.06	0.15
C22:0	46.6	62.5	54.4	58.9	2.38	<0.001	<0.001	0.19
C24:0	32.5	49.0	31.2	33.7	2.65	<0.001	0.75	0.51
Total unsaturated	81.8	77.7	82.0	80.6	1.47	-	-	-
Total saturated	23.7	58.8	56.6	62.6	2.24	<0.001	<0.001	0.07

[1]: Diets with hydrogenated sunflower oil mixed (50/50) with sunflower oil (HSO+SO), sunflower oil (SO), palm oil (PO) and randomized palm oil (RPO)
[2]: Only calculated when the P-value of the dietary effect was <0.05

Discussion

This study with broiler chickens confirms that saturated fatty acids are less digestible than unsaturated fatty acids. It also confirms that the intake of C18:2 is reflected in the fatty acid profiles of breast meat and of abdominal fat. The broilers fed with SO diet instead of the HSO+SO diet, which in essence is consumption of C18:0 at the expense of C18:1 and C18:2, had a higher fat digestibility. This outcome corroborates earlier studies (Renner and Hill, 1961; Katangole and March, 1980; Wiseman and Salvador, 1991; Ketels, 1994). It is known that in broiler chickens the digestibility of medium-chain saturated fatty acids is higher than that of long-chain saturated fatty acids (Renner and Hill, 1961; Young, 1961; Kussaibati *et al.*, 1982; Dänicke et al, 2000; Knarreborg *et al.*, 2004). There was a significantly higher crude fat digestibility for the RPO diet in comparison with the HSO+SO diet. Deduced from this and different other studies with broiler chickens (Young, 1961; Kussaibati *et al.*, 1982;

Ketels and De Groote, 1989; Smits *et al.*, 2000) the differences in digestibility between poly- and monounsaturated fatty acids with a chain length of 18 C-atoms are low. For the digestibility, the relevant difference in dietary fat composition between HSO+SO and RPO is therefore the C16:0 and the C18:0 concentration.

Birds fed the HSO+SO diet in comparison with those fed with the SO diet had a significant lower digestibility for the predominant long-chain saturated fatty acids (C16:0 and C18:0), but not for the unsaturated fatty acids. This agrees with results of studies in which tallow was compared with soybean oil (Dänicke *et al.*, 1999; Knarreborg *et al.*, 2004). However, Dänicke *et al.* (2000) estimated a significant linear dose dependent decrease for digestibility of both the saturated and unsaturated fatty acids after replacing soybean oil by tallow. The digestibility of saturated fat may be improved by the addition of an unsaturated fat source (Sibbald, 1978), but according to Wiseman *et al.* (1998) it is not plausible to suggest a synergy between dietary unsaturated and saturated fat in broiler chickens. There might a dose-effect and a limiting digestibility for the amount of long-chain saturated fat. Ketels and De Groote (1989) showed that the inclusion of increasing levels of a saturated fat source like tallow decreased its digestibility, but not such effect was seen with soybean oil. The dose level of long-chain saturated fatty acids in the HSO+SO group was relatively high being approximately 3 and 6% of the diet in the starter and grower-finisher period, respectively. This dietary long-chain saturated fat content corresponds with a dietary dose level of 7 and 14 % of a saturated fat source like tallow in the starter and the grower-finisher period, respectively. Thus, the low digestibility of C18:0 in the HSO+SO group may relate to the high inclusion level of C18:0. However, a high intake itself tends to raise the apparent digestibility because of the diminishing effect of the relatively constant excretion of endogenous origin will be smaller. The calculated C18:0 digestibility was negative for the HSO+SO group in the starter period. This is probably caused by the combination of a low digestibility and endogenous C18:0 production in the lower gut. In addition, the C18:0 digestibility coefficient may be underestimated due to bio hydrogenation of C18:1 and C18:2 in the large intestine.

In calculation models, the dietary U/S ratio is used to predict the digestibility and/or metabolizable energy value (Ketels, 1994; Wiseman *et al.*, 1998). The S in the formulas mainly reflects the sum of C16:0 and C18:0. The U/S ratios of the HSO+SO and RPO diets were similar. The S digestibility was significantly lower in the HSO+SO group compared with the RPO group, but there was no difference in total U digestibility between the groups. The S fraction consists of C18:0 with relatively low digestibility and C16:0 with higher digestibility, as was shown earlier (Kussaibati *et al.*, 1982; Dänicke *et al.*, 2000; Smits *et al.*, 2000).

Table 2.7. Effect of dietary fat characteristics on the fatty acid profile (% of methyl esters) of breast meat in broilers.

	Diet[1]				Pooled SEM	P-values for contrasts[2]		
Fatty acids	HSO+SO	SO	PO	RPO		HSO+SO vs SO	HSO+SO vs RPO	PO vs RPO
C14:0	0.23	0.18	0.36	0.43	0.033	0.23	<0.001	0.12
C16:0	16.1	14.5	20.9	22.2	0.284	<0.001	<0.001	<0.001
C16:1	1.77	0.99	2.13	2.43	0.114	<0.001	<0.001	0.07
C18:0	11.5	9.26	9.01	8.82	0.231	<0.001	<0.001	0.56
C18:1 n-7	2.64	1.89	3.11	3.03	0.064	<0.001	<0.001	0.37
C18:1 n-9	23.7	20.8	29.4	29.7	0.672	0.004	<0.001	0.79
C18:2 n-6	25.1	33.8	16.8	16.1	0.371	<0.001	<0.001	0.20
C18:3 n-3	0.70	0.69	0.65	0.66	0.013	-	-	-
C20:1 n-9	0.35	0.29	0.48	0.49	0.015	<0.001	<0.001	0.75
C20:2 n-6	1.12	1.34	0.62	0.54	0.062	0.016	<0.001	0.33
C20:3 n-6	1.15	0.79	1.22	1.19	0.066	<0.001	0.73	0.72
C20:4 n-6	6.79	6.82	5.66	5.16	0.329	0.94	0.001	0.30
C22:4 n-6	2.07	2.61	1.36	1.18	0.109	0.001	<0.001	0.26
C22:5 n-3	0.62	0.47	0.81	0.76	0.038	0.010	0.008	0.42
C22:6 n-3	0.55	0.43	0.73	0.73	0.040	0.036	0.004	0.97
Unsaturated (U)	67.1	71.5	63.6	62.6	0.303	<0.001	<0.001	0.023
Saturated (S)	27.8	23.9	30.2	31.5	0.241	<0.001	<0.001	<0.001
U/S ratio	2.42	3.01	2.10	1.99	0.034	<0.001	<0.001	0.026

[1]: Diets with hydrogenated sunflower oil mixed (50/50) with sunflower oil (HSO+SO), sunflower oil (SO), palm oil (PO) and randomized palm oil (RPO)

[2]: Only calculated when the P-value of the dietary effect was <0.05

Table 2.8. Effect of dietary fat characteristics on the fatty acid profile (% of methyl esters) of abdominal fat in broilers.

	Diet[1]				Pooled SEM	P-values for contrasts[2]		
Fatty acid	HSO+SO	SO	PO	RPO		HSO+SO vs SO	HSO+SO vs RPO	PO vs RPO
C14:0	0.39	0.30	0.72	0.77	0.007	<0.001	<0.001	<0.001
C16:0	17.5	13.7	27.4	28.7	0.213	<0.001	<0.001	<0.001
C16:1	2.98	1.55	3.44	3.50	0.134	<0.001	0.008	0.77
C18:0	8.68	5.60	5.27	5.48	0.133	<0.001	<0.001	0.27
C18:1 n-7	1.93	1.28	2.10	2.06	0.043	<0.001	0.044	0.46
C18:1 n-9	34.4	28.9	42.4	41.6	0.331	<0.001	<0.001	0.10
C18:2 n-6	31.2	44.9	15.9	15.0	0.344	<0.001	<0.001	0.08
C18:3 n-3	1.23	1.19	1.06	1.04	0.014	<0.001	0.60	0.90
C20:1 n-9	0.36	0.34	0.40	0.38	0.331	0.22	0.031	0.09
C20:4 n-6	0.25	0.52	0.08	0.09	0.024	<0.001	<0.001	0.79
Total U	72.5	79.2	65.3	63.6	0.259	<0.001	<0.001	<0.001
Total S	26.6	19.6	33.4	35.1	0.267	<0.001	<0.001	<0.001
U/S ratio	2.73	4.05	1.96	1.82	0.034	<0.001	<0.001	0.007

[1]: Diets with hydrogenated sunflower oil mixed (50/50) with sunflower oil (HSO+SO), sunflower oil (SO), palm oil (PO) and randomized palm oil (RPO)
[2]: Only calculated when the P-value of the dietary effect was <0.05

Palm oil contains a high content of C16:0 that is predominantly located at the sn-1 and sn-3 positions of the glycerol molecule. Randomization yielded palm oil with one third of the C16:0 at the sn-2 position. As indicated in the Introduction section, we hypothesized that randomization of palm oil would increase its digestibility, in particular that of its C16:0 component. There was no statistically significant effect of randomization on crude fat digestibility and the digestibility of individual fatty acids. Thus our hypothesis would be rejected. However, we did find a systematic (P-value between 0.05 and 0.1) increase in the group mean digestibilities of C16:0, C18:0 and S during the growing-finisher period. Randomization of palm oil had increased the group mean digestibility of C16:0 by 5.8% units. The randomization-induced increase of C16:0 digestibility is in line with results found in rats (Renaud *et al.*, 1995). The effect of randomization of palm oil on the digestibility of C16:0 is also supported by significantly higher concentration of C16:0 in breast meat and abdominal fat. It is not known to what extent the increase in C16:0 in breast meat and abdominal fat is caused by increased digestion of C16:0 or increased *de novo* synthesis. A large part of deposition of S in broiler chickens fed with lipid rich diets, does not originate from the *de novo* production (Villaverde *et al.*, 2006). The deposition of C16:0 will come predominantly from dietary C16:0. It is concluded that the raised level of C16:0 in breast meat and abdominal fat of the birds fed the RPO diet was caused by the increased digestibility of C16:0. Scheeder *et al.* (2003) have also reported that the position of saturated fatty acids in

the glycerol molecule influence their position in the body of poultry. Triglycerides are hydrolyzed in the lumen of the gastro-intestinal tract in free fatty acids and 2-monoglycerides. Free fatty acids have a lower digestibility than monoglycerides (Garrett and Young, 1975). Due to a randomization of palm oil, the composition of free fatty acids in the lumen of the intestine will be changed. The amount of free S decreased and free U increased. The decrease of the fat digestibility by increasing the amount of dietary free fatty acids is higher in case of saturated fat in comparison with an unsaturated fat source (Wiseman and Salvador, 1991). The increased content of unsaturated fatty acids at the sn-1,3 position after randomization did not affect its digestibility.

From the results of the grower-finisher period (Table 2.6), the digestibility of C16:0 at the sn-2 and sn-1,3 positions can be calculated using the following formulas:

(1) 0.67 sn-1,3 + 0.33 sn-2 = 63.7% (C16:0 digestibility for RPO group)

(2) 0.82 sn-1,3 + 0.18 sn-2 = 57.9% (C16:0 digestibility for PO group).

Multiplying the first formula by 1/0.67 gives the value for sn-1,3 being:

(3) sn 1,3 = - 0.493 sn-2 + 95.07

The digestibility of C16:0 at the sn-2 position can be calculated by using formula 2 and 3:

(4) 0.82 (-0.493 sn-2 + 95.07) +0.18 sn-2 = 57.9

The digestibility of C16:0 at sn-2 is calculated to be 89.5%. The use of this value in formula 1 gives a calculated digestibility of 51.0 % for C16:0 at the sn-1,3 position.
Randomization also resulted in a numerically (P=0.09) increased digestibility of C18:0 during days 31-33. The calculated digestibility for C18:0 at the sn-2 and sn-1,3 positions was 84 and 37%, respectively. Thus the positions of C16:0 and C18:0 at the glycerol molecule are important factors to determining the digestibility of palm oil.

In conclusion, the present data support the idea that randomization of palm oil, which raises the content of C16:0 at the sn-2 position, improves the digestibility of its C16:0 component. Randomization of palm oil increased the group mean C16:0 digestibility by 5.8% units during the grower-finisher period. It was calculated that C16:0 at the sn-2 position was digested much more efficiently than C16:0 at the sn-1,3 position. Randomization of palm oil increased the incorporation of C16:0 into breast meat and abdominal fat and lowered the U/S ratios in these tissues. Thus, randomized palm oil may be used a vegetable oil in broiler nutrition with positive effects on saturated fat digestibility and firmness of meat compared with native palm oil. For the determination of the digestibility or metabolic energy value of palm oil to replace animal fat, both the differences between C16:0 and C18:0 and the position of C16:0 on the glycerol molecule are relevant.

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Chapter 3

Effect of dietary fat sources on fatty acid deposition and lipid metabolism in broiler chickens

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3. Effect of dietary fat sources on fatty acid deposition and lipid metabolism in broiler chickens

Abstract

The hypothesis tested was that dietary vegetable fats rich in saturated fatty acids, when compared with a vegetable oil rich in linoleic acid, increase fat deposition in broiler chickens and affect synthesis and/or oxidation of individual fatty acids. Diets with native sunflower oil (SO), a 50/50 mix of hydrogenated and native sunflower oil (HSO+SO), palm oil (PO) and randomized palm oil (RPO) were fed to broiler chickens. Intake of digestible fat and fatty acids, whole body fatty acid deposition, hepatic fatty acid profile and hepatic enzyme activities involved in fatty acid oxidation and synthesis were measured. The fat deposition/digestible fat intake ratio was significantly lower for the SO group in comparison with the groups fed the vegetable fats rich in saturated fatty acids. The difference between digestible intake and deposition of C18:2, reflecting its maximum disappearance rate, was highest for the SO group and lowest for the PO and RPO fed birds. The calculated minimal rate of *de novo* synthesis of monounsaturated fatty acids (MUFA), calculated as deposition minus digestible intake, was more than 50% lower for the SO group than for the other three dietary groups. Based on the fatty acid profiles in the liver it would appear that increasing contents of C18:2 decrease the desaturation of saturated fatty acids (SFA) into MUFA. It is concluded that a diet rich in C18:2 in comparison with different kinds of vegetable saturated fatty acids decreases the deposition of fat, especially of MUFA. It appears to be caused by a higher ß-oxidation and a reduced *de novo* synthesis of MUFA, but this conclusion is not fully supported by the measured activities of enzymes involved in fatty acid synthesis and oxidation.

Keywords: chicken, fatty acid, metabolism, fat deposition, liver

Introduction

The replacement of beef tallow by vegetable fats rich in polyunsaturated fatty acids (PUFA) like sunflower oil, soybean oil or linseed oil resulted in a decrease of abdominal fat deposition in broilers (Sanz *et al.*, 1999; 2000ab; Bilal *et al.*, 2001; Crespo and Esteve-Garcia, 2002a; Newman *et al.*, 2002; Ferrini *et al.*, 2008; Wongsuthavas *et al.*, 2008). Abdominal fat represents a small part of the total fat deposition in chicken. Studies of fat sources on whole body fat content are scarce and show no statistically significant effect of PUFA source on fat deposition in broilers (Pinchasov and Nir, 1992; Crespo and Esteve-Garcia, 2002a). The saturated fatty acid-rich sources used in deposition and metabolism studies in chickens are mostly tallow or lard. These fat sources contain high levels of both palmitic (C16:0) and stearic acid (C18:0) as saturated fatty acids (SFA). Palm oil is often used as vegetable fat source to replace animal fat. We are not aware of studies on deposition and metabolism of

fatty acids in broiler chickens fed with palm oil (C16:0-rich) and hydrogenated sunflower oil (C18:0-rich source) in comparison with PUFA. A high fraction of palmitic acid in palm oil is bound at the sn-1 or sn-3 position of the glycerol molecule (Breckenridge, 1978; Mu and Høy, 2004). Long-chain saturated fatty acids that are esterified on the sn-1 and sn-3 positions are absorbed less efficiently than those bound on the sn-2 position. From results of our previous study (Smink *et al.*, 2008), the digestibility of the C16:0 and the proportion of C16:0 in abdominal fat and breast meat was found to be higher in broilers fed randomized palm oil in comparison with the birds fed with palm oil.
Fat accumulation is the net result of absorption, *de novo* synthesis and oxidation of fatty acids. Results of studies in rats (Shimomura *et al.*, 1990) and in broiler chickens (Sanz *et al.*, 2000b) indicate that dietary PUFA increase the ß-oxidation and inhibit *de novo* fatty acid synthesis (Shimomura *et al.*, 1990; Sanz *et al.*, 2000b). However, in a study using linseed oil as PUFA, both an increased oxidation and *de novo* synthesis were found (Crespo and Esteve-Garcia, 2002b). Thus, the effect of high PUFA intake on *de novo* synthesis of fatty acids is not clear. In this study on the effect of native sunflower oil versus vegetable fats rich in saturated fatty acids we re-addressed the issue of the relations between fatty acid deposition, synthesis and oxidation. It is well-known that PUFA versus SFA are preferentially oxidized (Beynen and Katan, 1985), which may explain the above-mentioned lowering of abdominal fat in broiler chickens fed diets rich in PUFA. Thus, in this study we tested the hypothesis that in broilers fed various dietary vegetable fats rich in SFA, instead of a vegetable oil rich in linoleic acid, there is more fat deposition in the body, which is associated with less fatty acid oxidation and an unpredictable change in fatty acid synthesis. Fatty acid oxidation and synthesis at the level of the whole body were assessed on the basis of calculated rates of net fatty acid disappearance and appearance. The liver plays a dominant role in deposition and oxidation of fatty acids. Thus, we also measured hepatic enzyme activities, liver fat content and hepatic fatty acid composition. The effects of the dietary treatments on fatty acid digestibility have been published elsewhere (Smink *et al.*, 2008).

Material and methods

The experimental protocol was approved by the animal experiments committee of the Faculty of Veterinary Medicine, Utrecht University Utrecht, The Netherlands.

Birds and housing
One-day old female broilers (Ross 308) were purchased from a local hatchery. On arrival, they were wing-banded, weighed and housed in wire-floor, suspended cages. There were 4 dietary treatments each consisting of 12 replicates (cages). The experiment started with 6 birds per cage during the starter period of 2 weeks. Then up to two randomly chosen birds in each cage were removed, leaving 4 birds for the grower-finisher period. Continuous lighting was provided throughout the experimental period. The temperature in the cage at arrival was 32°C and was decreased gradually to ambient temperatures during the course of the experiment.

Diets

The diets were mainly based on wheat and soybean meal and are given in detail elsewhere (Smink *et al.*, 2008). The birds received a starter feed until day 14 and a grower-finisher feed between days 15 to 35. Four different fat sources were used in the starter and grower-finisher diets. The diets were in pelleted form (2.5 mm). The diets were fed on an *ad libitum* basis and the birds had free access to tap water. The inclusion level of experimental fat was 4 and 8% (w/w) in the starter and grower-finisher period, respectively. The four experimental fats consisted of sunflower oil (SO), a 50/50 mix of fully hydrogenated sunflower oil and sunflower oil (HSO+SO), palm oil (PO) and chemical randomized palm oil (RPO). The macronutrient and the major fatty acids are summarized in Table 3.1.

Table 3.1. Analyzed macronutrient and fatty acid content (g/kg)[2] of the experimental diets[1] in the starter and grower-finisher period.

	Starter period (d 0-14)				Grower-finisher period (d 15-35)			
	HSO+SO	SO	PO	RPO	HSO+SO	SO	PO	RPO
Ash	49	49	53	52	45	45	53	52
Crude protein	216	214	217	213	204	201	203	197
Crude fiber	43	42	43	42	44	45	44	43
Crude fat	95	97	93	95	130	131	127	125
Fatty acid								
C14:0	1.5	1.4	0.51	0.63	0.19	0.09	1.01	1.06
C16:0	10.0	9.58	27.3	28.1	11.9	11.4	45.8	44.7
C16:1	0.00	0.13	0.15	0.15	0.00	0.00	0.19	0.20
C18:0	23.1	3.14	3.32	3.43	42.1	4.52	4.90	5.01
C18:1 n-9	15.6	20.9	25.4	26.2	18.2	28.1	36.9	36.2
C18:1 n-7	1.10	1.15	1.16	1.18	1.25	1.34	1.34	1.35
C18:2 n-6	35.1	5.12	25.7	25.7	44.8	73.2	25.3	24.5
C18:3 n-3	2.00	2.09	2.05	2.10	1.90	1.97	1.92	1.91

[1]: Diets with hydrogenated sunflower oil mixed (50/50) with sunflower oil (HSO+SO), sunflower oil (SO), palm oil (PO) and randomized palm oil (RPO)

[2]: Values are means of duplicate analysis.

Sampling and analysis

Excreta were collected in the starter period from days 10 to 14 and in the growing-finishing period during 17 to 21 and during 31 to 33 days of age. Excreta were collected quantitatively per cage, dried at 60°C, weighed and ground. On day 33, two birds per cage were euthanized to determine body composition. Whole bodies were cut into pieces and ground, and were for each replicate pooled, homogenized, sampled and then dried to a constant weight in a forced-air oven at 60°C for a period of three days. The dried birds were weighed again and the percentage of water was calculated. In order to determine the initial body composition, two broilers were euthanized at day 1, applying procedures as described above. Also at day 35, up to two birds per cage were euthanized and used to determine enzyme activities in the liver. Livers were removed, placed in ice-cold saline, divided into portions for the different assays,

snap frozen in liquid nitrogen, and stored at -70°C until analysis. Hepatic lipids were extracted according to the method of Bligh and Dyer (1959) from the homogenate used for the assay of citrate synthase (CS) and 3-hydroxyacyl-CoA dehydrogenase (3-HOAD). Part of the extract was also used for the determination of triacylglycerols and part for determination of it for the fatty acid composition of total hepatic lipids.

Crude fat concentration of diets and excreta were determined after acid hydrolysis (AOAC, 1990; nr 954.02). To determine the fatty acid composition of the diets, and the whole bodies, samples were extracted with a chloroform:methanol (2:1, v/v) mixture according to the method of Folch *et al.* (1957). Then, 20-25 mg of the extracted fat was saponified with 0.5 M methanolic sodium hydroxide and methylated with boronitrifluoride in methanol according to the method of Metcalfe *et al.* (1966). The fatty acid composition, dry matter, ash, crude fibre and nitrogen were analyzed as is given in the former study (Smink *et al.*, 2008).

Determination of enzyme activities

For the assays of acetyl-coenzyme carboxylase (ACC; EC 6.4.1.2) and fatty acid synthase (FAS; EC 2.3.1.85) activities, fresh liver pieces were homogenized immediately with five strokes of a loosely-fitted Dounce homogenizer in three volumes of ice-cold 250 mM mannitol, 50mM HEPES, 6.2 mM Na-EDTA, 4 mM potassium citrate and 2.5 mM ß-mercaptoethanol, pH 7.5. The crude homogenate was centrifugated at 12,000 x g for 5 min. The supernatant was stored at -70°C until analyzed for the activities of ACC and FAS as described (Tijburg *et al.*, 1988).

For the assay of CS (EC 2.3.3.1) and 3-HOAD (EC 1.1.1.35) activities, part of the frozen liver was placed in 9 volumes of 25 mM HEPES, 5 mM ß-mercaptoethanol, pH 8.0 and homogenized with an IKA-Ultra Turrax® T-5 tissue homogenizer. The CS and 3-HOAD activities in the homogenate were assayed as described (Geelen *et al.*, 2001).

Carnitine palmitoyltransferase-I (CPT-I; EC 2.3.1.21) activity was assayed in mitochondria isolated from a 20% homogenate in 250 mM sucrose, 20 mM Tris-HCl and 1mM EDTA (pH 7.4). The frozen liver was homogenized with 5 strokes of a glass-Teflon Potter-Elvehjem tissue homogenizer. The homogenate was centrifuged at 600 g for 5 min (4°C). The supernatant was again centrifuged at 10,000 g for 15 min (4°C). The pellet was resuspended in the homogenizing buffer and termed mitochondrial preparation. The CPT-I activity was monitored as the incorporation of radio-labelled carnitine into acylcarnitine as reported by Guzman *et al.* (1994). CPT-I activity that was insensitive to 100 µM malonyl-CoA was always subtracted from the CPT-I activity experimentally determined.

Measurement of diacylglycerol acyltransferase (DGAT; EC 2.3.1.20) activity was described previously (Tijburg *et al.*, 1988).

Calculations

The intake of digested fat and fatty acid intake was calculated as analyzed fatty acid intake x apparent fat and fatty acid digestibility (fraction of intake). The digestibility coefficient measured during days 10 to 14 was used to determine the digestible intake during the first 14 days. The digestibility coefficients determined during days 17 to 21 and during 31 to 33 days were used for the calculation of the digestible intake during days 15 to 22 and days 29 to 33,

respectively. Then average of the determined digestibility of the two periods was used to calculate the intake of digestible fat and fatty acids during the period of day 22 to 29.

The deposition of fat and fatty acids was calculated by difference between the fat mass per bird in each cage minus the initial quantity present, estimated as the average body fat mass of the one-day old broilers.

The minimal *de novo* synthesis rate of individual fatty acids was calculated by difference between the quantity of a particular fatty acid deposited in the carcass and the digested quantity. For essential fatty acids, the digested minus deposition was calculated. This number represents the maximum disappearance rate.

The hepatic fatty acid content per liver was calculated using a conversion factor (Geelen and Gibson, 1976) and an assumed molecular weight of 850 g per triglyceride and 95% fatty acids per triglyceride.

Statistical analysis

Data from animals housed together in one cage served as the experimental unit. The effects of dietary treatment were statistically analyzed by one-way ANOVA. The statistical differences between treatments were determined by a Tukey-test. The level of statistical significance was pre-set at $P<0.05$. Results are presented as least square mean (LSM) and a pooled standard error of the mean (pooled SEM). Statistical analysis was done with the SAS program (SAS JMP, 2000).

Results

The effects of the dietary treatments on body weight gain, feed intake and feed conversion and effects of dietary fat source are presented elsewhere (Smink *et al.*, 2008). Briefly, body weight gain was not affected by the dietary treatments. Feed intake and feed conversion were significantly decreased for the SO birds. The results of body fat and fatty acid composition are presented in Table 3.2. The RPO and PO fed birds showed the highest content of body fat on d 33. The content of unsaturated and saturated fat was significantly different between the four diets. The RPO showed the highest content of saturated fat followed by the groups PO, HSO+SO and SO. This difference was mainly related to differences in the concentration of C16:0. The concentration of unsaturated fatty acids was highest in SO birds, followed by HSO+SO, PO and RPO, and was mainly related to differences in C18:2 n-6.

The results on fat balance are given in Table 3.3. The intake of digestible fat was highest for the SO fed broilers. The different dietary fats had a clear effect ($P = 0.02$) on fat deposition, which was highest in the PO and RPO broilers and lowest in the SO broilers. The ratio of fat deposition/digestible fat intake was significantly lower for the SO broilers in comparison with the others. Effects of the dietary treatments on the digestible intake and deposition of individual fatty acids are presented in Table 3.4. Both digestible intake and deposition of fatty acids were significantly affected by the dietary treatments. The PO and RPO group showed a high deposition of C16:0 and C18:1 n-9. The predominant fatty acid in the HSO+SO and SO groups were C18:1 n-9 and C18:2 n-6. The ratio deposition/digestible intake for the essential fatty acids C18:2 n-6 and C18:3 n-3 was significant higher for the PO

and RPO group. Results of the calculated minimal synthesis (deposition – digestible intake) of non-essential fatty acids and the maximum disappearance (digestible intake – deposition) of essential fatty acids are given in Table 3.5. The calculated minimal synthesis of MUFA was significantly lower of the broilers in the SO group in comparison with the other groups. The minimal synthesis of SFA was highest in the SO and HSO+SO group. The calculated maximal disappearance of the essential fatty acids C18:2 n-6 and C18:3 n-3 was highest for the SO group and lowest in the broilers of treatment groups PO and RPO.

Effects of the dietary treatments on hepatic fat content and enzyme activities are shown in Table 3.6. There were no statistically significant effects of diet on hepatic enzyme activities. There were significant dietary effects on hepatic fatty acid content for MUFA (Table 3.7). The livers of the broilers in the SO group had the lowest content of MUFA, while the amount of PUFA was not affected. The ratios of C16:1/C16:0 and C18:1/C18:0 were lowest in the SO group and significantly different from those in the HSO+SO, PO and RPO groups. There is a negative correlation between hepatic PUFA profile of the birds (R^2= 0.85; P<0.001 and R^2=0.93; P<0.001 for C16:1/C16:0 and C18:1/C18:0, respectively; Figure 3.1).

