THE INFLUENCE OF DIETARY FATTY ACID COMPOSITION AND SEX ON PORK QUALITY

Effekt av fettsyresammensetning i fôrfett og kjønn på svinekjøttkvalitet

Philosophiae Doctor (PhD) Thesis 2011:4

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Ås 2010

Thesis number 2011:4
ISSN 1503-1667
ISBN 978-82-575-0968-2

Acknowledgements

The present work was carried out as a part of a project covering both breeding and feeding for a better understanding and improvement of pork quality. Collaborative members were Department of Animal and Aquacultural Sciences – Norwegian University of Life Sciences (UMB), Norsvin (Norwegian Pig Breeders Association), Animalia – Norwegian Meat and Poultry Research and Felleskjøpet Fôrutvikling (FKF). This part of the study was funded by the Foundation for Research Levy of Agricultural Products, The Research Council of Norway and Felleskjøpet Fôrutvikling.

First of all, I wish to thank my main supervisor Magny S. Thomassen. You have been an amazing encourager and an excellent contributor in planning and finishing the scientific work in addition sharing all your knowledge during all these years. Thank you!

I would also like to express my gratitude to my co-supervisors Nils Petter Kjos, Margareth Øverland and Odd Vangen. Thank you for all the scientific input, discussions and for your enthusiasm and help conducting experiments and writing the scientific papers. Anna Caroline Rehnberg, thank you for help conducting the experiments, being a co-author and for all the encouragement. Jeff Wood, thank you for making a memorable and a fruitful stay for me at the University of Bristol. Thanks are given to the slaughterhouse staff at both Nortura Hed-Opp and Nortura Tønsberg and to the Animalia staff for all the help and the enjoyable time during sampling and measurements.

Thanks to my former leader Torbjørn Auran having the idea about PhD-students at FKF and for giving me the opportunity. I am thankful to you Odd Vangen for your appreciated recommendation. I would also like to thank my excellent colleagues and my present boss at Felleskjøpet Fôrutvikling. You have encouraged and helped me along the way and made a nice working atmosphere. Special thanks go to Hallgeir and Randi, my fellow PhD students at FKF for help during the compulsory courses and for helping me during the experiments and writing periods. Randi, Line and Anne Stine: Thank you for keeping me in shape! Øydis Marie, thank you for all the smiles, encouraging talks and help to finish my work, you are a true friend. Thanks to all my fellow PhD-friends at Ås and to all other supporting friends.

My always supporting family has provided me with the interest for animals and food. You have been the main reason for my choice and wish to study. Warm “takk” goes to Ask, Tora Marie, Erlend, Katrine, Berit and Ragnhild for keeping in touch and always providing a roof

over my head when needed. Special thanks go to pappa/dad Kjell and mamma/mum Torgun for contributing during the experiments with storage of the experimental feeds, storage and sending samples together with “always being there for me”.

Last, but not least, thank you Frode for believing in me and for all the back up and discussions in a field you originally didn’t know.

Trondheim, November 2010

Elin Hallenstvedt

Contents

Abstract

Norway is an important fish catching and producing country and has traditionally used fish and fish by-products in feed for monogastric animals, the pig is no exception. Fish and fish by-products are excellent sources of essential amino acids, healthy fatty acids, vitamins and serves as a mineral supplier. Extended use has, however, caused negative effects on pork quality in terms of fat firmness, off-flavours and off-odours. During the last years feed recommendations, for most pigs in Norway, have been: no use of fish or fish by-products in feed for slaughter pigs, maximum iodine value product (IVP) 57/feed unit and minimum 200 mg/kg vitamin E. These limitations have challenged the formulation of pig feeds. The overall aim of this thesis was to evaluate the effects of different dietary fat level, fatty acid composition and of sex (entire male and female) on sensory, technological and nutritional pork quality parameters.

Three feeding experiments were conducted using entire males and females of the crossbreed [(Norwegian Landrace x Yorkshire) x (Duroc)]. Pigs were individually and restricted fed. Fat sources used were soybean oil, palm kernel oil and fish oil. Experimental diets varied in IVP: 31 to 118, fish oil inclusion in percent: 0, 0.25, 0.48, 0.52, 0.72 and 3.0 and fat level in percent of dry matter 2.9 up to 9.6. The ribs, pork chops and mince for meat balls were short-term frozen stored at -20 ˚C for 3 months, and belly and neck were short-term frozen stored for 2 and 4 months, respectively, at -80 ˚C. All products were tested by sensory profiling after short-term frozen storage. Long-term stored ribs, loins and meat balls were wrapped in plastic bags and frozen stored at -20 ˚C for additional 6 months and meat balls for additional 7 months. The ribs were long-term stored in darkness while the chops and meat balls were exposed to light simulating normal retail display. Long-term stored bellies were stored for 12 months at -80 ˚C with additional 6 months at -20 ˚C. Fatty acid composition was measured in shoulder fat (outer and inner layer), in backfat (outer and inner layer) at P2 location and in neutral lipids and phospholipids of M. *longissimus dorsi* (LD). Backfat firmness and colour, and meat colour were also evaluated.

The results from the present study showed that the fatty acid composition of backfat, inner and outer layer and neutral lipids of LD changed towards the dietary fatty acid composition. Feeding low fat diets seemed to enhance the *de novo* fatty acid synthesis, producing SFA and MUFA, especially C18:1. Pigs fed low fat diets had low percentages of the very long chain (VLC) *n*-3 fatty acids, mostly C22:5*n*-3 in backfat and in neutral fat of LD. These pigs had

also a high C20:4*n*-6 percentage indicating production from C18:2*n*-6. Dietary soybean oil, giving a high IVP, led to an increase in the percentage of C18:2*n*-6 linearly to the dietary contribution. These pigs were also low in VLC *n*-3 and still the C22:5*n*-3 was dominating. When introducing fish oil, high in VLC *n*-3 fatty acids, a substantial increase in the percentages of C20:5*n*-3, C22:5*n*-3 and C22:6*n*-3 in backfat and neutral lipids of LD was found indicating a higher nutritional quality. Increasing the fat level, by use of other added fat in combination with fish oil, seemed to improve the wanted incorporation of VLC *n*-3 fatty acids into both backfat and neutral fat of LD.

Our results further suggest that fish oil and high PUFA diets can be used for slaughter pigs without detrimental effects on sensory attributes like odour and flavour on short-term frozen stored products. After long-term frozen storage rib, meat balls and belly were not negatively influenced by high dietary PUFA, or dietary fish oil up to 0.7%. Only pork chops from the highest dietary IVP group showed increased odour and flavour rancidity and intensity. Long-term stored belly from pigs fed the second highest fish oil inclusion (0.5%) gave no off-flavour or off-odour. Only tendencies to higher fish oil flavour in the long-term stored belly was found in the highest fish oil group. After reheating, bellies from the highest fish oil group had a slightly increased fish oil flavour and odour together with a significant rancid flavour and odour.

Dietary IVP slightly influenced the backfat firmness. Higher firmness scores were obtained when the dietary IVP declined. Fat colour was less influenced by the dietary fatty acid composition.

Phospholipids had, as expected, high percentages of PUFA but were also influenced by the dietary treatments. Both low fat and soybean oil diets gave higher C20:4*n*-6 percentages and lower percentage of VLC*n*-3 fatty acids than fish oil fed pigs. The supply of C18:3*n*-3 seemed to enhance the desaturation and elongation into C20:5*n*-5 and C22:5*n*-3 but a decrease in the percentage of C22:6*n*-3 when feeding these diets strongly supports the view that the synthesis of this fatty acid is very limited in pigs. Providing dietary VLC *n*-3 fatty acids by using fish oil, these fatty acids were incorporated into phospholipids, now with the C20:5*n*-3 and C22:6*n*-3 as the prominent.

Sex had an impact on fatty acid composition. Males had higher PUFA and lower MUFA percentages in outer and inner layers of backfat and shoulder fat and in neutral fat of LD. The

percentage of C18:1 was in particular lower in males, indicating a lower delta-9-desaturase activity as compared to females.

In conclusion, it is possible to change the fatty acid composition of pork towards healthier products for human consumption without detrimental effects on important sensory traits. Care must, however, be taken when long term storage or reheating is intended.

Sammendrag

Norge er en stor fiskerinasjon og har tradisjonelt brukt fisk og biprodukter av fisk i fôr til enmaga dyr, grisen som intet unntak. Fisk og biprodukter av fisk er gode kilder til essensielle aminosyrer, gunstige fettsyrer, vitaminer og som mineralkilde. Utvidet bruk har imidlertid forårsaket negative effekter på svinekjøttkvalitet i form av dårlig spekkfasthet og uønska smak og lukt. De siste anbefalinger knytta til fôr, for de fleste griser i Norge, har vært: ingen bruk av fisk eller biprodukter av fisk i fôr til slaktegris, maksimum IVP 57/FEn og 200 mg vitamin E/kg. Disse begrensningene har utfordret optimering av svinefôr. Det overordna målet med denne avhandlinga var å evaluere effekten av ulik fettsyresammensetning i fôr, fettnivå i fôr og kjønnseffekten (råner og purker) på sensorisk, teknologisk og ernæringsmessig kvalitet av svinekjøtt.

Tre fôringsforsøk blei gjennomført med råner og purker av rasekryssingen [(norsk landsvin x yorkshire) x (duroc)]. Grisene blei individuelt og restriktivt fôra. Fettkilder som blei brukt var soyaolje, palmekjerneolje og fiskeolje. Forsøksfôra varierte i jodtallsprodukt (31 til 118), nivå av tilsatt fiskeolje i prosent (0, 0,48, 0,25, 0,52, 0,72 og 3) og fettnivået i prosent av tørrstoff (2,92 opp til 9,6). Produktene som blei testa sensorisk var ribbe, medisterkaker, svinekoteletter, side og nakke. Ribbe, svinekoteletter og råstoff til medisterkaker var kort tids fryselagra ved -20 ˚C i 3 måneder, og side og nakke blei kort tids fryselagra i henholdsvis 2 og 4 måneder, ved -80 ˚C. Alle produktene blei testa sensorisk etter kort tids fryselagring. Lang tids fryselagra ribbe, koteletter og medisterkaker var pakka i poser og fryselagra ved -20 ˚C for ytterligere 6 måneder for ribbe og koteletter, og medisterkaker i 7 måneder. Fettsyresammensetningen blei målt i nakkefettet (ytre og indre lag), ryggspekk (ytre og indre lag) fra P2, i nøytrale lipider og fosfolipider fra M. *longissimus dorsi* (LD). Spekkfasthet og farge, samt kjøttfarge blei vurdert.

Resultatene i denne avhandlinga viser at fettsyresammensetningen i spekk, indre og ytre lag, i nøytrale lipider og fosfolipider i LD gjenspeilte fettsyresammensetningen i fôret. Fôring med lavfettfôr så ut til å forsterke *de novo* syntesen av fettsyrer og ga en høy andel SFA og MUFA, særlig C18:1. Griser fôra med lavfettfôr hadde lavt innhold av svært lange *n*-3 fettsyrer, men C22:5*n*-3 var den som utpekte seg i spekk og i nøytralt fett i LD. Fôr med soyaolje, det vil si høyt jodtallsprodukt, økte andelen C18:2*n*-6. Disse grisene hadde også lavt innhold av de

svært lange *n*-3 fettsyrene og fremdeles var C22:5*n*-3 den viktigste. Ved å bruke fiskeolje, med høyt innhold av de svært lange *n*-3 fettsyrene, blei det funnet en betydelig økning i C20:5*n*-3, C22:5*n*-3 og C22:6*n*-3 i spekk og nøytrale lipider i LD, noe som indikerer en høyere ernæringsmessig kvalitet. Økt fettmengde i fôret, ved mer tilsatt fett, i kombinasjon med fiskeolje ga forbedra inkorporering av de svært lange *n*-3 fettsyrene i både spekk og nøytralt fett i LD.

Videre viste resultatene at det er mulig å bruke fiskeolje og høyt innhold av PUFA i fôr til slaktegris uten reduksjon i de sensoriske egenskapene som lukt og smak etter kort tids fryselagring. Etter lang tids fryselagring var ribbe, medisterkaker og side upåvirket av høyt PUFA eller opp til 0,7 % fiskeolje i fôret. Koteletter fra gris foret med det høyeste innholdet av PUFA hadde etter lang tids fryselagring noe mer harsk og intensiv lukt og smak. Sider fra griser fôret med det nest høyeste nivå av fiskeolje (0,5 %) ga ingen negativ smak eller lukt. Side fra gris gitt det høyeste fiskeoljenivået viste små tendenser til økt fiskeoljesmak. Først etter gjenoppvarming ga gruppa med det høyeste fiskeoljenivået i fôret mer smak og lukt av fiskeolje i tillegg til en harsk smak og lukt.

Jodtallsproduktet i fôr påvirka spekkfastheten; jo høyere jodtallsprodukt jo dårligere spekkfasthet. Fettfargen var mindre påvirket av jodtallsproduktet og fettsyresammensetningen i fôret.

Fosfolipider hadde som forventa høyt innhold av PUFA, men blei også påvirka av fettsyresammensetningen i fôret. Både lavfett- og soyaolje-fôra griser viste høyere prosent av C20:4*n*-6 enn griser som blei gitt fiskeolje. Dette indikerer en utstrakt elongering og desaturering av C18:2*n*-6. Griser fôra med lite fett eller soyaolje hadde også lavt innhold av de svært lange *n*-3 fettsyrene i motsetning til griser fôra med fiskeolje. Ved å tilsette de svært lange *n*-3 fettsyrene i fôret blei de også inkorporert i fosfolipidene; nå med C20:5*n*-3 og C22:6*n*-3 som de mest fremtredende.

Kjønn hadde innvirkning på fettsyresammensetningen. Råner hadde høyere PUFA innhold og lavere innhold av MUFA enn purker. Prosentandelen av C18:1 var lavere i råner, noe som tyder på en redusert delta-9-desaturase aktivitet sammenliknet med purker.

Det kan konkluderes med at det er mulig å endre fettsyresammensetningen i svinekjøtt mot sunnere produkter uten reduksjon i de viktigste sensoriske egenskapene. Ved lang fryselagringstid eller gjenoppvarming må det imidlertid være stor oppmerksomhet på mulige harskningsproblemer.

List of papers

The present thesis is based on the following four papers and will be referred to in the text as follows:

I. Hallenstvedt, E., M. Øverland, A.C. Rehnberg, N.P. Kjos & M. Thomassen. Sensory quality of short- and long-term frozen stored pork products. Influence of diets varying in polyunsaturated fatty acid (PUFA) content and iodine value. Submitted for publication in Meat Science.

II. Hallenstvedt, E., N.P. Kjos, A.C. Rehnberg, M. Øverland & M. Thomassen. (2010). Fish oil in feeds for entire male and female pigs: Changes in muscle fatty acid composition and stability of sensory quality. ***Meat Science. 85: 182 – 190***

III. Hallenstvedt, E., N.P. Kjos, M. Øverland & M. Thomassen. Changes in texture, colour and fatty acid composition of inner and outer layers of pig shoulder fat. Submitted for publication in Meat Science.

IV. Hallenstvedt, E., M. Øverland, N.P. Kjos & M. Thomassen. Production and deposition of very long chain *n*-6 and *n*-3 fatty acids in pigs as affected by dietary fat level and composition. Manuscript.

Abbreviations

FEg	The previously Norwegian feed unit
FO	Fish oil
FU	Feed unit, according to CVB 2003
IV	Iodine value
IVP	Iodine value product
LD	*M. longissimus dorsi*
LF	Low fat
MUFA	Monounsaturated fatty acids
PK	Palm kernel oil
PUFA	Polyunsaturated fatty acids
SBO	Soybean oil
SFA	Saturated fatty acids
VLC	Very long chain

1.1 Background

1.1.1 Norwegian considerations – history

Pig production has been common in Norway since the Stone Age. The pork quality has been a topic for a surprisingly long time. In a book published in 1879 meat and fish leftovers were recognized as good feed for pigs (Thesen, 1879). Christiana svineslagteri claimed already in 1890 that fish or fish by-products were reprehensible of floppy and oily fat with a fish oil flavour and odour.

In the fifties more knowledge about the positive effects of intra muscular fat content on juiciness was known (Altern, 1955). Further it was described how to obtain good quality lard, the backfat thickness along the back should become impressive 34mm in average. The use of herring meal as an excellent protein, calcium, phosphorous and vitamin D source was known but the negative off-flavours were also recognized. Already in early sixties the recommendation in Husdyrboka (Skjærvold, Presthegge, Eskilt, Skei & Brandt, 1962) was no use of herring meal 6 weeks before slaughter since it increased the incidences of soft fat and a fishy taste. Fish meal and fish by products were acknowledged as excellent protein sources.

In the early 90'ties consumers, slaughter houses and other meat processing industry reported vide variation in pork quality in terms of taste and odour as well as the technological quality. The recommended dietary fish fat inclusion was at that time maximum 3g fish fat per feed unit (FEg) for slaughter pigs. Based on this feedback a sensory test including a TBA test of LD was conducted to evaluate the Norwegian pork quality (Arnkværn & Bronken Lien, 1997). Meat samples from totally 180 slaughter pigs from five different slaughter houses and also samples with known feeding strategies (high vitamin E or a high food waste inclusion) were evaluated. The LD samples were frozen stored for 1, 4 and 8 months and analyzed for TBA values and sensory evaluated by a trained panel. The evaluation revealed that Norwegian pork was considered to have good meat flavour and being tender even during storage, however, rather low juiciness in short and long-term frozen stored products was also found. Mainly rancid taste and off-taste were pronounced in some samples and this was further supported by the high TBA values in these samples. It was concluded that the main reason to the undesired taste was the high content of the VLC *n*-3 fatty acids, C22:5*n*-3 and C22:6*n*-3 which ranged up to 2-3% of total fat. Pigs fed 80-100% food waste had also a high incidence of off-taste.

In another study the PUFA and VLC *n*-3 fatty acid content and the effect on storage stability and sensory quality was investigated (Bryhni, Kjos, Ofstad & Hunt, 2002b). After 1 month of frozen storage, chops of LD and sausages from the high dietary PUFA fed pigs showed higher rancid odour than pigs fed the low PUFA diet. No effect of C22:5*n*-3 and C22:6*n*-3 was found after 1 or 8 months frozen storage.