Table 3.2. Effect of dietary fat source on fat content and fatty acid profile (% of methyl esters) of the whole body of broilers.

	Diet[1,2]				Pooled SEM	P-value diet effect
	HSO+SO	SO	PO	RPO		
Crude fat (g/kg dm)	415[a]	417[a]	452[b]	443[ab]	6.9	<0.001
Fatty acids (%)						
C14:0	0.47[b]	0.41[a]	0.78[c]	0.83[d]	0.007	<0.001
C16:0	18.8[b]	15.6[a]	27.0[c]	28.5[d]	0.19	<0.001
C16:1	3.58[b]	1.97[a]	4.33[c]	4.27[c]	0.13	<0.001
C18:0	8.15[c]	6.28[b]	5.06[a]	5.16[a]	0.10	<0.001
C18:1 n-7	2.11[b]	1.51[a]	2.14[b]	2.10[b]	0.03	<0.001
C18:1 n-9	33.9[b]	29.6[a]	40.5[c]	39.6[c]	0.30	<0.001
C18:2 n-6	27.8[b]	37.9[c]	15.3[a]	14.6[a]	0.43	<0.001
C18:3 n-3	0.96[b]	0.84[a]	0.97[b]	0.97[b]	0.02	<0.001
C20:1 n-9	0.51[b]	0.46[a]	0.49[ab]	0.47[ab]	0.01	0.010
C20:4 n-6	0.66[b]	0.78[c]	0.53[a]	0.51[a]	0.03	<0.001
Unsaturated (U)	69.9[c]	73.6[d]	64.6[b]	62.9[a]	0.22	<0.001
Saturated (S)	27.9[b]	23.0[a]	33.4[c]	35.1[d]	0.21	<0.001
U/S ratio	2.51[c]	3.20[d]	1.93[b]	1.79[a]	0.03	<0.001

[1]: Diets with hydrogenated sunflower oil mixed (50/50) with sunflower oil (HSO+SO), sunflower oil (SO), palm oil (PO) and randomized palm oil (RPO)

[2]: Numbers of observations: HSO+SO, 9; SO, 12; PO, 11; RPO, 8.

[abcd]: Means in the same row with different superscript differ significantly (P<0.05)

Table 3.3. Effect of dietary fat source on the fat balance in broilers during days 1-33.

	Diet[1,2]				Pooled SEM	P-value diet effect
	HSO+SO	SO	PO	RPO		
Fat intake (g/bird)	373[b]	343[a]	359[ab]	348[a]	4.7	<0.001
Digestible fat intake (g/bird)	200[a]	256[c]	226[b]	234[b]	5.7	<0.001
Fat deposition (g/bird)	242[a]	254[a]	281[a]	284[a]	10.5	0.020
Digestible intake / fat intake	0.54[a]	0.75[c]	0.63[b]	0.67[b]	0.016	<0.001
Fat deposition / digestible fat intake	1.22[b]	0.99[a]	1.24[b]	1.21[b]	0.040	<0.001

[1]: Diets with hydrogenated sunflower oil mixed (50/50) with sunflower oil (HSO+SO), sunflower oil (SO), palm oil (PO) and randomized palm oil (RPO)
[2]: Numbers of observations: HSO+SO, 9; SO, 12; PO, 11; RPO, 8.
[abc]: Means in the same row with different superscript differ significantly (P<0.05)

Table 3.4. Effect of dietary fat source on the digestible fatty acid intake and deposition in broiler chickens.

	Diet[1,2]				Pooled SEM	P-value diet effect
	HSO+SO	SO	PO	RPO		
Digestible fatty acid intake (g/bird)						
C16:0	16.0^{a}	18.6^{a}	63.3^{b}	70.0^{c}	1.66	<0.001
C16:1	0.00^{a}	0.02^{a}	0.33^{b}	0.34^{b}	0.007	<0.001
C18:0	17.8^{b}	4.81^{a}	4.62^{a}	5.94^{a}	2.14	<0.001
C18:1 n-7	1.91^{b}	1.55^{a}	2.13^{b}	2.15^{b}	0.097	<0.001
C18:1 n-9	40.5^{a}	56.4^{b}	78.1^{c}	77.2^{c}	1.38	<0.001
C18:2 n-6	104.9^{b}	155.6^{c}	56.3^{a}	54.7^{a}	2.03	<0.001
C18:3 n-3	4.51^{b}	4.18^{a}	4.30^{ab}	4.32^{ab}	0.078	0.044
Fat deposition (g/bird)						
C14:0	1.07^{a}	0.97^{a}	2.08^{b}	2.21^{b}	0.060	<0.001
C16:0	42.6^{a}	37.4^{a}	71.6^{b}	76.3^{b}	2.34	<0.001
C16:1	8.2^{b}	4.8^{a}	11.6^{c}	11.5^{c}	0.51	<0.001
C18:0	18.5^{b}	14.8^{a}	13.3^{a}	13.8^{a}	0.65	<0.001
C18:1 n-7	4.8^{b}	3.6^{a}	5.7^{c}	5.6^{bc}	0.201	<0.001
C18:1 n-9	77.2^{a}	70.7^{a}	107.3^{b}	106.1^{b}	3.96	<0.001
C18:2 n-6	63.6^{b}	91.0^{c}	40.4^{a}	38.9^{a}	2.51	<0.001
C18:3 n-3	2.18^{a}	2.01^{a}	2.58^{b}	2.59^{b}	0.085	<0.001
C20:1 n-9	1.16^{ab}	1.10^{a}	1.29^{b}	1.26^{ab}	0.049	0.020
C20:4 n-6	1.43^{a}	1.78^{b}	1.33^{a}	1.29^{a}	0.055	<0.001
Deposition / digestible intake						
C16:0	2.68^{c}	2.01^{b}	1.14^{a}	1.09^{a}	0.087	<0.001
C18:0	1.50^{a}	3.23^{b}	2.94^{b}	2.38^{ab}	0.251	<0.001
C18:1 n-9	1.91^{b}	1.25^{a}	1.37^{a}	1.37^{a}	0.064	<0.001
C18:2 n-6	0.61^{a}	0.58^{a}	0.72^{b}	0.71^{b}	0.020	<0.001
C18:3 n-3	0.48^{a}	0.48^{a}	0.60^{b}	0.60^{b}	0.016	<0.001

[1]: Diets with hydrogenated sunflower oil mixed (50/50) with sunflower oil (HSO+SO), sunflower oil (SO), palm oil (PO) and randomized palm oil (RPO)

[2]: Numbers of observations: HSO+SO, 9; SO, 12; PO, 11; RPO, 8.

[abc]: Means in the same row with different superscript differ significantly (P<0.05)

Table 3.5. Effect of dietary fat source on the minimal synthesis (deposition- digestible intake) of non-essential and maximal disappearance rate (digestible intake – deposition) of essential fatty acids.

	Diet[1,2]				Pooled SEM	P-value diet effect
	HSO+SO	SO	PO	RPO		
Minimal synthesis (g/bird)						
C16:0	26.6[c]	18.8[b]	8.29[a]	6.24[a]	2.22	<0.001
C16:1	8.20[b]	4.74[a]	11.2[c]	11.1[c]	0.51	<0.001
C18:0	0.70[a]	10.0[b]	8.65[b]	7.84[b]	2.23	0.021
C18:1 n-9	36.7[b]	14.3[a]	29.2[b]	28.9[b]	3.59	<0.001
C18:1 n-7	2.87[b]	2.05[a]	3.53[c]	3.45[bc]	0.20	<0.001
MUFA[3]	47.8[b]	21.1[a]	43.9[b]	43.5[b]	3.48	<0.001
SFA[3]	27.3[b]	28.8[b]	16.9[ab]	14.1[a]	4.19	0.010
Maximum disappearance rate (g/bird)						
C18:2 n-6	41.3[b]	64.7[a]	15.8[c]	15.7[c]	2.30	<0.001
C18:3 n-3	2.35[a]	2.17[a]	1.74[b]	1.71[b]	0.073	<0.001

[1]: Diets with hydrogenated sunflower oil mixed (50/50) with sunflower oil (HSO+SO), sunflower oil (SO), palm oil (PO) and randomized palm oil (RPO)
[2]: Numbers of observations: HSO+SO, 9; SO, 12; PO, 11; RPO, 8.
[3]: MUFA: monounsaturated fatty acids; SFA: saturated fatty acids
[abc]: Means in the same row with different superscript differ significantly (P<0.05)

Discussion

Body deposition of fatty acids

Body weight gain was similar among treatment groups. However, in SO birds feed intake and feed conversion was lower (Smink *et al.*, 2008). This difference was quantitatively accounted for by a difference in the digestibility of the fat source. Fat digestibility of the SO group was much higher than in the other groups (75 % in SO versus 61 % as a mean in the other groups), which is in agreement with literature (Wiseman and Salvador, 1991; Ketels, 1994). Furthermore, the reduction in amino acid intake by SO broilers did not result in reduced body weight gain, indicating that the amino acid content of all diets was not limiting growth. This was expected because optimization of the diets was based on the composition of the SO diet.

The dietary fatty acid pattern clearly affected the fatty acid pattern of the body. The relation between dietary PUFA and their proportion of fatty acids in the body agrees with literature data (Bavelaar and Beynen, 2003; Waldroup and Waldroup, 2005). The deposition of fat was higher for the PO than for SO fed birds. This was expected as based on other investigators finding a decrease in abdominal fat deposition using PUFA-rich sources in comparison with tallow (Sanz *et al.*, 1999; 2000a; Newman *et al.*, 2002). However, the total concentration of fat in broilers found in other studies was not different between birds fed either saturated and unsaturated fat (Crespo and Esteve-Garcia, 2002a). There was no

difference in fat deposition between the HSO+SO and the SO group. When expressed relative to the digestible fat intake, fat deposition rates were markedly lower for the SO group when compared to all other treatments. These results indicate that both dietary C16:0 and C18:0 versus C18:2 increase fat deposition, implying that our hypothesis is confirmed.

Fatty acid deposition is the result of absorption, *de novo* synthesis and ß-oxidation of fatty acids. The deposition/digestible intake ratio reflects the net amount of synthesized or the proportion not oxidized or both. A deposition/digestible intake >1 of a certain fatty acid means a net *de novo* synthesis, whereas the ratio of <1 indicates a net oxidation or change or both, due to elongation and desaturation. All main non-essential fatty acids C16:0, C18:0, C18:1 show net synthesis, whereas the ratio is <1 for the essential fatty acids C18:2, C18:3 and total PUFA, pointing at net oxidation. However, the ratios of deposition/digestible intake for C18:2 and C18:3 in the PO and RPO groups were significantly higher than in the HSO+SO and the SO-fed birds. The reason for this is not clear, but it is probably due to the lower intake of C18:2 in PO and RPO birds. Therefore, the relative amount needed for synthesizing essential fatty acids from C18:2 was higher. The maximum disappearance rate of PUFA for the SO group was higher in comparison to the other groups, indicating more oxidation of PUFA. Studies with rats indicated that C18:2 and C18:1 have higher oxidation or elongation-desaturation rates or both, than C16:0 and C18:0 (Leyton *et al.*, 1987).

The calculated minimal synthesis of MUFA + SFA was highest for the HSO+SO group followed by RPO, PO and SO. The feed intake of the treatments with saturated fat is higher in comparison with the SO group (Smink *et al.,* 2008). This means that the birds in the latter group had a lower intake of carbohydrates to be used as substrate for the *de novo* synthesis of fatty acids. In addition, it should be noted also that the higher (calculated) rate of fatty acid synthesis of the HSO+SO group in comparison with the PO and RPO groups might be due to the relatively high oxidation or elongation-desaturation, or both, rates of PUFA. The results of the SO group showed a lower calculated synthesis of MUFA in comparison with the broilers in the other groups. This agrees with a lower MUFA synthesis recently found in pigs fed with a sunflower oil diet in comparison with a diet with 5% beef tallow (Mitchaothai *et al.*, 2008). Villaverde *et al.* (2006) suggested that in chickens a mechanism exists to change the ratio of *de novo* synthesis of SFA/(MUFA+SFA) in order to keep a specific range of these fatty acids in the membranes. Monounsaturated fatty acid synthesis is regulated in the liver. PUFA in the diet will inhibit the activity of the enzyme Δ9-desaturase in the liver (Kouba and Mourot, 1998), resulting in a reduced conversion of SFA into MUFA. Which agrees with the lower ratio of C16:1/C16:0 and C18:1/C18:0 in the liver in the SO group in comparison with the other groups, and appeared to have a strong negative correlation between hepatic PUFA of all birds. Such a negative correlation agrees with earlier work of Infield and Annison (1973).

Hepatic fatty acids and enzyme activities

The enzymes ACC and FAS are involved in fatty acid synthesis in the liver. In literature, a depressed activity of these enzymes after feeding C18:2 was found in studies with rmice (Javadi *et al.*, 2006) and chicken (Sanz *et al.*, 2000b). The negative correlation (R^2=0.25; P<0.001) that we found between PUFA concentration in the liver and enzyme activities of both ACC and FAS (results not shown) is in line with these observations, but the dietary effect did not reach statistical significance.

The activity of 3-HOAD, a key enzyme for fatty acid oxidation, was similar for the SO and the HSO+SO treatments. It was expected that the high dietary PUFA and low SFA in the SO group had increased ß-oxidation in comparison with HSO+SO group (Sanz *et al.*, 2000b). However, due to the very low digestibility of C18:0 in the HSO+SO group, the difference in the fatty acid pattern of digested fatty acids was limited between the two groups.

Liver fat mass tended (P=0.053) to be lower in SO birds. This agrees with Pinchasov and Nir (1992), who demonstrated a reduced liver fat content at a high inclusion level of PUFA in the diet. The reduction of fatty acids in the liver of the broilers in our study was significant for MUFA. This agrees well with others (Dänicke *et al.*, 1999; Crespo and Esteve-Garcia, 2002b). The reduced C16:1/C16:0 and C18:1/C18:0 in the SO group indicates a lower Δ9-desaturase activity. The inhibition of this enzyme will impair triacylglycerol secretion in hepatocytes (Legrand *et al.*, 1997).

Table 3.6. Effect of dietary fat source on hepatic fat content and hepatic enzyme activities in broiler chickens.

	Diet[1,2]				Pooled SEM	P-value diet effect
	HSO+SO	SO	PO	RPO		
Body weight (g/bird)	1776	1746	1767	1760	38.0	0.98
Liver weight (g/bird)	56.0	51.2	52.5	56.8	2.32	0.27
Liver fat (mg/g liver)	19.9	9.68	16.5	20.3	3.02	0.053
Enzyme activities[3]						
ACC[4]	3.51	2.41	2.75	3.16	0.33	0.12
FAS[4]	2.60	1.90	2.17	2.29	0.23	0.21
CPT-I[5]	12.7	15.5	15.2	12.9	1.36	0.32
3-HOAD[6]	547	567	510	475	28.2	0.11
Citraat synthase[5]	72.2	64.3	73.8	73.8	5.0	0.49
DGAT[6]	0.64	0.61	0.60	0.63	0.056	0.96

[1]: Diets with hydrogenated sunflower oil mixed (50/50) with sunflower oil (HSO+SO), sunflower oil (SO), palm oil (PO) and randomized palm oil (RPO)

[2]: Numbers of observations: HSO+SO, 12; SO, 12; PO, 12; RPO, 12.

[3]: ACC: Acetyl-CoA Carboxylase; FAS: fatty acid synthethase; CPT-I: Carnitin palmitoyltransferase-I; 3HOAD: 3-Hydroxy-acyl-CoA dehydrogenase; DGAT: Diacylglycerol acyltransferase. Enzyme activities are expressed as nmol per min per mg of protein.

[4]: measured in homogenate protein

[5]: measured in mitochondrial protein

[6]: measured in microsomal protein

Table 3.7. Effect of dietary fat source on hepatic fatty acids content (mg/bird) in broiler chickens.

	Diet[1,2]				Pooled SEM	P-value diet effect
Fatty acids	HSO+SO	SO	PO	RPO		
C14:0	4.55	1.34	3.08	4.41	0.916	0.053
C16:0	249[a]	83.0[a]	182[a]	243[a]	45.4	0.040
C16:1	29.2[a]	4.82[a]	21.2[a]	29.6[a]	6.94	0.046
C18:0	196	98.8	149	184	27.7	0.067
C18:1 n-7	20.4[ab]	5.72[a]	18.2[ab]	23.6[b]	4.05	0.016
C18:1 n-9	325[ab]	81.5[a]	241[ab]	346[b]	67.4	0.030
C18:2 n-6	177	112	123	146	25.5	0.22
C18:3 n-3	1.86[ab]	0.982[a]	1.44[ab]	2.53[b]	0.395	0.048
C20:1 n-9	3.88[ab]	1.61[a]	4.54[ab]	5.22[b]	0.753	0.009
C20:2 n-6	5.41	5.16	3.93	3.80	0.693	0.24
C20:3 n-6	9.52[ab]	4.39[a]	8.27[ab]	9.37[b]	1.36	0.032
C20:4 n-6	68.1	53.9	54.5	57.7	7.64	0.52
C22:4 n-6	5.91[a]	7.02[a]	4.34[a]	4.36[a]	0.757	0.044
C22:3	4.97[ab]	5.61[b]	3.35[ab]	3.13[a]	0.606	0.013
C22:6 n-3	6.44[ab]	3.51[a]	8.72[b]	8.64[b]	0.799	<0.001
SFA[3]	450[a]	184[a]	335[a]	433[a]	73.3	0.047
UNSFA[3]	680	296	510	662	112	0.063
MUFA[3]	378[ab]	94.1[a]	285[ab]	405[b]	80.7	0.028
PUFA[3]	302	202	225	257	37.7	0.27
C16:1/C16:0	0.099[b]	0.043[a]	0.092[b]	0.108[b]	0.0119	0.001
C18:1/C18:0	1.39[b]	0.67[a]	1.37[b]	1.68[b]	0.175	0.002

[1]: Diets with hydrogenated sunflower oil mixed (50/50) with sunflower oil (HSO+SO), sunflower oil (SO), palm oil (PO) and randomized palm oil (RPO)

[2]: Numbers of observations: HSO+SO, 12; SO, 11; PO, 11; RPO, 11.

[3]: MUFA: monounsaturated fatty acids; SFA: saturated fatty acids; USFA: unsaturated fatty acids; PUFA: polyunsaturated fatty acids

[ab]: Means in the same row with different superscript differ significantly ($P<0.05$)

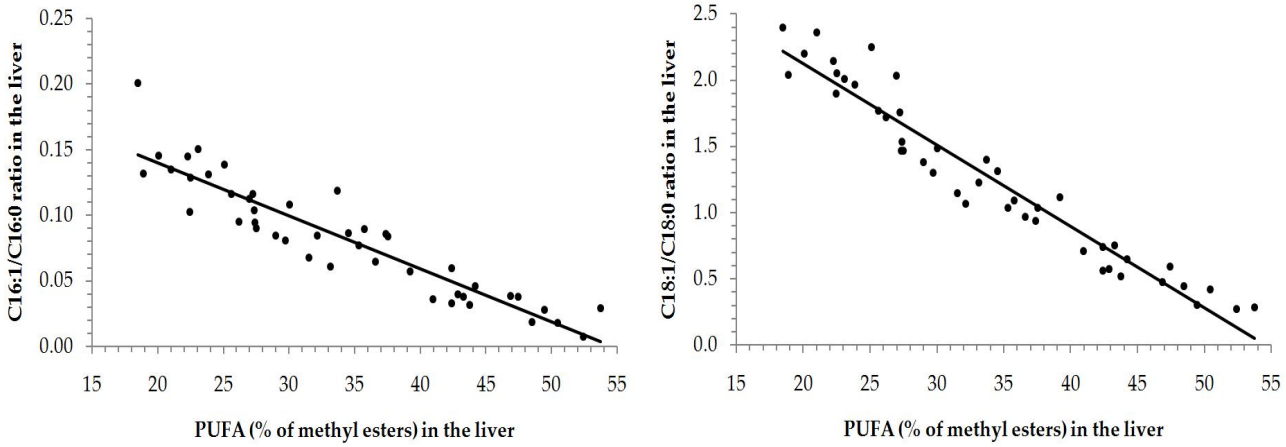

Figure 3.1. Effect of polyunsaturated fatty acids (PUFA; in % of methyl esters) in the liver on the ratio of C16:1/C16:0 and C18:1/C18:0 in the liver of broiler chickens fed with different fat sources (values are means of two chickens).
C16:1/C16:0 = 0.22 - 0.00405 PUFA (R^2 = 0.85; Intercept: P<0.0001); PUFA: P<0.0001)
C18:1/C18:0 = 3.36 - 0.0616 PUFA (R^2 = 0.93; Intercept: P<0.0001); PUFA: P<0.0001)

PO and RPO mainly differ with regard to the position of C16:0 and C18:1 on the glycerol molecule. The change from C16:0 from the sn-1,3 position to the sn-2 position increases its digestibility in chickens as was shown previously (Smink *et al.*, 2008). In Table 4, a significantly higher digestible intake of C16:0 in the RPO group is shown compared to PO. Contrary to C16:0 the quantitative effect of the position of C18:1 on the glycerol molecule on digestibility is small. The effect on digestibility agrees well with studies in rats (Reinaud *et al.*, 1995). Apart from an increased digestibility, there were no other effects of randomization of palm oil.

The deposition/digestible intake ratios for fatty acids and the calculated *de novo* synthesis and maximum disappearance rates of fatty acids were clearly affected by dietary treatment. Based on these results, it is likely that the SO diet resulted in a higher oxidation and/or desaturation/elongation rates of fatty acids. This is confirmed by the maximum disappearance rate of fatty acids which was highest for the SO group and lowest for the RPO and PO groups. The combination of a decreased synthesis of MUFA in the liver and the calculated reduction in the minimal synthesis of MUFA in the whole body of the broilers in the SO group, indicate a reduction of *de novo* fatty acid synthesis. However, the group mean activities of FAS and ACC were not significantly different from the other treatments.

In conclusion, the present data show that, like tallow, vegetable saturated fat sources increase body fat deposition in comparison with PUFA. A combination of a higher *de novo* synthesis of MUFA and a lower fatty acid oxidation rate seems to be responsible.

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Chapter 4

Equal net energy intake of starch, saturated and unsaturated fat in growing-finishing pigs in relation to performance, carcass and meat characteristics

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4. Equal net energy intake of starch, saturated and unsaturated fat in growing-finishing pigs in relation to performance, carcass and meat characteristics

Abstract

A study was conducted to compare the effect of an equal net energy (NE) intake of starch, unsaturated (UNSAT) and saturated (SAT) fat in growing-finishing pigs on their performance, carcass and meat characteristics. Each dietary group consisted of 100 pigs divided into 6 replicates with barrows and gilts mixed in each pen. Diets were fed at 2.7 x the energy requirement for maintenance. Performance parameters were similar among dietary treatments. Pigs fed the saturated fat diet showed a trend towards a higher lean meat percentage compared to the starch diet fed pigs (P=0.07). Backfat thickness was not affected by dietary treatment, but intramuscular fat (IMF) content tended to be lower in the pigs in the SAT group compared with the starch group (P=0.08). Feeding pigs the unsaturated fat diet increased the unsaturated/saturated (U/S) ratio in backfat, while pigs in the starch group had the lowest backfat U/S ratio. It can be concluded that, when compared at equal NE intake, high starch diets will increase firmness of fat and may increase the content of IMF.

Key words: Energy source – carcass – fatty acids - pigs

Introduction

Practical pig diets in the Netherlands are formulated using the net energy (NE) system as described by the CVB (2000). The NE system provides an estimate of the amount of dietary, nutrient-derived energy which can be used by the animal for body maintenance and growth. The efficiency of the conversion of nutrient-derived energy differs among nutrients. The partial efficiency of the conversion of glucose into body fat is lower than that of dietary fat into body fat. The energetic efficiency from dietary fat into body lipids in growing-finishing pigs is assumed to be approximately 92%, while that of enzymatically digested starch is 77% (CVB, 2000). Van Milgen *et al.* (2001) reported an energetic efficiency of energy utilisation in growing pigs of 84.2 and 88.3% for digestible starch and fat, respectively. These differences between starch and fat are included in the net energy system. In most commercial pig units around the world, the deposition of fat in growing-finishing pigs greatly exceeds the intake of digestible fat. Bikker *et al.* (1996) reported that daily fat deposition in growing-finishing pigs was approximately 400 g at a digestible fat intake of 150 g per day, implying that *de novo* fatty acid synthesis exceeds 250 g per day. Hillcoat and Annison (1974) showed that the efficiency of fat deposition increases when tallow replaces starch in the diet. This effect, however, was not found in the latter study when soybean oil replaced starch in the diets. The replacement of unsaturated fat by saturated fat has been reported to increase body fat in rats (Shimomura *et al.*, 1990) and abdominal fat in broilers (Crespo and Esteve-Garcia,

2002; Ferrini *et al.*, 2008; Wongsuthaves *et al.*, 2008). It has been suggested that dietary medium-chain fatty acids and polyunsaturated fatty acids (PUFA) are preferentially oxidized in rats (Beynen and Katan, 1985). Studies with broiler chickens indicate that a lower ß-oxidation of saturated fat is responsible for the effect on fat deposition (Sanz *et al.*, 2000). The Dutch NE system (CVB, 2000) for pigs does not discriminate between dietary fat sources, except in terms of digestibility. Unlike in broiler chickens, a comparison of dietary saturated and unsaturated fat in pigs showed no clear difference in fat deposition rate (Bee *et al.*, 2002; Teye *et al.*, 2006; Mitchaothai, 2007). Halas *et al.* (2010) showed no difference in fat deposition between pigs fed additional energy as starch or as unsaturated fat. Dietary energy value can affect the carcass quality. It is unclear whether the energy source in practical diets with an optimal NE/lysine level will affect the location of fat deposition in the body such as fat deposited as backfat or as intramuscular fat (IMF).
The current study was conducted to obtain insight in potential differences between performance, carcass quality and location of fat deposition (backfat vs IMF) of growing-finishing pigs. Diets with an equal NE intake in the form of starch, unsaturated and saturated fat rich diets were fed under practical circumstances.

Material and methods

Animals and housing
Three hundred Topics 20 x Topics Tempo pigs (150 barrows and 150 gilts) of approximately 50 kg body weight were accurately weighed and assigned to 30 pens containing 10 pigs per pen. Part of the pens contained one feeding unit for two pens. In that case one replicate consisted of two pens. There were a total of 12 replicates consisting of two pens and 6 replicates consisting of one pen. Each pen contained 50% gilts and 50% barrows. The pigs were housed in a practical farm (Lentelink in Ambt Delden, The Netherlands) and fed one of the three diets. The feed was provided to the pigs using a previously calibrated automatic feeding system (Fanco). The 18 replicates were distributed over three parts of the stable (departments). Each department contained two replicates per diet. The animals were individually weighed at the start and at the day before slaughter.

Diets and feeding
The ingredient and nutrient composition of the experimental diets is presented in Table 4.1. Pigs were fed restrictedly, and diets and feeding schedules were formulated to realize equal NE intakes according to CVB (2000). The NE content of the saturated, and unsaturated high fat diets (SAT and UNSAT, respectively) was 9.7, and that of the starch diet (STARCH) 9.0 MJ NE/kg diet. In order to provide pigs with the same amount of NE, the feed intake of the pigs fed with SAT and UNSAT diets was set at 92.5% of the intake of the pigs fed the STARCH diet. Daily intake of crude protein and crude fibre was the same in each treatment. The starch diet (STARCH) was formulated to contain 43% starch (Table 4.1) and had a fat content of approximately 2%. In the other two diets, starch was exchanged on a net energy basis by 4% unsaturated (UNSAT) or 4% saturated fat (SAT). The fat source in the UNSAT diet was soybean oil while palm oil was used to formulate the SAT diet. The digestibility of

soybean oil and palm oil were assumed to be 95 and 91%, respectively. Energy intake was fixed at 2.7 x the NE requirements for maintenance (293 kJ NE per kg metabolic weight; Verstegen *et al.*, 1973).

Table 4.1. Ingredient and nutrient (calculated and analysed) composition of the diets.