Based on this work new feeding recommendations were introduced. This was a maximum of 0.3g C22:5*n*-3 and C22:6*n*-3 per FU. When a new crossbreed was produced by the cooperative slaughter houses the new recommendations for most slaughter pigs in Norway became:

- No use of any fish by-products to ensure a good pork quality
- Maximum 57 IVP per FU in feed
- 200 mg Vitamin E

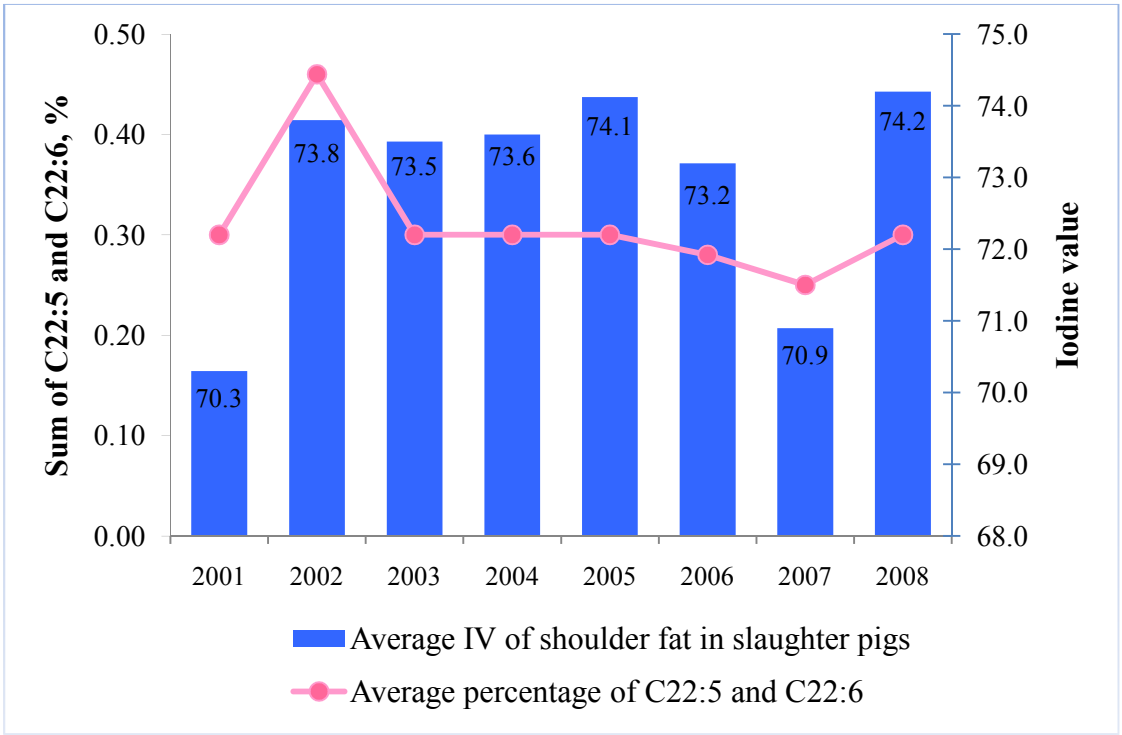

Figure 1. Development of IV and percentage of samples exceeding the maximum level of 0.5% of C22:5n-3 + C22:6n-3 of total fatty acids in shoulder fat from slaughter pigs from 2001 to 2008.

After these changes in feed the sum of C22:5*n*-3 and C22:6*n*-3 fatty acid content in shoulder fat has declined indicating a reduced nutritional quality the last years (Figure 1). The effort into reducing the iodine value has not been according to the desire of an iodine value below 70.

Figure 2. "Smaken kommer igjen" sa bonden han gav grisen sild (Skjærvold et al., 1962), (translated "The flavour comes back" said the farmer and gave the pig a herring).

Norway is the second largest fish exporter in the world (Eksportutvalget for fisk, 2010), this gives a high supply of fish and other fish by-products. The most common fish products are fish silage and fish meal. Fish silage is produced of newly dead fish, slaughter and fillet waste and processed by using acidifiers or enzymatic degrading of the protein. Fish silage is mostly used to monogastric animals due to the rather poor quality to ruminants. Fish products are excellent sources of minerals and essential amino acids and have a high digestibility. The producers of fish by product know that the products must have a good quality in terms of free nitrogen content and oxidation status. The today's fish by-products are therefore of a much higher quality than before. It can be anticipated that a regulated use of such products have a positive impact on the pigs performance and making healthier products for human consumption.

Use of some fish products in feed for pigs would be positive in several ways:

- High content of essential amino acids
- High content of minerals such as phosphorous
- Contributor of the VLC *n*-3 fatty acids
- Healthier fatty acid composition of pork

Norway is a protected market concerning agricultural production, Norwegian farmers produce mainly for the national market. The main pig breeds are Norwegian Landrace, Yorkshire, Duroc and Hampshire, the latter as the only one with halothane gene *RN*$^-$. The crossbreed of [(Norwegian Landrace x Yorkshire) x (Norwegian Landrace x Duroc)] has a market share of

more than 50%. Entire males are castrated with use of anaesthetics. A survey including 400 persons concluded that 40% of the Norwegian population is sensitive to boar taint, and half of this group experience it as unbearable (Nofima Mat, 2008). The goal is to produce pigs without castration but also no boar taint.

There are approximately 2900 pig farmers in Norway (Statistisk sentralbyrå, 2010) producing just below 1.5 million slaughter pigs annually (Animalia, 2009). The total meat consumption, poultry included, is in average 67.9 kg per capita per year while pork consumption stands for 25.9 kg per capita annually based on numbers from 2009 (Nortura SA, 2009). Average fish consumption is in comparison 22.3 kg (Helsedirektoratet, 2010). The meat consumption has increased the last decades, giving rise to concern by the Norwegian Directorate for Health, (Helsedirektoratet, 2010) since meat is the second largest contributor of saturated fat. Ideally the fish consumption should increase due to the content of VLC *n*-3 fatty acids with the positive implications for human health.

Among the Norwegian consumers 95% are pleased with the agricultural products of Norwegian origin (Norsk landbrukssamvirke, 2008), however, from 1997 to 2007 there has been an increased number of consumers avoiding meat, as beef and pork, due to the negative impact on the nutritional quality and health (Lavik, 2008).

1.2 General introduction

1.2.1 Fat and fatty acids

Adipose tissue is a loose connective tissue built up of mostly adipocytes and serves as an energy pool. The adipocytes consist of a large lipid droplet covered by a cytoplasm layer. The lipid consists of different fatty acids. A fatty acid is a straight chain of carbon atoms of usually an even number (Figure 3) and can be saturated with no double bonds or unsaturated with at least one double bond. A fatty acid has a methyl group and a carboxyl acid end and forms the basics to the counting of double bond position. One double bond in a fatty acid is most commonly Δ9 (nine carbon atoms from the carboxyl group) or *n*-9 (nine carbon atoms from the methyl end).

The animal cell has enzyme systems to produce the *n*-9 double bond fatty acid but the *n*-6 and *n*-3 fatty acids cannot be synthesized. The C18:2*n*-6 and C18:3*n*-3 fatty acids are essential fatty acids and must be provided through the diet.

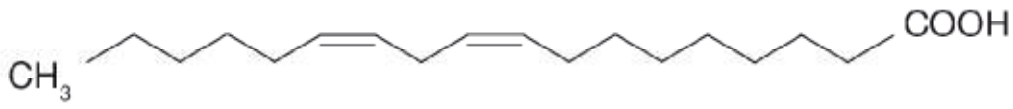

Figure 3. One of the most common fatty acid C18:2n-6, linoleic acid

1.2.2 Fatty acid biosynthesis

The main energy store in an animal is the fat tissue. Diets for pigs are composed of mostly grains, protein raw material and only few percentages of fat is added. The body cannot store the high supply of carbohydrates and therefore the *de novo* synthesis of fatty acids occurs. *De novo* synthesis is found primarily in the liver, adipose tissue, lactating mammary glands and in the central nervous system. The process uses acetyl groups (2C) from acetyl CoA and form palmitate, C16:0. Acetyl CoA is formed from glucose through pyruvate, amino acids, ketone bodies and from fatty acids. The newly formed C16:0 and dietary fatty acids can further be modified by both elongation to give longer fatty acids and desaturation to produce unsaturated fatty acids. Elongation takes place in most tissues and can use both saturated and unsaturated fatty acids. Desaturation is the other process in the biosynthesis of fatty acids and the first double bond is normally positioned at *n*-9. The process requires a chain length of 16 carbons.

The essential dietary fatty acids C18:2*n*-6 and C18:3*n*-3 may be changed by elongation and desaturation (Figure 4). C18:2*n*-6 can be converted to C20:4*n*-6 and serves as a precursor for eicosanoids and is abundant in phospholipids especially in the brain. The other essential fatty acid C18:3*n*-3 may be converted to the longer VLC *n*-3 C20:5, C22:5 and C22:6 fatty acids. To what extent these processes occur in the pig or the human body are somewhat unclear. There seems to be sex differences (Burdge, 2004) and also breed differences among the pig breeds (Zhang, Knight, Stalder, Goodwin, Lonergan & Beitz, 2007).

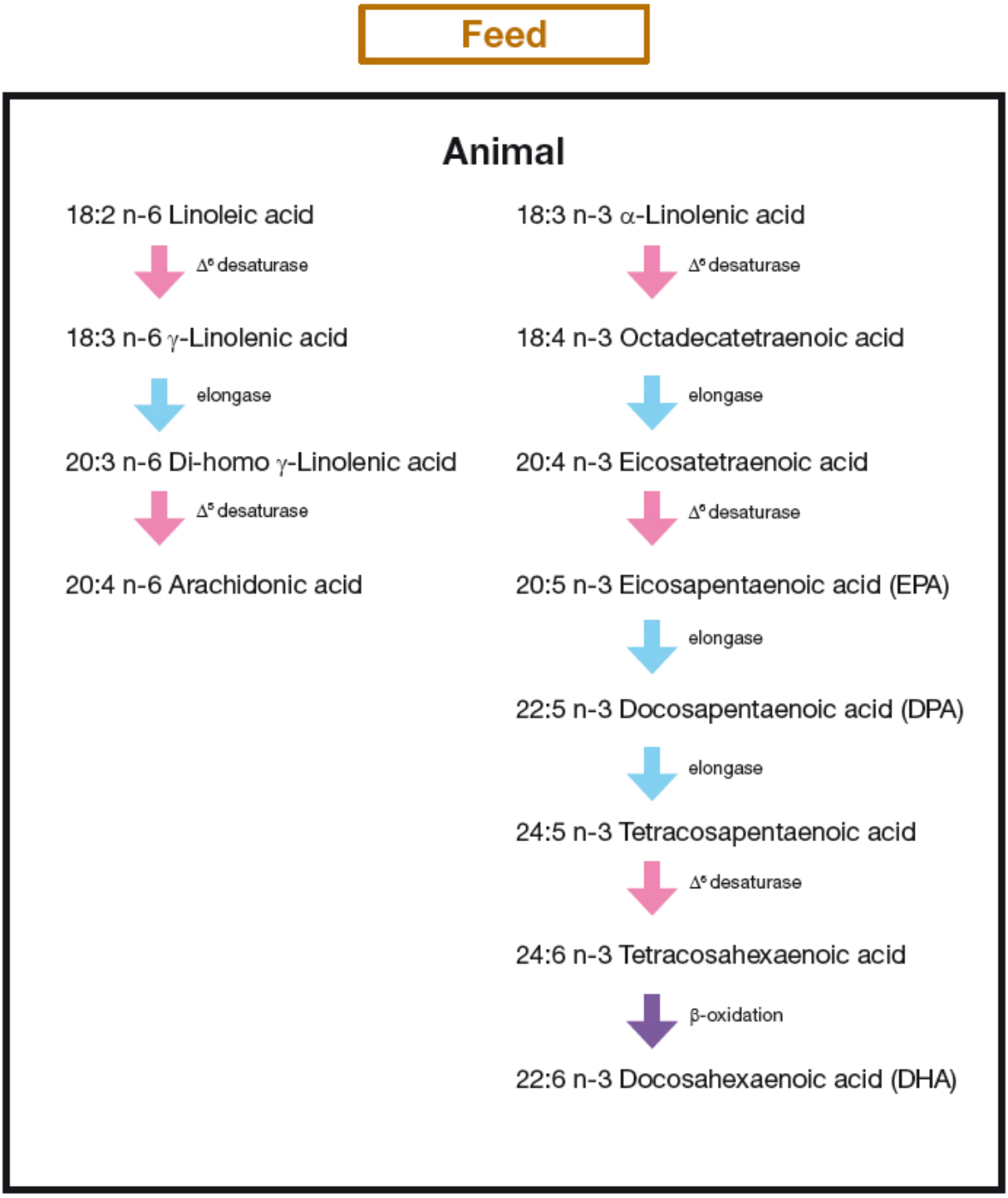

Figure 4. Overview of the VLC-PUFA biosynthesis of both n-6 and n-3 families (modified from Napier, 2007).

1.2.3 Fat digestion and deposition

Dietary fat is in general absorbed in the small intestine both in the distal duodenum and the jejunum. Lipase splits the triacylglycerols esters into mostly monoglycerides and free fatty acids. These smaller components are absorbed and reform the structure of triacylglycerols. Further these are incorporated into chylomicrones and transported through the lymphatic system to the circulatory system. Short- and medium-long fatty acids are absorbed directly to the blood, transported to the liver and oxidized.

In the formulation of diets information about digestibility of nutrients from the raw materials is used (CVB, 2003). This gives a more accurate picture of the net amount that is taken up from the mouth to anus. The digestibility of fat differs according to the fatty acid composition showing a higher digestibility with higher PUFA content (Leek, Beattie & O'Doherty, 2004).

As the dietary fat supply exceeds the need for maintenance and production the fat deposition increases. Single stomached animals incorporate the dietary fatty acids unchanged into the fat depot and therefore the dietary influence is great. Taugbøl & Saarem (1995) showed differences in the allocation of fatty acids, where dark red mm adductors showed a higher PUFA content compared to the lighter muscle *M. longissimus lumborum*. This is most probably due to the higher amount of mitochondria and thus more membranes. Also the depot fat differ in allocation and some studies have shown a difference in fatty acid composition in different backfat layers (Apple, Maxwell, Galloway, Hamilton & Yancey, 2008; Irie & Sakimoto, 1992; Wiseman & Agunbiade, 1998). The highest content of PUFA is in general seen in the outer backfat layer.

1.3 Meat quality

Meat quality can be defined in several terms according to the purpose. A definition made by Andersen, Oksbjerg, Young & Therkildsen (2005) described meat quality as a complex and multivariate property of meat, which is influenced by multiple interacting factors including the conditions under which the meat is produced. Warriss (2000) divided meat quality in two types of quality; the functional quality referring to the characteristics of the meat and secondly the conformance quality meeting the consumers requirements. In more detail this includes yield and gross composition, appearance and technological characteristics, palatability, wholesomeness and ethical quality. The different quality characteristics may conflict with

each other. A solution suggested was to further divide the quality traits into three distinct qualities, the first taking care of the wholesomeness and that the product should be safe to consume, the second meeting the processing demands and the third covering requirements as appearance, convenience and eating quality (Kaufmann et al. 1990 cited by Warriss (2000)).
It is when the consumers recognize a quality trait and appreciate it, that the intended quality trait may be a competitive trait for food producers (Grunert, 2005). The quality has gained focus among the consumers the recent years. To meet the market requirements it is necessary to know the factors affecting quality. There are several ways of changing the intrinsic pork quality; this includes nutrition, genetic breed, sex, age, body weight, hormones, pre-slaughter handling, carcass treatment and processing. This thesis will focus on the sensory attributes colour, odour and flavour, technological quality of meat and fat and finally the nutritional quality.

1.3.1 Sensory quality

When a consumer's decision to purchase pork is made, the appearance is very important since it is the only way acceptability can be judged. The appearance is mainly dependent on colour, fat and drip loss. Meat colour is determined by to main factors; the state and content of haem myoglobin and secondly the muscle structure. The state of myoglobin can appear as purple red deoxymyoglobin (Mb), bright red oxymyoglobin (MbO2) or the brownish metmyoglobin (MetMb). Available oxygen will affect the colour by reacting with Mb towards MbO2 and make a brighter red colour. This is why blooming is used to standardize the colour measurement in meat.

Fat colour is dependent on the composition of fat tissue and can vary from clear white to light pink. It is suggested that increased unsaturation of fatty acids leads to a greyer appearance of fat tissues (Lebret & Mourot, 1998). Young animals have generally a higher content of unsaturated fatty acids than older animals and the incidence of discoloration towards grey or yellow is more common (Wood, 1984). Warnants, Van Oeckel & Boucque (1996) found a pink backfat colour in thin bakcfat layers. A high content of linoleic acid has shown a yellow fat colour (Maw, Fowler, Hamilton & Petchey, 2003).

Bryhni et al. (2003) found a correlation between the consumers satisfaction and consumption indicating a higher consumption when a higher quality of pork is available. Consumers are influenced by the appearance of pork but the palatability traits texture and flavour are also

important sensory traits (Aaslyng et al., 2007). Juiciness and tenderness is according to Risvik (1994) an easy and good way of describing texture. A newly finished marketing study with students in Tromsø discovered that young consumers have a preference towards healthy products that are easy to prepare (Roaldstveit, 2010). This study also emphasises the sensory traits; good odour and flavour, appearance and texture as contributing factors when the consumers make a decision.

1.3.2 Technological quality

The technological quality of fat can best defined by the firmness and the preferred fat is normally firm and white compared to soft, yellow and oily fat. Soft fat may lead to increased fat layer separation in loins. Soft fat can be recognized by a high content of PUFA (Madsen, Jakobsen & Mortensen, 1992). Several quality criteria's has been proposed concerning fat quality. These are mainly linked to individual fatty acids, ratios of fatty acids or combinations of fatty acids.

Iodine value gives an estimate on the overall unsaturation and is being used as a quality parameter of backfat. Lea, Swaboda & Gatherum (1970) suggested an iodine value below 65 to obtain good quality while a value higher than 70 was considered as soft fat. Later Barton-Gade (1987) concluded that as long as the iodine value was below 70 it would be of acceptable quality.

The fatty acid C18:0 (Wood, Jones, Bayntun & Dransfield, 1985) or the C16:0/C18:2*n*-6 ratio (Whittington, Prescott, Wood & Enser, 1986) has been proposed as good indicators of firmness. Girard (1988) also claimed that fat should contain at least 12% C18:0 to obtain good fat quality.

A high content of PUFA may reduce the oxidative stability since PUFA are more susceptible to oxidation than saturated fatty acids. Limitations in feed of 50 g PUFA/kg feed have been suggested to reduce problems with oxidation (Bryhni et al., 2002b). In a study with different dietary PUFA levels it was concluded that the maximum threshold of dietary PUFA should be limited to 18 g/kg and a maximum of 22% PUFA in backfat (Warnants, Van Oeckel & Boucque, 1996)

1.3.3 Nutritional quality

The pig has been blamed for a high fat content with a high content of saturated fat. The heritability for lean meat percentage is high across sex with heritabilities between 0.40 and 0.57 (Hallenstvedt & Pedersen, 2004). Norwegian Landrace had in 1933-1944 a backfat thickness of 35.1 mm, this was reduced to 34.1 mm in 1952/53 (Altern, 1955) and nowadays backfat thickness is reported to be around 15 mm (Overland, Kjos, Olsen & Skrede, 2005). Due to breeding schemes towards leaner breeds most pigs produced are lean with reduced fat content. Lower backfat thickness has shown a higher PUFA content (Wood et al., 2008).

The fatty acids C18:2*n*-6 and C18:3*n*-3 are essential fatty acids for humans. C18:3*n*-3 can be elongated and desaturated into VLC *n*-3 fatty acids C20:5, C22:5 and C22:6 but this process seems to be limited to produce C20:5*n*-3 and C22:5*n*-3 in humans (Brenna, Salem, Sinclair & Cunnane, 2009). There may be gender effects, men being less capable than woman to produce C22:6*n*-3 (Burdge, 2006). The positive health effects of VLC *n*-3 fatty acids in connection with cardiovascular diseases are well established (Baghurst, 2004). Providing dietary *n*-3 fatty acids the content will be deposited in the pork and increase the nutritional quality. Increased dietary *n*-3 fatty acids will also reduce the C20:4*n*-6. This is considered to be positive since C20:4*n*-6 has been reported to enhance the blood supply to cancer cells (Hyde & Missailidis, 2009).

1.4 Factors affecting sensory, nutritional and technological quality

1.4.1 Sex

The production of slaughter pigs are mainly based on castrated males and females. A ban of castration has been suggested in Norway and there may be some effects on meat quality traits apart from the well known boar taint (Babol, Squires & Gullett, 2002).

There has been shown significant differences in fatty acid composition in entire males and females, where females have shown a higher percentage of C18:1 and a lower percentages of C18:2*n*-6 and C18:3*n*-3 (Wood, Enser, Whittington, Moncrieff & Kempster, 1989). This difference may give reduced firmness in males compared to females.

The colour of fat may differ in the sexes. Tikk, Tikk, Karlsson & Andersen (2006) found lighter backfat colour in castrates than females and Sterten, Frøystein, Ekker & Kjos (2009) reported lighter meat colour in castrates than females indicating a sex differences in colour.

1.4.2 Feeding

Manipulation of meat quality by dietary means is possible and especially the effect of fatty acids composition is profound. Diets for pigs are generally low in fat, however, the fatty acid composition influence the firmness of adipose tissue, shelf-life of meat and fat, which further have an impact on flavour and colour (Wood et al., 2008).

There are a vide selection of fat sources used in pig diets, varying in fatty acid composition. The dietary fatty acid composition will readily change the fat composition in adipose tissue and the muscle of the animal. Fat sources are of vegetable or animal origin. The most common fat raw material used are summarised in Table 1. The different fat sources are suppliers of saturated, monounsaturated and polyunsaturated fatty acids of both the *n*-6 and *n*-3 families. Feeding saturated fat as coconut oil, palm and palm kernel oil or animal fat, with low iodine value, will increase fat firmness compared to unsaturated fat. This will also affect the storage ability of products from such fed pigs. Unsaturated fatty acids are more susceptible to oxidation and formation of off-flavours than saturated fat (Kanner, Shegalovich, Harel & Hazan, 1988). There are several compounds produced during oxidation. The components responsible for the characteristic rancid odour and flavour are, among others aldehydes, ketones and epoxides (Faustman, Sun, Mancini & Suman, 2010).

A reduction in human consumption of saturated fat is recommended. This is possible by using fat with more unsaturated fatty acids, however, care must be shown due to the higher risk of oxidation. Several research groups have studied the possible fortification of meat products with *n*-3 fat sources. Vegetable sources as linseeds, linseed oil, rapeseeds or rapeseed oil contribute with C18:3*n*-3 and improves the nutritional quality. An even better way is to use sources with high content of the very long chain *n*-3 fatty acids such as fish. This improves the nutritional quality of pork by a higher VLC*n*-3 fatty acid composition but an unwanted fish odour and flavour may occur, especially when the dietary fish oil inclusion is high (3%) (Overland, Taugbol, Haug & Sundstol, 1996).

Table 1. Fatty acid composition in different fat sources used in swine diets

	C12:0	**C14:0**	**C16:0**	**C16:1**	**C18:0**	**C18:1**	**C18:2 *n*-6**	**C18:3 *n*-3**	**C20:5 *n*-3**	**C22:6 *n*-3**
Coconut oil[1]	47.1	18.1	9.1		2.8	6.8	1.9	0.1		
Linseed oil[1]			5.3		4.1	20.2	12.7	53.3		
Olive oil[1]			9.0	0.6	2.7	80.3	6.3	0.7		
Palm oil[1]	0.3	1.1	42.9	0.2	4.6	39.3	10.7	0.4		
Palm kernel oil[1]	48.2	16.2	8.4		2.5	15.3	2.3			
Rapeseed oil[2]		0.1	4.4	0.4	1.8	59.4	19.8	9.6		
Soybean oil[1]		0.1	10.6	0.1	4.0	23.2	53.7	7.6		
Sunflower oil[1]		0.1	7.0	0.1	4.5	18.7	67.5	0.8		
Animal fat[2]	0.1	2.2	22.1	3.1	18.9	37.9	6.1	0.9		0.1
Fish oil - cod liver oil[1]		6.2	10.5	7.4	1.6	14.3	0.9	0.5	12.8	8.0
Fish oil - Salmon oil[1]		5.3	15.8	9.3	3.3	15.5	3.4	1.0	16.6	13.4

[1]Modified from Reese, 2003
[2]Unpublished data, Felleskjøpet Fôrutvikling

2. Objectives of the study

The main objective of this thesis was to evaluate the impact of feed on pork quality. This includes two sexes; entire males and females, raw materials of different fatty acid composition including *n*-6 and *n*-3 fatty acids and their metabolism in the pig. Based on this overall aim the specific objectives were:

- To determine a maximum dietary inclusion of *n*-3 fatty acid to obtain appropriate sensory quality and a healthier product for human consumption
- To examine the effect of fat level on deposition of the fatty acid
- To evaluate the sensory quality of short- and long-term frozen stored pork products with different fatty acid composition
- To evaluate the change in fat texture and fat colour due to fatty acid composition and sex (entire male and female)
- To gain more knowledge in fatty acid metabolism, especially elongation and desaturation within the *n*-6 and *n*-3 families of fatty acids

3. Materials and methods

Three feeding experiments (Exp) were conducted at the Animal Production Experimental Centre at Ås, Norway. A total of 72 pigs were used in each experiment including entire male and female pigs of the crossbred [(Norwegian Landrace x Yorkshire) x Duroc]. They were allotted according to litter, live weight and sex in a randomised block design. Individual and restricted feeding according to a feeding scale for growing-finishing pigs (Øverland, 1997) was used in all experiments. To obtain the dietary treatments fat raw materials such as soybean oil, fish oil and palm kernel oil were used (Table 2). Iodine value was calculated according to analyzed fatty acid profile (AOCS, 1998). Iodine value product (IVP) was calculated using the formula: IVP = percentage of fat x iodine value x 0.1

When reaching a target live weight the pigs were slaughtered at a commercial abattoir (Nortura Rudshøgda or Nortura Tønsberg).

Table 2. Use of low fat diet and different fat raw materials in the three experiments

	Low fat	**Soybean oil**	**Fish oil**	**Palm kernel oil**
Exp 1	+	+	+	–
Exp 2	–	+	–	+
Exp 3	+	–	+	+

Sampling procedures varied among experiments and included both meat and fat samples (Table 3). Fatty acid composition of outer and inner layers of fat taken from P2 location and shoulder fat were analyzed. The M. *longissimus dorsi* was analyzed for fatty acid composition divided in neutral lipids and phospholipids. Fat quality parameters as colour was measured by using two different methods, the traditional Minolta Chromameter and the PhotoBox, a fairly new method mainly used to evaluate colour of salmon fillets.

Table 3. Analyzed tissues to evaluate fatty acid composition and shoulder fat quality in the three experiments (Exp)

	Fatty acid profile						**Shoulder fat quality**			
	Backfat (P2)		**Shoulder fat**		***M. longissimus dorsi***		**Colour**		**Texture**	
Exp	Outer layer	Inner layer	Outer layer	Inner layer	Neutral lipids	Phospho lipids	Minolta	PhotoBox	TA-TX2	Firmness
1	+	+	–	–	+	+	–	–	–	–
2	+	+	+	+	–	–	+	+	+	+
3	–	–	+	+	+	–	+	+	+	+

Sensory profiling by a trained panel (Nofima Mat, Ås, Norway) was used to evaluate the meat quality. Different pork products; ribs, chops, meat balls and belly with two different storage times; short- and long-term have been used (Table 4).

Table 4. Products evaluated by sensory profiling in Exp. 2 and 3

	Meat balls		**Rib**		**Chops**		**Belly**		**Neck**
Storage	**Short**	**Long**	**Short**	**Long**	**Short**	**Long**	**Short**	**Long**	**Short**
Exp 2	+	+	+	+	+	+	–	–	–
Exp 3	–	–	–	–	–	–	+	+	+

The aim of feeding experiment 1 was to evaluate the biosynthesis of fatty acids, especially the *n*-6 and *n*-3 fatty acids. The production of experimental animals was without any supply of raw materials of animal origin from insemination of the sows until start of the feeding experiment. This included gestation diet, lactation diet and the piglet diet. This procedure with no animal raw material was to obtain piglets with as low percentages of VLC *n*-3 fatty acids as possible. At start of experiment 1, 12 young siblings of the experimental pigs were sacrificed. Fatty acid composition of backfat and of neutral and of phospholipids of LD were measured.

4. Main results and discussion

4.1 Influence of dietary fatty acid composition

Feeding low-fat diets in experiment 1 and 3 gave in our study in descending order C18:1, C16:0, C18:0, C16:1 and C14:0 in backfat and in neutral fat of *M. longissimus dorsi* (Paper II and IV). This is clearly in agreement with earlier results when low-fat diets have been used (Busboom, Rule, Colin, Heald & Mazhar, 1991; Ding, Lapillonne, Heird & Mersmann, 2003; Kloareg, Bellego, Mourot, Noblet & van Milgen, 2005; Leat, Cuthberson, Howard & Gresham, 1964). The reason is the increased *de novo* synthesis of fatty acids. The enhanced *de novo* synthesis when feeding low fat diets is most likely due to an increased insulin secretion when starch rich diets are given (Hillgartner, Salati & Goodridge, 1995; Jump, Botolin, Wang, Xu, Christian & Demeure, 2005). In our experiment a substantial *de novo* synthesis seems to occur even with a small dietary inclusion of fish oil (0.48%). Indications of a small decline in the synthesis in terms of reduced backfat content of the classical *de novo* synthesised fatty acids was, however, seen compared to diet with no added fat (Paper II). The reason might be that PUFA may be a suppressor of the *de novo* synthesis of fatty acids (Hillgartner et al., 1995; Jump et al., 2005; Sessler & Ntambi, 1998) .

In Experiment 3 two diets with the same fish oil inclusions but with different fat level were tested, one with only fish oil as added fat (LFF2) and one with palm kernel oil added (PK3F2) (Paper II). The low-fat diet resulted in lower percentages of VLC *n*-3 fatty acids in muscle and in backfat than the diet with added fat. A possible reason may be a higher digestibility of fat when higher dietary fat level is provided (Jorgensen, Jakobsen & Eggum, 1992) or less use of the *n*-3 fatty acids for energy production.

Soybean oil is rich in the essential fatty acid C18:2*n*-6, more than half of the fatty acids are found as C18:2. Feeding soybean oil clearly changed the fatty acid composition in fat and meat in pigs giving high content of C18:2*n*-6 (Paper I, III & IV). This has been shown previously when adding soybean oil to pig diets (Bee, Gebert & Messikommer, 2002). Our data also show an increased C20:4*n*-6 percentage when feeding soybean oil as shown by Bee et al. (2002) and Morgan, Noble, Cocchi & Mccartney (1992). Since soybean oil lack the C20:4*n*-6 the deposited C20:4*n*-6 is presumably synthesised from C18:2*n*-6.

Fish oil is the best source of the VLC *n*-3 fatty acids like C20:5*n*-3, C22:6*n*-3 and to less extent C22:5*n*-3. Using fish oil in pig diets all the VLC *n*-3 fatty acids, and especially C20:5*n*-

3 and C22:6*n*-3 increased in both backfat and muscle (Paper I, III & IV). This agrees with results from a recent study when tuna meal was used and a clear increase of the VLC *n*-3 fatty acids in steak, mince and sausages was found (Sioutis, Coates, Buckley, Murphy, Channon & Howe, 2008). The percentages of C20:5*n*-3 and C22:6*n*-3 was highest among the VLC *n*-3 fatty acids but also C22:5*n*-3 was found, indicating elongation from C20:5*n*-3. Also Overland et al. (1996) found a linear increase in the VLC *n*-3 fatty acids with dietary fish oil inclusion. Dietary fish oil also gave a substantial C22:5*n*-3 content indicating a synthesis from the other *n*-3 fatty acids.

Palm kernel oil consists of mostly saturated fatty acids and especially the medium short chain fatty acids. When feeding such fat to pigs (Paper I, II and III) we found rather low percentages of the medium short chain fatty acids in the products. Due to low incorporation in the body these fatty acids are most probably oxidized for energy use (Leyton, Drury & Crawford, 1987) or being metabolised into longer fatty acids.

4.2 Sensory quality

The trained sensory panel found no difference in the short-term (frozen stored up to 3 months, at -20˚C or up to 4 months at -80˚C) stored meat cuts or the processed product even if the fatty acid composition varied among the dietary groups (Exp 2 and 3, Paper I & III). Similar results have been shown by others evaluating fresh or short-term frozen stored products with different C18:3*n*-3 percentages (Van Oeckel, Casteels, Warnants, Van Damme & Boucque, 1996) and soybean and EPAnoil (EPA and DHA rich oil) (Morgan, Noble, Cocchi & Mccartney, 1992).

Ribs, pork chops, meat balls and belly were frozen stored for 6-9 months in experiment 2 and 3 (Paper I & II). Pork chops and meat balls were exposed to light to simulate normal retail conditions. The trained sensory panel still detected only minor effects between the dietary treatments. After long-term frozen storage ribs from pigs fed high IVP tended to be more rancid and have a more intense flavour, as well a significant more oily flavour. The lean pork chops were the product most affected by long-term storage. This was an unexpected result since at least C22:6*n*-3, as an indication of PUFA level, has shown reduced storage stability when bound in triacyl glycerols than in phospholipids (Lyberg, Fasoli & Adlercreutz, 2005;

Song, Inoue & Miyazawa, 1997). Higher total odour and flavour of the pork chops increased most probably due to the increased rancidity in meat from the highest dietary IVP value. Since the storage conditions with exposure to light for several hours a day the difference in sensory quality was somewhat expected. The findings are in agreement with earlier results by Bryhni, Kjos, Ofstad & Hunt (2002b) where loins from pigs fed approximately 50% dietary PUFA and frozen stored for 8 months showed a higher rancid odour and flavour compared to a low dietary PUFA fed group. In the same experiment sausage showed similar sensory trends. This is somewhat conflicting with the results obtained with meat balls in experiment 2 (Paper I). We found no effect of dietary treatment even if the dietary IVP varied from 48 to 99. Meat balls were in our study exposed to light for several hours daily and stored one month longer than the recommended maximum storage time to produce the worst case scenario. The mincing process to produce meat balls is expected to reduce the storage stability since the process increases the surface and thereby the exposure to oxygen leading to easier oxidation (Gray, Gomaa & Buckley, 1996). Oxidation has further been found as the main detrimental process in meat (Buckley, Morrissey & Gray, 1995). The meat balls were, however, seasoned and this may perhaps have disguised the off-flavour and –odour. In a study a trained panel was used to test and categorize loins and sausages into the categories "normal" and "rancid". The products were then evaluated by consumers. The consumers could not discriminate the categories of loin, however, "normal" sausages was preferred over "rancid" (Bryhni, Hunt & Ofstad, 2002a).

In experiment 3 (Paper II), with varying dietary fish oil level, belly meat was evaluated by the sensory panel. This showed only numerical differences among the dietary treatments, with increased fish oil odour and flavour and a decrease in meat flavour with higher fish oil inclusion. Using 1 and 3% fish oil in diets to pigs has previously given off-flavours and off-odour of flank, both fresh and stored frozen at -20 (Overland et al., 1996). In the same study the lard showed less undesired off-flavours and -odours than meat. Jonsdottir, Valdimarsdottir & Baldursdottir (2003) produced diets with up to 9 g fish fat/kg by using low-fat fishmeal and found higher incidences of off-flavour and –odour of meat and fat after 6 months frozen storage. They concluded that a maximum inclusion of fish oil should not exceed 3 g/kg feed in diets for finishing pigs to ensure appropriate pork quality. In another study fish silage was used in combinations with fish oil until slaughter and the highest content of 9.5 g fish oil/kg feed gave, after 6 months frozen storage increased off-taste and –odours of loin and off-taste

of bacon (Kjos, Skrede & Overland, 1999). In the same experiment the flank was sensory tested, but no indication of reduced sensory quality was found.

Belly from pigs fed the highest content of dietary fish oil was after reheating identified with higher rancid and fish oil flavour, however, no warmed over flavour (WOF) was observed. Warmed-over flavour has been suggested to be one of the main parameters to evaluate meat quality since the consumers are able to detect this parameter (Bryhni et al., 2002a). The WOF parameter is related to the oxidative status of the product and it is likely than WOF intensity increase with PUFA content (Jensen, Flensted-Jensen, Skibsted & Bertelsen, 1998). One possible explanation has been that the first heating partly degrades the antioxidants such as vitamin E and the product is less protected against oxidation.