Ingredient (g/kg)	Dietary treatment		
	STARCH	UNSAT	SAT
Rye	141	152	152
Barley	100	108	108
Wheat	390	249	248
Peas	115	124	124
Palm kernel expeller	12	13	13
Rapeseed meal	100	108	108
Soybean meal	45	48	49
Wheat bran	33	71	71
Wheat gluten	-	19	19
Cane molasses	40	43	43
Soybean oil	-	31.4	-
Palm oil	4.0	10.3	42.6
Lysine 50%	2.3	2.5	2.5
Methionine analog (Alimet)	0.33	0.36	0.36
Limestone	10.0	12.3	11.4
Salt	3.4	3.70	3.70
Premix	4.12	4.42	4.42
Calculated (CVB, 2000) and NIR analysed (between brackets) nutrient composition			
Crude protein (g/kg)	155 (156)	168 (168)	169 (165)
Crude fat (g/kg)	21.2 (19.8)	59.9 (57.1)	60.8 (57.7)
Crude fibre (g/kg)	44.3 (42.6)	47.1 (47.1)	47.1 (45.5)
Starch (g/kg)	434 (426)	374 (362)	373 (362)
Ash (g/kg)	45.4 (42.8)	49.6 (47.4)	48.8 (47.8)
Net energy (MJ/kg)	9.0	9.7	9.7
Apparent ileal digestible (g/kg)			
lysine	6.7	7.2	7.3
methionine+cystine	4.6	5.0	5.0

Measurements, sampling and fatty acid analysis

Analysis of the diets was performed using standard near infrared spectroscopy procedures. Pigs were slaughtered at a body weight of approximately 105-110 kg. The regular carcass characteristics such as slaughter weight, lean meat percentage, backfat and muscle thickness, were determined for each pig at the slaughter house. In addition, backfat thickness was determined via a probe and measured manually after splitting the carcass longitudinally. Two gilts and two barrows from each experimental unit were selected for sampling; per sex, one was randomly selected of the heaviest part and one from the part with the lowest body weight. Visible intermuscular fat was removed from the muscle (*longissimus thoracis*) before the

determination of intramuscular fat content and the specific weight density of the meat. This specific weight was calculated according to the formula: weight of meat / (weight of meat – weight of meat under the water surface). In addition, this meat sample was used to determine the marbling score of 1-5 (NPPC pork quality standards; Jones *et al.*, 1992). A sample of the inner layer of backfat was obtained to determine the fatty acid composition.
The content of intramuscular fat (IMF) was measured following muscle extraction with a choloroform:methanol (2:1, v/v) Folch mixture. The fatty acid composition of backfat was determined by gas chromatography as described previously (Smink *et al.*, 2008).

Statistical analysis
The effects of dietary treatments were statistically analyzed by one-way ANOVA. The statistical differences between treatments were determined by a post-hoc Tukey-test. The level of statistical separation of means was pre-set at $P<0.05$. Results are presented as least square mean (LSM) and a pooled standard error of the mean (pooled SEM). Statistical analysis was performed using SAS (SAS JMP, 2000).
For general performance data, means per experimental unit (n=6 for each treatment) were calculated and analysed using the following model:
$Y = \mu + Department_i + Diet_j + error_{ij}$
where Y = Feed intake, daily weight gain, feed conversion, net energy intake and net energy conversion conversion (net energy intake in MJ / kg body weight gain); i=3 departments, j = 3 diets (Table 4.2)

For carcass quality parameters, means were used per sex for each experimental unit (n=6 for each treatment). To account for the effect of variation in slaughter weight on carcass characteristics, slaughter weight was included as a covariate:
$Y = \mu + Department_i + Sex_j + Diet_k + \beta*Slaughter\ weight + error_{ijk}$
whereby Y = carcass and meat characteristics; i=3 departments; j=2 sexes (gilt or barrow); k= 3 diets (Table 4.4).

For fatty acid composition of the backfat, the same model was used as for the carcass characteristics, with the exception of the effect of department and the covariate because these do not affect the fatty acid composition.
$Y = \mu + Sex_i + Diet_j + error_{ij}$
where: Y = fatty acids in backfat; i=2 sexes (gilt or barrow); j = 3 diets (Table 4.5).

Results

The experiment went well, with only minor health problems and a mortality rate below one percent. Performance data of the pigs are presented in Table 4.2. Rates of body weight gain and net energy conversion were similar among treatments. The feed conversion ratio (FCR), however, was significantly higher for the pigs fed the STARCH diet with no difference being observed in FCR between pigs in the SAT and UNSAT groups.

The results of the carcass characteristics of sexes are presented in Table 4.3. The comparison of barrows and gilts showed that barrows had a significantly (P<0.01) lower meat percentage and a tendency (P<0.1) for a lower muscle thickness. Backfat thickness and IMF were significantly (P<0.05) higher in barrows. The weight density of lean meat was significantly higher in gilts.

A comparison of carcass characteristics between dietary treatments is presented in Table 4.4. Meat percentage and muscle thickness tended (P<0.1) to be higher in the SAT group in comparison to the STARCH group. Backfat thickness was not different between treatments. There was a trend (P<0.08) for a difference in IMF content between groups with the lowest (2.01 %) IMF content recorded for the SAT group and the highest for the STARCH group. Marbling score was the lowest in the SAT group although results between groups were not statistically significant.

Fatty acid profiles of the inner layer of backfat in the STARCH, UNSAT and SAT group are presented in Table 4.5. Nearly all fatty acids were significantly affected by the dietary treatments. Backfat of pigs in the UNSAT group had a much higher contents of C18:2 and C18:3 which were almost double compared to pigs in the STARCH and SAT group. The content of saturated fatty acids was lower and the unsaturated/saturated fatty acid (U/S) ratio was higher in the UNSAT group. The differences for most fatty acids between the SAT and the STARCH group were much smaller than between SAT or STARCH and UNSAT. However, the highest content of C14:0 and C18:0 and the lowest U/S ratio was measured in the pigs fed the STARCH diet.

Table 4.2. Performance of pigs fed equal net energy from either starch, unsaturated fat (UNSAT) and saturated fat (SAT).

Parameter	Dietary treatment			Pooled	P-value
	STARCH	UNSAT	SAT	SEM	
Initial weight (kg)	49.8	48.2	49.0	0.96	0.48
Body weight gain (g/pig/day)	759	774	798	11.4	0.17
Feed intake (kg/pig/day)	2.37 (100)	2.16 (91.1)	2.22 (93.7)	-	-
Feed conversion ratio (g/g)	3.10[b]	2.79[a]	2.77[a]	0.078	0.015
Net energy intake (MJ)	21.2	20.9	21.4	-	-
Net energy/gain ratio	27.7	26.9	26.6	0.72	0.55

[a,b] Means in the same row with different superscript differ significantly (P<0.05).

Discussion

Performance

Body weight gain and NE conversion were not affected by the dietary treatments in the present study. This was expected because the NE and nutrients intake of the pigs in the three different groups were kept the same. Backfat thickness was not significantly different between dietary treatments (Table 4.4). This agrees with other studies where the effect of dietary

unsaturated and saturated fats on adipose tissue in pigs were compared (Bee *et al.*, 2002; Teye *et al.*, 2006; Mitchaothai, 2007). Although body protein and body fat deposition rates were not measured in this experiment, from the performance and carcass data it appears unlikely that these would have been different among treatments.

Table 4.3. Carcass and meat characteristics of the barrows and gilts[1].

Parameter	Barrow	Gilt	Pooled SEM	P-value
Meat percentage (%)	55.9	57.8	0.22	<0.001
Muscle thickness (mm)	55.4	56.9	0.58	0.087
Backfat thickness (probe) (mm)	16.7	14.5	0.24	<0.001
Backfat thickness (hand) (mm)	20.5	17.9	0.39	<0.001
Marbling score	1.97	1.73	-	0.10
Weight density lean meat (g/g)	1.0663	1.0680	0.00046	0.011
Intramuscular fat (%)	2.46	2.08	0.112	0.023

[1]LS means, interactions between dietary treatment and sex were absent (P>0.10); measurements averaged from 2 barrows and 2 gilts, sampled randomly from each experimental unit.

Table 4.4. Carcass and meat characteristics of the pigs fed equal net energy from either a high starch, unsaturated fat (UNSAT) or saturated fat (SAT) diet.[1]

Parameter	Dietary treatment			Pooled SEM	P-value
	STARCH	UNSAT	SAT		
Meat percentage (%)	56.4	56.9	57.3	0.28	0.065
Muscle thickness (mm)	54.8	56.6	57.0	0.72	0.088
Backfat thickness (probe) (mm)	16.1	15.6	15.2	0.30	0.15
Backfat thickness (hand) (mm)	19.1	19.4	19.2	0.48	0.92
Marbling score	1.92	2.00	1.64	-	0.10
Weight density meat (g/g)	1.0665	1.0670	1.0680	0.00055	0.16
Intramuscular fat (%)	2.43	2.37	2.01	0.136	0.076

[1]measurements taken from two barrows and two gilts per experimental unit

Table 4.5. Fatty acid composition (% of methyl esters) and unsaturated/saturated fatty acid (U/S) ratio of the inner layer of backfat in the pigs fed starch, unsaturated fat (UNSAT) and saturated fat (SAT) diets.

Fatty acid	Dietary treatment			Pooled SEM	P-value
	STARCH	UNSAT	SAT		
C10:0	0.049[b]	0.042[a]	0.041[a]	0.0016	<0.01
C12:0	0.11	0.12	0.11	0.0047	0.62
C14:0	1.30[b]	1.18[a]	1.17[a]	0.033	<0.05
C16:0	24.8[b]	22.7[a]	24.6[b]	0.23	<0.001
C16:1	1.84[c]	1.32[a]	1.49[b]	0.055	<0.001
C18:0	15.3[c]	13.1[a]	14.0[b]	0.24	<0.001
C18:1 n-9	38.1[b]	33.8[a]	38.9[b]	0.31	<0.001
C18:2 n-6	10.7[a]	19.5[c]	12.5[b]	0.39	<0.001
C18:3 trans	0.117[c]	0.051[a]	0.078[b]	0.0092	0.001
C18:3 cis	0.96[a]	1.88[b]	0.99[a]	0.040	<0.001
C20:0	0.24[b]	0.22[ab]	0.20[a]	0.078	<0.01
C20:1 n-9	0.99[b]	0.82[a]	0.93[b]	0.034	<0.01
U/S ratio	1.33[a]	1.61[c]	1.43[b]	0.026	<0.001

[a,b,c] Means in the same row with different superscript differ significantly (P<0.05).

Fat content and fatty acid composition

Intramuscular fat content has been associated with eating quality of meat with an IMF content of at least 2.5% being preferred in pig meat (Fernandez *et al.*, 1999). The fatness of the meat in the present study was determined by analyzing the IMF content, a visual assessment of the marbling score and measurement of the specific weight of a cut of the *longissimus thoracis*. All of these parameters indicated a higher fatness of meat in barrows compared to gilts, which agrees with other studies (e.g. Warnants, 1999).

The IMF content was not different between UNSAT and STARCH pigs, but tended to be lower in the SAT pigs (P=0.08), which was numerically confirmed by lower marbling scores and a higher weight density in these pigs. Replacing starch by animal fat or palm oil in earlier studies also resulted in a decrease in the intramuscular fat content of loin, while the use of rapeseed oil did not affect the IMF content (Madsen, 1983; Madsen *et al.*, 1992). These results were difficult to explain according to these authors. A higher dietary fat content without changing the dietary digestible energy will decrease the *de novo* synthesis of fat in adipose tissue of pigs (Chilliard, 1993). It can therefore be speculated that a higher rate of *de novo* fatty acid synthesis, as is expected with feeding starch compared to fat, coincides with a higher IMF content. In cattle adipocytes, glucose has been demonstrated as the preferred substrate for intramuscular fat synthesis (Smith and Crouse, 1984). The pigs in the SAT group had a lower IMF content than those in the UNSAT group. A difference between saturated and unsaturated fat sources on IMF content is confirmed by unpublished work. If the high C18:2 n-6 content in the UNSAT diet is preferentially oxidized, as is showed in broilers (Sanz *et al.*, 2000; Smink *et al.*, 2010), there should be a higher *de novo* synthesis of fat and therefore stimulating the IMF content.

As expected, the UNSAT group clearly showed the lowest concentration of saturated fat in backfat. The clear correlation of linoleic acid intake in the UNSAT diet and high linoleic acid content in the backfat of pigs fed this diet agrees with observations by others (Warnants, 1999; Nguyen *et al.*, 2003). A potential disadvantage of dietary UNSAT however, is that it decreases the firmness of fat (Gläser *et al.*, 2004; Wood *et al.*, 2008). The STARCH diet, which was low in fat, resulted in the lowest U/S ratio. Kloareg *et al.* (2005) reported that fatty acids, synthesized *de novo* consist of C14:0 (2%), C16:0 (30%) C16:1, (2%), C18:0, (20%) and C18:1 (46%). This would imply an U/S ratio of approximately 1.0. As such, a high *de novo* synthesis contributes to a lower U/S ratio. The *de novo* synthesis explains also the higher C14:0 in the STARCH group. In our study the fatty acid profile was measured in the inner layer of backfat. Fat in the outer layer has generally a slightly higher U/S ratio due to an increased deposition of oleic acid (Bee *et al.*, 2002). It can be speculated that this is related to migration of unsaturated fatty acids to the outer layer or to Δ9 desaturase activity in the outer layer (Wiseman and Agunbiade, 1998).

The results in the present study confirm similar animal performance of diets formulated to result in identical NE intake, originating from either starch, saturated or unsaturated fatty acids. It was demonstrated that potential improvements in palatability of meat through a higher firmness and IMF content can be achieved by the use of dietary starch. An increase of unsaturated fat in the carcass can be achieved with a high intake of unsaturated fat. Backfat thickness was not affected by iso-energetic intake of starch, saturated or unsaturated fat.

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Chapter 5

Linoleic and α-linolenic acid as precursor and inhibitor for the synthesis of long-chain polyunsaturated fatty acids in liver and brain of growing pigs

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5. Linoleic and α-linolenic acid as precursor and inhibitor for the synthesis of long-chain polyunsaturated fatty acids in liver and brain of growing pigs

Abstract

Studies suggested that in human adults, linoleic acid (LA) inhibits the biosynthesis of n-3 long-chain polyunsaturated fatty acids (LC-PUFA), but their effects in growing subjects are largely unknown. We used growing pigs as a model to investigate whether high LA intake affects the conversion of n-3 LC-PUFA by determining fatty acid composition and mRNA levels of Δ5- and Δ6 desaturase and elongase 2 and -5 in liver and brain. In a 2x2 factorial arrangement, 32 gilts from 8 litters were assigned to one of four dietary treatments, varying in LA and α-linolenic acid (ALA) intake. Low ALA and LA intake were 0.15 and 1.31, and high ALA and LA intake were 1.48 and 2.65 g/(kg $BW^{0.75}$/d), respectively. LA intake increased arachidonic acid (ARA) in liver. ALA intake increased eicosapentaenoic acid (EPA) concentrations, but decreased docosahexaenoic acid (DHA) (all $P<0.01$) in liver. Competition between the n-3 and n-6 LC-PUFA biosynthetic pathways was evidenced by reductions of ARA (>40%) at high ALA intakes. Concentration of EPA (>35%) and DHA (>20%) was decreased by high LA intake (all $P<0.001$). Liver mRNA levels of Δ5- and Δ6 desaturase were increased by LA, and that of elongase 2 by both ALA and LA intake. In contrast, brain DHA was virtually unaffected by dietary LA and ALA. Generally, dietary LA inhibited the biosynthesis of n-3 LC-PUFA in liver. ALA strongly affects the conversion of both hepatic n-3 and n-6 LC-PUFA. DHA levels in brain were irresponsive to these diets. Apart from Δ6 desaturase, elongase 2 may be a rate-limiting enzyme in the formation of DHA.

Key words: Linoleic acid – linolenic acid – liver – brain – enzymes

Introduction

Arachidonic acid (ARA; C20:4 n-6), eicosapentaenoic acid (EPA; C20:5 n-3) and docosahexaenoic acid (DHA; C22:6 n-3) are long-chain polyunsaturated fatty acids (LC-PUFA) and well known to play important regulatory functions in the immune, nervous and cardiovascular system (Innis, 2007; Calder, 2009; Calder and Yaqoob, 2009; Russo, 2009). The n-6 LC-PUFA can be synthesized from linoleic acid (LA; C18:2 n-6) and those of the n-3 family from α-linolenic acid (ALA; C18:3 n-3). Synthesis of EPA and DHA from ALA is achieved by a sequence of desaturation and chain elongation steps, controlled by the enzymes Δ5- (FADS1) and Δ6 desaturase (FADS2) and presumably elongase 2 (ELOVL2) and -5 (ELOVL5) (see Figure 5.1). DHA can be generated from EPA which requires an additional chain-shortening step (i.e. ß-oxidation) in the peroxisome (Sprecher, 2000; Igarashi *et al.,* 2007a). The conversion of n-6 LC-PUFA from LA (resulting in the production of ARA) sharing the same enzymes resulting in enzymatic competition between the biosynthetic pathways of n-3 and n-6 LC-PUFA.

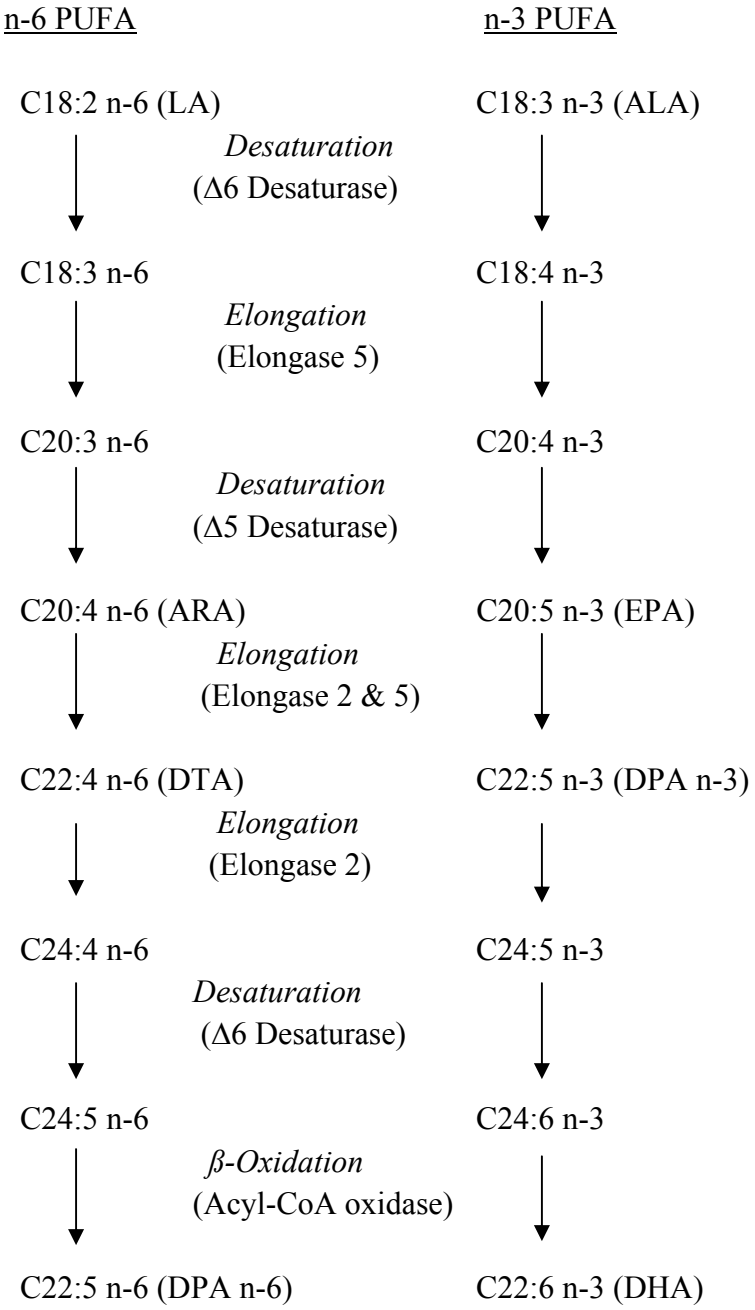

Figure 5.1. General metabolic pathway of omega 6 and -3 fatty acids (adapted from Igarashi *et al.,* 2007a). LA, linoleic acid; ALA, α-linolenic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DTA, docosatetraenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.

The requirement of LC-PUFA for brain growth and development is of current interest. LC-PUFA, in particular DHA and ARA, accumulate rapidly during the brain growth spurt, the most critical stage of brain development which takes place during the later part of gestation and early postnatal life. ARA is essential for normal brain growth, playing a role in synaptic transmission (Bazan, 2003; Sang and Chen, 2006). Changes in brain DHA concentrations have been demonstrated to be positively correlated with cognitive development (Innis, 2007). In addition, the dietary supplementation of DHA could improve the cognitive dysfunction due

to aging (McCann and Ames, 2005; Cole and Frautschy, 2006). The regulatory mechanisms of the conversion of LC-PUFA into ARA, EPA and DHA, and the consequences of LA- and ALA-rich diets consumed during childhood remain to be elucidated. In adult humans, it has been estimated that the ß-oxidation of ALA is ±20-30%, which is much higher than that of LA which was estimated to be 12-19 % (DeLany *et al.,* 2000). The oxidative losses may partly explain why the conversion of ALA to EPA and DHA in adults is low (Burdge and Calder, 2005). In addition, human food sources like cereals, sunflower and soybean oil are rich in LA (>50% of the fat) and have been dominant in the Western diet over the last decades. Consumption of LA-rich diets could also result in inadequate conversion of ALA to DHA and reduced concentrations of DHA in the developing brain (Russo, 2009). Dietary studies about the effect of LA are mostly carried out at an isocaloric basis. LA is then replaced by other energy sources, like oleic acid (18:1 n-9). However, it has been reported recently that oleic acid itself can reduce Δ6 desaturase activity (Portolesi *et al.,* 2008).
The present study focuses on the interactions between the n-3 and n-6 LC-PUFA biosynthetic pathways in liver and brain using young growing pigs as a model for human infancy. This animal model has three major advantages above rodents when studying lipid metabolism. Firstly, brain anatomy and morphology and the timing of the brain growth spurt in pigs and humans are similar (Pond *et al.,* 2000; Duhaime, 2006). Secondly, the anatomy of the digestion system, including liver, stomach and intestine, and also many of the pathways of lipid metabolism in pigs are rather similar to human (Moughan *et al.,* 1991; Innis, 1993). Thirdly, the risk for obesity in young, growing pigs is low, enabling large study contrasts in absolute intakes of LC-PUFA precursors, rather than exchanging for other nutrients to maintain isocaloric intake. Here, we have fed young growing pigs either low or high amounts of LA and ALA, with equal difference between low and high in the intake within the respective fatty acid, and investigated the effect on the fatty acid composition in liver and brain. In the same tissues, we have determined the mRNA levels of Δ6- and Δ5 desaturase and ELOVL2 and -5 by quantitative PCR.

Material and methods

Animals and housing
Thirty two female pigs were selected from 8 litters. From each litter, 4 gilts were allotted to one of the 4 dietary treatments. On arrival, pigs had a body weight of 16 kg (SD=1.8). The pigs were housed individually during an experimental period of 4 weeks. They were weekly weighed and feed intake was recorded daily. The experimental protocol was approved by the animal experimental committee of Wageningen University, The Netherlands.

Diets
The study was carried out as a 2 x 2 factorial design with daily intakes of LA and ALA as independent variables. Differences between low and high intake were designed to be identical for LA and ALA: Low ALA and LA intakes were 0.16 and 1.32, and high ALA and LA intakes were 1.48 and 2.65 g/(kg $BW^{0.75}$/d). The dietary energy% from LA was 3.4, 3.3, 6.6 and 6.4 and those of ALA was 0.4, 3.7, 0.4 and 3.5 for the diets low LA / low ALA, low LA /

high ALA, high LA / low ALA and high LA / high ALA, respectively. The size of the experimental contrasts was, in part, based on recent studies with humans showing an effect of dietary LA on EPA in plasma phospholipids (Goyens *et al.,* 2006). In addition, the low LA and ALA intakes were slightly above minimal requirement figures for pigs (National Research Council, 1998; Schellingerhout, 2002). The addition of LA and ALA was on top of a basal diet. The realized intakes of fatty acids are presented in Table 5.1. With the exception of LA and ALA, the intake of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) were kept constant. This was realized by optimizing the dosages of analyzed sunflower oil, linseed oil, high oleic acid sunflower oil (HOSF) and palm oil. The diets did not contain n-3 or n-6 LC-PUFA other than LA and ALA. The composition of the basal diet and the inclusion rates of the oils in the treatments are presented in Table 5.2. The low LA / low ALA diet was formulated to contain sufficient ileal digestible amino acids in relation to energy (CVB, 2007).

As the LA and ALA increments were not exchanged for other nutrients but dosed on top of the basal diet, digestible energy intake varied among experimental treatments. The diets were fed twice daily as mash (2.6 to 2.8 x maintenance requirement). To allow pigs to express at least some natural foraging behaviour, straw (20 g/d) was provided daily, which has been demonstrated to alleviate the stress of individual housing (De Jong *et al.,* 1998; Chaloupková *et al.,* 2007). Water was provided *ad libitum*.

Sampling and fatty acid analysis

At the start of the experimental period, a blood sample was taken from each pig at 2.5 hours after feeding. At the end of the experimental period, pigs were anesthetized 2.5 hours after feeding with an administration of ketamine (10 mg/kg body weight) and xylazine (1 mg/kg body weight). Blood was collected by cardiac puncture before euthanasia was performed by an intracardiac administration of pentobarbital (100 mg/kg body weight). Brain and liver were immediately removed after euthanasia. The total brain, frontal cortex and hippocampus were weighed. For gene expression measurements, samples (~1 mg) of liver and hippocampus were rapidly frozen in liquid nitrogen and stored at -80°C until analysis. Other parts of the collected tissues were stored at -20°C pending fatty acid analysis. Total lipids were extracted from diets, liver, frontal cortex and hippocampus with a choloroform:methanol (2:1, v/v) Folch mixture and then saponified and methylated to determine fatty acid composition by gas chromatography as described previously (Smink *et al.,* 2008).

Table 5.1. Experimental design: Average intake of digestible energy (DE), starch, ileal digestible lysine, fat and fatty acids[1] in g/(kg $BW^{0.75}$/d)

	Low LA		High LA	
	Low ALA	High ALA	Low ALA	High ALA
C18:2 n-6 (LA)	1.32	1.31	2.64	2.67
C18:3 n-3 (ALA)	0.15	1.49	0.16	1.47
C12:0	0.03	0.03	0.03	0.03
C14:0	0.02	0.02	0.02	0.02
C16:0	0.69	0.66	0.64	0.60
C16:1	0.01	0.01	0.01	0.01
C18:0	0.13	0.17	0.17	0.22
C18:1 n-9	1.34	1.32	1.31	1.31
C18:1 n-7	0.03	0.04	0.04	0.05
C20:0	0.01	0.01	0.02	0.02
C20:1 n-9	0.01	0.01	0.01	0.01
C22:0	0.02	0.01	0.03	0.03
C24:0	0.01	0.01	0.01	0.01
Saturated fatty acid (SFA)	0.90	0.91	0.92	0.92
Monounsaturated fatty acid (MUFA)	1.39	1.38	1.37	1.38
Total fat	4.00	5.42	5.42	6.85
Digestible energy (MJ/kg $BW^{0.75}$/d)[2]	1.24	1.30	1.30	1.35
Ileal digestible lysine[2]	0.89	0.89	0.89	0.89
Starch[2]	34.7	34.7	34.7	34.7

LA, linoleic acid; ALA, α-linolenic acid

[1]based on analyzed values

[2]based on calculated values (CVB, 2007)

Table 5.2. Dietary ingredients (in g/kg, relative to the Low LA / low ALA diet) of the dietary treatments with differences in LA and ALA intake

	Low LA		High LA	
	Low ALA	High ALA	Low ALA	High ALA
Basal diet[1]	967.4	967.4	967.4	967.4
Sunflower oil	10.5	3.36	40.5	33.6
Linseed oil	1.92	34.1	1.85	34.2
HOSF[2] oil	10.3	6.61	3.76	-
Palm oil	9.89	6.07	4.05	-
Total[3]	1000	1018	1018	1035

[1]: The basal diet contained (g/kg, as fed) maize starch, 160.1; wheat, 160.5; barley, 321.0; wheat bran, 53.5; molasses, 32.1; soybean meal, 64.2; peas, 53.5; potato protein, 53.5 wheat gluten, 53.5; limestone, 14.1; monocalcium phosphate, 13.7; salt, 5.4; vitamin-mineral mix, 10.7; L-lysine HCl, 3.0; , L-Threonine, 0.32; tryptophan, 0.32; DL-methionine, 0.54.
[2]High oleic sunflower oil
[3]The sum of feed ingredients adds up to 1000 g/kg for the Low LA / low ALA diet only. A total exceeding 1000 reflects an increased feed intake of the respective treatment group as explained in the text.