When using a trained panel they may find differences a common consumer would have difficulties in detecting. Bryhni, Hunt & Ofstad (2002a) found that consumers did not detect a difference of 0.8 by the trained panel in rancid taste of LD samples. Based on our results and previously results it seems possible to used fish oil at an inclusion level of 0.5% without any reduced sensory quality even when the products are frozen stored for at least 6 months.

4.3 Backfat quality

The backfat quality has been evaluated using iodine value (calculated from the fatty acid composition), texture and colour. The iodine values in shoulder fat or backfat from the experiments ranged from 58.6 to 92.6. We have in all experiments found a good and linear correlation between the dietary iodine value and iodine value in backfat and in neutral lipids of LD. The correlation was, however, best when some added fat was used in the diets. Feeding very low dietary IVP diets seems to give a higher iodine value in the tissues (Paper II & III) while the extremly high dietary IVP gave slightly lower iodine values than in the feed (Paper III). Our results agrees with Warnants, Van Oeckel & Boucque (1996) who increased the dietary PUFA level and found a linear relationship to iodine value. Our results are also in accordance to a Danish experiment that found a good correlation between dietary IVP and the IV in backfat of pigs (Madsen et al., 1992).

Feeding influenced, as expected, the firmness scores of backfat. Feeding low IVP diets in experiments 2 and 3 gave firmer backfat than higher dietary IVP values (Paper III). Diets with

added fat gave higher firmness scores than the low fat diet even if the dietary IVP values were similar. This is an indication that the IVP to predict fat firmness gives a better estimate when a certain dietary fat level is used. Experiment 3 with different fat levels and fish oil inclusion showed a firmer backfat when added fat was used. The increased dietary fish oil inclusion did not affect the firmness. This is in accordance with a study using dietary linseed oil where no effects on fat firmness was found (D'Arrigo et al., 2002).

Few consistent differences in colour of shoulder fat in relation to the dietary treatments were found, even when a vide range in dietary fatty acid composition was used. Only the diet with rather extremely low IVP gave a slightly higher L*-value after 1 and 15 days of storage suggesting a whiter appearance than fat with higher dietary IVP values. Low fat diet gave higher Hue values after 15 days of storage with both colour measurement methods. In accordance with our results Teye, Wood, Whittington, Stewart & Sheard (2006) found no difference due to dietary means. Studies using 1 and 3 % dietary fish oil inclusion resulted in no colour change due to the altered fatty acid composition (Leskanich, Matthews, Warkup, Noble & Hazzledine, 1997; Overland et al., 1996). It seems that a dietary IVP of up to 99 or dietary fish oil inclusions up to 0.7% have no impact on the fat colour.

Meat colour was measured in the experiment with different fish oil inclusions but gave no colour change (Paper II) according to the dietary treatments as found when dietary PUFA content varied (Van Oeckel et al., 1996).

4.4 Fatty acid metabolism and deposition

In experiment 1 backfat and neutral lipids in muscle of young siblings contained a substantial percentage of C18:1 compared to the sows milk and the piglet diet provided (Paper IV). This is an indication of a *de novo* synthesis of fatty acids in the relatively small pigs. The piglet diet consisted of nearly 50 % of C18:2*n*-6 and a high deposition was seen in the phospholipids. Further, the longer C20:4*n*-6 was quite high in the phospholipids and also detected in the neutral fat of LD and in backfat. Since the piglet diet lacked C20:4*n*-6 and the sow milk was low in this fatty acid, the deposited C20:4*n*-6 most probably was a metabolite from C18:2*n*-6. In carcass a slight increase in the percentage of C20:4*n*-6 compared to the sow milk has been reported earlier (Bazinet, McMillan & Cunnane, 2003). Also backfat, muscle and especially plasma fatty acids composition have had detectable C20:4*n*-6

percentgaes even without any dietary supply of the fatty acid (Amusquivar, Sanchez, Hyde, Laws, Clarke & Herrera, 2008). This strongly suggest that piglets have a fairly good capacity for elongation and destauration of C18:2*n*-6.

Also the VLC *n*-3 fatty acids were found in the backfat, and in the neutral fat and especially in the phospholipids in LD muscle, even if no C22:6*n*-3 and only negligible percentages of C22:5*n*-3 and C20:5*n*-5 were contributed by the piglet diet or the sow milk. This further suggests an elongation and desaturation in piglets also of of the C18:3*n*-3 available from the diet as earlier found by Amusquivar et al. (2008).

In experiment 1 muscle lipids were analyzed for fatty acid composition in both neutral and in phospholipids (Paper IV). Young siblings of the experimental pigs were analyzed to give the general fatty acid composition in backfat and in neutral and phospholipids of LD at start of the experiment. Pigs fed on the low fat diet in our feeding experiment showed a higher percentage of MUFA, in particular C18:1, than the young siblings, and also than the soybean oil or the soybean oil and fish oil fed pigs. This indicates that *de novo* synthesis of fatty acids influence also the fatty acid composition of phospholipids, as seen earlier in backfat and neutral lipids (Paper II & III). The percentages of the VLC *n*-3 fatty acids C20:5 and C22:5 were slightly higher than at start of the experiment. Because the diet only contributed with C18:3*n*-3 of the *n*-3 fatty acids the ability to keep the level of these fatty acids in the muscle phospholipids suggest the presence of a capacity for elongation and desaturation of C18:3*n*-3 also in the growing pigs. As discussed below, the percentage of C22:6*n*-3, on the other hand seemed to be reduced as compared to the percentage found in the young siblings at start of the experiment.

Feeding soybean oil clearly increases the percentage of PUFA and C18:2*n*-6. The soybean oil also contributed with C18:3*n*-3. This fatty acid was highest in the soybean oil fed group and similar to the percentage at start. The percentages of the longer *n*-3 fatty acids C20:5 and C22:5 were, as in the low fat group, kept at similar levels as at start, while again a reduction in C22:6*n*-3 was seen. These results support the view that these pigs have a very limited capacity to produce C22:6*n*-3 from C18:3*n*-3. This has been suggested by Raes, De Smet & Demeyer (2004), while Enser, Richardson, Wood, Gill & Sheard (2000) concluded that C22:6*n*-3 also was produced and deposited in phospholipids.

The fish oil added diet introduced all the VLC *n*-3 fatty acids and they were deposited in muscle phospholipids at higher percentages than seen both at start and with the two other experimental diets. The main VLC *n*-3 fatty acids were now C20:5 and C22:6. That the ratio of C22:5*n*-3 to C20:5*n*-3 was now significantly reduced may suggest that the process of elongation from C20:5*n*-3 to C22:5*n*-3 was either saturated or inhibited by C22:6*n*-3. A slightly lower percentage of C20:4*n*-6 was observed when the dietary VLC *n*-3 fatty acid contribution was high.

So when no dietary VLC *n*-3 fatty acids are present in the diet it seems that C22:5*n*-3 is the main fatty acid produced from C18:3*n*-3. This is similar to a study with guinea pigs (Fu & Sinclair, 2000) and this same pattern of change in ratio between 20:5*n*-3 and 22:5*n*-3 can also clearly be seen from our results on analysis of total lipids from LD of slaughter pigs in experiment 3 (Paper II) .

4.4.1 Deposition of fatty acids into different tissues

Backfat from locations P2 (Paper I & IV) and shoulder (Paper II & III), divided in outer and inner layers were analyzed separately for fatty acid composition. The PUFA percentage was similar in both layers, while the outer layer had higher percentage of MUFA and a lower SFA percentage. The main fatty acids contributing to these differences were C18:1 and C18:0 with higher and lower percentage, respectively, in outer compared to inner layer. These results partly concur the results reported from another study where outer layer had the least SFA percentage but greatest MUFA, and the inner layer had the greatest proportion of PUFA (Apple et al., 2008).

4.5 The sex effect on meat quality and fatty acid composition

Only female pigs were selected for sensory analysis. This was because of the known boar taint in males that most probably would have influenced the sensory properties, as seen in a study with consumers (Babol et al., 2002).

Intra-muscular fat showed no clear differences due to sex. Females were found to have a higher intra muscular fat content in experiment 1 (Paper IV) while no such difference was found in experiment 3 (Paper II). Females have been reported to have a higher intra muscular

fat content (Channon, Kerr & Walker, 2004), but as inidicated by the differences in our study, this may perhaps not always be the rule.

Backfat quality in terms of firmness and colour (Paper III) was not consistently affected by sex and differed also among the experiments. These results confirms the study by Kempster, Dilworth, Evans & Fisher (1986) finding only slightly floppier fat in entire males but concluding that these effects were negligible. Fatty acid composition showed, however, some interesting differences. Males had in both backfat layers and neutral lipids of LD a higher PUFA percentage, in particular C18:2*n*-6 and C18:3*n*-3. The fatty acid composition in females was characterized by a consistently higher MUFA percentage with C18:1 as the prominent fatty acid, in outer and inner layer and neutral lipids of LD. Such differences have been reported by others but the main explanation has been the lower backfat thickness seen in males compared to females (Wood et al., 1989). In our three experiments no consistent difference in backfat thickness due to the sex was detected. It can thus be suggested that females have a higher delta-9 desaturase activity than males as seen in rats (Thorling & Hansen, 1995). The phospholipids showed very few significant differences in fatty acid composition between males and females.

4.6 Production and carcass parameters

The overall average daily gain ranged in our experiments from 891 to 1046 g pr day and the total feed consumption varied according to the energy density in the diets. It seems that pigs are able to adjust the feed intake according to energy content, as also observed earlier (Chiba, Peo, Jr., Lewis, Brumm, Fritschen & Crenshaw, 1985). The FCR (kg feed/kg gain) seemed to be slightly improved when using some added fat.

Backfat thickness measured at the cut surface at the ham lateral to the aitchbone in females was significantly higher in females than in males. Channon et al., (2004) reported similar results but they measured in the P2 location. Also earlier studies report females to have a higher backfat thickness than entire males as found by Ellis, Smith, Clark & Innes (1983).

No significant difference in lean meat percentage in male and female pigs was, however, seen in any of the three experiments conducted. In the early work by Wood (1982) and the results review by Lundström, Matthews & Haugen (2009) it was concluded that entire males are leaner than castrated males. Further females has been reported to be leaner than castrates

(Sterten, Frøystein, Ekker & Kjos, 2009). Högberg, Pickova, Stern, Lundström & Bylund (2004) reported a higher backfat thickness in castrates than both entire males and females. It can thus be suggested that the lean meat percentage is highest in males and possibly females, than in castrated males.

5. Conclusions and future perspectives

Generally, it can be concluded from the present study that the endogenous fat synthesis in growing pigs is lowered by fat added to the diets. It is also quite clear, and perhaps not very surprisingly, that the fatty acid composition of backfat, outer and inner layer, and neutral fat and phospholipids of LD changed according to the dietary treatments. The nutritional value of the pig products will therefore rely strongly on the type and amount of fat given in the diets. Of special interest is the capacity of the pig to elongate and desaturate the C18 *n*-6 and *n*-3 fatty acids available from the diet. Our results strongly suggest that the pig has the capacity to produce C20:4*n*-6 and C20:5*n*-3, and especially C22:5*n*-3, from the shorter chain precursors. But of importance are our findings a decline in the percentage of C22:6*n*-3 when this fatty acid was not present in the diet, suggesting that the capacity of the further process from 22:5*n*-3 to 22:6*n*-3 is, at the best, very limited. To ensure a good nutritional quality of the pig products, addition of low percentages (0.5%) of fish oil will therefore, as shown in our experiments, be a good solution.

Due to the higher risk of unwanted oxidation when pig contains increased amounts of PUFAs care must, however be taken in the use and storage of such products. In the sensory evaluation of short-term stored (-20 ˚C, 3 months) pork rib, chops, meat balls and short-term (-80 ˚C for two or four months) neck and belly from pigs given feeds with a wide variation in IVP and PUFA content or up to 0.7% dietary fish oil inclusion, no difference were, however, found. Only for the pigs fed the highest level of fish oil (0.7%), long-termed stored (-80˚ C for 12 months, -20 ˚C for 6 months) belly showed a slight increase in fish oil flavour, and reheating resulted in increased fish oil and meat odour and flavour.

Entire male pigs had in general higher PUFA and lower MUFA, in particular C18:1, in backfat and neutral lipids of LD than female pigs. This suggests a lower delta-9-desaturase activity in males. The fatty acid composition in phospholipids was less influenced by sex.

In general the outer backfat layer had higher percentages of PUFA and MUFA while the SFA was slightly lower than the inner backfat layer. The effects of different diets or sex on the backfat firmness and colour were low.

Some future perspectives linked to pork quality:

- To further define the possible use of dietary PUFA or fish oil inclusions in feed to ensure a healthy fat quality, especially in relation to antioxidants, types and levels
- To further check the possibilities to use other fish products like fishmeal and fish silage
- To gain more knowledge of the initial quality of raw materials used on the impact of pork quality. Although in broilers this has been studied and the fat quality of feed ingredients seemed to have little effect on meat quality, this is still an open question when considering pig feed
- The apparent higher deposition of *n*-3 fatty acids and thereby a conserving effect when given with added fat observed in our experiments is interesting, and should be further studied in more detail

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Papers I – IV

Paper I

Sensory quality of short- and long-term frozen stored pork products.
Influence of diets varying in polyunsaturated fatty acid (PUFA) content and iodine value

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Keywords: Pork quality; iodine value; fatty acid composition; storage stability; sensory

Abstract

Predicting aspects of pork quality is becoming increasingly important from a nutritional as well as a technological point of view. Here, the influence of increasing PUFA and iodine values (IV) in feed and pigs on sensory qualities of short- and long-term frozen stored products was investigated. Entire male and female grower finisher pigs were fed diets with iodine value products of 48 (LowIVP), 77 (MedIVP) or 99 (HighIVP) according to a restricted feeding scale. Ribs, chops and meat balls were short- (0-3 months) and long-term (6-9 months) frozen stored before sensory profiling. C18:2*n*-6 increased linearly in backfat with increased dietary inclusion. No negative effect on sensory quality was found in short-term stored products. After long-term storage the lean chops was the product most affected. Increasing the dietary IVP led to an increased rancid and total odour and flavour intensity, and to reduced meat and sour odour and flavour.

1. Introduction

Due to the importance of colour and technological quality of pig fat, especially in relation to ham production (Bosi, Cacciavillani, Casini, Lo Fiego, Marchetti & Mattuzzi, 2000), several limitations have been proposed to control the dietary inclusion of unsaturated fat. Iodine value gives an overall estimate of fatty acid unsaturation (Davenel, Riaublanc, Marchal & Gandemer, 1999) and has been used to control fat firmness (Lea, Swaboda & Gatherum, 1970). Originally the iodine number has its name after a titration method where halogen was added to the double bonds in unsaturated fatty acids. Methods of calculating the iodine value from the fatty acid profile from gas chromatography has been developed and has become more commonly used (Cd 1c-85 AOCS, 1998). In feed formulation the iodine value product, which is iodine value adjusted for the fat level, has shown good correlation to the iodine value in adipose tissue (Madsen, Jakobsen & Mortensen, 1992). Iodine value in backfat below 65 (Lea et al., 1970) or 70 (Barton-Gade, 1987) has been proposed to give appropriate fat

quality. The same iodine value can be obtained by using fats high in monounsaturated fatty acids (MUFA) or in PUFA. Since linoleic acid has been shown to be twelve times more susceptible to oxidation than oleic acid (Enser, 1974 as cited by Wood, 1984), storage stability of products from pigs with similar iodine values may, however, be expected to be significantly different depending on the contents of MUFA and PUFA.

The pig is a monogastric animal and changes the fatty acid composition in meat and adipose tissue according to the dietary fat composition (Wood et al., 2004), and the fatty acid composition of different pork products can affect the sensory quality as reviewed by Wood et al., (2003).

Bryhni et al., (2002) concluded that flavour was one of the most important sensory traits in pork, while Risvik (1994) stated that very low levels of off-flavours are essential for the consumers acceptance. Lipid oxidation is one of the main factors to deteriorate the colour, texture and nutritive value, and producing off-flavour and off-odour (Gray, Gomaa & Buckley, 1996; Kanner, 1994). Lipid oxidation is influenced by species, muscle type, type of dietary fat, antioxidant status, processing, storage time and storage conditions as reviewed by Morrissey, Sheehy, Galvin, Kerry & Buckley (1998). Since muscles and other pork products differ in fibre structure, fat content and antioxidant status there is reason to believe that they also differ in storage stability. Ground products, often with added salt are found to be more prone to oxidation (Gray et al., 1996) with reduced storage stability, as also seen for products low in antioxidant such as E-vitamin (Jensen, Lauridsen & Bertelsen, 1998).

Focus on technological quality has for many years led to requirements of low iodine value and high content of saturated fatty acids (SFA) in pig products. More recently, however, human health concerns have gained increasing consumer attendance, and from a nutritionally

53 point of view more unsaturated fatty acids are desirable. The polyunsaturated/saturated fatty
54 acid ratio in human consumption has been suggested to be between 0.4 to 1 to ensure a human
55 healthy lifestyle as reviewed by Hugo & Roodt (2007). Since pork is relatively lean it can,
56 with a somewhat higher PUFA content, actually be considered as a healthy protein source.
57
58 The overall aim of this experiment was to investigate the importance of dietary PUFA
59 contents on the sensory quality and storage stability different lean, fat and restructured pork
60 products. The role of iodine value as a feed and fat quality parameter, and its relation to
61 product storage stability was further of interest.
62
63 **2. Materials and methods**
64 **2.1 Animals and management**
65 The animals in the experiment were 72 crossbred [(Norwegian Landrace x Yorkshire) x
66 Duroc], 36 entire male and 36 female grower finisher pigs at an initial weight of 26.5 kg. The
67 pigs were allotted according to litter, initial weight and gender and placed in 12 pens, giving
68 24 animals per treatment. Pigs were individually fed according to a restrictive feeding regime
69 (Øverland, 1997), each pig was considered as an experimental unit. After each meal, excess
70 feed was collected for each pig, weight and withdrawn from the total individual feed intake.
71 At a live weight of approximately 106 kg the pigs were slaughtered at a commercial abattoir
72 (Nortura Rudshøgda, Norway). To reach less variation in live weight at slaughter, pigs were
73 slaughtered in three groups according to when they reached the targeted slaughter weight. The
74 animals were treated in accordance with the guidelines outlined in Norway (the Animal
75 Protection Act of December 20th, 1974).
76
77
78

2.2 Dietary treatments

Three experimental diets were formulated to meet the requirements of grower-finisher pigs, and with three different levels of iodine value products. Iodine value product (IVP) was calculated as IVP = percentage of fat x iodine value x 0.1. The basal diet consisted of barley, oat and soybean meal, and different blends of soybean oil and palm kernel oil: 1.7/39.7, 24.0/17.4 and 34.0/3.3, resulted in IVP levels of 48 (LowIVP), 77 (MedIVP) and 99 (HighIVP). The compositions, analyzed and calculated contents of the experimental diets are presented in Table 1 and Table 2. The content of Vitamin E (*dl*-α-tocopheryl acetat) was 210 mg/kg in all the experimental diets. The diets were analyzed for water content (EU DIR 71/393 m), crude protein (EU DIR 93/28 m), crude fat (EU DIR 98/64 m), crude fiber (EU DIR 92/89 m), total ash (EU DIR 71/250 m), fatty acid composition (AOCS, Ce 1b-89) and iodine value was calculated based on the fatty acid composition (AOCS, Cd 1c-85).

2.3 Collection of samples

One day after slaughter the carcasses were cut into primal cuts. Outer backfat layer of approximately 3x3 cm from the P2 location (Overland, Rorvik & Skrede, 1999) was collected, vacuum packed and frozen at -80 °C until fatty acid. Analysis was conducted within six weeks after the first slaughter day.

Samples of pork rib, deboned loin (M. *Longissimus dorsi*) and minced fat and lean (230 g/kg fat, for production of meat balls) were taken from female carcasses for sensory analysis. Rib (n=18), loin (n=16) and minced fat and meat (n=18) were wrapped in plastic bags and stored at -20 °C for 3 months. After 3 months 6 ribs two from each dietary treatment, were sensory tested. The second sensory test was conducted after additional 6 months at -20 °C without any light. After 3 months all loins were cut into pork chops and wrapped in plastic bags and the first sensory test was conducted on chops from 4 animals. The rest of the chops were stored

105 for additional 6 months at -20 °C in light (2000 lux illumination) each day from 9h to 23h to
106 simulate normal retail display. The minced fat and lean, 3 months frozen stored, was mixed
107 according to dietary treatment and used to produce meat balls. The meat balls contained 179
108 g/kg fat and 17 g/kg salt. The first sensory test was conducted after processing. The rest of the
109 meat balls were wrapped in plastic bags and stored at -20 °C for 7 months in light (2000 lux
110 illumination) each day from 9h to 23h to simulate normal retail display.
111
112 **2.4 Fatty acid composition**
113 Backfat samples from the P2 location were separated in outer and inner layers, and the outer
114 layer was analysed for fatty acid composition by gas chromatography (Hallenstvedt, Kjos,
115 Rehnberg, Øverland & Thomassen (2010). Each fatty acid was calculated from the
116 chromatograms including an internal standard, and given as g/100g of fatty acids. The iodine
117 value was calculated on each sample using the fatty acid profile (AOCS, Cd 1c-85).
118
119 **2.5 Sensory analysis**
120 In each sensory test conducted at Nofima Food, Norway, a trained panel of 10 members was
121 used to judge the odour, flavour and texture parameters of the products. Sensory tests
122 (Modified ISO 6564) of pork rib, pork chops and meat balls were conducted after both short-
123 and long-term frozen storage. The responses of the panellists were expressed in numerical
124 values ranging from 1 = no intensity to 9 = the highest intensity. Each panellist was served the
125 product on white plates twice in each test, randomized according to treatment and panel
126 member.
127
128 Two days before the sensory test the ribs were thawed over-night in refrigeration room at
129 about 4 °C. The day before the test the ribs were roasted to a core temperature of 75 °C and
130 quickly cooled down. The whole rib was further cut into standardized pieces, vacuum packed,

individually numbered and stored over night at 4 °C in a refrigeration room. Before serving, the rib pieces were heated in water bath at 75 °C for 40 min.

One day before the sensory test, pork chops and meat balls were thawed over-night in a refrigeration room at about 4 °C. On the day of sensory profiling the pork chops and meat balls were vacuum-packed separately in plastic bags and numbered. The samples were heated in water bath at 72 °C for 30 minutes and cut in two before serving.

2.6 Statistics

All the statistics were carried out with a general linear model in Minitab 15 software (Minitab Inc, PA, USA) with the factors sex (entire male, female) and diet (LowIVP, MedIVP, HighIVP). For the production parameters start weight and experimental days were included as covariates in the model. Only female pigs were used for sensory profiling so only diet was included in the statistical model used. The Tukey test was used to reveal differences among treatments. *P*-values below 0.05 are considered as significant, but for the sensory parameters the trends with *P*-values below 0.1 are also indicated.

3.0 Results

3.1 Growth and feeding performance

The main pig production parameters are shown in Table 3. Generally few sex differences were observed. A higher feed intake (both kg and feed units) in female than in entire male pigs was seen ($P<0.01$). Pigs given the LowIVP diet had slightly lower feed conversion ratio (FCR, measured as kg feed/kg weight gain) than the MedIVP and HighIVP diet fed pigs.

157 **3.2 Fatty acid composition**

158 The fatty acid composition in outer backfat layer was significantly influenced by sex (Table
159 4). The total SFA was similar, but males had significantly (P<0.001) higher C12:0 and C14:0
160 than female pigs. Males had also significantly less C18:1 and total MUFA and higher
161 proportion of C18:2*n*-6, C18:3*n*-3 and total PUFA (P<0.001). In general females had slightly
162 lower (P<0.05) iodine value than males in the outer backfat layer.

163

164 The dietary treatments had a great impact on the fatty acid composition in backfat, reflecting
165 the dietary fatty acid composition in a dose-dependent manner for several fatty acids. The
166 LowIVP diet group had significantly higher proportions of the medium-chain fatty acids
167 C12:0, C14:0 and C16:0 and thereby SFA content. The fatty acid C16:1 (originating from *de*
168 *novo* synthesis) was significantly different among all groups and highest in the LowIVP
169 group. Total MUFA was highest (P<0.001) in the LowIVP group compared to the MedIVP
170 and HighIVP groups. The percentage of C18:1 was also higher (P<0.001) in the LowIVP
171 group compared to MedIVP and HighIVP. By increasing the dietary IVP, the proportions of
172 C18:2*n*-6 (Fig. 1), C18:3*n*-3 and total PUFA increased. No C20:4*n*-6 was present in the diets,
173 but a significant increase in outer backfat layer was found with increasing dietary IVP. The
174 iodine value showed a linear effect giving low IV in the LowIVP fed group and highest IV in
175 the HighIVP group (P<0.001).

176

177 **3.3 Sensory analysis**

178 The results of sensory profiling are shown for short- and long-term frozen stored pork ribs
179 (Table 5), pork chops (Table 6) and meat balls (Table 7). No differences were found in any of
180 the short-term stored products. Long-term stored pork ribs showed only few differences due
181 to diet. The HighIVP fed group had sweeter and more oily flavour (P<0.05) than the LowIVP
182 fed group. The chops, the leanest product tested, were the most affected by long-term storage.

The HighIVP group scored highest on the less preferred sensory attributes after long-term storage. The rancid odour increased linearly and significantly ($P<0.001$) with increasing dietary IVP values, while the rancid flavour was lower ($P<0.01$) in the LowIVP than in the MedIVP and HighIVP fed groups. Chops from HighIVP fed pigs had significantly lower metal odour intensity ($P<0.05$) and lower meat and sour ($P<0.01$) odour intensity than the other groups. The flavour parameters meat, sweet, salty and metal were all significantly lower in the HighIVP group. Both odour and flavour intensity ($P<0.01$) showed linear increase with dietary iodine values with highest score in the HighIVP group. Somewhat unexpectedly long-term stored meat balls were not significantly influenced by dietary treatments. Only a slight tendency of higher odour and flavour intensity ($P<0.10$) could be seen in the HighIVP compared to the MedIVP and LowIVP groups.

4.0 Discussion

4.1 Growth and feeding performance

Few significant dietary effects were observed on growth and feed intake in the present study. Only a slight increase in feed conversion ratio was seen with increasing PUFA content, which may suggest that the pigs react metabolically to the fatty acid composition of the diets. Our results further showed that female pigs had a higher daily feed intake in both kilos and feed units than entire males. Several studies have shown that entire males eat less than castrated males, as sited by Lundström, Matthews & Haugen (2009), and other studies have shown that females eat less than castrated males (Serrano, Valencia, Nieto, Lázaro & Mateos, 2008; Sterten, Frøystein, Ekker & Kjos, 2009; Latorre, Lazaro, Gracia, Nieto & Mateos, 2003). It thus seems that feed intake is generally highest in castrates, followed by females while entire males have lowest intake. In most cases the excess energy intake in females than in entire males is found to be deposited as fat. Male pigs often show a higher lean content than females (Babol & Squires, 1995) and females have been observed to have a higher capacity for lipid

synthesis (Eguinoa, Brocklehurst, Arana, Mendizabal, Vernon & Purroy, 2003). Such differences were, however, not observed in our experiment.

4.2. Fatty acid composition in backfat

The analysis of backfat fatty acid composition revealed a considerably lower percentage of MUFA, and especially C18:1, in males than females. This has previously been found in backfat (Wood, Enser, Whittington, Moncrieff & Kempster, 1989) and also in M. *longissimus dorsi* (Cameron & Enser, 1991; Hallenstvedt et al., 2010). Wood et al., (1989) suggested that the difference in MUFA content was because of the the higher backfat level in females. In the current experiment no such difference in backfat thickness was, however, found. Another possible explanation can be that females have a higher delta-9-desaturase activity than entire males. Female steroid hormones have in rats been shown to influence the fatty acid composition, including higher percentages of 18:1 (Thorling & Hansen, 1995), and similar effects were observed in the fatty acid profile in foals (Sarriés, Murray, Troy & Beriain, 2006). No similar difference has been observed in intramuscular fatty acid composition (Ntawubizi, Raes, Buys & De Smet, 2009; Zhang, Knight, Stalder, Goodwin, Lonergan & Beitz, 2007) or in subcutanous fat (Peinado, Medel, Fuentetaja & Mateos, 2008) when castrated male and female pigs were compared.

As shown also by others (Barton-Gade, 1987), increasing the dietary iodine value led to increased iodine value in the pig backfat. This is most probably related to the a high PUFA uptake in backfat (Kloareg, Noblet & van Milgen, 2007; Warnants, Van Oeckel & Boucque, 1996) and especially the essential fatty acid C18:2*n*-6 (Kouba, Enser, Whittington, Nute & Wood, 2003; Wood, 1984). In the present study we demonstrated a linear deposition of C18:2*n*-6 with no indication of saturation even at the highest dietary C18:2*n*-6 percentage. High dietary IVP due to increased PUFA content, resulted in lower proportions of C16:0 and

C18:1, most possibly due to the previously observed suppression of fatty acid synthesis and lipogenic enzymes by PUFA (Hillgartner, Salati & Goodridge, 1995). The percentage of the traditionally *de novo* synthesised fatty acids C16:0, C16:1, C18:0 and C18:1 were, however, still higher than in the diets used in our study, indicating a significant endogenous fatty acid synthesis even with rather high dietary fat content. Despite feeding the pigs high proportions of C10:0 and C12:0 only small amounts were found in backfat, as also reported previously (Teye, Wood, Whittington, Stewart & Sheard, 2006). This may be because of low incorporation of short chain fatty acids into triacylglyserols (Knittle & Hirsch, 1965) in backfat, but elongation and desaturation of these fatty acids can perhaps also have contributed to the proportion of the *de novo* synthesised fatty acids.

4.3 Sensory quality

The sensory analysis of the short-term frozen stored pork rib and pork chops, and meat balls freshly made from frozen stored minced fat end lean were not influenced by our feeding treatments, in spite of the significant difference in dietary fatty acid composition and iodine value, and the corresponding changes in backfat fatty acid composition and iodine value. Similarly Houben & Krol (1980) found no negative off-flavours in freshly prepared products but storage up to two months gave higher incidence of rancid off-flavours after feeding pigs diets containing up to 8,5 % soybean oil. In two other studies reported, linseeds were used to reach 35 g/kg dietary α-linolenic acid (Ahn, Lutz & Sim, 1996), and a combination of 4.5 g/kg dietary α-linolenic acid and 10 g/kg dietary linoleic acid (Sheard, Enser, Wood, Nute, Gill & Richardson, 2000). In the first study freshly cooked loins were stored three or 48 hours at 4 °C before sensory profiling and only minor difference to control were found after 3 hours, however, 48 hours storage led to a detrimental effect on the acceptability of pork loins. In the study by Sheard et al. (2000) loin chops, bacon and sausage were frozen stored up to 6 months and only minor differences to the controls were found in the sensory evaluation. Even

fresh pork chops from pigs fed high fishmeal levels (13%) were comparable to chops from the negative control group in the sensory test (Valaja, Suomi, Alaviuhkola & Immonen, 1992). Based on the earlier findings and the current experiment, it may be concluded that a substantial difference in dietary fatty acid composition and IV values can be used without any detrimental effects on sensory attributes when short-term frozen storage is used.

In our experiment only minor effects on sensory attributes were observed on long-term frozen stored pork ribs. Meat from pigs given the diet with the highest IVP appeared to be somewhat sweeter and had an increased oily flavour, while no significant difference in rancidity was detected.

Somewhat surprisingly the lean chops were the product mostly affected by frozen storage. In this product increasing the dietary iodine value resulted in significantly higher total odour and flavour intensity, mainly due to increased rancidity. Similar results were found by Bryhni, Kjos, Ofstad & Hunt (2002). Loin samples from pigs fed a high PUFA diet (50% PUFA of total fat) were slightly more rancid (odour and flavour) after 8 months storage than the low PUFA (31% PUFA of total fat) samples. It seems that the higher rancidity is followed by reduced meat odour and flavour, this has also been reported earlier (Bryhni, Hunt & Ofstad, 2002), but also the sour odour and flavour were reduced in our study. To what extent the increased rancidity may camouflage other taste parameters is not clarified.

The long-term stored processed meat balls showed surprisingly few negative effects even when produced from meat and fat from the highest iodine product value group. Only a tendency towards rancid odour and reduced meat flavour with increasing dietary iodine value was seen. Gray, Gomaa & Buckley (1996) suggested restructured meat to be more susceptible to lipid oxidation, which has been shown to be the most affecting process to reduced meat

quality (Buckley, Morrissey & Gray, 1995). Only a few reports are found on this kind of products, but Tikk, Haugen, Andersen & Aaslyng (2008) tested meat balls from pigs fed palm oil or rapeseed oil and found higher incidents of rancid taste in the rapeseed group after only 4 days of cold storage. They also found reduced metal flavour and sour taste. It is possible that the low incidence of off-flavour and odour after frozen storage observed in our study can be due to the seasoning used in the processing. The flavours can perhaps to some extent hide less desired flavour and odour.

The iodine values in diet and fat are dependent on the proportion of unsaturated fatty acids. To what extent diets and products with similar iodine values obtained by different combinations in MUFAs and PUFAs relates to differences in storage stability is at present not completely clarified. Most probably will increased PUFA give products more prone to lipid oxidation. Linoleic acid is, as shown in this study, easily deposited in pigs and linoleic acid has been shown to be susceptible to oxidation (Wood, 1984). Why the leanest product tested in our experiment gave the most negative results is not clear, but Hernández, Navarro & Toldrá (1999) concluded that phospholipids in M. *longissimus dorsi* were the most susceptible to lipid oxidation and produced free fatty acids, while the non-polar lipids were rather stable when the products were vacuum packed and frozen stored for 6 months. This observation does, however, partly conflict with earlier results where several frozen stored products with different fat levels were tested and no correlation between oxidation and fat level was found. Sheard et al., (2000) found highest incidence of oxidation in bacon but not in loin chops, liver and sausages produced from pigs fed a high linseed diet. In our experiment a relatively high dietary vitamin E content was used and it may have prevented lipid oxidation as reported by Guo et al., (2006) with similar dietary vitamin E inclusion and high PUFA content.

Conclusions

In conclusion, pig backfat reflects the dietary fatty acid composition, with a linear incorporation of C18:2*n*-6 even with the highest dietary inclusion. The backfat iodine value further increased with increased dietary iodine value, but with a slight deviation from linearity, mainly due to less endogenous production of C18:1. The sensory quality of short-term stored pork rib, chops and meat balls were not influenced by the dietary fatty acid composition or the iodine value. Only small influences of long-term storage on rib and meat balls were observed. Pork chops, the leanest product tested were negatively influenced by long-term frozen storage giving higher rancidity and reduced meat and sour odour and flavour with increasing dietary IVP. The results obtained in the present study, comparing the influence of increasing the PUFA content in the diet on the frozen storage stability of several pig products, further show that this important improvement in nutritional quality is possible. Care must, however, be taken when long-term frozen storage is intended, and more detailed knowledge on the relationship between PUFA contents and product stability is necessary.

Acknowledgement

We gratefully acknowledge financial support by The Research Council of Norway, The Foundation for Research Levy on Agricultural Products, Animalia, Norwegian Meat and Poultry Research Centre and Felleskjøpet Fôrutvikling. Acknowledgments are also given to Norsvin and The Animal Production Experimental centre staff at the Norwegian University of Life Sciences, Ås, Norway.

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Table 1

Experimental diets, calculated and analyzed contents (g/kg)

	LowIVP	MedIVP	HighIVP
Ingredients			
Barley	553	548	579
Oat	200	200	169
Soybean meal	156	161	159
Soybean oil	1.7	24.0	34.0
Palm kernel oil	39.7	17.4	3.3
Limestone	15.0	15.0	15.0
MCP	11.4	11.5	12.1
NaCL	6.1	6.1	6.2
Maize gluten meal	0.0	0.0	5.0
DL-Methionin	0.8	0.8	0.8
L-Lysine	3.0	3.0	3.2
L-Threonin	1.2	1.2	1.2
Acid	10.0	10.0	10.0
Vitamin-mineral premix[A]	2.1	2.0	2.2
Calculated content			
Metabolizable energy MJ kg^{-1}	12.7	12.9	13.1
Net energy (FU)[B]	1.06	1.07	1.09
Analyzed content			
DM g/kg	872	87.1	87.4
Crude protein g/kg DM	178	181	181
Crude fat g/kg DM	80.3	78.2	80.7
Crude fibre g/kg DM	64.2	60.8	56.1
Total ash g/kg DM	58.5	59.7	60.6

[A]Vitamin-mineral premix provided the following amounts per kg of feed: 134 mg Fe, 125 mg Zn, 75 mg Mn, 18 mg Cu, 1 mg I, 0.3 mg Se, 9000 I.U. vitamin A, 1125 I.U. vitamin D_3, 5.6 mg B_2, 19 mg pantothenic acid and 25µg B_{12}.