Quantitative PCR

Total RNA was isolated from the hippocampus and liver tissue using TRIzol reagent (Invitrogen). Reverse transcription of 1 µg of total RNA was performed in a 20-µl reaction using Superscript III reverse transcriptase (Invitrogen), dNTPs (Roche) and random hexamer primers (Roche) for 1 h at 50 ºC according to manufacturer's protocol (Invitrogen). Real-time PCR was performed on a LightCycler 2.0 Real-Time PCR System by using FastStart DNA Master SYBR Green I reagents (Roche). The primers used are presented in Table 5.3. All primer pairs, except for 18S RNA, were designed in such a way that they span an intron of their corresponding genomic sequence or that its sense or reverse primer anneals on an exon-intron junction. Templates were amplified after a preincubation for 10 min at 95 ºC, followed by amplification for 40 cycles (10 s at 95 ºC, 5 s at 60 ºC, 5 s at 72 ºC). PCR efficiencies for the genes were established to be between 97 and 100%. Expression levels of FADS1, FADS2, ELOVL2 and ELOVL5 were normalized using the corresponding values of 18S RNA. All reactions revealed a single product as determined by melting curve analysis and specificity of the primer sets were verified by sequencing of the generated amplicons.

Statistical analysis

The trial was conducted as a 2 x 2 factorial arrangement of treatments with 8 replicates per treatment. The individually housed pig served as the experimental unit. The results were analyzed by ANOVA using the software program SAS version 9.1 (Statistical Analysis Systems Institute Inc., Cary, NC, USA). The fatty acid concentrations in all tissues and enzyme RNA concentrations were tested using the following model:

$Y = \mu + LA_i + ALA_j + (LA \times ALA)_{ij} + litter_k + e_{ijk}$,

With LA = daily LA intake i (i = low or high); ALA = daily ALA intake j (j= low or high); litter k (k = 1, .., 8).
For fatty acid concentrations in blood plasma, the initial concentrations, measured at the start of the experiment were included as a covariate.
Homogeneity of variance was tested using the Shapiro-Wilk test. When model residuals were not distributed normally ($P<0.05$), the statistical analysis was performed on transformed data. The level of statistical difference was preset at $P<0.05$.

Table 5.3. Q-PCR primers

Gene	Forward primer	Reverse primer
Δ5 desaturase	CAGGATGCTACGGATCCCTTT	GCTGCTCCGGAGACAGTTCT
Δ6 desaturase	CAGCACGATTACGGCCATCT	AGTTGGCAGAGGCACCCTTT
ELOVL2	TGACGCTGGTCATCCTGTTC	CGGCACGTCCGTATCTTTCT
ELOVL5	CCTCTCGGCTGGCTGTACTT	GAGAGGCCCCCTTCTTGTTG
18S RNA	GTTCAAAGCAGGCCCGAG	CGCCGCCGCATCGCCA

Results

All pigs remained healthy throughout the study and realized rates of BW gain were within the normal range. The effects of LA and ALA intake on body weight and brain weight are presented in Table 5.4. The average body weight at slaughter was 29 kg and did not significantly differ among treatments. Fresh brain weights (g/kg BW) were significantly ($P=0.012$) lower for the pigs fed the high LA diet. The hippocampus weight was lower ($P=0.046$) for the high ALA groups.

The effects of LA and ALA intake on the content of liver fatty acids are presented in Table 5.5. The results of the dietary treatments on the composition of plasma fatty acids largely resembled the effects on liver fatty acid composition (data not shown). Nearly all fatty acid contents were affected by LA and ALA intake. High LA pigs had a significant ($P<0.0001$) higher content of LA and ARA in liver. The content of n-3 fatty acids such as ALA, docosapentaenoic acid (DPA; C22:5 n-3) and DHA were decreased ($P<0.01$) in the high LA pigs. A high ALA intake resulted in an increased ($P<0.0001$) ALA and EPA content in liver. The proportion of DHA, however, was decreased ($P=0.01$) by high intake of ALA. High ALA pigs had increased content of LA in the liver but ARA and docosatetraenic acid (DTA; C22:4 n-6) contents decreased ($P<0.0001$) by about 50%.

The fatty acid profiles of the frontal cortex and the hippocampus are presented in Tables 5.6 and 5.7, respectively. LA and ALA were hardly present in both brain tissues. ARA (9%), C22:4 n-6 (3-4%) and DHA (7-8%) were the main PUFA in the two brain tissues. High LA intake tended ($P<0.1$) to increase the ARA content and significantly ($P<0.05$) increased the proportion of C22:4 n-6 in both the hippocampus and frontal cortex. High LA intake resulted in a significant ($P<0.001$) decrease of DPA and a tendency ($P=0.09$) of a decreased DHA content in the frontal cortex. Increased ALA intake did not affect the ARA and C22:4 n-6 contents but lowered C22:5 n-6 in the frontal cortex. High ALA increased DPA, but did not

affect DHA contents in the frontal cortex significantly. High LA intake resulted in a decreased content of DPA but did not affect that of DHA in the hippocampus. A high ALA intake significantly decreased all n-6 LC-PUFA, but increased the LA content in the hippocampus. High ALA intake increased the DPA content, but did not affect other n-3 fatty acids.

The change in mRNA expression levels of desaturase and elongase enzymes were measured in liver and hippocampus (Table 5.8). In liver, both LA and ALA intake significantly ($P<0.05$) increased the transcript level of ELOVL2. On the contrary, ELOVL5 mRNA was not significantly increased. In addition, both hepatic transcript levels of Δ5- and Δ6 desaturase were increased following high LA intake ($P<0.01$). In hippocampus, high ALA intake significantly ($P=0.022$) increased the mRNA expression of ELOVL2, but not of ELOVL5, Δ6- and Δ5 desaturase. In contrast, variation in LA intake had no effect on the mRNA expression of these four genes.

Table 5.4. Body weight (BW) at the start and the end of the trial and the effect of LA and ALA intake on brain weight in growing pigs

	Low LA		High LA		Pooled SEM	P-values for effect		
	Low ALA	High ALA	Low ALA	High ALA		LA	ALA	LA*ALA
BW start (kg)	16.3	16.4	16.1	16.4	0.68	0.85	0.75	0.85
BW end (kg)	28.5	28.8	29.3	29.7	0.63	0.20	0.61	0.96
Brain (g/ BW end)	2.14	2.13	2.00	1.99	0.051	0.012	0.78	0.97
Frontal cortex (g/BW end)	0.48	0.48	0.46	0.44	0.019	0.13	0.56	0.67
Hippocampus (g/BW end)	0.076	0.073	0.076	0.070	0.002	0.37	0.046	0.63

LA, linoleic acid; ALA, α-linolenic acid

Discussion

The formulated diets contained LA and ALA and were free of other n-3 or n-6 LC-PUFA. Once consumed, LA and ALA can be converted to other LC-PUFA by desaturation and elongation. The route and assumed enzymes involved in the biosynthesis of LC-PUFA are given in Figure 5.1.

In this study we supplied LA or ALA maintaining identical intakes of other nutrients, an approach which allows independent evaluation of the effects of LA and ALA in liver and brain. Importantly, we did not observe a difference in body weight of the gilts among the experimental groups.

Liver

A high LA intake increased the content of LA and n-6 LC-PUFA such as ARA in liver lipid. This agrees well with other studies in pigs (Romans *et al.,* 1995; Schellingerhout, 2002). Increasing the ALA intake by 1.3 g /kg $BW^{0.75}$ per day increased the EPA content in liver lipid from less than 1% to 8%. In contrast to other n-3 LC-PUFA, the DHA content was decreased by high ALA intake. Interestingly, a similar effect was seen on the content of ALA, EPA and DHA in plasma phospholipids in humans (Goyens *et al.,* 2006), indicating that the composition of these plasma lipids is a reflection of the lipid metabolism of the liver. Other earlier studies showed that plasma DHA level was not affected or marginally increased in humans by dietary ALA (Blank *et al.,* 2002; Burdge and Calder, 2005). In contrast to our study, these authors compensated the higher intake of ALA with a decrease in the intake of other fatty acids. We believe that caution should be to taken in choosing this exchange approach, as there is evidence that other lipids like LA, oleic acid and myristic acid (C14:0) can alter hepatic desaturase activity as well (Bézard *et al.,* 1994; Jan *et al.,* 2004; Portolesi *et al.,* 2008; Rioux *et al*., 2008). These fatty acids may contribute to the observed opposite effects on plasma DHA in their studies.

This study shows that high LA intake increased levels of both Δ5- and Δ6 desaturase mRNA in liver. Similar effects have been found in rats (Bézard *et al.,* 1994) and piglets (Theil and Lauridsen, 2007). In contrast, in a human hepatoma cell line a suppression of Δ6 desaturase mRNA content was seen after the addition of LA or ALA (Portolesi *et al.,* 2008). Increased ALA intake had no effect on Δ5- and Δ6 desaturase. The rate limiting enzyme Δ6 desaturase has been reported to have a higher affinity for ALA than for LA (Rodriguez *et al.,* 1998; Sprecher, 2002). Although not measured in our model, it is tempting to speculate that the surfeit of ALA in liver is preferentially converted above LA down the cascade (Figure 5.1) or alternatively catabolized (via β-oxidation) and that the LA-elevated expression of Δ6- (and Δ5) desaturase may be necessary to metabolize the surplus of LA provided by the diet. Indeed, we found a strong increase in the EPA content in liver lipid in response to ALA intake, suggesting that the enzymatic activity of both desaturases and ELOVL5, at least for ALA, were not rate limiting.

In our study, high LA and high ALA intake increased the transcript of ELOVL2 but not of ELOVL5 in the liver of growing piglets. These findings indicate that the conversion of ARA and EPA to longer elongation products became more efficient (see Figure 5.1). Indeed, we found an increased proportion of DPA and DTA in liver lipid in response to ALA and LA

intake, respectively. Unfortunately, we did not have the standards in order to establish whether this was paralleled by a positive effect on the content of C24:5 n-3 and C24:4 n-6 as well. Fatty acid-dependent changes in hepatic ELOVL2 expression have also been reported in rat and salmon (Igarashi *et al.,* 2007a; Morais *et al.,* 2009).

Interestingly, DHA levels were significantly reduced, while ELOVL2 mRNA expression was increased in response to ALA intake. This finding suggests a rate-limiting enzyme downstream of ELOVL2. As Δ6 desaturase has a higher affinity for ALA than for C24:5 n-3 (D'Andrea *et al.,* 2002; Portolesi *et al*., 2007), it is conceivable that Δ6 desaturation of C24:5 n-3 is inhibited when ALA is abundant, preventing DHA synthesis. We would suggest that in the liver of piglets, Δ6 desaturase rather than ELOVL2 is the rate-limiting enzyme downstream of EPA in the biosynthesis of DHA. Whether ALA has any inhibitory effect on the peroxisomal β-oxidation of C24:6 n-3 remains to be elucidated.

Increased intake of LA decreased the proportion of total n-3 LC-PUFA and a high intake of ALA decreased the total n-6 LC-PUFA in liver lipid. This confirms the general concept that competition between ALA and LA occurs due to sharing of the same desaturation and elongation enzymes. In addition, this study illustrates that for EPA and DHA, the effects of LA intake are stronger at high ALA intakes and the effects of ALA intake are stronger at low LA intakes (interaction LAxALA for EPA, DHA, $P<0.001$ and $P=0.07$, respectively). The first concern is the effect of high LA as the Western diet is generally rich in LA and poor in n-3 fatty acids. High LA intake in our study decreased the proportion of EPA and DHA in liver fat by >35% and >20%, respectively. A similar effect was also observed in blood plasma fat. On the other hand, high ALA intake drastically decreased the proportion of ARA by roughly 50%. This inhibitory effect provides a strong indication that ALA and LA competes for the same desaturases and elongases.

Taken together, our experimental design allows a direct comparison of the effects of identical increments in daily intakes of LA and ALA (both 1.3 g/kg $BW^{0.75}$), which is rarely found in literature. The effect of LA both as a substrate of the n-6 chain and as an inhibitor of the n-3 chain was much lower in comparison with that of ALA. Generally, the effect of ALA on LC-PUFA appeared to be four times stronger than that of LA. Our findings in growing piglets are in agreement with human intervention studies showing a strong positive correlation of ALA intake and blood EPA, but weaker between LA intake and blood ARA (Mantzioris *et al.,* 1995).

Table 5.5. Effect of LA and ALA intake on the fatty acid profile (% of total fatty acids) of the liver in growing pigs

Fatty acids	Low LA		High LA		Pooled SEM	P-values of effects		
	Low ALA	High ALA	Low ALA	High ALA		LA	ALA	LA*ALA
C16:0	15.1	11.7	13.0	10.5	0.33	<0.0001	<0.0001	0.18
C17:0	1.31	1.17	1.29	1.03	0.040	0.49	0.07	0.55
C18:0	23.5	26.7	24.6	24.0	0.43	0.08	<0.01	<0.0001
SFA	40.3	39.6	38.9	35.6	0.40	<0.0001	<0.001	<0.01
C16:1	0.53	0.43	0.38	0.26	0.040	<0.001	0.013	0.75
C18:1 n-7	1.48	1.26	1.11	1.20	0.043	<0.001	0.17	<0.01
C18:1 n-9	13.5	10.8	10.7	9.51	0.31	<0.01	<0.001	0.79
MUFA	15.7	12.5	12.2	11.1	0.36	<0.0001	<0.0001	<0.01
C18:2 n-6 (LA)	16.4	16.8	21.2	24.3	0.33	<0.0001	<0.0001	<0.001
C20:3 n-6	0.81	0.77	0.68	0.80	0.047	0.28	0.38	0.10
C20:4 n-6 (ARA)	17.4	8.86	19.3	11.1	0.33	<0.0001	<0.0001	0.57
C22:4 n-6 (DTA)	0.73	0.13	1.02	0.19	0.036	<0.0001	<0.0001	<0.01
PUFA n-6	35.4	26.6	42.3	36.4	0.36	<0.0001	<0.0001	<0.001
LC n-6	19.0	9.77	21.0	12.1	0.32	<0.0001	<0.0001	0.62
C18:3 n-3[1] (ALA)	0.48	5.09	0.44	4.18	0.030	<0.01	<0.0001	<0.05
C20:3 n-3	ND	0.87	ND	0.98	0.066	-	-	-
C20:5 n-3 (EPA)	0.58	8.13	0.28	5.28	0.155	<0.0001	<0.0001	<0.0001
C22:5 n-3 (DPA n-3)	2.42	3.23	1.67	2.66	0.079	<0.0001	<0.0001	0.26
C22:6 n-3 (DHA)	2.63	2.33	2.10	1.71	0.123	0.0001	0.010	0.07
PUFA n-3	6.16	19.7	4.50	14.8	0.25	<0.0001	<0.0001	<0.0001
LC n-3	5.67	14.6	4.06	10.6	0.181	<0.0001	<0.0001	<0.0001
C16:1 / C16:0	0.035	0.037	0.029	0.024	0.0028	<0.01	0.56	0.24
C18:1 n-9 / C18:0	0.58	0.40	0.44	0.40	0.019	<0.001	<0.001	<0.01

LA = linoleic acid, ALA = α-linolenic acid, ARA = arachidonic acid, EPA = eicosapentaenoic acid, DTA = docosatetraenoic acid, DPA = docosapentaenoic acid, DHA = docosahexaenoic acid, SFA = saturated fatty acid, MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid, LC = Long-chain (fatty acids with 20 or 22 C-atoms)

[1]: P value and SEM are obtained from Y=log(1+x) function

Table 5.6. Effect of LA and ALA intake on the fatty acid profile (% of total fatty acids) of the frontal cortex in growing pigs

Fatty acids	Low LA		High LA		Pooled SEM	P-values of effects		
	Low ALA	High ALA	Low ALA	High ALA		LA	ALA	LA*ALA
C16:0	20.0	19.4	19.2	19.3	0.234	0.06	0.30	0.20
C18:0	18.2	17.9	17.8	17.8	0.184	0.24	0.58	0.32
SFA	39.4	38.5	38.2	38.3	0.406	0.13	0.33	0.25
C16:1	0.89	0.94	0.90	0.92	0.018	0.76	0.07	0.26
C17:1	1.48	1.60	1.72	1.59	0.099	0.27	0.93	0.23
C18:1 n-7	4.91	4.93	5.03	5.02	0.097	0.30	0.97	0.23
C18:1 n-9	16.8	17.4	17.7	17.1	0.482	0.56	0.96	0.18
MUFA	24.9	25.8	26.3	25.4	0.733	0.48	0.97	0.89
C18:2 n-6 (LA)	0.69	0.80	0.79	0.90	0.030	<0.01	<0.01	0.91
C20:4 n-6 (ARA)	8.85	8.40	8.92	9.28	0.237	0.06	0.85	0.10
C22:4 n-6 (DTA)	3.30	3.04	3.39	3.36	0.086	0.027	0.10	0.21
C22:5 n-6 (DPA n-6)	1.18	0.59	1.02	0.61	0.094	0.46	<0.0001	0.34
PUFA n-6	14.5	13.4	14.6	14.7	0.330	0.015	0.17	0.08
LC n-6	13.8	12.6	13.8	13.8	0.330	0.07	0.09	0.08
C18:3 n-3 (ALA)	0.62	0.68	0.72	0.63	0.066	0.70	0.72	0.10
C22:5 n-3 (DPA n-3)	0.24	0.56	0.21	0.46	0.015	<0.001	<0.0001	<0.05
C22:6 n-3 (DHA)	7.77	8.14	6.81	7.69	0.393	0.09	0.13	0.52
PUFA n-3	8.56	9.39	7.68	8.74	0.356	0.044	0.015	0.74
LC n-3	8.16	8.91	7.20	8.30	0.388	0.06	0.027	0.65

LA = linoleic acid, ALA = α-linolenic acid, ARA = arachidonic acid, EPA = eicosapentaenoic acid, DTA = docosatetraenoic acid, DPA = docosapentaenoic acid, DHA = docosahexaenoic acid, SFA = saturated fatty acid, MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid, LC = Long-chain (fatty acids with 20 or 22 C-atoms)

Table 5.7. Effect of LA and ALA intake on the fatty acid profile (% of total fatty acids) of the hippocampus in growing pigs

Fatty acids	Low LA		High LA		Pooled SEM	P-values of effects		
	Low ALA	High ALA	Low ALA	High ALA		LA	ALA	LA*ALA
C14:0	0.46	0.44	0.45	0.43	0.0059	0.13	<0.05	0.90
C16:0	17.6	17.5	18.3	17.8	0.36	0.17	0.47	0.61
C17:0	0.21	0.21	0.21	0.20	0.0040	0.35	0.12	0.17
C18:0	18.5	18.4	18.6	18.6	0.14	0.42	0.60	0.73
SFA	37.6	37.2	38.4	37.7	0.47	0.20	0.27	0.80
C16:1	0.83	0.86	0.80	0.52	0.011	<0.01	0.015	0.50
C17:1	1.15	1.28	1.06	1.17	0.073	0.17	0.12	0.91
C18:1 n-7	4.93	4.80	4.72	4.80	0.047	0.031	0.57	0.041
C18:1 n-9	18.5	19.0	17.6	18.3	0.54	0.15	0.29	0.80
C20:1 n-9	0.74	0.76	0.62	0.68	0.050	0.07	0.45	0.63
MUFA	26.5	27.0	25.0	26.0	0.72	0.12	0.31	0.74
C18:2 n-6 (LA)	0.53	0.70	0.56	0.94	0.10	0.19	0.010	0.29
C20:4 n-6 (ARA)	8.90	8.33	9.35	8.81	0.24	0.07	0.033	0.96
C22:4 n-6 (DTA)	4.18	3.87	4.42	4.12	0.098	0.018	<0.01	0.96
C22:5 n-6 (DPA n-6)	0.70	0.43	0.88	0.50	0.034	0.03	<0.0001	0.14
PUFA n-6	14.1	13.5	14.8	14.5	0.37	0.02	0.06	0.81
LC n-6	13.6	12.8	14.2	13.5	0.32	0.03	0.01	0.97
C18:3 n-3[1] (ALA)	0.45	0.48	0.38	0.43	0.194	0.56	0.29	0.06
C20:3 n-3	0.04	0.04	0.03	0.06	0.027	0.86	0.59	0.82
C20:5 n-3 (EPA)	0.07	0.06	0.06	0.06	0.0051	0.13	0.25	0.67
C22:5 n-3 (DPA n-3)	0.29	0.67	0.26	0.54	0.011	<0.0001	<0.0001	<0.001
C22:6 n-3 (DHA)	6.38	6.65	6.96	6.93	0.31	0.18	0.71	0.63
PUFA n-3	7.23	7.90	7.69	8.02	0.29	0.32	0.10	0.55
LC n-3	6.78	7.42	7.31	7.58	0.31	0.29	0.17	0.56

LA = linoleic acid, ALA = α-linolenic acid, ARA = arachidonic acid, EPA = eicosapentaenoic acid, DTA = docosatetraenoic acid, DPA = docosapentaenoic acid, DHA = docosahexaenoic acid, SFA = saturated fatty acid, MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid, LC = Long-chain (fatty acids with 20 or 22 C-atoms)

[1]: P value and SEM are obtained from an inverse function

Table 5.8. Effects of LA and ALA intake on the mRNAs of Δ5- and Δ6 desaturases and elongase 2 and -5 in liver and hippocampus of growing pigs. mRNA levels were quantified by quantitative PCR and normalized to 18S ribosomal RNA. They are expressed relative to the Low LA/Low ALA diet group (1.00)

	Low LA		High LA		Pooled	P-values for effect		
	Low ALA	High ALA	Low ALA	High ALA	SEM	LA	ALA	LA*ALA
Liver								
ELOVL2	1.00	2.44	2.40	3.53	0.412	0.044	0.037	0.24
ELOVL5	1.00	1.13	1.20	1.24	0.108	0.11	0.27	0.92
Δ5 desaturase	1.00	1.08	1.60	1.78	0.219	<0.01	0.57	0.81
Δ6 desaturase	1.00	1.15	1.54	1.71	0.158	<0.01	0.33	0.96
Hippocampus								
ELOVL2	1.00	2.81	1.80	2.14	0.421	0.89	0.022	0.09
ELOVL5	1.00	1.59	1.02	1.22	0.248	0.50	0.13	0.43
Δ5 desaturase	1.00	1.60	1.14	1.05	0.393	0.58	0.51	0.36
Δ6 desaturase	1.00	1.73	1.00	1.14	0.264	0.27	0.12	0.27

LA, linoleic acid; ALA, α-linolenic acid

Brain

The timing of the pig brain growth spurt is similar to that in humans (Dobbing and Sands, 1979; Pond *et al.,* 2000). Several trials with young piglets were conducted to study the effect of dietary fat on brain fatty acid composition (Arbuckle *et al.,* 1991; De la Presa-Owens *et al.,* 1998; Arbuckle and Innis, 2003). These nutritional intervention studies were mainly focused on whole brain. In the present study we selected the frontal cortex, representing a rather large region of the brain, with primary functions (Ng and Innis, 2003), and in addition, the hippocampus, which is involved in the memory storage and retrieval. Although it has been reported that deprivation of essential fatty acids causes a decrease in brain weight (Odutuga, 1981), we found to our surprise a decreased brain weight in response to increased LA intakes, irrespective of ALA intake. This is in contrast with the study of Hrboticky et al. (1990), showing that higher LA at the expense of oleic acid did not affect brain weight in young piglets. In our study, an increased LA intake tended ($P<0.1$) to increase ARA and significantly increased C22:4 n-6 in fat extracts of both hippocampus and frontal cortex. Moreover, high ALA intake increased DPA, but not DHA concentrations in the fat of those brain tissues. Earlier studies in piglets demonstrated an increase in brain DHA by dietary ALA (Arbuckle *et al*., 1991). The difference in the effect on DHA between the latter and our study may be due to their use of younger piglets, knowing that younger piglets respond stronger to dietary PUFA (Cheon *et al.,* 2000). However, the lack of the effect on brain DHA was also found in adult rats where ALA intake exceeded the low ALA groups in our study (Bourre *et al.,* 1993). The elongation of n-3 fatty acids in brain astrocytes did not extend beyond DPA (Innis and Dyer, 2002). Although high ALA intakes drastically increased the ALA content in plasma lipids (an increase of the proportion from ±1 to 10%), which is a

reflection of the increase of ALA content in liver (Table 5.5), the ALA and DHA content in frontal cortex and hippocampus did not increase (Tables 5.6 and 5.7). Other studies in rat brain showed that ALA after uptake from the circulation across the blood-brain barrier will be mainly oxidized and only a small fraction is converted to DHA (DeMar *et al.,* 2005; Igarashi *et al.,* 2007b). The activity of desaturation and elongation are higher in liver in comparison with brain (Igarashi *et al.,* 2007a). This suggests that most DHA in the brain is derived from another source, like liver, intestine (diet) and/or adipose tissue. Surprisingly, the DHA concentration in the pig liver lipid decreased with increasing ALA intake. High ALA intake resulted in a significant increase in ELOVL2 mRNA expression which may relate to the strong increase in the content of DPA, a product of ELOVL2, in both frontal cortex and hippocampus. On the other hand, we did not observe an effect on mRNA expression of Δ6 and Δ5 desaturase and ELOVL5 in the hippocampus which agrees with studies in rat brain (Igarashi *et al.,* 2007a).

Similarly to liver, high dietary ALA intake increased the concentration of LA in fat extracts of both frontal cortex and hippocampus. The proportion of ALA, however, was not increased. The latter may be due to a high rate of oxidation in brain (DeMar *et al.,* 2005), or to elongation and desaturation, resulting in increased concentrations of n-3 LC-PUFA in the frontal cortex. The higher LA concentration in high ALA diets might be both due to competition for enzymes and sparing of LA oxidation in the presence of ALA. The rate of oxidation of ALA is higher than that of LA (DeLany *et al.,* 2000).

In conclusion, the effect of LA and ALA are important both as precursor and inhibitor for the synthesis of LC-PUFA. LA as a substrate is increasing mRNA expression of Δ6- and Δ5 desaturase and all n-6 PUFA in liver. High ALA intake did increase EPA in liver lipid but decreased the proportion of DHA. This study supports the idea that high dietary LA intake inhibits the conversion of ALA into n-3 LC-PUFA and that ALA inhibits the conversion of LA into n-6 LC-PUFA. The magnitude of the effect of ALA in liver is higher than that of LA. This suggests that manipulation of ARA and EPA availability by dietary interventions should be optimized varying both dietary LA and ALA. DHA in brain tissue is hardly affected by both dietary LA and ALA. mRNA expression of ELOVL2 was up-regulated by dietary treatment and was, apart from Δ6 desaturase, identified as a potentially rate-limiting step.

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Chapter 6

Effect of intake of linoleic acid and α-linolenic acid level on conversion into long-chain polyunsaturated fatty acids in backfat and in intramuscular fat of growing pigs

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6. Effect of intake of linoleic acid and α-linolenic acid level on conversion into long-chain polyunsaturated fatty acids in backfat and in intramuscular fat of growing pigs

Abstract

A study was conducted to determine the effect of two levels of linoleic acid (LA) intake at either high or low α-linolenic acid (ALA) intake on their conversion and subsequent deposition into long-chain (20-22 C atoms) polyunsaturated fatty acids (LC-PUFA) in muscle and backfat in growing pigs. In a 2 x 2 factorial arrangement, 32 gilts from 8 litters were assigned to one of four dietary treatments, varying in LA and ALA intake. Low ALA and LA intake were 0.15 and 1.31 g/(kg $BW^{0.75}$/d), respectively and high ALA and LA intake were 1.48 and 2.65 g/(kg $BW^{0.75}$/d), respectively. There was a close positive relation between intake of ALA and the concentration of ALA in backfat and in intramuscular fat. Dietary ALA did not affect the concentration of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), but increased docosapentaenoic acid (DPA) in backfat. High ALA intake did not significantly affect DHA but significantly increased EPA, C20:3 n-3 and DPA concentration in intramuscular fat. The n-3 LC-PUFA proportion in backfat was increased from approximately 1 to 3%, which may be useful to enrich meat with these fatty acids. The effect of ALA intake on n-3 LC-PUFA was suppressed by LA intake. Dietary ALA suppressed the concentration of n-6 LC-PUFA in blood plasma by more than 50%. When compared at equal incremental dose, the inhibiting effect of ALA on blood arachidonic acid was stronger than the stimulating effect of LA as precursor.