[B]Feed units (FU) estimated from CVB (2005) tables

Table 2

Fatty acid composition (g/100 g fatty acids) in the experimental diets

	LowIVP	MedIVP	HighIVP
C10:0	1.7	0.8	0.2
C12:0	26.4	11.6	2.2
C14:0	9.5	4.1	0.9
C16:0	13.4	13.5	14.1
C16:1*n*-7	0.1	0.1	0.1
C18:0	2.2	2.4	2.5
C18:1[A]	20.9	23.1	23.6
C18:2*n*-6	21.4	38.5	49.6
C18:3*n*-3	1.7	3.8	5.1
C20:1*n*-9	0.4	0.4	0.4
C20:4*n*-6	<0.01	<0.01	<0.01
C20:5*n*-3	<0.01	<0.01	<0.01
C22:5*n*-3	<0.01	<0.01	<0.01
C22:6*n*-3	<0.01	<0.01	<0.01
SFA	53.2	32.4	19.9
MUFA	21.4	23.6	24.1
PUFA	23.1	42.3	54.7
*n*6:*n*3	12.6	10.1	9.7
P:S[B]	0.5	1.4	3.2
Iodine value (IV)	60	97	120
Iodine value product (IVP)	48.3	76.8	98.6

[A]Sum of *n*-7 and *n*-9

[B]P = C18:2 + C18:3, S = C12:0 + C14:0 + C16:0

Table 3
Production parameters showed for sex and diet

	Sex				Diet				
	Entire male	Female	Sed[A]	Sign[B]	LowIVP	MedIVP	HighIVP	Sed[A]	Sign[B]
Number of pigs	34	23			16	21	20		
Final weight, kg	106	107	0.97	ns	108	106	106	1.14	ns
Growth pr day, g	1023	1036	14.4	ns	1046	1026	1018	18.0	ns
Feed intake, kg	164	171	2.21	**	167	168	167	3.58	ns
Feed intake, FU[C]	175	183	2.35	**	177	180	180	3.83	ns
FCR kg/kg weight gain[D]	2.20	2.26	0.02	ns	2.17[a]	2.25[b]	2.27[b]	0.04	*
FCR FU/kg weight gain[D]	2.06	2.12	0.03	ns	2.05	2.11	2.10	0.03	ns

[A]Standard error of difference
[B]Significance of differences between samples in each row. ns: $p>0.05$; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$
[C]Feed units (FU) estimated from CVB (2005) tables
[D]Feed conversion ratio

Table 4
The fatty acid composition (g/100 g fatty acids) in outer backfat layer from entire males and females fed different experimental diets

	Sex				Diet				
	Entire Male	Female	Sed[A]	Sign[B]	LowIVP	MedIVP	HighIVP	Sed[A]	Sign[B]
C10:0	0.10	0.10	0.01	ns	0.10	0.10	0.09	0.01	ns
C12:0	1.20	1.03	0.04	***	2.04[c]	1.02[b]	0.30[a]	0.05	***
C14:0	3.60	3.33	0.06	***	5.67[c]	3.22[b]	1.52[a]	0.07	***
C16:0	21.8	22.1	0.27	ns	24.2[c]	21.7[b]	19.9[a]	0.35	***
C16:1*n-7*	2.30	2.30	0.10	ns	3.11[c]	2.11[b]	1.69[a]	0.12	***
C18:0	11.0	11.1	0.29	ns	11.3	10.9	11.0	0.37	ns
C18:1[C]	33.6	35.7	0.33	***	36.2[b]	34.1[a]	33.6[a]	0.42	***
C18:2*n-6*	21.1	19.4	0.40	***	12.9[a]	21.7[b]	26.1[c]	0.50	***
C18:3*n-3*	1.71	1.56	0.03	***	0.93[a]	1.76[b]	2.21[c]	0.04	***
C20:1*n-9*	0.65	0.66	0.02	ns	0.67	0.64	0.64	0.02	ns
C20:4*n-6*	0.25	0.25	0.01	ns	0.23[a]	0.26[ab]	0.27[b]	0.02	*
C20:5*n-3*	<0.01	<0.01			<0.01	<0.01	<0.01		
C22:5*n-3*	0.09	0.09	0.01	ns	0.08	0.09	0.10	0.01	ns
C22:6*n-3*	0.05	0.07	0.01	ns	0.04[a]	0.05[ab]	0.09[b]	0.02	*
∑ SFA	37.8	37.6	0.49	ns	43.4[c]	36.9[b]	32.8[a]	0.62	***
∑ MUFA	36.5	38.6	0.37	***	40.0[b]	36.8[a]	35.9[a]	0.47	***
∑ PUFA	23.2	21.3	0.43	***	14.2[a]	23.8[b]	28.8[c]	0.55	***
*n*6:*n*3 ratio	11.8	11.7	0.19	ns	12.6[c]	11.6[b]	11.0[a]	0.25	***
P:S ratio[D]	0.92	0.85	0.03	*	0.43[a]	0.90[b]	1.31[c]	0.04	***
Iodine Value	76.2	74.6	0.77	*	62.3[a]	77.6[b]	86.4[c]	0.98	***

[A]Standard error of difference
[B]Significance of differences between samples in each row. ns: $p>0.05$; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$
[C]Sum of *n-7* and *n-9*
[D]P = C18:2 + C18:3, S = C12:0 + C14:0 + C16:0

Table 5
Sensory profile of ribs after short- and long-term frozen storage

	Short-term					Long-term				
	LowIVP	MedIVP	HighIVP	Sed[A]	Sign[B]	LowIVP	MedIVP	HighIVP	Sed[A]	Sign[B]
Odour										
Intensity	6.15	6.09	6.25	0.12	ns	6.14[a]	6.60[b]	6.26[ab]	0.16	*
Meat	4.31	4.34	4.12	0.08	ns	4.04	3.39	4.09	0.44	ns
Sour	3.37	3.70	3.37	0.26	ns	3.09	2.57	3.22	0.43	ns
Stale	2.22	2.17	2.36	0.15	ns	2.55	2.97	2.51	0.36	ns
Metal	3.98	3.94	3.90	0.11	ns	3.71	3.62	3.65	0.13	ns
Rancid	1.69	1.84	2.01	0.14	ns	2.29	3.58	2.51	0.39	ns
Flavour										
Intensity	6.14	6.27	6.22	0.10	ns	6.17	6.52	6.38	0.11	t
Meat	4.52	4.40	4.47	0.31	ns	4.01	3.60	4.14	0.45	ns
Sour	3.69	3.46	3.40	0.32	ns	3.07	2.54	3.24	0.43	ns
Sweet	2.90	2.93	2.92	0.07	ns	2.49[a]	2.48[a]	2.76[b]	0.09	*
Salty	2.27	2.51	2.47	0.05	ns	1.83	1.81	1.89	0.03	ns
Bitter	3.41	3.52	3.42	0.07	ns	3.66	3.69	3.55	0.16	ns
Metal	3.95	4.05	3.88	0.06	ns	3.82	3.80	3.83	0.12	ns
Rancid	1.77	2.56	2.08	0.26	ns	2.47	4.03	2.79	0.50	t
Oily	2.32	2.86	2.40	0.15	ns	2.60[a]	3.51[b]	3.24[b]	0.27	*
Texture										
Hardness	4.74	4.55	6.33	0.44	ns	4.84	4.45	4.43	0.17	ns
Tenderness	5.15	5.46	5.02	0.31	ns	4.91	5.38	5.58	0.32	ns
Fattiness	5.52	5.98	5.38	0.22	ns	5.22	5.74	5.63	0.18	ns
Juiciness	5.05	5.42	4.93	0.23	ns	4.86	5.18	5.38	0.25	ns

[A]Standard error of difference
[B]Significance of differences between samples in each row, ns: $p>0.10$; t: $p<0.10$; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$

Table 6
Sensory profile of pork chops after short- and long-term frozen-storage

	Short-term				Long-term				
	LowIVP	MedIVP	HighIVP	Sign[B]	LowIVP	MedIVP	HighIVP	Sed[A]	Sign[B]
Odour									
Intensity	6.42	6.24	6.69	ns	6.56[a]	6.97[ab]	7.44[b]	0.18	**
Meat	4.25	4.65	3.79	ns	3.74[b]	3.13[b]	2.30[a]	0.31	**
Sour	3.44	3.96	3.09	ns	2.99[b]	2.48[b]	1.79[a]	0.26	**
Stale	2.45	2.05	2.52	ns	2.55[a]	3.10[ab]	3.45[b]	0.28	*
Metal	3.79	3.84	3.89	ns	3.36[b]	3.37[b]	2.99[a]	0.10	*
Piggy	2.80	2.24	2.84	ns	3.48	3.50	3.28	0.22	ns
Rancid	1.96	1.69	2.99	ns	2.91[a]	4.41[b]	5.72[c]	0.45	***
Flavour									
Intensity	6.27	6.15	6.36	ns	6.50[a]	6.89[ab]	7.38[b]	0.16	**
Meat	4.50	4.85	4.25	ns	4.15[c]	3.41[b]	2.53[a]	0.26	***
Sour	3.92	4.33	3.34	ns	3.24[b]	2.46[a]	1.89[a]	0.24	**
Sweet	2.69	2.67	2.75	ns	2.72[b]	2.62[b]	2.32[a]	0.05	**
Salty	1.93	2.01	1.97	ns	2.31[b]	2.34[b]	2.11[a]	0.06	*
Bitter	3.45	3.45	3.52	ns	3.84	3.94	4.29	0.15	t
Metal	3.85	3.81	3.92	ns	3.52[b]	3.42[b]	3.09[a]	0.07	**
Piggy	2.12	1.92	2.28	ns	3.03	3.28	3.11	0.18	ns
Rancid	2.12	2.17	3.14	ns	3.44[a]	5.45[b]	6.21[b]	0.59	**
Oily	2.08	2.77	2.80	ns	2.91	3.59	3.55	0.31	ns
Texture									
Hardness	4.68	4.82	4.49	ns	4.62	4.71	4.65	0.12	ns
Tenderness	5.04	4.98	5.26	ns	4.89	4.83	4.85	0.19	ns
Fattiness	4.64	5.03	5.27	ns	4.32	4.48	4.32	0.22	ns
Juiciness	4.55	4.36	4.71	ns	4.41	4.25	4.18	0.23	ns

[A]Standard error of difference
[B]Significance of differences between samples in each row. ns: $p>0.10$; t: $p<0.10$; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$

Table 7
Sensory profile of meat balls after short- and long-term frozen storage

	Short-term					Long-term				
	LowIVP	MedIVP	HighIVP	Sed[A]	Sign[B]	LowIVP	MedIVP	HighIVP	Sed[A]	Sign[B]
Odour										
Intensity	6.02	6.15	6.10	0.04	ns	5.90	5.97	6.13	0.04	t
Meat	4.12	3.90	3.90	0.10	ns	3.97	3.96	4.37	0.10	ns
Sour	4.00	3.93	3.91	0.06	ns	3.74	3.68	3.89	0.06	ns
Stale	2.00	1.96	2.04	0.14	ns	2.29	2.23	2.14	0.17	ns
Metal	3.14	3.16	3.21	0.01	ns	3.31	3.24	3.37	0.01	ns
Piggy	2.13	2.14	2.33	0.17	ns	2.45	2.66	2.45	0.17	ns
Rancid	1.23	1.51	1.63	0.04	ns	1.38	1.54	1.35	0.04	ns
Flavour										
Intensity	6.39	6.37	6.45	0.03	ns	6.26	6.23	6.35	0.03	t
Meat	4.08	3.87	4.00	0.15	ns	4.08	4.27	4.26	0.15	ns
Sour	3.93	3.75	3.84	0.13	ns	3.68	3.94	3.92	0.13	ns
Sweet	2.69	2.63	2.59	0.08	ns	2.92	2.91	2.90	0.08	ns
Salty	5.68	5.48	5.60	0.13	ns	5.84	5.55	5.50	0.13	ns
Bitter	3.35	3.39	3.36	0.03	ns	3.87	3.75	3.69	0.03	ns
Metal	3.17	3.26	3.15	0.06	ns	3.45	3.31	3.37	0.06	ns
Piggy	2.03	2.06	2.12	0.14	ns	2.21	2.49	2.06	0.14	ns
Rancid	1.35	1.62	1.51	0.04	ns	1.28	1.56	1.34	0.04	ns
Oily	2.19	2.31	2.42	0.05	ns	2.19	2.40	2.06	0.05	ns
Texture										
Hardness	2.92	3.23	3.16	0.14	ns	3.62	3.56	3.78	0.14	ns
Tenderness	7.29	7.08	7.01	0.17	ns	6.69	6.53	6.52	0.17	ns
Fattiness	4.63	4.92	4.86	0.07	ns	4.89	5.10	5.10	0.07	ns
Juiciness	5.47	5.57	5.69	0.09	ns	5.23	5.61	5.51	0.09	ns

[A]Standard error of difference
[B]Significance of differences between samples in each row. ns: $p>0.10$; t: $p<0.10$; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$

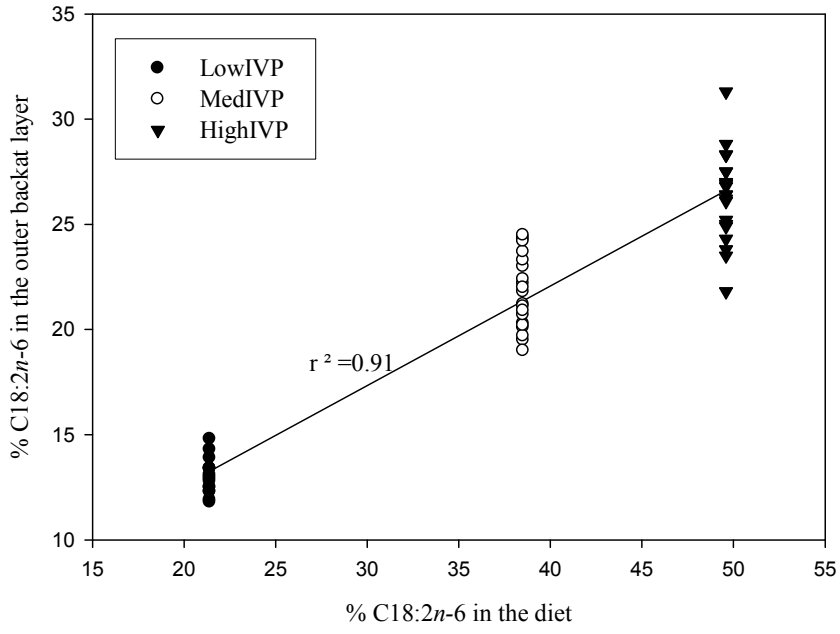

Fig. 1. Dietary and backfat percentage of C18:2*n*-6

Paper II

Meat Science 85 (2010) 182–190

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Fish oil in feeds for entire male and female pigs: Changes in muscle fatty acid composition and stability of sensory quality

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ARTICLE INFO

Article history:
Received 3 June 2009
Received in revised form 11 December 2009
Accepted 21 December 2009

Keywords:
Pigs, Sex
Fat level
Fish oil
Fatty acid deposition
Pork quality
Sensory properties

ABSTRACT

A total of 72 crossbred [(Norwegian Landrace × Yorkshire) × Duroc] male and female growing-finishing pigs were restrictedly fed diets containing fish oil to study the fatty acid composition of *Musculus longissimus dorsi* and sensory quality of belly and neck. Six diets were used: two low-fat diets with or without 0.5% fish oil added, and four medium-fat diets with palm kernel oil to fish oil in ratios given as % inclusion: 4.1:0.0, 3.9:0.3, 3.6:0.5 and 3.4:0.7. Feeding fish oil gave a dose-dependent response between fatty acids in the diets and in the *M. longissimus dorsi* and increased the level of very long chain *n*−3 fatty acids, especially the C22:5*n*−3 (DPA). A more efficient *n*−3 fatty acids deposition was obtained when given as a medium-fat diet rather than the low-fat diet. Female pigs had a significant higher percentage of monounsaturated fatty acids and C18:1 than males suggesting a gender related difference in the delta-9-desaturase activity. No significant differences were found in sensory attributes for short-term stored neck and belly. For pigs fed the highest level of fish oil (0.7%) long-term stored (12 months at −80 °C, 6 months at −20 °C) belly showed a slight increase in fish oil flavour. After warmed-over treatment, fish oil odour and flavour as well as rancid flavour were increased in this group. The results suggest levels of dietary fish oil up to 0.5% produce a healthier meat fatty acid composition, without negative effects on sensory attributes, even in long-termed stored belly.

1. Introduction

Many experiments have shown effects of fatty acid composition on meat quality, as reviewed by Wood et al. (2004). An efficient way of influencing the fatty acid composition in pork is by feeding fat sources with varying fatty acid composition. The desired fatty acid composition in meat products should give appropriate pork quality; shelf life, flavour and high nutritional value.

In recent years there has been a change in the fatty acid profile in pork from highly saturated (SFA) to more polyunsaturated (PUFA) fatty acids. In particular the level of very long chain (VLC) *n*−3 fatty acids elicits beneficial health effects for humans. An intake of C20:5*n*−3 (eicosapentaenoic acid, EPA) and C22:6*n*−3 (docosahexaenoic acid, DHA) of approximately 500 mg/day has shown a reduced risk of cardio-vascular diseases (Gebauer, Psota, Harris, & Kris-Etherton, 2006). EPA and DHA, and to a lesser extent DPA, are mainly found in marine products, and fish oil additions to pig diets have been evaluated in several experiments with different inclusion levels. Øverland, Taugbøl, Haug, and Sundstøl (1996) fed pigs with 1% and 3% dietary fish oil until slaughter and found a dose-dependent increase in VLC *n*−3 fatty acids in fat and muscle. VLC *n*−3 fatty acids are, however, easily oxidized, especially during storage (Kanner, 1994), and Øverland et al. (1996) found high off-flavour and off-odour with both 1% and 3% fish oil. In a similar experiment feeds were added 0.2% and 0.4% fish oil, and no difference was found in sensory properties as compared to control (Bryhni, Kjos, Ofstad, & Hunt, 2002). Fish oil and other marine raw materials are limited, expensive and the quality varies. The best way to utilize the marine raw materials and the VLC *n*−3 fatty acids content into high deposition rate in meat is of economical interest. When fed low-fat, starch-rich diets, pigs efficiently produce saturated and monounsaturated fatty acids by de novo synthesis (Kloareg, Bellego, Mourot, Noblet, & van Milgen, 2005). Although in pigs the effect of varying dietary fat level is little investigated, it might be anticipated that by using more fat in the feeds, a reduction in endogenous produced fatty acids, and a difference in deposition of feed fatty acids can occur.

The market for convenience foods is increasing and the trait warmed-over flavour (WOF) is of great importance for pork quality. The term WOF describes the off-flavour caused by rapid development of oxidation in refrigerated cooked meats within 48 h

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doi:10.1016/j.meatsci.2009.12.023

(Cross, Leu, & Miller, 1987). Bryhni et al. (2003) stated WOF to be the most important sensory trait. More detailed knowledge about dietary fish oil inclusions and the effect on quality parameters, including sensory evaluation, is needed.

Animal welfare has become more important, and a ban on castration of entire males may be introduced in several European countries within the near future. A review by Babol and Squires (1995) concluded that entire males have a higher nutritional and commercial value due to higher lean content compared to other genders. Several studies have confirmed a gender effect on the fatty acid composition as reviewed by (Lebret & Mourot, 1998; Nurnberg, Wegner, & Ender, 1998) showing unsaturation of fatty acids in order males > females > castrated males. In an experiment with castrated male and female pigs the fatty acid composition in *Musculus longissimus dorsi* confirmed gilt, when compared to castrated males, have more PUFA and less MUFA and SFA (Zhang et al., 2007). The effect of entire male on fatty acid composition is less investigated and more knowledge on meat quality from entire males is needed.

The specific objectives in this experiment were thus (1) to investigate the possibilities of enhancing the inclusion of VLC $n-3$ fatty acids from fish oil without compromising meat quality, (2) to detect any possible effect of the feed fat level on deposition rate of $n-3$ fatty acids (3) to evaluate the effect of sex in this context.

2. Materials and methods

2.1. Experimental diets

Six experimental diets were formulated and produced by pelleting. All feed ingredients, calculated and analyzed values for the experimental diets are presented in Table 1. The diets consisted of barley, soybean meal and inclusion of different levels of $n-3$ fatty acid rich fish oil (F), produced from mackerel, sardines and anchovy families, and palm kernel oil (PK). Palm kernel oil contain a high proportion of saturated fatty acids and has reduced nutritional value by low ratio of polyunsaturated to saturated fatty acids (P/S). The oils were analyzed according to the methods described by AOCS for fatty acid composition (AOCS,Ce 1b-89) and for oxidation by peroxide (AOCS,Cd 8b-90) and anisidin (AOCS, Cd 18–90) values. Nearly 80% of the fatty acids in PK were saturated and consisted mainly of C12:0 and C14:0. The fish oil contained 38% $n-3$ fatty acids, mainly C20:5$n-3$ and C22:6$n-3$ and anisidine and peroxide values of the fish oil were 9.6 and 6.6 respectively. All diets were formulated to contain the same amount of amino acids/MJ and 212 mg/kg of Vitamin E (*dl*-α-tocopherol acetate).

Two low-fat diets were used; one without added fat (LF) and one with 0.5% fish oil (LFF2). Four diets had a medium-fat level and the % inclusion of palm kernel oil and fish oil were: 4.1:0.0 (PK1), 3.9:0.3 (PK2F1), 3.6:0.5 (PK3F2) and 3.4:0.7 (PK4F3). The feeds were analyzed for water content (EU DIR 71/393 m), crude protein (EU DIR 93/28 m), crude fat (EU DIR 98/64 m), crude fiber (EU DIR 92/89 m), ash (EU DIR 71/250 m) and fatty acid composition (AOCS, Ce 1b-89) (Table 2). The anisidin and peroxide values of the diets were measured at the start and end of the experiment according the methods described by AOCS.

2.2. Animals

Seventy-two crossbred [(Norwegian Landrace × Yorkshire) × Duroc] pigs were produced with two different boars, and entire males and females from 10 litters were used in the experiment. They were allotted according to litter, live weight and sex in a randomized block design with six animals of the same sex per pen and 12 animals per treatment. The live weights at start and end of the experiment were 24.3 kg and 95.5 kg, respectively. The animals were fed according to a restricted scale for growing-finishing pigs described by Øverland (1997).

2.3. Carcass and sampling

The pigs were slaughtered in three groups when they reached a live weight of approximately 95 kg and all diets were represented equally on each slaughter day. At the slaughter line, a commercially GP2Q pistol (Hennessy System) was used to determine the lean meat percentage as described by Kjos, Øverland, Bryhni, and Sørheim (2000).

Table 1
The ingredients, calculated and analyzed composition of the experimental diets.

	Low fat level		Medium-fat level			
	LF	LFF2	PK1	PK2F1	PK3F2	PK4F3
Ingredients, g/kg						
Barley	832	822	754	750	750	749
Soy bean meal	109	114	144	148	148	149
Fish oil	0	4.8	0	2.5	5.2	7.2
Palm kernel oil	0	0	41.2	38.7	36.1	34.0
Limestone	14.6	14.6	14.6	14.7	14.6	14.6
MCP	8.1	8.2	9.2	9.2	9.2	9.3
NaCL	5.8	5.9	6.2	6.2	6.2	6.2
DL-Methionin	0	0	0.2	0.2	0.2	0.2
L-Lysine	0.5	0.5	0.5	0.4	0.4	0.4
L-Threonin	0	0	0.1	0.1	0.1	0.1
Acid	10	10	10	10	10	10
Vitamin-mineral premix[a]	20	20	20	20	20	20
Calculated content						
Metabolizable energy (MJ/kg)	11.9	12.1	13.0	13.0	13.0	13.0
Analyzed content						
DM, %	89.1	89.4	89.5	89.7	89.5	89.5
Crude protein g/kg DM	159.4	160.0	167.6	163.9	173.2	170.9
Crude fat g/kg DM	29.2	33.6	67.0	72.5	72.6	72.6
Total ash g/kg DM	48.3	53.7	46.9	49.1	52.5	49.2
Crude fiber g/kg DM	50.5	52.6	54.7	54.6	55.9	51.4

[a] Vitamin-mineral premix provided the following amounts per kg of feed: 134 mg Fe, 125 mg Zn, 75 mg Mn, 18 mg Cu, 1 mg I, 0.3 mg Se, 9000 I.U. vitamin A, 1125 I.U. vitamin D_3, 5.6 mg B_2, 19 mg pantothenic acid and 25 μg B_{12}.

Table 2
Fatty acid composition (% of total fatty acids) and oxidation status of the fat sources and experimental diets at start.

	Fat sources		Low-fat diets		Medium-fat diets			
	Palm kernel oil	Fish oil	LF	LFF2	PK1	PK2F1	PK3F2	PK4F3
C10:0	3.5	<0.1	<0.1	0.1	2.3	2.2	2.1	2.0
C12:0	49.1	<0.1	0.1	1.2	33.5	31.3	29.5	28.0
C14:0	16.3	7.4	0.3	1.8	11.9	11.3	10.9	10.6
C16:0	8.3	17.3	22.3	20.0	12.4	12.6	12.8	12.9
C16:1 *n*−7	<0.1	7.9	0.2	1.6	0.1	0.4	0.8	1.0
C18:0	2.3	3.1	1.6	2	2.3	2.3	2.4	2.4
C18:1	14.7	13.8	13.2	14	15.7	15.7	15.5	15.5
C18:2 *n*−6	2.4	1.8	53.4	42.1	16.7	16.7	16.4	16.2
C18:3 *n*−3	<0.1	0.2	5.7	4.8	1.7	1.8	1.8	1.9
C20:1 *n*−9	0.1	2.0	0.9	1.1	0.3	0.4	0.5	0.5
C20:4 *n*−6	<0.1	0.7	<0.1	0.2	<0.1	<0.1	0.1	0.1
C20:5 *n*−3	<0.1	17.4	0.1	3.5	<0.1	0.9	1.6	2.2
C22:5 *n*−3	<0.1	1.9	<0.1	0.3	<0.1	0.1	0.2	0.2
C22:6 *n*−3	<0.1	13.5	<0.1	2.7	<0.1	0.5	1.4	1.6
SFA	79.5	27.9	24.3	25.1	62.4	59.7	57.7	55.9
MUFA	14.8	23.7	14.3	16.7	16.1	16.5	16.8	17.0
PUFA	2.4	35.6	59.2	53.6	18.4	20.0	21.5	22.2
n−6/*n*−3	∞	2.6	9.2	3.7	9.8	5.1	3.3	2.8
P/S[a]	0.03	33.1	2.6	2.3	0.3	0.4	0.4	0.4
Iodine value	18	195	120	131	50	54	62	67
Anisidin value meq/kg	1.6	9.6	138	140	43	40	41	40
Peroxide value meq/kg	<2	6.6	27.5	32.0	4.9	7.2	9.7	10.9

LF: Low fat, LFF2: Low fat + 4.8 g fish oil, PK1: 41.2 g palm kernel oil, PK2F1: 38.7 g palm kernel oil + 2.5 g fish oil, PK3F2: 36.1 g palm kernel oil + 5.2 g fish oil, PK4F3: 34.0 g palm kernel oil + 7.2 g fish oil.
[a] P = C18:2, C18:3, C20:4, C20:5, C22:5 and C22:6, S = C12:0, C14:0, C16:0.

An 8 cm sample of *M. longissimus dorsi* around P2 location (Øverland, Rørvik, & Skrede, 1999) was collected and cut perpendicular to the fibre direction. Fat and connective tissue were removed and the sample was divided into three sub-samples. The first sub-sample was taken from the anterior end and used for bloomed colour, protein, fat and moisture analysis. The second sub-sample was used for drip loss measurements. The last sub-sample, in the posterior end, was used for fatty acid composition and oxidative stability analysis.

Whole belly and neck, the front extension of the chop strand to the fifth rib, from the left side of the female carcasses were collected for sensory analysis. Neck and belly samples frozen-stored at −80 °C for two or four months are named "short-term stored", and belly samples frozen-stored at −80 °C for 12 months and then for an additional 6 months at −20 °C are named "long-term stored". Each neck was cut in slices, individually vacuum packed in plastic bags and frozen for short-term storage for 2 months. Bellies were cut in slices, individually vacuum packed in plastic bags and frozen for short-term storage for four months and for long-term storage. After short-term storage of neck and belly samples and long-term storage of bellies, the samples were analyzed for sensory attributes.

2.4. Fatty acid analysis

Meat samples of approximately 200 g from *M. longissimus dorsi* were ground with an Ultra Mill Retch mill from Braun with a 0.5 mm screen and approximately 5 g were used in the analysis. The meat samples were hydrolysed with HCl (4 M) and mixed, and then diethyl ether and petroleum ether were added and the samples were left for at least 3 h. The supernatant was evaporated to dryness in a water bath at 33 °C and under nitrogen. Samples of 200 mg, to which 1 ml of internal standard (tricosanoic, C23:0, fatty acid) was added, were methylated in H_2SO_4 in methanol at 60–70 °C for 3 min. The resulting methyl esters were cooled and extracted into n-hexane and transferred to vials containing anhydrous Na_2SO_4. Hexane/dimethylcarbonate and sodium methylate solutions were added and mixed. A gas chromatograph, Varian Star 3400 C_x (Varian Inc., Palo Alta, USA), with auto injector and equipped with flame ionisation detector, split injector and Nordion fused silica column NS-351 (25 m, 0.32 mm, 0.25 μm) (HNU Systems Inc., Helsinki, Finland) and helium as carrier gas was used. The injector temperature was set to 270 °C, the detector temperature at 260 °C and the column temperature set at 110 °C for 2 min. The GC was then programmed to increase at a rate of 10 °C per minute up to 220 °C and to maintain this temperature for 27 min. Each fatty acid methyl ester was identified by comparing the retention time to authentic lipid standard acquired from Larodan Fine Chemicals AB (Malmö, Sweden). Percentages of each fatty acid (of total sum of fatty acids) were calculated.

2.5. Colour, chemical composition, drip loss and oxidative stability

Meat colour was measured after one hour blooming at 4 °C. Duplicate measurements on three sites of the loin using a Minolta CR400 (Minolta Co. Ltd., Osaka, Japan) were taken. Mean values for the CIELAB L^*, a^*, b^*, Hue and Chroma values were calculated and used in the statistical analysis. Values for Hue were calculated as Hue = $\tan^{-1}$ (b^*/a^*) and the Chroma as Chroma = $\sqrt{(a^{*2} + b^{*2})}$. The Minolta apparatus was standardised according to instrumental procedures. Protein, fat and moisture content was determined using a FOSS Foodscan™ (Anderson, 2007). The samples were ground according to AOAC Official Method 983.18 (AOAC, 1989). The ground meat was placed in a round sample dish, placed in the FoodScan and scanned. Results were shown as percent (g/100 g) for protein, fat and moisture. Drip loss was conducted according to the EZ-Drip loss method described by Christensen (2003).

Oxidative stability was measured using TBARS test described by Tarladgis, Watts, Younathan, and Dugan (1960) with modification made by Crackel, Gray, Pearson, Booren, and Buckley (1988). The sample for TBARS test was split in two and each sample was vacuum packed. One sample was stored at 4 °C for three days before analysed. The second sample was frozen-stored for five months at −18 °C before the test was conducted. TBARS values were expressed as mg malondialdehyde/kg muscle.

2.6. Sensory analysis

Meat samples from the neck were thawed one day before the sensory assessment. Each slice was cut in two, vacuum packed and marked randomly with a three-digit number. All samples were stored in a cooler (4 °C) overnight. Neck samples were cooked in a 72 °C water bath for 40 min and put in steel containers on hot plates and served to the assessors in random order with regards to treatment and assessor. Belly samples followed the same procedure as neck samples except cooking for 20 min.

After long-term storage half of the bellies were thawed one day before sensory profiling. Each belly sample was cut in two, vacuum packed and marked randomly with a three-digit number. All samples were stored in a cooler overnight. The samples were cooked in an air-o-stem oven at 72 °C for 12 min before serving to the assessors in steel containers on hot plates. Samples were given in random order regarding treatment and assessor.

The last long-stored bellies followed the former procedure, but after cooking all samples were cooled in ice water for 15 min and a core temperature of 6 °C was reached. Then all samples were stored in a cooler at 4 °C for 24 h. The samples were then warmed at 72 °C for 12 min and served randomly to each assessor in steel containers on hot plates.

The members for the sensory assessments were trained and familiar with evaluation of pork and followed international standards for sensory analysis (ISO 6564). The panel evaluated the samples randomly and used a scale from 1 to 9, where 1 was the lowest and 9 the highest intensity for all parameters. A single score for each sample was calculated as an average of all panellists. Before each sensory assessment, the taste panel was calibrated with three samples from different treatments in the experiment. Short-stored neck and belly samples were evaluated for 21 attributes: odour intensity, meat odour, sour odour, stale odour, metal odour, piggy odour, rancid odour, flavour intensity, meat flavour, sour flavour, sweet flavour, salty flavour, bitter flavour, metal flavour, piggy flavour, rancid flavour, oily flavour, hardness, tenderness, fattiness and juiciness. Eleven assessors evaluated neck samples and eight assessors evaluated belly samples.

Long-stored bellies were evaluated by an 8-membered panel for 24 attributes: odour intensity, meat odour, sour odour, stale odour, metal odour, piggy odour, rancid odour, oily odour, fishy odour, flavour intensity, meat flavour, sour flavour, sweet flavour, salty flavour, bitter flavour, metal flavour, piggy flavour, rancid flavour, oily flavour, fishy flavour, hardness, tenderness, fattiness and juiciness. For evaluating warmed-over flavour (WOF), long-term stored bellies were evaluated by an 8-member panel for the same 24 attributes in addition to WOF odour and flavour.

2.7. Statistics

Statistical analysis was conducted using a general linear model (GLM) of analysis of variance (ANOVA) in Minitab 15 software (Minitab Inc., PA, USA). The factors used in the model were sex (entire male and female), diet (LF, LFF2, PK1, PK2F1, PK3F2 and PK4F3) and the interaction between sex and diet. Tukey test was used to show differences among treatments.

3. Results

No significant interactions between diet and sex were found for any of the parameters measured. One entire male in the LF group died during the feeding experiment, giving $n = 35$ and $n = 36$ for males and females, respectively.

3.1. Animal performance

The production parameters (Table 3) showed no significant differences between dietary treatments or sex in daily weight gain, feed intake or lean meat content. Feed conversion ratio (FCR) in MJ/weight gain was not affected by dietary treatments, but male pigs had lower feed conversion ratio ($p < 0.001$) than female pigs. Pigs fed combinations of palm kernel oil and fish oil had lower FCR in kg/weight gain than the LF and LFF2 fed pigs.

3.2. Fatty acid composition

Fatty acid composition of *M. longissimus dorsi* given in % of total fatty acids is shown in Table 4. As expected, the fatty acid composition was affected by dietary treatments. A clear dose–response relationship between several dietary fatty acids and meat fatty acids was found. The percentages of C12:0, C14:0, C16:0 and total sum of saturated fatty acids (SFA) were generally higher in pigs fed the four PK diets. Furthermore, the percentage of C18:1 and total monounsaturated fatty acids (MUFA) were higher in the LF groups as compared to all PK groups.

Most PK groups had a significantly higher percentage of C18:2*n*−6 than LF groups, and a tendency to higher percentage of C18:3*n*−3, significantly only for the group given the highest inclusion of fish oil.

In general an increase in individual VLC *n*−3 fatty acids was observed with higher dietary inclusion of fish oil (Fig. 1). Interestingly, the PK3F2 group had significantly higher percentage of C20:5*n*−3 compared to the LFF2 group getting the same dietary fish oil inclusion, and a similar tendency was clearly seen for

Table 3
Effects of diet and sex on growth and carcass composition.