Keywords: Linoleic acid – α-linolenic acid – muscle – backfat - pigs

Introduction

Arachidonic acid (ARA; C20:4 n-6), eicosapentaenoic acid (EPA; C20:5 n-3) and docosahexaenoic acid (DHA; C22:6 n-3) are the most abundant long-chain (20-22 C- atoms) polyunsaturated fatty acids (LC-PUFA) in mammals. These LC-PUFA have important regulatory functions in the immune system, nervous and cardiovascular system (Calder, 2008; Russo, 2009). ARA can be synthesized from linoleic acid (LA; C18:2 n-6) and EPA and DHA from α-linolenic acid (ALA; C18:3 n-3) in subsequent elongation and desaturation reactions. In infants dietary supplementation of ARA and DHA improved development (Birch et al., 1998) and dietary requirements for DHA and ARA have been proposed (Hoffman *et al*., 2009). Dietary supplementation of EPA and DHA, however, decreases the ARA status of infants (Carlson, 1996). Consumption of EPA and DHA are known to have a positive impact on human health and a dosage of 250 mg per day is advised to prevent cardiovascular diseases (EFSA, 2010). In addition to EPA and DHA, docosapentaenoic acid (DPA; C22:5 n-3) has a high potency to play a beneficial role in heart disease (Kaur *et al.*, 2011). Mammals are able

to synthesize DHA, DPA and EPA from ALA. The efficiency of these conversions is, however low in several tissues. To some extent, this may be related to the Western-style diets, with generally high consumption of n-6 fatty acids (Burdge and Calder, 2005). There is interest to increase the consumption of EPA and DHA via an enrichment of those fatty acids in meat. This enrichment can be realized through nutrition via (1) direct deposition of dietary n-3 LC-PUFA and (2) synthesis of n-3 LC-PUFA by the pig from dietary ALA. It has been established that the first route is possible, as there are strong correlations between EPA and DHA in the diet and these fatty acids in backfat and muscle (Hertzman et al, 1988; Nguyen *et al.*, 2003). The second route is more attractive, but the potential is less clear. Studies showed that an increase in dietary ALA will increase the concentration EPA in liver of piglets (Schellingerhout, 2002) and in fattening pigs (Roman *et al.*, 1995). In addition, the content of n-3 LC-PUFA in muscle and backfat seemed to be increased (Wood *et al.*, 2004; Mitchaothai, 2007), but the results vary. Several dietary factors such as protein intake (Bézard, 1994) and dietary fatty acids including LA and myristic acid (Bézard et al, 1994; Goyens *et al.*, 2006; Jan *et al.*, 2008) may affect enzymes responsible for the conversion of ALA into n-3 LC-PUFA. Information of the interaction between dietary LA and ALA on backfat and intramuscular fat concentration of n-3 LC-PUFA is not readily available.
This study was designed to quantify the effect of the addition of ALA to a low and a high LA diet, and of LA to a low and high ALA diet, on the LC-PUFA concentrations in backfat and intramuscular fat. Effects of dietary treatments on brain and liver LC-PUFA concentrations and gene expression are published elsewhere (Smink *et al.*, 2012).

We hypothesize that additional dietary LA will reduce the conversion of ALA into n-3 LC-PUFA and as a result this decreases n-3 LC-PUFA concentrations in intramuscular fat and adipose tissue. In addition, the effect of ALA as a potential inhibitor of the concentration of ARA in blood and tissues was assessed.

Material and methods

Animals and housing
Thirty-two female pigs were selected from 8 litters. From each litter, 4 gilts were selected and allotted to one of the 4 dietary treatments. The pigs (20-30 kg) were housed individually during an experimental period of 4 weeks. The pigs were weighed weekly and feed intake was recorded daily. The experimental protocol was approved by the animal experimental committee of Wageningen University, The Netherlands.

Diets
The study was carried out as a 2 x 2 factorial design with daily intakes of LA and ALA as independent variables. The incremental differences between low and high intake were designed to be identical for LA and ALA: Low ALA and LA intakes were 0.15 and 1.31, and high ALA and LA intakes were 1.48 and 2.65 g/(kg $BW^{0.75}$/d). Feed intake adjusted daily and was expressed per kg metabolic weight because that figure is fairly constant in *ad lib* fed pigs. The concentrations of LA and ALA in the diet low LA / low ALA were 12 and 1.4 g/kg,

respectively. Concentrations of LA and ALA in the high LA / high ALA diet were 28 and 17.5 g/kg for LA and ALA, respectively, i.e. 16 g/kg higher for both LA and ALA compared with the low LA/low ALA diet. The low LA and ALA intakes were slightly above minimal requirement figures for pigs (NRC, 1998; Schellingerhout, 2002). The addition of LA and ALA was on top of a basal diet. The realized intakes of fatty acids are presented in Table 6.1. With the exception of LA and ALA, the intake of all nutrients including saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) were kept constant. This was realized by optimizing the dosages of analyzed sunflower oil, linseed oil, high oleic acid sunflower oil (HOSF) and palm oil. The diets did not contain n-3 or n-6 LC-PUFA other than LA and ALA. The data on composition of the basal diet and the inclusion rates of the oils in the treatments are given in Table 6.2. The low LA / low ALA diet was formulated to contain sufficient ileal digestible amino acids in relation to energy (CVB, 2007). The diets were fed twice daily as mash. To allow pigs to express some natural foraging behavior, straw (20 g/d) was provided daily, which has been demonstrated to alleviate the stress of individual housing (De Jong *et al.*, 1998; Chaloupková et al, 2007). Water was provided *ad libitum*.

Sampling and fatty acid analysis

At the start of the experimental period, a blood sample from the Jugular vein was taken of each pig at 2.5 hours after feeding. At the end of the experimental period, pigs were anesthetized 2.5 hours after feeding with an administration of ketamine (10 mg/kg body weight) and xylazine (1 mg/kg body weight). Blood was collected by cardiac puncture before euthanasia was performed by an intracardiac administration of pentobarbital (100 mg/kg body weight). In addition, backfat and muscle samples were collected. These tissues and blood plasma were stored at -20°C pending fatty acid analysis. Total lipids were extracted from diets, blood plasma and muscle with a choloroform:methanol (2:1, v/v) Folch mixture and then saponified and methylated to determine fatty acid composition by gas chromatography as described previously (Smink *et al.*, 2008).

Statistical analysis

The trial was conducted as a 2 x 2 factorial arrangement of treatments with 8 replicates per treatment. The individually housed pig served as the experimental unit. The results were analyzed by ANOVA using the software program SAS. The fatty acid concentrations in blood and tissues were tested using the following model:

$Y = \mu + LA_i + ALA_j + (LA \times ALA)_{ij} + litter_k + e_{ijk}$,

With LA = daily LA intake i (i = low or high); ALA = daily ALA intake j (j= low or high); litter k (k = 1, .., 8).

For fatty acid concentrations in blood plasma, the initial concentrations, measured at the start of the experiment were included as a covariate.

Homogeneity of variance was tested using the Shapiro-Wilk test. When model residuals were not distributed normally ($P<0.05$), the statistical analysis was performed on transformed data. The level of statistical difference was preset at $P<0.05$.

Table 6.1. Experimental design: average intake of digestible energy (DE), starch, ileal digestible lysine, fat and fatty acids[1] in g/(kg $BW^{0.75}$/d).

	Low LA		High LA	
	Low ALA	High ALA	Low ALA	High ALA
18:2 n-6 (LA)	1.32	1.31	2.64	2.67
18:3 n-3 (ALA)	0.15	1.49	0.16	1.47
C12:0	0.03	0.03	0.03	0.03
C14:0	0.02	0.02	0.02	0.02
C16:0	0.69	0.66	0.64	0.60
C16:1	0.01	0.01	0.01	0.01
C18:0	0.13	0.17	0.17	0.22
C18:1 n-9	1.34	1.32	1.31	1.31
C18:1 n-7	0.03	0.04	0.04	0.05
C20:0	0.01	0.01	0.02	0.02
C20:1 n-9	0.01	0.01	0.01	0.01
C22:0	0.02	0.01	0.03	0.03
C24:0	0.01	0.01	0.01	0.01
Saturated fatty acid (SFA)	0.90	0.91	0.92	0.92
Monounsaturated fatty acid (MUFA)	1.39	1.38	1.37	1.38
Total fat	4.00	5.42	5.42	6.85
Digestible energy (MJ/kg $BW^{0.75}$/d)[2]	1.24	1.30	1.30	1.35
Ileal digestible lysine[2]	0.89	0.89	0.89	0.89
Starch[2]	34.7	34.7	34.7	34.7

LA, linoleic acid; ALA, α-linolenic acid

[1]based on analyzed values

[2]based on calculated values (CVB Table of Dutch Bureau of Livestock Feeding)

Table 6.2. Dietary ingredients (in g/kg, relative to the Low LA / low ALA diet) of the dietary treatments with differences in LA and ALA intake.

	Low LA		High LA	
	Low ALA	High ALA	Low ALA	High ALA
Basal diet[1]	967.4	967.4	967.4	967.4
Sunflower oil	10.5	3.36	40.5	33.6
Linseed oil	1.92	34.1	1.85	34.2
HOSF[2] oil	10.3	6.61	3.76	-
Palm oil	9.89	6.07	4.05	-
Total[3]	1000	1018	1018	1035

[1]: The basal diet contained (g/kg, as fed) maize starch, 160.1; wheat, 160.5; barley, 321.0; wheat bran, 53.5; molasses, 32.1; soybean meal, 64.2; peas, 53.5; potato protein, 53.5; wheat gluten, 53.5; limestone, 14.1; monocalcium phosphate, 13.7; salt, 5.4; vitamin-mineral mix, 10.7; L-lysine HCl, 3.0; L-Threonin, 0.32; tryptophan, 0.32; DL-methionin, 0.54.
[2]High oleic sunflower oil
[3]The sum of feed ingredients adds up to 1000 g/kg for the Low LA / low ALA diet only. A total exceeding 1000 reflects an increased feed intake of the respective treatment group as explained in the text.

Results

All pigs remained healthy throughout the study. The effect of LA and ALA intake on the fatty acid profile in blood plasma is presented in Table 6.3. Nearly all concentrations of fatty acids in plasma were affected by the level of LA and ALA intake. High LA pigs had significantly higher proportions of LA and ARA in plasma compared with low LA pigs. The concentration of n-3 fatty acids such as ALA, DPA and DHA were decreased by a high intake of LA. A high ALA intake resulted in increased ($P<0.0001$) ALA and EPA in blood plasma. The proportion of DHA was decreased by high intake of ALA. High ALA intake decreased the proportion of LA and ARA in plasma by 5 and 50%, respectively.
The fatty acid profile of backfat is presented in Table 6.4. High LA intake increased the concentration of n-6 fatty acids in backfat. The concentration of n-3 PUFA in backfat was decreased in the high LA pigs. High ALA intake increased ALA and total n-3 LC-PUFA levels in backfat, but the concentration of DHA was not affected. There was a significant interaction between LA and ALA for most n-3 PUFA. The effect of LA on n-3 PUFA was stronger at a high ALA intake. Both high LA and high ALA intake decreased the MUFA proportion in backfat. There was a significant interaction of LA and ALA. The effect of high LA was lower at a high ALA intake.
The effect of LA and ALA intake on intramuscular fatty acid profile is presented in Table 6.5. High LA intake increased intramuscular LA concentration significantly, but did not affect the concentration of ARA. The concentrations of ALA, C20:3 n-3 and EPA were decreased at a high LA intake, but those of DPA and DHA were not affected. High ALA intake of pigs resulted in an increase ($P<0.0001$) in all n-3 fatty acids, except for DHA. The concentration of

ARA was not affected by ALA. There was a significant interaction between LA and ALA intake on total n-3 PUFA concentration in muscle. High LA intake decreased intramuscular n-3 fatty acids in muscle in pigs fed a high intake of ALA, but not those of the low ALA pigs. Both high LA and high ALA intake decreased the proportion of MUFA and the ratio of C18:1 n-9/C18:0 in muscle. The effect of LA on this ratio was lower at a high ALA intake (interaction, $P<0.05$).

Table 6.3. Effect of LA and ALA on the fatty acid profile (% of total fatty acids) of blood plasma in growing pigs.

Fatty acids	Low LA		High LA		Pooled SEM	P-values of effects		
	Low ALA	High ALA	Low ALA	High ALA		LA	ALA	LA*ALA
C14:0	0.26	0.14	0.22	0.08	0.060	0.40	0.05	0.86
C16:0	20.6	17.1	17.7	14.5	0.351	<0.0001	<0.0001	0.64
C17:0	0.68	0.58	0.68	0.55	0.041	0.80	0.012	0.66
C18:0	9.91	10.8	10.3	10.8	0.413	0.67	0.12	0.63
SFA	31.8	29.0	28.8	26.0	0.600	<0.001	<0.001	0.94
C16:1	0.61	0.46	0.38	0.30	0.034	<0.0001	<0.01	0.30
C18:1 n-7	1.28	1.13	0.96	0.98	0.054	<0.001	0.29	0.12
C18:1 n-9	21.4	17.3	16.5	13.6	0.601	<0.0001	<0.0001	0.33
MUFA	23.5	18.9	17.9	15.2	0.637	<0.0001	<0.0001	0.14
C18:2 n-6	29.8	27.9	39.5	38.5	0.608	<0.0001	0.043	0.44
C20:4 n-6	5.20	2.41	5.93	2.79	0.233	0.027	<0.0001	0.46
PUFA n-6	35.2	30.5	45.7	42.2	0.659	<0.0001	<0.0001	0.42
LC n-6	5.43	2.50	6.07	2.92	0.252	0.047	<0.0001	0.012
C18:3 n-3	1.24	12.9	1.25	9.71	0.376	<0.001	<0.0001	<0.001
C20:5 n-3	0.21	3.08	0.08	2.00	0.113	<0.0001	<0.0001	<0.001
C22:5 n-3	0.57	0.71	0.36	0.60	0.048	<0.01	<0.01	0.33
C22:6 n-3	0.44	0.38	0.39	0.26	0.039	0.038	0.025	0.40
PUFA n-3	2.72	17.0	1.99	12.6	0.394	<0.0001	<0.0001	<0.001
LC n-3	1.25	4.21	0.89	2.99	0.150	<0.0001	<0.0001	<0.05
C16:1/ C16:0	0.030	0.027	0.020	0.022	0.002	<0.01	0.72	0.36
C18:1 n-9/ C18:0	2.17	1.66	1.62	1.29	0.096	<0.001	<0.001	0.38

LA = linoleic acid, ALA = α-linolenic acid, SFA = saturated fatty acid, MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid, LC = Long-chain (fatty acids with 20 or 22 C-atoms)

Table 6.4. Effect of LA and ALA on the fatty acid profile (% of total fatty acids) of backfat in growing pigs.

Fatty acids	Low LA		High LA		Pooled SEM	P-values of effects		
	Low ALA	High ALA	Low ALA	High ALA		LA	ALA	LA*ALA
C14:0	1.50	1.36	1.45	1.36	0.030	0.51	<0.01	0.40
C16:0	21.6	20.5	20.7	19.7	0.190	<0.001	<0.0001	0.84
C17:0	0.36	0.32	0.35	0.27	0.022	0.16	0.015	0.36
C18:0	8.63	9.57	8.76	8.80	0.272	0.26	0.09	0.11
SFA	32.7	32.3	31.9	30.7	0.389	<0.01	0.07	0.30
C16:1	3.00	2.29	2.44	2.42	0.121	0.09	<0.01	0.010
C17:1	0.33	0.24	0.27	0.20	0.025	0.06	<0.01	0.71
C18:1 n-7	2.99	2.44	2.41	2.51	0.082	<0.01	0.012	<0.001
C18:1 n-9	39.0	35.1	33.9	32.7	0.484	<0.0001	<0.0001	<0.01
C20:1 n-9	0.84	0.66	0.66	0.62	0.043	0.020	0.025	0.10
MUFA	46.2	40.7	39.7	38.5	0.632	<0.0001	<0.0001	<0.01
C18:2 n-6	16.7	15.8	23.7	20.4	0.468	<0.0001	<0.001	0.028
C20:4 n-6	0.28	0.20	0.33	0.22	0.011	<0.01	<0.0001	0.16
C22:4 n-6	0.08	0.05	0.10	0.06	0.003	<0.01	<0.0001	0.18
PUFA n-6	17.3	16.1	24.2	20.8	0.479	<0.0001	<0.001	0.028
LC n-6	0.48	0.35	0.56	0.39	0.016	<0.01	<0.0001	0.26
C18:3 n-3	1.59	7.98	1.65	6.50	0.173	<0.001	<0.0001	<0.001
C20:3 n-3	0.24	0.88	0.20	0.69	0.031	<0.01	<0.0001	0.018
C22:5 n-3	0.15	0.23	0.11	0.20	0.010	<0.01	<0.0001	0.68
C22:6 n-3	0.10	0.10	0.09	0.09	0.006	0.025	0.65	0.86
PUFA n-3	2.08	9.32	2.06	7.56	0.198	<0.001	<0.0001	<0.001
LC n-3	0.49	1.34	0.40	1.07	0.035	<0.0001	<0.0001	0.015
C16:1/ C16:0	0.14	0.11	0.12	0.12	0.006	0.41	0.07	0.011
C18:1 n-9/ C18:0	4.54	3.71	3.92	3.76	0.141	0.06	<0.01	0.026

LA = linoleic acid, ALA = α-linolenic acid, SFA = saturated fatty acid, MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid, LC = Long-chain (fatty acids with 20 or 22 C-atoms)

Table 6.5. Effect of LA and ALA on the fatty acid profile (% of total fatty acids) of intramuscular fat in growing pigs.

Fatty acids	Low LA		High LA		Pooled SEM	P-values of effects		
	Low ALA	High ALA	Low ALA	High ALA		LA	ALA	LA*ALA
C14:0	1.20	0.92	0.98	0.95	0.065	0.17	0.031	0.07
C16:0	21.2	19.0	19.4	18.3	0.304	<0.001	<0.0001	0.12
C17:0	0.36	0.31	0.35	0.29	0.017	0.52	<0.01	0.74
C18:0	9.38	10.0	9.86	10.0	0.270	0.38	0.15	0.39
SFA	32.6	30.6	31.0	29.9	0.401	0.023	<0.01	0.39
C16:1	2.72	1.93	2.07	1.83	0.123	<0.01	<0.001	0.037
C17:1	0.39	0.16	0.27	0.33	0.070	0.78	0.23	0.047
C18:1 n-7	3.02	2.57	2.65	2.42	0.068	<0.01	<0.0001	0.13
C18:1 n-9	34.3	27.1	27.0	25.5	1.117	<0.001	<0.01	0.045
C20:1 n-9	0.52	0.38	0.41	0.36	0.017	<0.001	<0.0001	0.046
MUFA	41.0	32.2	32.4	30.4	1.279	<0.001	<0.0001	0.015
C18:2 n-6	17.3	18.5	24.2	23.5	0.642	<0.0001	0.71	0.17
C20:4 n-6	2.50	3.07	4.11	2.96	0.400	0.08	0.47	0.045
C22:4 n-6	0.35	0.28	0.58	0.31	0.047	0.015	<0.01	0.046
PUFA n-6	20.6	22.3	29.4	27.2	1.025	<0.0001	0.81	0.07
LC n-6	3.18	3.72	5.11	3.62	0.474	0.07	0.33	0.045
C18:3 n-3	1.21	6.73	1.09	5.73	0.178	<0.01	<0.0001	0.023
C20:3 n-3	0.16	0.68	0.15	0.59	0.017	<0.01	<0.0001	0.037
C20:5 n-3	0.19	1.13	0.18	0.66	0.062	<0.01	<0.0001	<0.01
C22:5 n-3	0.51	1.08	0.61	0.91	0.081	0.62	<0.0001	0.11
C22:6 n-3	0.33	0.44	0.35	0.38	0.045	0.70	0.12	0.37
PUFA n-3[1]	2.41	10.1	2.38	8.27	0.022	<0.001	<0.0001	<0.01
LC n-3	1.19	3.33	1.29	2.54	0.163	0.047	<0.0001	0.014
C16:1/ C16:0	0.13	0.10	0.11	0.10	0.006	0.06	0.010	0.10
C18:1n-9/ C18:0	3.71	2.71	2.75	2.59	0.165	<0.01	<0.01	0.020

LA = linoleic acid, ALA = α-linolenic acid, MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid, LC = Long-chain (fatty acids with 20 or 22 C-atoms)

[1]: P-value and SEM are obtained from Y=log(1+x) function

Discussion

In designing this study, we chose to increase LA and ALA intake by addition, rather than exchanging them with other nutrients such as non-essential fatty acids. There is evidence that fatty acids like myristic acid and oleic acid do affect desaturase activity (Jan *et al.*, 2008; Portolesi *et al.*, 2008) and therefore may influence the conversion of n-3 and n-6 fatty acids. Furthermore, the difference in LA intake between the low and high LA treatments was identical to the difference in ALA intake between low- and high-ALA treatments (1.16 $g/kg^{0.75}/d$). This design allows direct comparison of the effect of precursor intake and antagonistic effects of LA and ALA, at intake levels above requirements (NRC, 1998; Schellingerhout, 2002). As a consequence, treatments were not iso-energetic. As the extra energy intake was less than 5%, this would, according to Madsen *et al.* (1992), not have a measurable impact on intramuscular fat content. An increase of ALA intake significantly increased its concentration in blood, muscle and backfat. This was expected from earlier studies in growing and fattening pigs (Roman *et al.*, 1995; Schellingerhout, 2002; Kouba *et al.*, 2003). It can be expected that the efficiency of ALA deposition in pigs is higher than 50% (Mitchaothai, 2007). In addition to the deposition, some of the ALA is used for the ß-oxidation and some is converted into n-3 LC-PUFA. Predictive equations have been developed for the relation between dietary ALA and backfat or intramuscular ALA concentrations by Hertzman *et al.* (1988) and Nguyen *et al.* (2003). Predicted ALA concentrations for our study in backfat and muscle by the formula of Hertzman *et al.* (1988), were 8 and 4%, respectively, and 6 and 4% when predicted by formulas developed by Nguyen *et al.* (2003). These values are in line with our results of approximately 7 and 6% ALA in backfat and intramuscular fat, respectively. EFSA (2010) proposed a minimum requirement for EPA plus DHA in order to prevent cardiovascular diseases. The only essential n-3 fatty acid in the diets in this study is ALA. Therefore all n-3 LC-PUFA found were produced by the pigs. The n-6 and n-3 LC-PUFA are synthesized from LA and ALA by sequential desaturation and elongation steps. Total n-3 LC-PUFA was increased in backfat and intramuscular fat by dietary ALA. This was a result of an elevated concentration of C20:3 n-3, EPA and DPA, but not of DHA. The total n-3 LC-PUFA was increased from 0.5 to 1.3% in backfat and from 1.2 to 3.3% in intramuscular fat. The increase in backfat was due to an increased C20:3 n-3 and DPA. This indicates a low efficiency of EPA and DHA synthesis in backfat via dietary ALA. Some have shown small effects (Raes *et al*., 2004; Duran-Montgé *et al.*, 2008), but others have found an increase in EPA (Guillevic *et al.*, 2009; Juarez *et al.*, 2010) and DHA (Enser *et al.*, 2003) with dietary ALA supplementation. The increase in EPA concentration in intramuscular fat is about 50% of the increase in total n-3 LC-PUFA. Increasing ALA intake increased muscle fat EPA concentration from 0.19 into 1.13%. Approximately 16 g ALA per kg diet was needed for this 1% increase. Some studies were carried out to determine the effect of dietary EPA on intramuscular EPA (Duran-Montgé *et al.*, 2008; Hallenstvedt *et al.*, 2010). From their results it can be calculated that an EPA increase from 0.1 to 1.1% in intramuscular fat can be achieved by 3 to 5 g of EPA per kg of feed. It would imply that the potential to increase EPA in muscle via dietary ALA is 25% of that of dietary EPA. Apart from this increase in EPA, dietary ALA increases the concentration of intramuscular ALA, C20:3 n-3 and DPA. The concentration of DHA in meat was not

affected by dietary ALA. Dietary LA did also not affect DHA concentrations of muscle fat. Dietary LA did, however, decrease the DHA concentration in backfat, but the effect was rather small. Recommended intakes of LC-PUFA, generally only specify EPA and DHA (e.g EFSA, 2010). However, studies indicated that DPA has an important role in preventing platelet aggregation and is even more potent then EPA (Kaur *et al.*, 2011). DPA can also being metabolized into EPA (Holub *et al.*, 2011; Kaur *et al.*, 2011). The concentration of DPA in both backfat and muscle of pigs fed the high ALA diets were doubled. This enrichment of DPA in backfat is interesting. The latter is a large pool of fat and the enrichment of ALA derived EPA and DHA are lacking. The beneficial effect of EPA and DHA in humans is based on studies using fish oil. The major n-3 LC-PUFA in fish oil are EPA and DHA, while the concentration of DPA is generally much lower.

Interactions between dietary ALA and LA intake on the fatty acid profiles studied were significant for many of the LC-PUFA, indicating antagonism between n-3 and n-6 pathways. This antagonism was most evident in FA profiles of plasma, but was also reflected in FA profiles in muscle and backfat. The effect of ALA on intramuscular concentration of EPA was lower at a high LA intake than at a low LA intake.

A high LA intake resulted in a lower intramuscular EPA level, particularly at the high ALA diet. This is a relevant hampering production of EPA at a high dietary LA. Dietary ALA diminished the concentration of ARA in plasma to a much higher extent than the increasing effect by dietary LA. A high affinity of desaturation enzymes for ALA instead of LA might be responsible (Rodriguez *et al.*, 1998). The gene expression of desaturase in the liver was increased in the pigs fed the high LA diets (Smink *et al.*, 2012). This might indicate a limitation of the production of ARA. ARA concentrations in muscle fat were not affected by dietary LA nor by ALA intake. The absence of the effect on muscle ARA by dietary ALA and LA agrees well with other studies in fattening pigs (Duran-Montgé *et al*., 2008). However, in both backfat and muscle, the highest ARA concentration was found in the high LA /low ALA group.

The intake of SFA, MUFA and carbohydrates from the diet were similar among treatments. Both supplementation of LA and ALA decreased the concentration of MUFA and SFA. High LA and high ALA pigs had decreased ratios of C18:1/C18:0 in blood and muscle indicating that LA and ALA decreased the desaturation of SFA into MUFA. It can be expected that a suppression of Δ9 desaturase is responsible for the relative strong effect on MUFA (Kouba *et al.*, 2003). A decrease of *de novo* synthesis of MUFA by dietary LA or ALA was also found in pigs by Mitchaothai (2007) and in broiler chickens by Smink *et al.* (2010).

In conclusion, it is possible to increase n-3 LC-PUFA in pork by feeding its dietary precursors. Effects on DHA are limited, but a relevant increase of EPA in intramuscular fat is possible. In addition, an increase of DPA is possible in both muscle and backfat. High ALA diets in pigs for pork EPA enrichment should be low in LA. The inhibitory effect of LA on n-3 LC-PUFA confirms our hypothesis. The concentration of ARA in tissues is affected by the diet. Compared at an identical incremental dose, the inhibition of ARA synthesis by ALA is stronger than its stimulation by LA as precursor.

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Chapter 7

General discussion

7. General discussion

Fats in diets for animals kept for meat production are important as an energy source, but also provide the precursors for long-chain polyunsaturated fatty acids (LC-PUFA) synthesis. Dietary fatty acids can strongly influence the fatty acid profile of the end-product, thereby potentially contributing to the provision of essential fatty acids to the consumer. This discussion focuses on the digestion process, integrating results of the experiments described in Chapter 2 to 6 with some new, unpublished work performed in veal calves. A new approach is proposed to predict the digestibility of fat sources in animal nutrition, taking the effect of positional distribution of fatty acids on the glycerol molecule into account. Secondly, in this general discussion some results regarding the correlation of LC-PUFA and behaviour of pigs are summarized. The third part of the discussion focuses on the potential contribution of essential fatty acids in animal diets, influencing the fatty acid profile in meat products, to meeting the recommended dietary intake in humans. The focus is here on the contribution of feeding of precursors (i.e. linoleic acid, LA and α-linolenic acid, ALA), as LA and ALA are available in common feed ingredients. The potential contribution of enrichment of meat directly by provision of LC-PUFA in the diets is thereby disregarded. The latter has been demonstrated to have potential (Nguyen *et al.,* 2003; Raes *et al.,* 2004) but the sensibility of doing so depends on the efficiency of incorporating dietary LC-PUFA into meat compared with the efficiency of producing LC-PUFA sources for human consumption directly.

Towards an improved prediction of digestibility of fat sources from chemical properties

Chain length and saturation of fatty acids

It is widely accepted that the digestibility of dietary fatty acids decreases with a higher degree of saturation. The ratio of unsaturated:saturated fatty acids was used as a proxy in the models developed by Ketels (1994) and Wiseman *et al.* (1998) for broiler chickens and pigs. The effect of chain length on fatty acid digestibility is less clear. Although the digestibility of medium-chain fatty acids (≤C12) clearly exceeds that of the longer chain fatty acids, the difference between C16:0 and C18:0 is subject to debate. While the digestibility of C16:0 was demonstrated to exceed that of C18:0 in broilers (Kussaibati *et al.*, 1982; Ketels, 1994; Dänicke *et al.*, 2000) and pigs (Jørgenson *et al.*, 2000), biohydrogenation of C16:1 and C18:1 by hindgut microbiota, likely causes an underestimation of the apparent total tract digestibility of C16:0 and C18:0, respectively. The higher content of C18:1 compared with C16:1 in most dietary sources generally leads to a larger underestimation of C18:0 digestibility compared with that of C16:0. In calculation models by Wiseman *et al.* (1998) and CVB (2011), differences in C16:0 and C18:0 digestibility are ignored and these fatty acids are combined.

Position of fatty acids on the glycerol molecule

Information about the effect of the position of fatty acids on the glycerol molecule on their digestibility in animals for meat production is scarce. In rats, long-chain saturated fatty acids, esterified at the sn-2 position are digested to a higher extent in comparison to those fatty acids esterified at the sn-1,3 position (Brink *et al.*, 1995; Renaud *et al.*, 1995). The study with broiler chickens (Chapter 2) confirmed these differences. In addition, a study with veal calves was carried out with feeding non randomized and randomized palm oil (Text box 7.1). Palmitic acid in palm oil is predominantly situated at the sn-1,3 positions and a relatively low amount is situated at the sn-2 position (Table 1.2 of Chapter 1). Randomization of palm oil leads to equal distribution of fatty acids among the three positions of the glycerol molecule. Randomizing palm oil was expected to increase the digestibility as was seen in rats (Renaud *et al.*, 1995). In the veal calf study, the digestibilities of total fat and that of C16:0 were at a similar level.

From the measured digestibility of C16:0 in the native and randomized palm oil group, the digestibility of C16:0 esterified at the sn-1,3 and sn-2 positions were calculated as presented in Chapter 2. In analog, digestibilities of C18:0 and C18:1 situated at the sn-2 or sn-1,3 position of the glycerol molecule were calculated. The results, as well as data for human infants (Filer *et al.*, 1969), rats (Mattson *et al.*, 1979; Brink *et al.*, 1995) and broilers (Smink *et al.,* 2008; Chapter 2) are presented in Table 7.1.

Text box 7.1 Experiment determining fatty acid digestion in veal calves. (Smink *et al*., unpublished)[1]

Twenty eight individually housed (Klaremelk BV, Speuld, The Netherlands) veal calves exclusively fed milk replacer, were assigned to one of two dietary treatments, comprising of either randomized or native palm oil, included at 13% in the milk replacer dry matter (DM). The dietary fat level was 20 % in the DM and the composition of the fat was based on palm oil (randomized and not randomized), coconut oil, lecithin and a synthetic emulsifier. The basal diet was also based on whey (42%), 25% skim milk powder, soybean concentrate, wheat gluten and starch. The content of protein, ash and fat was 19.1, 6.0 and 20.5%, respectively. The calves on a diet with native and the randomized palm oil showed a daily growth of 955 and 980 g, respectively in a weight range between 45 and 70 kg. A digestibility trial was carried out at a body weight of approximately 60 kg. The faeces were collected during 4 days and after collection frozen at -18°C until DM, fat and fatty acid analysis. The digestibility of fat in both groups of calves was very high; 96.4 and 96.9% for the diets with native and randomized palm oil, respectively.

[1:] internal report

Table 7.1. Calculated apparent total tract digestibility of the long-chain saturated fatty acids C16:0, C18:0 and of oleic acid (C18:1) at the sn-1,3 and sn-2 position of the glycerol molecule of the triglyceride.

Species	Fatty acid	Digestibility (%)		Reference
		Sn-1,3 position	Sn-2 position	
Infant (human)	C16:0	46	82	Filer *et al.*, 1969
	C18:0	-19	136	
	C18:1	101	69	
Rat	C18:0	46	98	Mattson *et al.*, 1979
	C18:1	97	97	
Rat	C18:0	46	115	Brink *et al.*, 1995
	C18:1	94	96	
Broiler chicken	C16:0	51	90	Smink *et al.*, 2008
	C18:0	37	84	
	C18:1	79	87	
Veal calf	C16:0	92	100	Smink *et al.*, unpublished text box 7.1
	C18:1	98	103	

From Table 7.1 it appears that the calculated digestibility for C16:0 and C18:0 are in all cases clearly higher when these fatty acids are esterified at the sn-2 position in comparison to esterification at the sn-1,3 positions. The effect of positional distribution on C18:1 digestibility, however, is rather small. Triglycerides are enzymatically hydrolyzed in the intestine and this results in a mix of sn-2 monoglycerides and free fatty acids (FFA) which are released from the sn-1,3 positions. Monoglycerides have a hydrophilic character while long-chain saturated fatty acids are hydrophobic and are therefore less available for absorption in comparison to the monoglycerides. Monoglycerides with C18:1 at the sn-2 position might be slightly better absorbed than when the C18:1 comes from the sn-1,3 positions. Overall, there are large differences in the digestibility of saturated fatty acids between the sn-2 and sn-1,3 position in triglycerides but the differences for unsaturated fatty acids are small. This implies that fat sources with saturated fatty acids at the sn-2 position are likely to be better digested compared with fat sources in which these saturated fatty acids are esterified at sn-1 or sn-3. Current calculation models (Wiseman *et al.*, 1998; CVB, 2011) do not take this effect into account.

Free fatty acids

Several studies have shown that in pigs and poultry, dietary FFA have a lower digestibility compared with the same fatty acids ingested as triglycerides. From studies in broilers (Wiseman and Salvador, 1991; Vila and Esteve-Garcia, 1996) and pigs (Powles *et al.*, 1993; 1994), it appears that the effect of FFA on digestibility strongest for saturated fatty acids. Based on these studies it is justified to separate the digestibility of various FFA according to type of FFA. It should be emphasized that the results in Table 7.1 indicate also that the digestibility of FFA from the lumen of the intestine will be much lower for the long-chain

saturated fatty acids compared to the unsaturated fatty acids. After hydrolysis of FFA from the sn-1 and -3 position of glycerol, two free fatty acids plus one monoglyceride will become available. These FFA can be expected to act in a similar way as the FFA in a mixture of blended fatty acids. When lipase activity in the intestinal lumen is not rate limiting, the digestibility of FFA and fatty acids at the sn-1,3 position are likely similar. Although the validity of this assumption remains to be investigated, there are studies confirming its credibility. Lipase supplementation, for example, did not result in a systematic improvement of the fat digestibility in 3 weeks old broilers (Meng *et al.*, 2004; Brenes *et al.*, 2008). The calculation model of Wiseman *et al.* (1998) includes an effect of FFA, but does not discriminate between different types of FFA.

Proposed model to predict fat digestion

Based on the discussion above and data presented in this thesis, a new model was developed taking into account:

1. The difference in digestion between unsaturated and saturated fatty acids.
2. The effect of chain length to account for the relevant differences in digestion between C16:0 and C18:0.
3. The influence of positional distribution of fatty acids at the glycerol molecule.
4. The influence of the proportion of free fatty acids and the effect of the composition of free fatty acids.

To this end, the digestibility of C16:0, C18:0 and unsaturated (UNSAT) fatty acids, each of them esterified at the sn-2 or at the sn-1,3 position or present as FFA was estimated, the results of which are presented in Table 7.2. The digestibility of monoglycerides is very high and there is no indication that this digestibility differs between the fatty acids in the monoglyceride. Based on Table 7.1 and studies presented in broilers (e.g. Wiseman and Salvador, 1991; Ketels, 1994) and pigs (e.g. Powles *et al.*, 1993; Jørgenson *et al.*, 2000), the digestibility coefficient of monoglycerides for broilers and pigs was estimated to be 97 and 98%, respectively. The digestibility of individual fatty acids at the sn-1,3 position or as FFA is affected by chain length and degree of saturation. The lowest digestibility is observed for C18:0, followed by C16:0 and the highest digestibility for unsaturated fatty acids. The latter includes then also fatty acids shorter than C14:0. The difference in digestion of fatty acids at the sn-1,3 and sn-2 position was taken from the studies summarized in Table 7.1. The differences between C16:0, C18:0 and unsaturated fatty acids in broilers was mainly based on results of the study reported in Chapter 2 of this thesis and studies with broilers reported in the literature (Kussaibati *et al.*, 1982; Vila and Esteve-Garcia, 1996; Dänicke *et al.*, 1999; 2000; Smits *et al.*, 2000). The digestibility values of C16:0, C18:0 and of unsaturated FFA were based on results by Renner and Hill, (1961), Young (1961) and Wiseman and Salvador (1991). The digestibility of fat in pigs is less affected by dietary factors in comparison with broilers. The estimation of the values for pigs in Table 7.2 are mainly based on results as presented in Table 7.1 and on ileal digestibility studies in pigs by Jørgenson *et al.* (2000) and of Duran-Montgé *et al.* (2007). The values for FFA are mainly based on the studies by Powles *et al.* (1993; 1994).

Unlike in broilers, increased fat intake in pigs will not reduce its digestibility (Powles *et al.*, 1994). It may even increase its apparent digestibility (Jørgenson and Fernandez, 2000) due to reduced contribution of endogenous fat to faecal or ileal fat excretion. In broiler chickens, however an increased dietary concentration of long-chain saturated fat in broiler chickens decreases its digestibility (Ketels, 1994). Therefore, the digestibility coefficients for broiler chickens presented in Table 7.2 were chosen to represent digestibility coefficients in diets with approximately 6% of added fat.

Table 7.2. Apparent total tract digestibility (%) of individual fatty acids in growing/fattening pigs and in 4 weeks old broiler chickens. Digestibility figures are given for FFA and at different positions on the glycerol molecule.

Fatty acid	Broilers		Growing/fattening pigs	
	Sn-2 position[2]	Sn-1,3 position or FFA	Sn-2 position[2]	Sn-1,3 position or FFA
C16:0	97	60	98	80
C18:0	97	45	98	70
Unsaturated[1]	97	91	98	93

[1]: Includes C8-C12
[2]: Including the glycerol molecule

Based on the chemical composition of fats (Table 1.2, Chapter 1) and on the digestibility coefficients for fatty acids as presented in Table 7.2, the digestibility coefficients of total fats can be calculated for broiler chickens (equation 1) and pigs (equation 2).

Equation 1. DC FAT (%) broiler chickens =

(97 ((C16:0 sn-2 + C18:0 sn-2 + UNSAT sn-2) x 1.15) + C16:0 sn-1,3 x 60
+ C18:0 sn-1,3 x 45 + UNSAT sn-1,3 x 91) / ((C16:0 + C18:0 + UNSAT) x 1.05)

Equation 2. DC FAT (%) pigs =

(98 ((C16:0 sn-2 + C18:0 sn-2 + UNSAT sn-2) x 1.15) + C16:0 sn-1,3 x 80
+ C18:0 sn-1,3 x 70 + UNSAT sn-1,3 x 93) / ((C16:0 + C18:0 + UNSAT) x 1.05)

Where:
-DC FAT = apparent total tract digestibility coefficient of fat in %
-C16:0, C18:0 and UNSAT are presented in g/kg
-1.05 and 1.15 are a correction for the content of glycerol in the triglycerides and monoglycerides (fatty acid at the sn-2 position of the glycerol molecule), respectively. The contribution of glycerol to the molecular weight in a triglyceride is about 5% and 15% in a monoglyceride.

Equation 1 means for example, that a complete randomised triglyceride which contains 5% glycerol and 31.7% C16:0 at each position of the glycerol molecule has a digestibility coefficient of 97 (317 x 1.15) + (60 x 634) / (951 x 1.05) = 73.5%.

The differentiation between the kind of free fatty acids and the interaction with the position of fatty acids can be illustrated in two figures (Figure 7.1 and 7.2). The negative effect of FFA on the digestibility is lower for unsaturated fatty acid sources like soybean oil in comparison with saturated fatty acid sources like tallow (Figure 7.1). The same digestibility for fatty acids at the sn-1,3 position of the glycerol molecule and as FFA is assumed. The digestibility of fatty acids at the sn-1,3 position of the triglycerides or as FFA is lower than those fatty acids at the sn-2 position of triglycerides. The exchange of fatty acids at the sn-2 position into FFA will have an impact on the digestibility, while an exchange of fatty acids from the sn-1,3 position into FFA will not have an effect. The fatty acid C16:0 in lard is predominantly situated at the sn-2 position, while those in palm oil are mainly at the sn-1,3 position. This means that an exchange of C16:0 from triglyceride into FFA will have a strong negative effect on the digestibility (Figure 7.2)

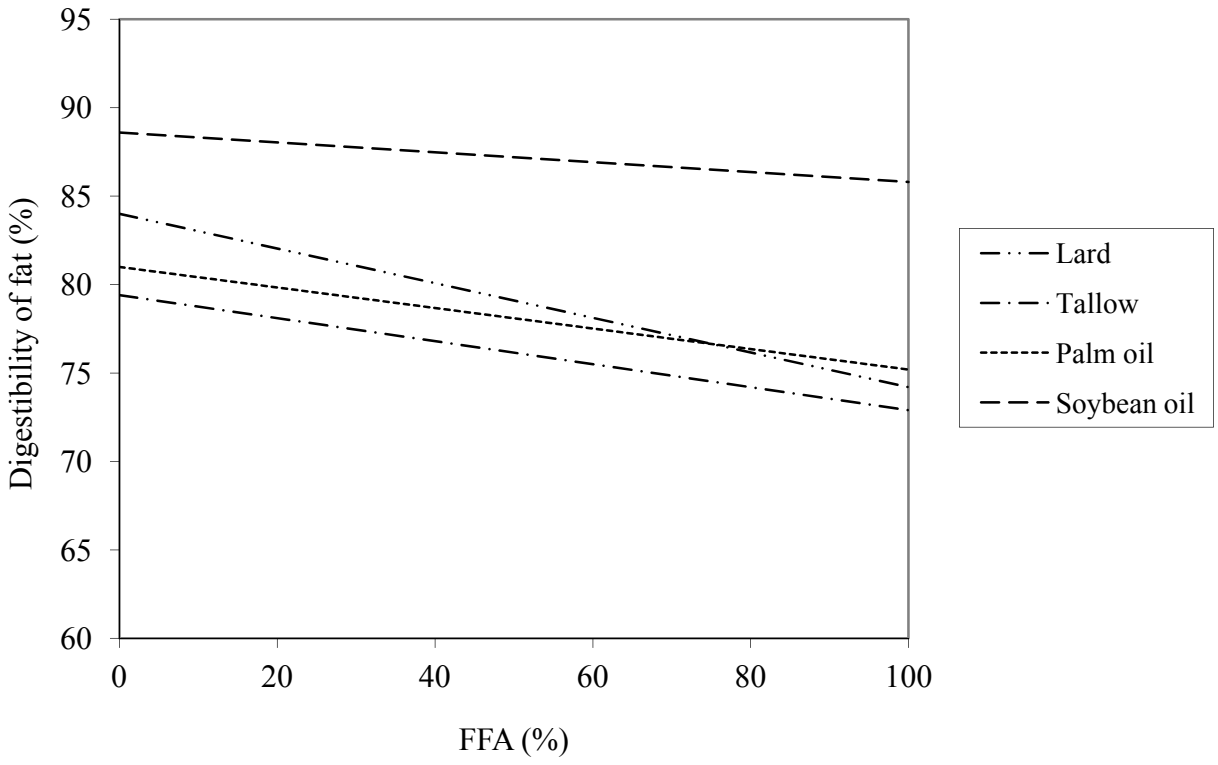


Figure 7.1 Predicted fatty acid digestibility of C16:0, C18:0 and unsaturated fatty acids in lard and palm oil as affected by the proportion present as free fatty acid (FFA, expressed as a % of total extractable fat) in broiler chickens.

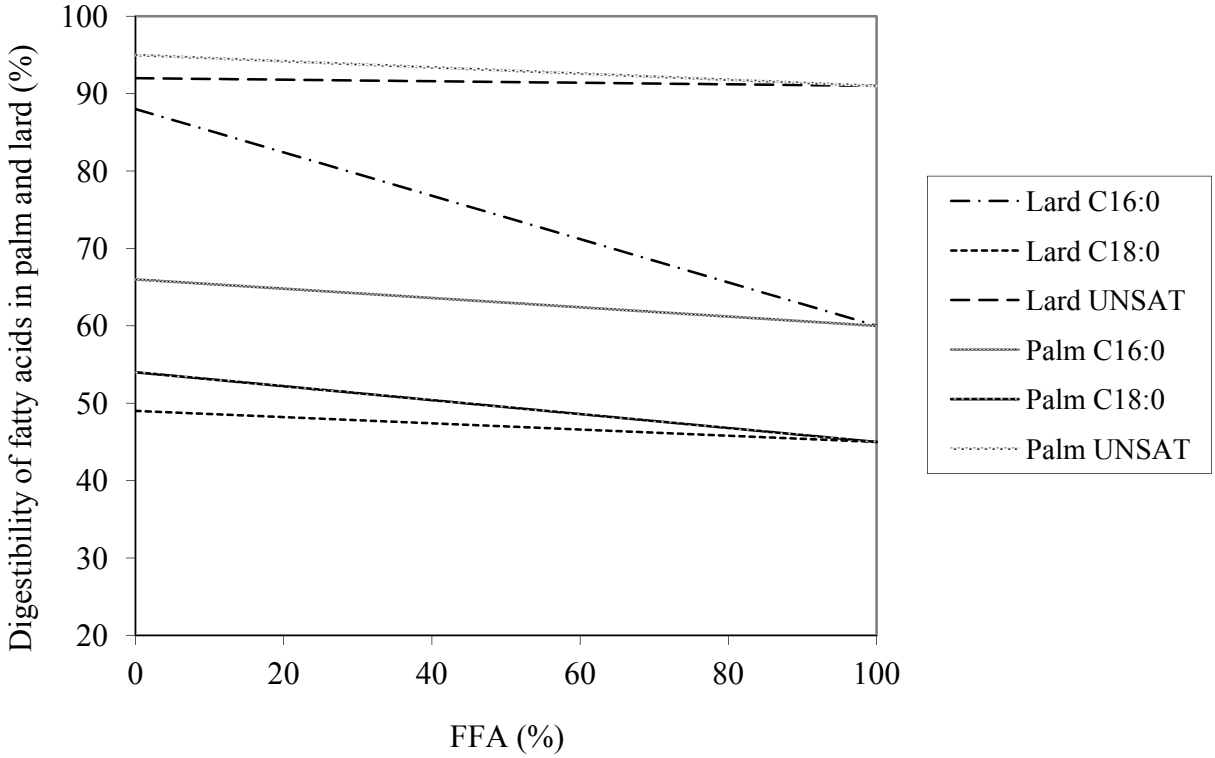


Figure 7.2 Predicted fatty acid digestibility of C16:0, C18:0 and unsaturated fatty acids (UNSAT) in lard and palm oil as affected by the proportion present as free fatty acid (FFA, expressed as a % of total extractable fat) in broiler chickens.

The results of some fats are presented and compared with the coefficients reported by others (Ketels, 1994; Wiseman and Salvador, 1991; Wiseman *et al.*, 1998; CVB, 2011).

Table 7.3 Apparent faecal digestibility of various fats in broiler chickens calculated using a new model and the values derived from digestibility studies or models presented in the literature.

Fat source	CVB, 2011 Model[1]	Ketels, 1994 Measured[1]	Wiseman and Salvador, 1991 Measured[2]	Wiseman *et al.*, 1998 Model[2]	***Values new model 2012***[1]
Soybean oil	90	91	94	96	***89***
Rapeseed oil	93	-	-	96	***92***
Palm oil	78	-	85	86	***81***
Lard	79	82	-	86	***84***
Tallow	79	76	81	86	***79***
Poultry fat	88	-	-	95	***88***
Coconut oil	93	91	-	-	***92***
Palm kernel oil	-	-	-	-	***92***
Soybean oil 70% FFA	-	-	90	86	***87***
Palm oil 70% FFA	-	-	74	75	*77*
Tallow 70% FFA	-	-	71	75	***75***

[1]: 4 weeks old broilers in studies with an added fat content of 5-6%

[2]: 7 weeks old broilers with 8% fat added fat

The difference of the new approach taken here is the additional inclusion of a deviation between 1) C16:0 and C18:0, 2) a differentiation in the kind of FFA and 3) the effect of fatty acids at the glycerol molecule. The latter aspect will lead to an increase in the accuracy of the prediction of the digestibility of fat sources with a high content of long-chain saturated fatty acids at the sn-2 position. The effect of FFA on the digestibility of soybean oil is low in comparison to saturated fat sources. This agrees with the measured values of Wiseman and Salvador (1991; see Table 7.3). The largest effect of FFA with the new model was seen in lard. This is because lard has a relatively beneficial position of fatty acids at the glycerol molecule. This benefit will disappear when the content of triglycerides decreases. Especially the large effect of C16:0 FFA in lard, as is illustrated in Figure 7.2, is responsible for the large difference between lard as triglyceride and lard as FFA. The new model proposes a higher digestibility for lard in comparison with tallow, while the fatty acid composition hardly differs. The higher digestibility of lard in comparison with tallow agrees well with measurements of earlier studies in broilers (Renner and Hill, 1961; Ketels, 1994).

Dietary α-linolenic acid and linoleic acid in relation to behaviour

The LC-PUFA, docosahexaenoic acid (DHA) is important for optimal visual and neural development. Changes in brain DHA concentrations have been demonstrated to be positively correlated with cognitive development (Innis, 2007). Although animals do not completely parallel humans, the timing of the development of the pig's brain growth is similar to that of humans. Dullemeijer *et al.* (2008) showed that feeding fish oil resulted in an increased DHA in the frontal cortex and whole brain of juvenile pigs. Consumption of LA-rich diets could also result in an inadequate conversion of ALA to DHA and reduced concentrations of DHA in the developing brain (Russo, 2009). The study described in Chapter 5, showed that DHA concentration in the frontal cortex was not significantly affected by dietary ALA. High LA tended to decrease the DHA concentration and decreased brain weight. The piglets in this trial were also used for a behaviour study. The results of that study have been published as an abstract by Bolhuis *et al.*, 2010 (Text box 7.2). As mentioned in Text box 7.2, high LA intake

Text box 7.2 Effects of linoleic and α-linolenic acid intake on pig behavior, and its relationship with brain DHA. J. E. Bolhuis, I. van Kerkhof, and W. J. J. Gerrits, *Wageningen Univerisity, Wageningen, the Netherlands.*

Effects of linoleic acid (LA) and α-linolenic acid (ALA) on behavior of individually housed pigs (15-30 kg BW) were studied. In a 2x2 factorial arrangement, 32 gilts from 4 litters were assigned to one of four dietary treatments, varying in LA and ALA intake. Differences between low and high intake levels were designed to be identical for LA and ALA: Low ALA and LA intakes were 0.15 and 1.30, and high ALA and LA intakes were 1.45 and 2.60 g/(kg $BW^{0.75}$.d), respectively. Intakes of saturated and mono-unsaturated fatty acids (FA), and other nutrients were kept constant. Pigs were subjected to an open field test (d 15) and a novel object test (d 16). In addition, behavior in the home pen was observed using 2-min instantaneous scan sampling for 5 h per day (d 12 and d 18). After 28 d on the dietary treatments, pigs were sacrified and brain tissues were sampled and analysed for FA composition. The latencies to approach and touch the novel object were reduced by ALA intake, but at the low LA intake only (LA*ALA, $P<0.05$). The low LA-high ALA combination also tended to reduce standing alert in the open field ($P=0.08$). The percentage of time spent nosing in the open field, and exploratory behaviours in the home pen were reduced by LA intake ($P<0.05$). Although dietary treatments did not greatly influence DHA concentrations in the hippocampus and frontal cortex ($P>0.05$), DHA concentrations in the frontal cortex were positively correlated with explorative behavior ($r = 0.56$, $P<0.001$). In conclusion, an increase in ALA intake, specifically at low LA intake levels, causes consistent changes in behavioural patterns, indicating reduced fear and increased exploration. It is unclear to what extent DHA concentrations in the brain are important for mediating these effects.

[1] Published as abstract in J. Anim. Sci. 87: E suppl. 2, p 565

reduced exploratory behavior of the piglets. It appears that DHA concentrations were positively correlated to explorative behaviour. In can be concluded that a potential beneficial effect of dietary ALA should be achieved with a low LA intake.

Potential contribution of α-linolenic acid in pig diets to dietary n-3 LC-PUFA requirements in humans

Enrichment of LC-PUFA in meat via nutrition of the animal can be achieved via 1) feeding LC-PUFA from fish oils or specific algae and 2) via provision of precursors (i.e. linoleic acid, LA and α-linolenic acid, ALA). The potential of the latter will be discussed here.

1) Deposition of ALA from dietary sources

In pigs as well as in broiler chickens, the vast majority of fatty acids deposited originate from *de novo* fatty acid synthesis, using glucose as the main precursor. In pigs, Kloareg *et al.* (2007) estimated that 86% of the non-essential fatty acids deposited originated from *de novo* fatty acid synthesis. Their diets contained 4.4% of fat. In chickens (Chapter 3), the ratio between fat deposition to digestible fat intake was used as a proxy for the minimal amount of *de novo* fatty acid synthesis and was about 25%. This agrees with results of Villaverde *et al.* (2006) in broiler chickens. These authors estimated that the *de novo* synthesis of fat decreases with increasing fat intake, but was still 25% when the dietary fat content was 10%. The efficiency of deposition of dietary LA and ALA in pigs and broilers are interdependent. As demonstrated in Chapter 3, 48-60% of the dietary digestible ALA was deposited in broiler chickens with the highest efficiency at the low LA diet. In pigs, Mitchaothai (2007) recovered in most cases between 40-80% of the dietary ALA in the body of growing pigs. Although from the data presented in Chapter 5 and 6 quantitative recoveries cannot be calculated, these data clearly show that the deposition of ALA in body tissues depends on the dietary LA concentration. There is a linear relation between the content of dietary ALA and the content of ALA in backfat of pigs (Nguyen *et al.*, 2003, Chapter 1). In addition, the content of dietary ALA is also clearly positively related to the ALA content in intramuscular fat (IMF). Results from Chapter 6, showed that dietary LA decreases the concentration of ALA in muscle. Figure 7.3 summarizes the data from this thesis and the available data in literature, illustrating the strong positive relationship between ALA intake and ALA concentration in muscle.

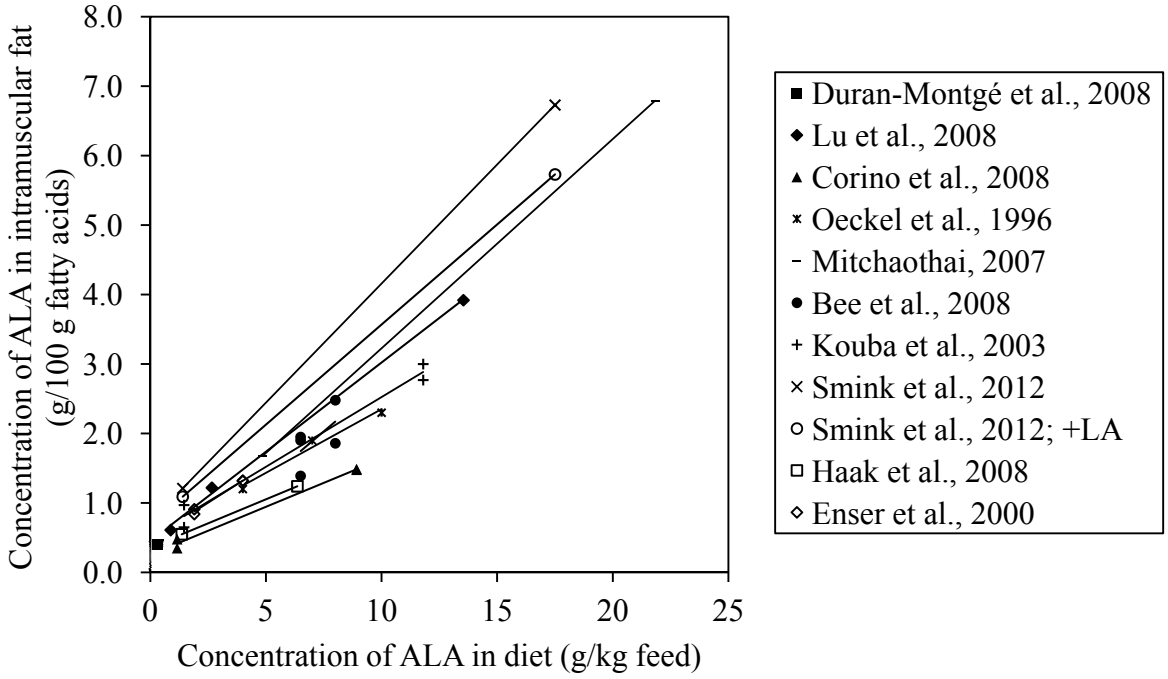


Figure 7.3. Effect of dietary α-linolenic acid (ALA) on the ALA content in intramuscular fat of pigs. LA = linoleic acid.

Most studies were carried out using two dietary dosage levels. Effects are presented as a linear effect. However, it appears that the effect of ALA is stronger at a high ALA intake, suggesting that a linear effect may not be appropriate but that a curvilinear regression would better fit the data.

2) Influencing deposition of n-3 LC-PUFA by varying the intake of precursors

Increasing the intake of ALA potentially increases the rate of deposition of n-3 LC-PUFA in the body. Results of various studies on the possibility to increase EPA in muscle by increasing ALA intake are summarized in Figure 7.4. An elevated intake of ALA in the study reported here did, however, increase the concentration of EPA in IMF and the magnitude of this increase was reduced by a high LA diet. A high intake of 15 g ALA per kg feed increases the content of EPA in IMF from 0.2 to approximately 0.8%. In the study described in Chapter 6, EPA was not detected in backfat. The concentration of DHA in both backfat and IMF was not affected by ALA intake. This is generally also the case in other studies (see Figure 7.5 and 7.6). There is enzyme competition within the same chain of elongation and desturation steps. The preference for enzymes in the first step after C18:3 n-3 is discussed in Chapter 5 and is probably the main reason for the lack of an effect of ALA on DHA in backfat and in IMF. Alternatively, docosapentaenoic acid (DPA; C22:5 n-3), an intermediate in the n-3 LC-PUFA chain (see Figure 1.4), can be produced. DPA can be present in considerable quantities in fish and meat, but much less is known about its biological effect. The DPA concentration in IMF can be increased by dietary ALA intake. In addition, unlike for EPA and DHA, DPA can be elevated in backfat after a high ALA intake in pigs (Figure 7.7 and 7.8).

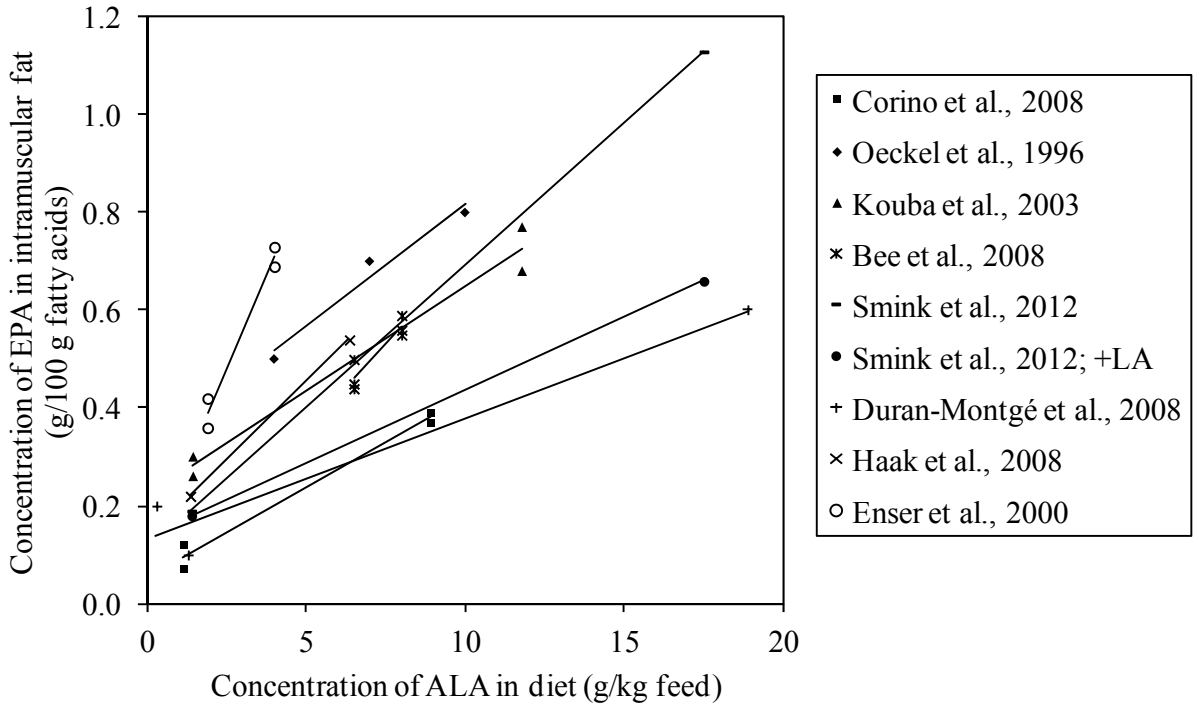


Figure 7.4 Effect of dietary α-linolenic (ALA) on eicosapentaenoic acid (EPA) in intramuscular fat of pigs. LA = linoleic acid

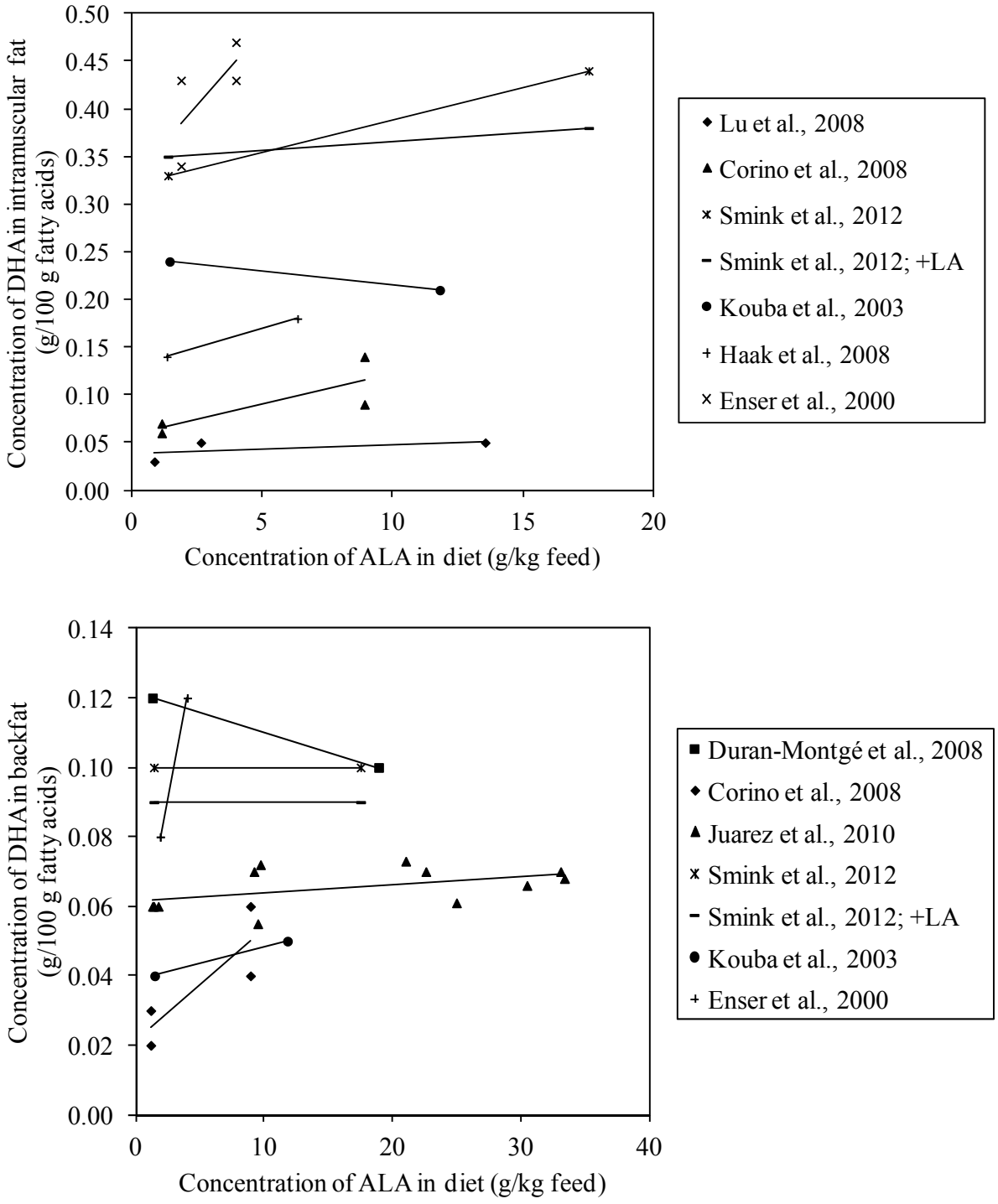


Figure 7.5 and 7.6. Effect of α-linolenic acid (ALA) intake on docosahexaenoic acid (DHA) in fat in as intramuscular fat in muscle and in backfat of pigs. LA = linoleic acid.

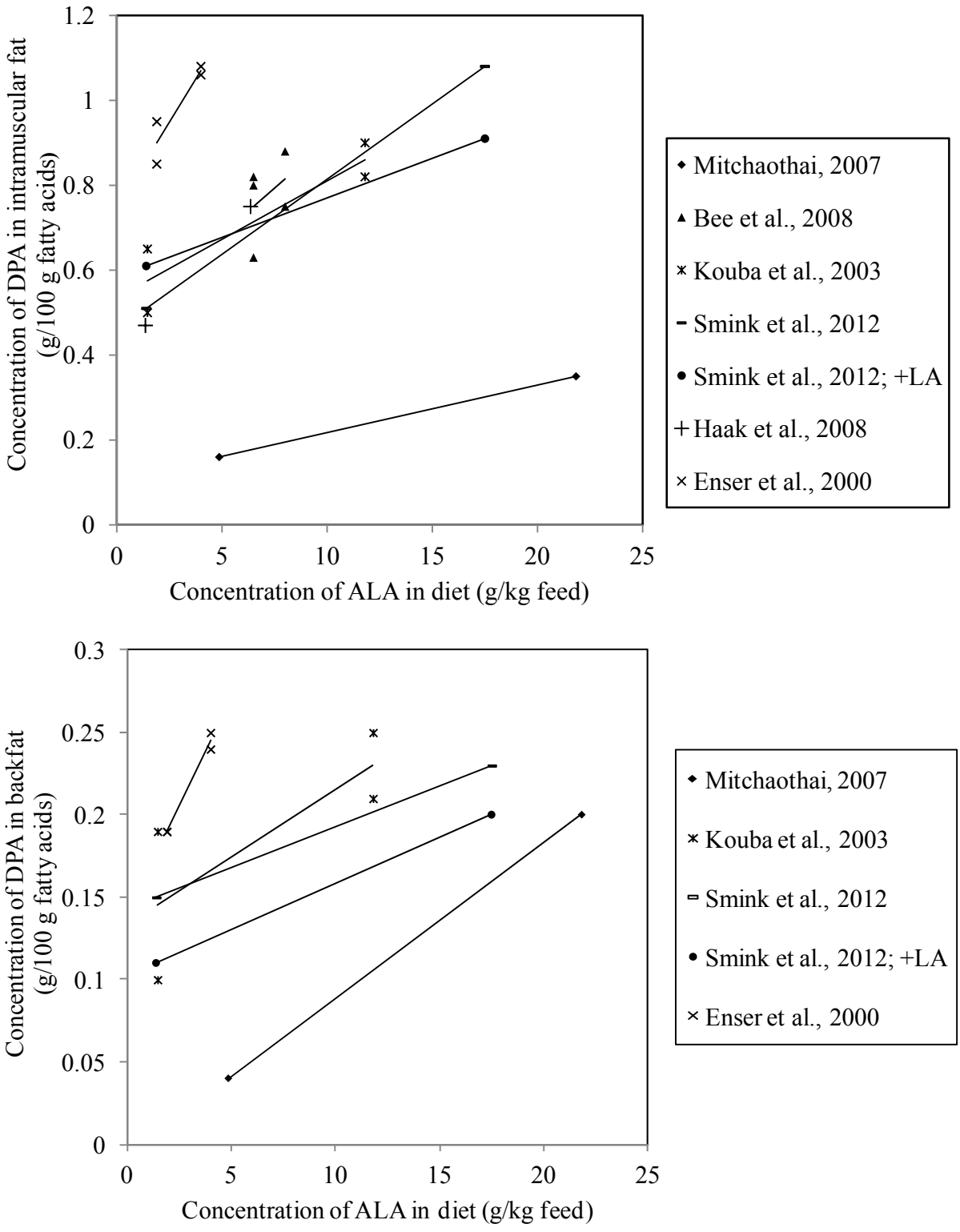


Figure 7.7 and 7.8. Effect of α-linolenic acid (ALA) intake on docosapentaenoic acid (DPA) in fat as intramuscular fat in muscle and in backfat in pigs. LA = linoleic acid.

3) Potential contribution of meat products to meet the dietary recommended intakes of ALA and LC-PUFA in humans

The recommended daily allowance (RDA) of ALA for humans is 0.5% of the daily energy intake (EFSA, 2010) which is comparable to an intake of approximately 1300 mg per day. EFSA assumes this level to be sufficient because this is a practical level that is at least achievable in every European country. The intake in the Netherlands is about 0.6% of the daily energy intake while the Dutch recommended ALA intake is 1% of the daily energy intake (www.voorlichtingmvo.nl). From Figure 7.3 it can be estimated that an intake of 15 g ALA per kg feed intake by pigs will result in a ALA concentration in intramuscular fat of about 5%. Lean meat consists of approximately 2% intramuscular fat (Chapter 4). This means that consumption of 100 g lean meat or 2 g IMF delivers 100 mg ALA if the pigs were fed with 15 g ALA per kg feed. The content of ALA in backfat of pigs fed 15 g ALA/kg is about 6%. Consumption of 10 g fat as backfat of those pigs delivers 600 mg ALA. A portion of 100 g meat with 2% IMF and 5% lard (composition assumed to be similar to backfat) contains then 400 mg ALA. A 30 g portion ingested via a sausage with one third of fat and two third lean meat consists of 620 mg ALA. Figure 7.9 illustrates that by manipulating dietary ALA content of pigs, the contribution of a normal consumption of 100 g of fresh pig meat to the RDA, can increase the RDA of ALA from 8 to 30%. For the consumption of a 30 g sausage, this contribution can be increased from 13 to 48% of the RDA.

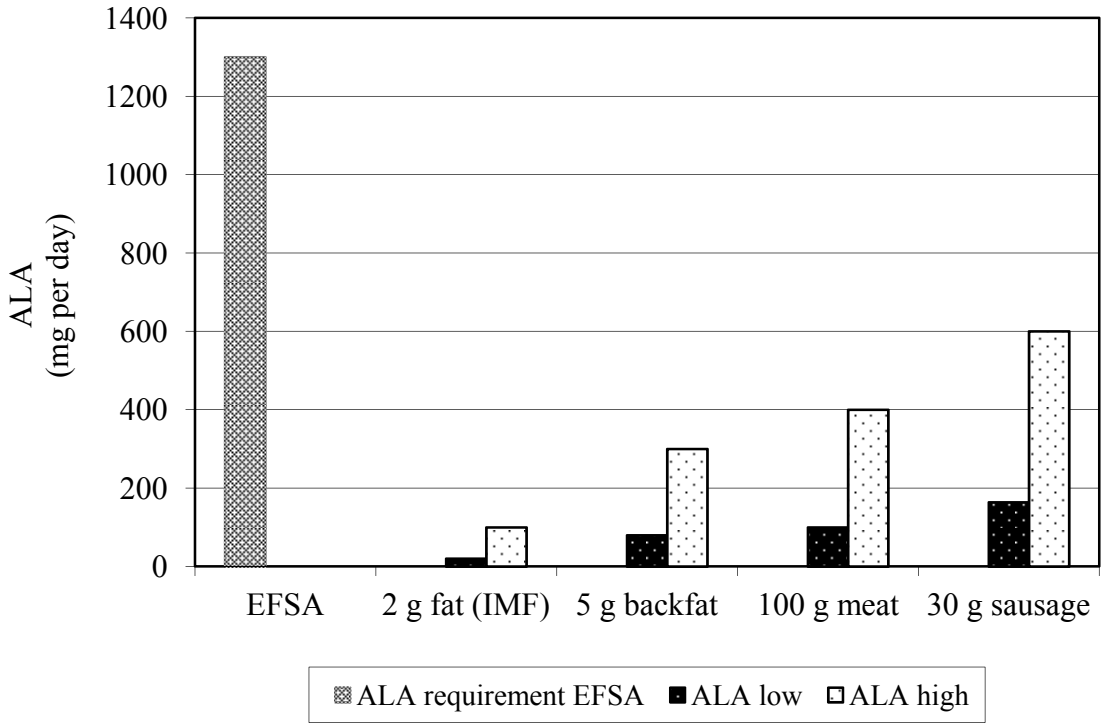
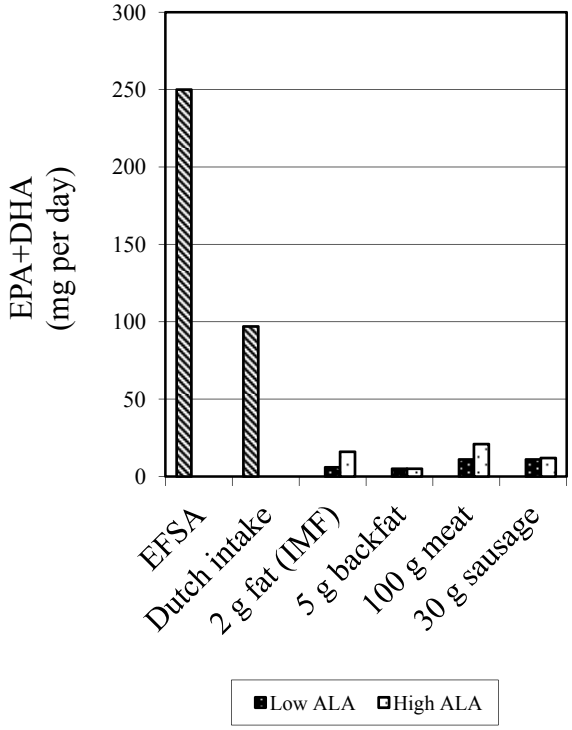


Figure 7.9. α-Linolenic acid (ALA) consumption in mg per day with 2 g fat as intramuscular fat (IMF) and 5 g fat as backfat of pigs fed with a low ALA and a high ALA diet. In this example it is assumed that 100 g meat contains 2 g IMF and 5 g fat as backfat and 30 g sausage contains approximately 10 g backfat, as documented in the text. In addition the recommended level of EFSA (2010) is presented.

The RDA of humans for EPA and DHA as set by EFSA (2010) is 250 mg EPA+DHA, based on considerations for a good cardiovascular health. Dutch RDA values for EPA and DHA (MVO; www.voorlichtingmvo.nl) are 450 mg EPA+DHA, while the average intake of adults in the Netherlands is less than 100 mg/d. As dietary intakes of LA or ALA did not significantly increase DHA concentrations in IMF and backfat (Figure 7.5 and 7.6), changes in IMF (Figure 7.4) by changing the precursor intake via the pig diet is a more successful route to influence DHA and EPA intake. Figure 7.10 illustrates that by manipulating dietary ALA sources, the contribution of the consumption of 100 g/d of fresh pig meat, the RDA can increase from 4 to 8% for the RDA of EPA+DHA. For the consumption of a 30 g sausage, the contribution is hardly increased yielding a 5% of the RDA of EPA+DHA.

An intermediate product, DPA, which is formed by elongation of EPA can be increased in backfat with a high intake of ALA. Some studies indicate that DPA is a biological active component and could give stronger or comparable effects as EPA on aggregation of platelets (Kaur *et al.*, 2011). In addition, DPA can be metabolized to EPA (Holub *et al.*, 2011; Kaur *et al.*, 2011). If DPA has similarly importance for cardiovascular health as EPA, a dietary intake of ALA is important. The intake of DPA with 10 g backfat is 90 mg and considerable higher than EPA+DHA. It appears that increasing the dietary concentration of ALA in diets for pigs potentially increases the contribution by consumption of 100 g of meat or one 30 g sausage to the RDA of ALA and EPA+DHA up to 48 and 8%, respectively. It should be noted that DHA concentrations in meat products are largely insensitive to dietary ALA intakes. The potential of meat products to supply DPA is considerable, but it remains to be investigated to what extent DPA can replace the dietary requirements for other n-3 LC-PUFA like EPA.



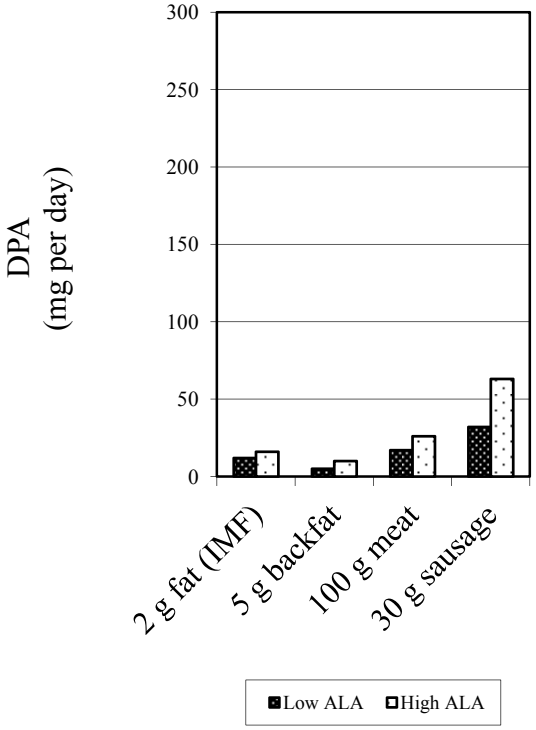


Figure 7.10 (left). Eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) consumption in mg per day with 2 g intramuscular fat (IMF) and 5 g fat as backfat of pigs fed with a low α-linolenic acid (ALA) and a high ALA diet. In this example it is assumed that 100 g meat contains 2 g IMF and 5 g fat as backfat and 30 g sausage contains approximately 10 g backfat. The EFSA (2010) recommendation and the average Dutch intake of EPA+DHA is also presented.

Figure 7.11 (right). Docosapentaenoic acid (DPA) consumption in mg per day with 2 g intramuscular fat (IMF) and 5 g fat as backfat of pigs fed with a low α-linolenic acid (ALA) and a high ALA diet. In this example it is assumed that 100 g meat contains 2 g IMF and 5 g fat as backfat and 30 g sausage contains approximately 10 g backfat.

It is clear that the potential of manipulation of EPA+DHA via dietary ALA is limited (Figure 7.10). However, the effect of dietary ALA on the DPA is considerable. If the biological function of DPA is at the same level as EPA as is suggested by Kaur *et al.* (2011), the contribution of pig meat derived from ALA fed pigs is considerable.

Conclusions

The main conclusions of the work presented in this thesis are:

Digestibility
For the determination of the digestibility of palm oil to replace animal fat, both the differences between C16:0 and C18:0 and the position of C16:0 on the glycerol molecule are relevant. Randomization of palm oil increases the digestibility of C16:0 in broiler chickens (Chapter 2).

Models predicting fat digestibility should take into account an estimation of the digestibility of C16:0, C18:0 and unsaturated fatty acids at the sn-2 and sn-1, or -3 positions of the glycerol molecule and their proportion present as free fatty acid (General discussion).

Fat deposition
Saturated fat sources increase body fat deposition in comparison with unsaturated fat sources in broilers. An increase in the rate of *de novo* synthesis of monounsaturated fatty acids and a reduced rate of fatty acid oxidation appears to be responsible for this effect (Chapter 3).

Backfat thickness of pigs is not affected by energy source, but the amount of intramuscular fat tends to be higher in pigs fed high starch diets compared with those fed diets with high amounts of saturated fat (Chapter 4).

Conversion of n-6 and n-3 fatty acids into LC-PUFA
Increasing the intake of LA increases mRNA expression of Δ6- and Δ5 desaturase and all n-6 PUFA in the liver of pigs. mRNA expression of elongase 2 was up-regulated by both high dietary LA and high ALA. Δ6 desaturase and elongase-2 activity are postulated to be rate-limiting in the conversion of ALA into DHA in pigs (Chapter 5).

Increasing the intake of LA inhibits the conversion of ALA into n-3 LC-PUFA. Increasing the intake of ALA inhibits the conversion of LA into n-6 LC-PUFA. When compared at equal incremental intake, the magnitude of the effect of ALA in liver is higher than that of LA (Chapter 5 and 6).

Docosahexaenoic acid (C22:6 n-3; DHA) and arachidonic acid (C20:4 n-6; ARA) are the predominant LC-PUFA in brain tissue. The effect of dietary LA and ALA on DHA and ARA are limited, but there is a correlation between brain DHA concentrations of pigs and their behaviour (Chapter 5 and General discussion).

Feeding high ALA diets to pigs increases ALA concentrations in backfat and intramuscular fat. Eicosapentaenoic (C20:5 n-3; EPA) and DHA are not or hardly present in backfat and only EPA was increased in intramuscular fat. Docosapentaenoic acid (C22:5 n-3; DPA) might have comparable biological functions as EPA and there is a possibility to increase the concentration of n-3 DPA considerable via dietary ALA in both muscle and backfat (Chapter 6 and General discussion).

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Summary

Fat is an important energy source in animal diets. A common inclusion level of 5% in pigs, 10% in broilers and 20% in veal calves corresponds with 15, 30 and 60% of the dietary digestible energy, respectively. Dietary fat consists mainly of triglycerides, which consist of one glycerol molecule connected to three fatty acids. The composition of fats differs among sources. The location of attachment of fatty acids at the position of the glycerol molecule can vary which may have consequences for their digestibility. Animals are not able to synthesize omega-6 (n-6) and omega-3 (n-3) fatty acids and therefore linoleic acid (LA) and α-linolenic acid (ALA) as n-6 and n-3 fatty acid, respectively, are essential. Both LA and ALA are precursors for the synthesis of long-chain polyunsaturated fatty acids. As such, it is important to understand factors affecting the digestibility of these dietary fatty acids as well as the deposition in animal products. The studies presented in Chapter 2-6 were carried out to determine effects of variation in dietary fat properties, mainly fatty acid composition, on fatty acid digestibility, fat metabolism, deposition of fat and elongation and desaturation of n-3 and n-6 fatty acids.

In **Chapter 1**, the variation in digestion and metabolism of various dietary fat sources in poultry and pigs is reviewed. With regard to fat digestion, this chapter focuses on saturation and the chain length of fatty acids, their position on the glycerol molecule and the proportion of dietary fat which is present as free fatty acids. After absorption, fatty acids are used for energy, further elongated and desaturated and/or deposited in animal tissues. The dietary fatty acid composition influences several metabolic processes related to their oxidation or deposition, consequently influencing the fatty acid composition of animal tissues.

Chapter 2 described a study with broiler chickens where the effect of variation in the degree of saturation, chain length (C16:0 vs. C18:0) and positioning of fatty acids at the glycerol molecule on fatty acid digestion was studied. For the latter effect, it was hypothesized that randomization of the location of palmitic acid on the glycerol molecule in palm oil would increase its digestibility, especially that of its palmitic acid component. This would result in changes in the fatty acid composition of body tissues. To this end, broiler chickens were fed diets containing either native or randomized palm oil. Diets with either native or a 50/50 mixture of native and hydrogenated sunflower oil were also fed to study the difference between saturated and unsaturated fat and the difference between C16:0 and C18:0. Randomization of palm oil raised the fraction of C16:0 at the sn-2 position of the glycerol molecule from 14 to 32%. Hydrogenation of sunflower oil reduced fat and total saturated fatty acid digestibility, while no change in the digestibility of total unsaturated fatty acids was found. Randomization of palm oil raised the mean apparent digestibility of C16:0 by 2.6 and 5.8% units during the starter and grower-finisher phase, respectively. On the basis of the observed digestibilities in the grower-finisher period, it was calculated that the digestibility for C16:0 at the sn-2 and sn-1,3 position were 90 and 51%, respectively. The feeding of randomized instead of native palm oil significantly raised the palmitic content of breast meat

and abdominal fat and lowered the ratio of unsaturated to saturated fatty acids. It was concluded that randomized palm oil may be used as a vegetable oil source in broiler nutrition with positive effects on saturated fatty acid digestibility when compared with native palm oil and positive effects on meat firmness when compared with vegetable oils rich in unsaturated fatty acids.

The study reported in **Chapter 3** describes a comparison of different vegetable fats in relation to fat and fatty acid deposition and their metabolism in the liver of broiler chickens. The hypothesis tested was that dietary vegetable fats rich in saturated fatty acids, when compared with a vegetable oil rich in linoleic acid, increase fat deposition in broiler chickens and affects synthesis and/or oxidation of individual fatty acids. Diets with native sunflower oil (SO), a 50/50 mixture of hydrogenated and native sunflower oil (HSO+SO), palm oil (PO) and randomized palm oil (RPO) were fed to broiler chickens. Intake of digestible fat and fatty acids, whole body fatty acid deposition, hepatic fatty acid profile and hepatic enzyme activities involved in fatty acid oxidation and synthesis were measured. The deposition/digestible fat intake ratio was significantly lower for the SO group in comparison with the groups fed the vegetable fats rich in saturated fatty acids. The difference between digestible intake and deposition of LA, reflecting its maximum disappearance rate, were highest for the SO fed birds and lowest for the birds fed PO and RPO. The calculated minimal rate of *de novo* synthesis of monounsaturated fatty acids (MUFA), calculated as deposition minus digestible intake, was more than 50% lower for the SO group than for the other three dietary groups. Based on the fatty acid profiles in the liver, it would appear that increasing contents of LA decreases the desaturation of saturated fatty acids (SFA) into MUFA. It is concluded that a diet rich in LA, in comparison with vegetable derived saturated fatty acids, decreases the deposition of fat especially of MUFA. The latter may be caused by a higher ß-oxidation and a reduced *de novo* synthesis of MUFA, but this conclusion is not fully supported by the measured activities of enzymes involved in fatty acid synthesis and oxidation.

In **Chapter 4**, the effect of equal net energy (NE) intake of starch, unsaturated and saturated fat on the performance, carcass and meat characteristics of pigs was studied. Each dietary group consisted of 100 pigs divided into 6 replicates with barrows and gilts mixed in each pen. Diets were fed at 2.7 x energy requirement for maintenance. Performance parameters were similar among dietary treatments. Pigs fed the saturated fat diet showed a trend towards a higher lean meat percentage compared to the starch diet fed pigs (P=0.07). Backfat thickness was not affected by dietary treatment, but intramuscular fat (IMF) content tended to be lower in the pigs in the saturated fat group compared with the starch group (P=0.08). Feeding pigs the unsaturated fat diet increased the unsaturated/saturated (U/S) ratio in backfat. Pigs in the starch group had the lowest backfat U/S ratio. It was concluded that high starch diets increase firmness of fat and may increase the content of IMF.

Studies suggested that in human adults, LA inhibits the biosynthesis of n-3 long-chain polyunsaturated fatty acids (LC-PUFA), but their effects in growing subjects are largely unknown. In **Chapter 5**, growing pigs were used as a model to investigate whether high LA

intake affects the conversion of n-3 LC-PUFA by determining fatty acid composition and mRNA levels of Δ5- and Δ6 desaturase and elongase 2 and 5 in liver and brain. In a 2x2 factorial arrangement, 32 gilts from 8 litters were assigned to one of four dietary treatments, varying in LA and ALA intake. Low ALA and LA intake were 0.15 and 1.31, and high ALA and LA intake were 1.48 and 2.65 g/(kg $BW^{0.75}$/d), respectively. LA intake increased arachidonic acid (ARA) in the liver. ALA intake increased hepatic eicosapentaenoic acid (EPA) concentrations, but decreased docosahexaenoic acid (DHA) (all $P<0.01$). Competition between the n-3 and n-6 LC-PUFA biosynthetic pathways was shown by reductions of ARA (>40%) at high ALA intakes. Concentration of EPA (>35%) and DHA (>20%) was decreased by high LA intake (all $P<0.001$). Hepatic mRNA levels of Δ5- and Δ6 desaturase were increased by LA, and that of elongase 2 by both ALA and LA intake. In contrast, brain DHA was virtually unaffected by dietary LA and ALA. Generally, dietary LA inhibited the biosynthesis of n-3 LC-PUFA in the liver. ALA clearly affects the conversion of both hepatic n-3 and n-6 LC-PUFA. DHA levels in brain tissue were irresponsive to these diets. Apart from Δ6 desaturase, elongase 2 may be a rate-limiting enzyme in the formation of DHA.

The study reported in **Chapter 6** was conducted utilising the same design as the study reported in Chapter 5 to determine the effect of two levels of LA intake at either high or low ALA intake on their conversion into LC-PUFA and its subsequent deposition in muscle and backfat in growing pigs. There was a close positive relation between intake of ALA and the concentration of ALA in backfat and in IMF. Dietary ALA did not affect the concentration of EPA and DHA, but increased docosapentaenoic acid (DPA) content in backfat. High ALA intake did not significantly affect DHA but significantly increased EPA, C20:3 n-3 and DPA concentration in IMF. The n-3 LC-PUFA proportion in backfat was increased from approximately 1 to 3%. This increase may be useful to enrich meat with these fatty acids. The impact of ALA intake on n-3 LC-PUFA was suppressed by increased LA intake. Dietary ALA suppressed the concentration of n-6 LC-PUFA in blood plasma by more than 50%. When compared at an equal incremental dose, the inhibiting effect of ALA on blood arachidonic acid was stronger than the stimulating effect of LA as precursor.

The general discussion (**Chapter 7**) focuses on two aspects; the digestibility of fat sources and the potential contribution of LC-PUFA intake by humans through meat or meat products from pigs fed with precursor fatty acids. In addition, some attention is paid to a third aspect; the role of ALA and LA on behaviour of pigs.

Current models to predict digestibility of fat sources are based on the content of saturated fatty acids or on the ratio of saturated versus unsaturated fatty acids and the proportion of free fatty acids. A new model is proposed for poultry and pigs which takes into account: 1) the content of long-chain saturated fatty acids, 2) the proportion of fatty acids present as free fatty acids, 3) discrimination between the digestion of C16:0 and C18:0 and 4) the differences in digestion between fatty acids situated at the different positions of the glycerol molecule. In this model it is assumed that the digestion of free fatty acids is similar to that of the same fatty acid esterified at the sn-1 or sn-3 position of the glycerol molecule. The model provides a simple way of representing the major sources of variation in the digestion of high fat feed ingredients into a value for its digestibility.

DHA and ARA are the predominant LC-PUFA in brain tissue. The effect of dietary LA and ALA on DHA and ARA are limited, but there is a correlation between brain DHA concentrations of pigs and their behaviour.

Subsequently, responses of ALA and the n-3 LC-PUFA in various tissues to variation in ALA intake are discussed, combining the results presented in Chapter 6 with literature data. The potential contribution of meat or meat products from pigs fed diets varying in ALA and LA to meet the human recommended daily allowances of ALA, EPA and DHA was investigated. There is a clear positive correlation between ALA intake via the diet of pigs and content of ALA in IMF and backfat. The content of EPA in IMF of pigs can be increased with ALA intake, but DHA is not affected in both IMF and backfat. The potential contribution of EPA+DHA intake via meat from pigs fed with a high ALA diet is rather low since their concentration in backfat is not affected. With a daily consumption of 100 g pig meat, 30 and 8% of the recommended daily intake for ALA and EPA+DHA would be met, respectively. The intermediate product DPA, however, can be increased in backfat and IMF of pigs by feeding high ALA diets to considerable proportions. There are indications that DPA is a biologically active component and can be metabolized into EPA. The potential of meat products to supply DPA is considerable and therefore of interest.

Conclusions

The main conclusions of the work presented in this thesis are:

Digestibility

For the determination of the digestibility of palm oil to replace animal fat, both the differences between C16:0 and C18:0 and the position of C16:0 on the glycerol molecule are relevant. Randomization of palm oil increases the digestibility of C16:0 in broiler chickens (Chapter 2).

Models predicting fat digestibility should take into account an estimation of the digestibility of C16:0, C18:0 and unsaturated fatty acids at the sn-2 and sn-1, or -3 positions of the glycerol molecule and their proportion present as free fatty acid (General discussion).

Fat deposition

Saturated fat sources increase body fat deposition in comparison with unsaturated fat sources in broilers. An increase in the rate of *de novo* synthesis of monounsaturated fatty acids and a reduced rate of fatty acid oxidation appears to be responsible for this effect (Chapter 3).

Backfat thickness of pigs is not affected by energy source, but the amount of intramuscular fat tends to be higher in pigs fed high starch diets compared with those fed diets with high amounts of saturated fat (Chapter 4).

Conversion of n-6 and n-3 fatty acids into LC-PUFA

Increasing the intake of LA increases mRNA expression of Δ6- and Δ5 desaturase and all n-6 PUFA in the liver of pigs. mRNA expression of elongase-2 was up-regulated by both high

dietary LA and high ALA. Δ6 desaturase and elongase 2 activity are postulated to be rate-limiting in the conversion of ALA into DHA in pigs (Chapter 5).

Increasing the intake of LA inhibits the conversion of ALA into n-3 LC-PUFA. Increasing the intake of ALA inhibits the conversion of LA into n-6 LC-PUFA. When compared at equal incremental intake, the magnitude of the effect of ALA in liver is higher than that of LA (Chapter 5 and 6).

Docosahexaenoic acid (C22:6 n-3; DHA) and arachidonic acid (C20:4 n-6; ARA) are the predominant LC-PUFA in brain tissue. The effect of dietary LA and ALA on DHA and ARA are limited, but there is a correlation between brain DHA concentrations of pigs and their behaviour (Chapter 5 and General discussion).

Feeding high ALA diets to pigs increases ALA concentrations in backfat and intramuscular fat. Eicosapentaenoic (C20:5 n-3; EPA) and DHA are not or hardly present in backfat and only EPA was increased in intramuscular fat. Docosapentaenoic acid (C22:5 n-3; DPA) might have comparable biological functions as EPA and there is a possibility to increase the concentration of n-3 DPA considerable via dietary ALA in both muscle and backfat (Chapter 6 and General discussion).

Samenvatting

Vet is een belangrijke energiebron in de diervoeding. Gangbare vetgehaltes van 5% bij varkens, 10% bij vleeskuikens en 20% bij vleeskalveren leveren respectievelijk 15, 30 en 60% van de hoeveelheid energie. Vet in de diervoeding bestaat met name uit triglyceriden: een glycerol molecule gekoppeld aan drie vetzuren. De vetzuursamenstelling is afhankelijk van de vetbron. Daarnaast varieert de positie van vetzuren op het glycerolmolecule hetgeen mogelijk ook de verteerbaarheid kan beïnvloeden. Dieren kunnen zelf geen zogenaamde omega-6 (n-6) of omega-3 (n-3) vetzuren synthetiseren, en moeten daarom voldoende linolzuur (LA, C18:2 n-6) en α-linoleenzuur (ALA, C18:3 n-3) via de voeding opnemen. Deze vetzuren worden dan ook essentiële vetzuren genoemd. LA en ALA zijn precursors voor de vorming van langketen poly-onverzadigde vetzuren (LC-PUFA) binnen respectievelijk de n-6 en de n-3 keten. Deze LC-PUFA met docosahexaanzuur (DHA; C22:6 n-3), arachidonzuur (ARA; C20:4 n-6) en eicosapentaanzuur (EPA; C22:5 n-3) als belangrijkste vervullen tal van belangrijke functies in het lichaam.

Hoofdstuk 1 geeft een overzicht van de processen die bij vertering en metabolisme van vetbronnen bij pluimvee en varkens belangrijk zijn en de manier waarop deze door de samenstelling van de vetbron worden beïnvloed. De vertering van vet hangt af van de verzadigingsgraad (aanwezigheid van een dubbele binding in de koolstofketen van het vetzuur), de ketenlengte van vetzuren, de plaats van vetzuren op het glycerolmolecule (op de buitenste posities, respectievelijk sn-1 en 3 of in het midden: sn-2) en het aandeel ongebonden ofwel vrije vetzuren in het verstrekte voedingsvet. Na absorptie worden vetzuren geoxideerd, opgeslagen of omgezet naar andere vetzuren middels enzymatische processen. De vetzuursamenstelling heeft invloed op verschillende metabole processen en de vetzuursamenstelling van weefsels.

In **Hoofdstuk 2** is een onderzoek beschreven over de invloed van verzadigingsgraad, ketenlengte (C16:0 vs C18:0) en de positie van vetzuren op het glycerol molecule op de vertering van vetzuren bij vleeskuikens. Met name de moeilijker verteerbare, verzadigde vetzuren op de buitenste positie van het glycerol molecule zijn slecht verteerbaar. Palmolie bevat een hoog aandeel moeilijk verteerbaar palmitinezuur (C16:0) op de buitenste posities. Verwacht werd dat chemische randomisering van palmolie de verteerbaarheid van C16:0 verbetert, hetgeen ook een gevolg zal hebben op de vetzuursamenstelling van weefsels. Voor dit onderzoek werden kuikens gevoerd met palmolie en gerandomiseerde palmolie. Daarnaast werden voeders verstrekt met zonnebloemolie en een 50/50 mengsel van geharde en natieve zonnebloemolie om het effect van verzadiging en het verschil in vertering tussen C16:0 en C18:0 te testen. Randomisering van palmolie verhoogde het aandeel C16:0 op de sn-2 positie van het glycerolmolecule van 14 naar 32%, en verhoogde de vertering van C16:0 met 2.6 en 5.8% eenheden in respectievelijk de startperiode en groei-afmestperiode. Hieruit kon berekend worden dat de verteerbaarheid van C16:0 op de sn-2 en sn-1,3 positie respectievelijk

90 en 51% was. Hydrogenatie van zonnebloemolie gaf een verlaging van de vertering van vet en verzadigde vetzuren maar de vertering van onverzadigde vetzuren werd niet beïnvloed. Het randomiseren van palmolie verhoogde het gehalte aan C16:0 en de ratio verzadigd: onverzadigd vet in borstvlees en buikvet. Er werd geconcludeerd dat gerandomiseerde palmolie als plantaardige olie voor vleeskuikens een positieve invloed heeft op de vertering van verzadigde vetzuren in vergelijking met palmolie en steviger vlees geeft in vergelijking met onverzadigde plantaardige oliën.

Hoofdstuk 3 geeft een beschrijving van de invloed van plantaardige vetbronnen op vet- en vetzuuraanzet en het vetmetabolisme in de lever van vleeskuikens. Verwacht werd dat het voeren van verzadigde, plantaardige vetten in vergelijking met linolzuurrijke oliën tot een hogere vetaanzet leidt. Daartoe werden voeders met zonnebloemolie, een 50/50 mengsel van geharde en natieve zonnebloemolie, palmolie en gerandomiseerde palmolie verstrekt aan vleeskuikens. De opname aan verteerbaar vet en vetzuren, vet- en vetzuuraanzet werd bepaald. In de lever werd het vetzuurprofiel en de enzymactiviteiten die betrokken zijn in de oxidatie en synthese van vet gemeten. De verhouding tussen de aanzet van een vet(zuur) en de hoeveelheid die verteerd is geeft een indruk van de balans tussen het verbruik van dat vetzuur voor ATP productie en de *de novo* synthese van dat vetzuur. De ratio vetaanzet:verteerbaar vetopname was significant lager bij de vleeskuikens die gevoerd werden met zonnebloemolie in vergelijking met de drie groepen die rijk waren aan verzadigde vetzuren. Het verschil tussen het verteerde en opgeslagen linolzuur was het grootst voor de zonnebloemoliegroep en het laagst voor de palmoliegroepen. De minimale *de novo* synthese van mono-onverzadigde vetzuren was meer dan 50% lager voor de zonnebloemoliegroep in vergelijking met de andere groepen. Uit de vetzuurprofielen van de lever bleek dat een verhoging van linolzuur een verlaging geeft van de omzetting van verzadigde vetzuren naar mono-onverzadigde vetzuren. Er werd geconcludeerd dat de verstrekking van vetbronnen rijk aan linolzuur een lagere aanzet van vet en met name mono-onverzadigde vetzuren geeft in vergelijking met de verstrekking van verzadigde vetbronnen. Ondanks dat de gevonden effecten van de enzymen niet statistisch significant waren kan gesteld dat de lagere aanzet van vet waarschijnlijk een gevolg is van zowel een hogere ß-oxidatie als een verlaagde *de novo* synthese bij een verstrekking van linolzuurrijk vet.

In **Hoofdstuk 4** worden drie voeders, met zetmeel, verzadigd of onverzadigd vet als belangrijkste energiebronnen vergeleken in een experiment op praktijkschaal met varkens. Van alle drie de voeders werd een gelijke opname aan netto energie (NE) gerealiseerd (2.7 x de energiebehoefte voor onderhoud). Elke groep bestond uit 100 varkens, verdeeld over 6 herhalingen. Energiebron had geen invloed op de technische resultaten. Er was een tendens (P=0.07) voor een hoger mager vleespercentage bij de varkens die gevoerd werden met verzadigd vet in vergelijking met de zetmeelgroep. Er was geen invloed van energiebron op de spekdikte, maar een trend (P=0.08) voor een lager intramusculair vetgehalte voor varkens die gevoerd werden met verzadigd vet in vergelijking met zetmeel. De verstrekking van onverzadigd vet leidde tot de hoogste onverzadigd/verzadigd vet ratio in rugspek en zetmeel tot de laagste ratio. De conclusie van het onderzoek was dat hoog zetmeel in het voer leidt tot harder vet en mogelijk een verhoging van het gehalte aan intramusculair vet bij vleesvarkens.

Vanuit de literatuur is bekend dat bij volwassen mensen hoge LA opname de synthese van n-3 LC-PUFA, zoals DHA en EPA uit ALA kan remmen. Deze informatie is echter vooral verkregen bij een lage ALA opname. Omgekeerd is het mogelijk dat een hoge ALA opname de synthese van n-6 LC-PUFA, zoals ARA uit LA remt. Het is bekend dat bij de synthese van n-3 en n-6 LC-PUFA deels dezelfde enzymsystemen gebruikt worden. Het identificeren van het beperkende enzymsysteem kan helpen om bruikbare voedingsadviezen voor LA en ALA te ontwikkelen. In **Hoofdstuk 5** is een onderzoek beschreven waarbij jonge groeiende varkens werden gebruikt om het effect van een toename in LA opname op de conversie van n-3 vetzuren, en van een toename in ALA opname op de conversie van n-6 vetzuren te testen. Naast de invloed op de vetzuursamenstelling werd ook de invloed op de expressie (mRNA) van Δ5- and Δ6 desaturase en elongase 2 en 5 in de lever en de hersenen bestudeerd. In een 2x2 factoriële proef werden 32 gelten gebruikt, afkomstig van 8 tomen. Elk van de vier varkens per toom werd toegewezen aan elk van de vier behandelingen. De behandelingen bestonden uit combinaties van laag en hoog LA en ALA. De opname aan laag ALA en LA was 0,15 en 1,31 en die van hoog ALA en LA was 1,48 en 2,65 g/kg metabool gewicht/d. Verhoging van de LA opname verhoogde het percentage arachidonzuur (ARA) in de lever. Verhoging van de ALA opname gaf een een verhoging van EPA, maar een verlaging van DHA in de lever ($p<0.01$). Er lijkt een competitie aanwezig te zijn tussen de n-3 en n-6 LC-PUFA synthese. Verhoging van de ALA opname verminderde de vorming van ARA (>40%) en verhoging van de LA opname verlaagde het EPA (>35%) en DHA (>20%) gehalte in de lever (allen $p<0,001$). De mRNA niveaus van Δ5- and Δ6 desaturase waren verlaagd bij een hoge LA opname. Elongase 2 mRNA was verhoogd zowel bij een verhoogde ALA als verhoogde LA opname. DHA in hersenweefsel werd nauwelijks beïnvloed door veranderingen in LA of ALA in de voeding. Geconcludeerd werd dat verhoging van LA opname, vorming van n-3 LC-PUFA in de lever uit ALA remt. ALA heeft een duidelijke invloed op de vorming van zowel n-3 als n-6 LC-PUFA in de lever. DHA niveaus in de hersenen werden niet beinvloed. Naast Δ6 desaturase is mogelijk ook elongase 2 beperkend in de vorming van DHA.

De beschreven studie in **Hoofdstuk 6** is gebaseerd op het dierexperiment beschreven bij Hoofdstuk 5. Er werd onderzocht wat de invloed is van een verhoging van ALA en LA opname op de conversie naar LC-PUFA is door de vetzuursamenstelling te meten in spier en spek bij varkens. Er was een duidelijk positieve relatie tussen de opname van ALA en het gehalte aan ALA in spek en in intramusculair vet. Verhoging van ALA opname gaf geen verhoging van EPA en DHA, maar wel een verhoging van docosapentaanzuur (C22:5 n-3; DPA) in spek. Een hogere ALA opname had geen significant effect op intramusculair DHA, maar gaf wel een verhoging van EPA, C20:3 n-3 en DPA in de spier. Het totale n-3 LC-PUFA gehalte was verhoogd van ongeveer 1 naar 3%. Dit effect is mogelijk interessant voor het verrijken van vlees met dergelijke vetzuren. Het effect van ALA op n-3 LC-PUFA werd geremd door een hoge LA opname. Een hoge ALA opname in het voer gaf een verlaging van concentratie aan n-6 LC-PUFA in bloed met meer dan 50%. Bij een gelijke dosering is de remming van de vorming van ARA door ALA sterker dan de verhoging door de precursor LA.

De algemene discussie (**Hoofdstuk 7**) is gericht op twee aspecten; de verteerbaarheid van vetbronnen en de potentiële bijdrage van LC-PUFA opname door de consument via vlees en vleesproducten afkomstig van varkens die gevoerd zijn met precursors van n-3 LC-PUFA. Daarnaast is enige aandacht besteed aan de rol van ALA en LA op het gedrag van jonge varkens.

Huidige modellen die gebruikt worden om een inschatting geven van de verteerbaarheid van vetbronnen zijn veelal gebaseerd op het gehalte aan verzadigde vetzuren of de ratio aan onverzadigde/verzadigde vetzuren, aangevuld met het gehalte aan vrije vetzuren. In de discussie wordt een nieuw model voorgesteld met de volgende factoren: 1) het gehalte aan verzadigde vetzuren, 2) het aandeel en de soort vrije vetzuren, 3) een onderscheid tussen C16:0 en C18:0 en 4) het verschil in vertering van vetzuren op verschillen posities van het glycerol molecule. In dit model wordt verondersteld dat de vertering van vrije vetzuren gelijk is aan die van diezelfde vetzuren veresterd op de sn-1 en -3 positie van het glycerol molecule. Via dit model kan op een eenvoudige manier alle vier bovengenoemde factoren gebruikt worden voor een berekening van de vertering van vetten.

Tenslotte zijn de resultaten van Hoofdstuk 6 gecombineerd met literatuurgegevens. De potentiële bijdrage van vlees en vleesproducten afkomstig van varkens die gevoerd worden met een variatie in gehaltes aan LA en ALA aan de aanbevolen humane consumptie van ALA, EPA en DHA werd geschat. Omdat het DHA gehalte in spier en spek nauwelijks door ALA en LA opname werd beïnvloed (Hoofdstuk 6) kan de behoefte aan DHA via de consumptie van varkensvlees niet worden gedekt. EPA in intramusculair vet kan verhoogd worden door ALA in de voeding van varkens. Echter, de mogelijke bijdrage in de EPA behoefte door varkensvlees afkomstig van varkens die gevoerd worden met extra ALA is vrij beperkt omdat het gehalte aan EPA in spek laag is en nauwelijk verhoogd wordt. Consumptie van 100 g varkensvlees kan 30% van de ALA behoefte en 8% van de EPA+DHA behoefte dekken. Het gehalte aan DPA kan echter duidelijk verhoogd worden in zowel in de spier als in spek door de opname van ALA in varkensvoer. Er zijn aanwijzingen dat DPA ook een biologisch actieve component is en gemakkelijk gemetaboliseerd kan worden in EPA en daarmee kan bijdragen aan het voorzien in de EPA behoefte.

Conclusies

De belangrijkste conclusies van dit proefschrift zijn:

Verteerbaarheid

Voor de inschatting van de verteerbaarheid van palmolie ter vervanging van dierlijk vet is zowel het onderscheid tussen C16:0 en C18:0 als de positie van C16:0 op het glycerolmolecule van belang. Randomisatie van palmolie verhoogt de verteerbaarheid van C16:0 bij vleeskuikens (Hoofdstuk 2).

Voor de inschatting van de vetverteerbaarheid in modellen is het van belang om een inschatting te geven van de verteerbaarheid van C16:0, C18:0 en onverzadigde vetzuren op de

sn-2 en sn-1,3 positie van het glycerolmolecule en het aandeel dat als vrij vetzuur aanwezig is (Hoofdstuk 7).

Vetdepositie
Verzadigde vetbronnen geven een verhoging van de vetaanzet bij vleeskuikens ten opzichte van de verstrekking van onverzadigde vetbronnen. Dit wordt veroorzaak door zowel een verhoging van de *de novo* synthese van mono-onverzadigde vetzuren als een verlaging van de vetzuuroxidatie (Hoofdstuk 3).

Rugspekdikte van varkens wordt niet beïnvloed door energiebron, maar er is een tendens dat het gehalte aan intramusculair vet van varkens die gevoerd worden met zetmeel hoger is dan die gevoerd worden met verzadigd vet (Hoofdstuk 4).

Conversie van n-6 en n-3 vetzuren naar LC-PUFA
Verhoging van de opname van linolzuur (LA) geeft een verhoging van de mRNA expressie van Δ5- and Δ6 desaturase en alle n-6 LC-PUFA in de lever van varkens. mRNA expressie van elongase 2 was verhoogd bij zowel een hoge LA als een hoge α-linoleenzuur (ALA) opname. Naast Δ6 desaturase lijkt ook elongase 2 beperkend te zijn voor de vorming van DHA uit ALA bij varkens (Hoofdstuk 5).

Verhoging van de opname van LA geeft een remming van de conversie van ALA naar n-3 LC-PUFA. Een hogere ALA opname geeft een remming op de conversie van LA naar n-6 LC-PUFA. Het effect van een verhoogde ALA opname op de remming is sterker in vergelijking met de verhoging door een gelijke opname van de precursor LA (Hoofdstuk 5 en 6).

Docosahexaanzuur (C22:6 n-3; DHA) en arachidonzuur (C20:4 n-6; ARA) zijn de belangrijkste LC-PUFA in hersenweefsel. Het effect van LA en ALA opname op ARA en DHA was beperkt. Echter er was een correlatie tussen DHA concentratie in de hersenen en het gedrag van de varkens (Hoofdstuk 5 en 7).

De verstrekking van voeders met hoge ALA-gehaltes geeft een verhoging van ALA in spek en intramusculair vet. Eicosapentaanzuur (C20:5 n-3; EPA) en DHA zijn nauwelijks aanwezig in spek en alleen EPA was verhoogd in intramusculair vet. Docosapentaanzuur (C22:5 n-3; DPA) heeft mogelijk biologische functies vergelijkbaar met EPA. Het is mogelijk om de gehaltes aan DPA aanzienlijk te verhogen in spek en intramusculair vet middels ALA verhoging in de voeding (Hoofdstuk 6 en 7).

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Curriculum Vitae

Willem Smink werd geboren op 28 november 1968 te Sondel (Frl) en is opgegroeid in het naburige Oudemirdum. Na het volgen van de MAVO te Balk, werd de middelbare landbouwschool in Sneek in 1988 afgerond. In 1992 behaalde hij het diploma van de studierichting Nederlandse Landbouw aan de Agrarische Hogeschool Friesland (AHoF) te Leeuwarden. In september 1992 startte hij met de studie Zoötechniek aan de Landbouwuniversiteit van Wageningen en studeerde af in de vakken Veevoeding en Agrarische bedrijfseconomie. Na afstuderen werd gewerkt bij TNO Voeding (afdeling diervoeding en -fysiologie; ILOB) in Wageningen en Cavo Latuco in Utrecht aan projecten over voeding van pluimvee, varkens en kalveren. In 1997 was hij werkzaam bij het Centraal Veevoederbureau (CVB) in Lelystad op het gebied van energiewaardering van grondstoffen voor herkauwers en aminozuren bij varkens. Vanaf december 1997 is hij in dienst als nutritionist bij Feed Innovation Services (FIS) BV in Wageningen. Daar is hij actief in onderzoek, advisering en productontwikkeling op het gebied van de diervoeding.

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