	Diet								Sex			
	LF	LFF2	PK1	PK2F1	PK3F2	PK4F3	Sed[a]	Sign[b]	Entire male	Female	Sed[a]	Sign[b]
Number of pigs	11	12	12	12	12	12			35	36		
Daily weight gain (g/d)	924	947	928	923	952	963	22.4	ns	937	942	12.8	ns
Feed intake, MJ	1826	1800	1931	1847	1894	1890	56.4	ns	1859	1870	32.0	ns
FCR, kg/weight gain[c]	2.16[b]	2.13[b]	2.06[ab]	2.01[a]	2.03[a]	2.00[a]	0.04	***	2.03	2.10	0.02	***
FCR, MJ/weight gain[c]	26.0	25.7	26.9	26.2	26.4	26.1	0.46	ns	25.8	26.7	0.26	***
Lean meat (%)	57.3	57.0	57.2	56.7	57.3	57.1	0.78	ns	57.0	57.2	0.43	ns

LF: Low fat, LFF2: Low fat + 4.8 g fish oil, PK1: 41.2 g palm kernel oil, PK2F1: 38.7 g palm kernel oil + 2.5 g fish oil, PK3F2: 36.1 g palm kernel oil + 5.2 g fish oil, PK4F3: 34.0 g palm kernel oil + 7.2 g fish oil.

[a] Standard error of difference.

[b] Significance of differences between samples in each row. ns: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

[c] Feed conversion ratio.

Table 4
The effect of diet on fatty acid composition (% of total fatty acids) in *M. longissimus dorsi* at P2 location.

	Diet								Sex			
	LF	LFF2	PK1	PK2F1	PK3F2	PK4F3	Sed[A]	Sign[B]	Entire male	Female	Sed[A]	Sign[B]
C10:0	0.16	0.13	0.16	0.16	0.15	0.17	0.02	ns	0.14	0.16	0.01	ns
C12:0	0.16[a]	0.14[a]	0.77[b]	0.68[b]	0.68[b]	0.72[b]	0.07	***	0.61	0.44	0.04	***
C14:0	1.49[a]	1.43[a]	2.96[b]	2.81[b]	2.78[b]	2.91[b]	0.16	***	2.59	2.21	0.09	***
C16:0	24.2[b]	23.0[a]	24.1[b]	24.3[b]	24.0[ab]	24.5[b]	0.36	***	24.2	23.9	0.20	ns
C16:1 *n*−7	4.5	3.53	4.01	3.84	3.96	3.98	0.19	ns	3.76	4.03	0.11	*
C18:0	12.7	13.8	13.0	13.4	12.9	13.0	0.51	ns	13.5	12.8	0.28	*
C18:1	47.2[b]	47.3[b]	43.4[a]	43.3[a]	43.2[a]	42.6[a]	0.91	***	42.9	46.1	0.51	***
C18:2 *n*−6	6.08[a]	6.09[a]	7.31[b]	7.05[ab]	7.39[b]	7.29[b]	0.40	***	7.58	6.16	0.22	***
C18:3 *n*−3	0.46[a]	0.48[ab]	0.52[ab]	0.56[ab]	0.57[ab]	0.58[b]	0.04	**	0.58	0.47	0.02	***
C20:1 *n*−9	0.75[bc]	0.81[c]	0.68[ab]	0.68[ab]	0.64[a]	0.62[a]	0.04	***	0.69	0.70	0.02	ns
C20:4 *n*−6	0.61	0.64	0.68	0.56	0.58	0.50	0.08	ns	0.64	0.55	0.04	ns
C20:5 *n*−3	0.05[a]	0.18[b]	0.08[ab]	0.16[b]	0.30[c]	0.38[c]	0.04	***	0.23	0.16	0.02	***
C22:5 *n*−3	0.09[a]	0.23[bcd]	0.13[a]	0.22[bc]	0.29[cd]	0.31[d]	0.03	***	0.24	0.18	0.02	***
C22:6 *n*−3	0.06[a]	0.20[b]	0.11[ab]	0.19[b]	0.28[bc]	0.36[c]	0.04	***	0.22	0.18	0.02	*
∑SFA	38.8[ab]	38.5[a]	40.9[c]	41.3[c]	40.5[bc]	41.3[c]	0.70	***	41.0	39.4	0.39	***
∑MUFA	52.0[b]	51.6[b]	48.1[a]	47.8[a]	47.8[a]	47.2[a]	1.01	***	47.4	50.8	0.56	***
∑PUFA	7.35[a]	7.82[a]	8.83[ab]	8.73[ab]	9.41[b]	9.41[b]	0.54	***	9.49	7.69	0.30	***
n−6/*n*−3 ratio	10.8[c]	6.51[ab]	9.88[c]	6.97[b]	5.78[ab]	4.82[a]	0.70	***	7.23	7.69	0.39	ns
P/S ratio[C]	0.28	0.32	0.32	0.31	0.34	0.33	0.02	ns	0.35	0.29	0.01	***
Iodine value	60.8	62.7	60.9	60.7	62.8	62.2	0.97	ns	62.2	61.1	0.54	*

LF: Low fat, LFF2: Low fat + 4.8 g fish oil, PK1: 41.2 g palm kernel oil, PK2F1: 38.7 g palm kernel oil + 2.5 g fish oil, PK3F2: 36.1 g palm kernel oil + 5.2 g fish oil, PK4F3: 34.0 g palm kernel oil + 7.2 g fish oil.
[A] Standard error of difference.
[B] Significance of differences between samples in each row. ns: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.
[C] P = C18:2, C18:3, C20:4, C20:5, C22:5 and C22:6, S = C12:0, C14:0, C16:0.

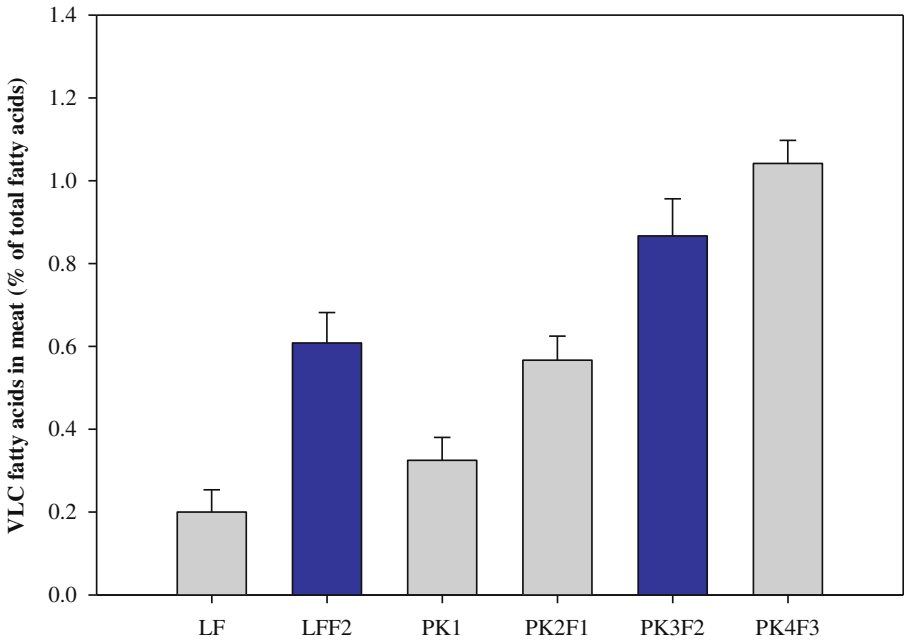

Fig. 1. The sum of percent very long chain (VLC) *n*−3 fatty acids in *M. longissimus dorsi* from pigs fed the experimental diets. (LF: Low fat, LFF2: Low fat + 4.8 g fish oil, PK1: 41.2 g palm kernel oil, PK2F1: 38.7 g palm kernel oil + 2.5 g fish oil, PK3F2: 36.1 g palm kernel oil + 5.2 g fish oil, PK4F3: 34.0 g palm kernel oil + 7.2 g fish oil). The two diets marked in blue had the same amount of VLC *n*−3 fatty acids/MJ added, but different fat levels.

22:5*n*−3 and 22:6*n*−3. All groups given fish oil had significantly lower *n*−6/*n*−3 ratio.

Sex had an effect on the fatty acid composition. The entire males had slightly higher percentage of C12:0, C14:0, C18:0 and total saturated fatty acids. The percentage of C16:1*n*−7 was significantly lower, and the percentage of C18:1 and total monounsaturated fatty acids considerably lower ($p < 0.001$) in males than in females. Percentages of C18:2*n*−6, C18:3*n*−3, C20:5*n*−3, C22:5*n*−3, total polyunsaturated fatty acids (PUFA) and *n*−6/*n*−3 ratio were all substantially higher ($p < 0.001$) and C22:6*n*−3 slightly higher ($p < 0.05$) in males than females. The iodine value was slightly higher in males compared to females.

3.3. Colour, chemical composition, drip loss and oxidative stability

Other meat quality parameters measured included bloomed colour, fat, protein and moisture content, drip loss and oxidation stability (TBARS) (Table 5). There were no effects of diet for any of the measured parameters except protein which was significant in the model ($p < 0.05$) but the Tukey test did not show any differ-

Table 5
The effect of diet and sex on colour (CIE L^*, a^*, b^* system), content (g/kg), driploss (g/kg) and oxidation (TBARS, mg malonaldehyde/kg) in *M. longissimus dorsi.*

		Diet								Sex			
		LF	LFF2	PK1	PK2F1	PK3F2	PK4F3	Sed[a]	Sign[b]	Entire male	Female	Sed[a]	Sign[b]
Colour	L^*	49.9	49.5	49.5	49.5	49.1	49.2	0.78	ns	49.2	49.6	0.44	ns
	a^*	6.73	6.64	6.61	6.99	6.66	6.80	0.41	ns	7.01	6.47	0.23	*
	b^*	3.09	2.90	2.84	3.12	2.70	2.98	0.31	ns	3.28	2.60	0.18	***
	Hue	24.6	23.4	22.8	23.9	21.9	23.1	1.92	ns	24.9	21.6	1.09	**
	Saturation	7.43	7.27	7.24	7.70	7.22	7.46	0.45	ns	7.77	7.00	0.25	**
Fat		15.5	16.0	15.8	17.5	14.9	16.3	0.12	ns	15.5	16.5	0.07	ns
Protein		221	223	224	224	225	223	0.15	*	222	224	0.06	***
Moisture		754	751	751	750	754	754	0.16	ns	754	751	0.09	***
EZ-driploss		71.9	72.0	66.4	55.1	58.7	70.5	0.75	ns	65.5	66.0	0.42	ns
TBARS 0		0.06	0.07	0.06	0.08	0.08	0.09	0.02	ns	0.07	0.08	0.01	ns
TBARS 5		0.05	0.05	0.04	0.06	0.08	0.08	0.02	ns	0.06	0.06	0.01	ns

LF: Low fat, LFF2: Low fat + 4.8 g fish oil, PK1: 41.2 g palm kernel oil, PK2F1: 38.7 g palm kernel oil + 2.5 g fish oil, PK3F2: 36.1 g palm kernel oil + 5.2 g fish oil, PK4F3: 34.0 g palm kernel oil + 7.2 g fish oil.
[a] Standard error of difference.
[b] Significance of differences between samples in each row. ns: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Table 6
The effect of diet on sensory attributes of short-term stored pork belly.

	Low fat level		Medium-fat level					
	LF	LFF2	PK1	PK2F1	PK3F2	PK4F3	Sed[a]	Sign[b]
Odour								
Intensity	6.72	6.54	6.39	6.62	6.56	6.55	0.28	ns
Meat	3.25	3.07	2.70	2.53	2.80	3.49	0.37	ns
Metal	3.42	3.49	3.54	3.42	3.53	3.47	0.09	ns
Pork	4.27	3.97	4.38	4.19	4.00	3.88	0.52	ns
Rancid	2.30	2.55	2.57	2.98	3.08	2.38	0.73	ns
Flavour								
Intensity	6.52	6.33	6.07	6.39	6.41	6.26	0.27	ns
Meat	3.88	3.76	3.45	3.22	3.25	3.80	0.38	ns
Pork	4.19	3.79	3.62	3.97	3.70	3.53	0.48	ns
Sour	3.01	3.01	2.79	2.76	2.67	3.08	0.39	ns
Sweet	2.86	2.93	2.90	2.84	3.04	2.94	0.10	ns
Metal	3.80	3.84	3.80	3.89	3.89	3.97	0.13	ns
Rancid	2.24	2.76	2.29	3.24	3.24	2.47	0.75	ns
Texture								
Tenderness	5.11	5.41	5.58	5.81	5.65	5.26	0.30	ns
Juiciness	5.14	5.36	4.55	5.46	5.16	4.98	0.34	ns

LF: Low fat, LFF2: Low fat + 4.8 g fish oil, PK1: 41.2 g palm kernel oil, PK2F1: 38.7 g palm kernel oil + 2.5 g fish oil, PK3F2: 36.1 g palm kernel oil + 5.2 g fish oil, PK4F3: 34.0 g palm kernel oil + 7.2 g fish oil.
[a] Standard error of difference.
[b] Significance of differences between samples in each row. ns: $p > 0.1$; t:$p < 0.1$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

ences among groups. A significant difference in meat colour between sexes was observed, with the entire males having highest a^*, b^*, Hue and Chroma values ($p < 0.01$). No difference in fat content due to sex was found. The protein content was, however, slightly but significantly higher and the moisture content lower in females compared to entire males.

3.4. Sensory

The sensory properties of short-term stored female neck (results not presented) and belly (Table 6) were not affected by diet.

Long-term stored bellies (Table 7), however, had a significantly lower meat odour in the fish oil groups than in the LF and PK1 groups ($p < 0.01$). Fish oil flavour was significant in the model but the pairwise Tukey test was not able to detect differences between the groups. Numerically the fish oil flavour increased with increasing dietary fish oil level. No significant differences in fish oil odour, rancid odour or rancid flavour were detected ($p > 0.05$).

Warmed-over treatment of long-term stored bellies resulted in significantly higher fish oil odour and flavour in the PK4F3 than the LF group ($p < 0.001$) (Table 8). The two groups with highest inclusion of palm kernel oil, (PK1 and PK2F1) had significantly lower scores for rancid flavour compared to the PK4F3 group. Metal odour and flavour was more pronounced in the PK1 group than the LFF2 group.

4. Discussion

4.1. Animal performance

The experimental diets differed in energy source but this did not affect the growth, FCR MJ/weight gain or lean meat percentage in accordance to earlier studies by Øverland et al. (1996). This shows that the low-fat diets were as good as diets with palm kernel oil and fish oil at the levels used in the current experiment. The observed improvement in FCR for males compared to females is also in agreement with an earlier study (O'Connell, Lynch, & O'Doherty, 2005) showing improved FCR from 20 to 60 kg live weight. Regarding the lean meat percentage there was no difference between the two sexes. In a review by Babol and Squires (1995) no difference or a higher lean meat percentage in entire males was reported.

4.2. Fatty acid composition in M. longissimus dorsi

Pigs fed diets containing palm kernel oil had markedly higher levels of C12:0 and C14:0 reflecting the dietary fatty acid composition compared to low-fat diets. This is in agreement with earlier studies when palm kernel oil has been used in pig diets (Teye et al., 2006).

The VLC $n-3$ fatty acids increased in muscle from pigs fed increasing levels of fish oil as found earlier (Bryhni et al., 2002; Irie & Sakimoto, 1992; Jaturasitha, Wudthithumkanaporn, Rurksasen, & Kreuzer, 2002; Øverland et al., 1996) and as also seen when using other fish raw materials (Hertzman, Gøransson, & Ruderus, 1988; Howe, Downing, Grenyer, Grigonis-Deane, & Bryden, 2002; Jonsdottir, Valdimarsdottir, & Baldursdottir, 2003). The levels of C20:5$n-3$, C22:5$n-3$ and C22:6$n-3$ increased in a dose-dependent manner when dietary fish oil increased up to 0.7%. Interestingly, the percentages of C22:5$n-3$ in muscle was almost doubled compared to the percentage in the diet. The high levels of C22:5$n-3$ compared to a markedly lower percentage of C20:5$n-3$ than seen in the diet, suggests a strong activity of C20:5$n-3$ elongation. The decrease in the ratio between C22:5$n-3$ and C20:5$n-3$ with in-

Table 7
The effect of diet on sensory attributes of long-term stored pork belly.

	Low fat level		Medium-fat level					
	LF	LFF2	PK1	PK2F1	PK3F2	PK4F3	Sed[a]	Sign[b]
Odour								
Intensity	6.31	6.44	6.19	6.31	6.50	6.53	0.18	ns
Meat	3.46	2.86	3.45	3.32	2.92	2.96	0.20	**
Pork	4.19	3.85	4.10	3.97	4.26	3.53	0.35	ns
Metal	3.95	3.93	4.13	4.08	4.09	4.02	0,13	ns
Fish oil	1.42[a]	1.72[ab]	1.46[a]	1.49[ab]	1.75[ab]	2.28[b]	0.30	t
Rancid	1.86	2.16	2.00	1.91	2.28	2.19	0.33	ns
Flavour								
Intensity	5.86	6.39	6.01	6.00	6.08	6.36	0.21	ns
Meat	4.14[b]	3.32[ab]	3.79[ab]	3.96[ab]	3.68[ab]	3.27[a]	0.31	t
Pork	3.60	3.41	3.43	3.32	3.45	3.18	0.28	ns
Sour	3.62[b]	2.48[a]	3.06[ab]	3.32[ab]	2,78[ab]	2.74[ab]	0.33	*
Sweet	3.11	2.93	3.07	2.99	2.82	2.89	0.13	ns
Metal	4.11	4.19	4.31	4.11	4.08	4.19	0.13	ns
Fish oil	1.41	3.00	1.85	1.62	2.64	3.13	0.60	*
Rancid	1.77	2.96	2.33	2.15	2.44	2.39	0.43	ns
Texture								
Tenderness	5.89	5.54	5.79	5.80	5.74	5.82	0.14	ns
Juiciness	5.49	5.37	5.37	5.33	5.39	5.50	0.11	ns

LF: Low fat, LFF2: Low fat + 4.8 g fish oil, PK1: 41.2 g palm kernel oil, PK2F1: 38.7 g palm kernel oil + 2.5 g fish oil, PK3F2: 36.1 g palm kernel oil + 5.2 g fish oil, PK4F3: 34.0 g palm kernel oil + 7.2 g fish oil.
[a] Standard error of difference.
[b] Significance of differences between samples in each row. ns: $p > 0.05$; t: $p < 0.1$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Table 8
The effect of diet on sensory attributes including warmed-over flavour (WOF) of long-term stored pork belly.

	Low fat level		Medium-fat level					
	LF	LFF2	PK1	PK2F1	PK3F2	PK4F3	Sed[a]	Sign[b]
Odour								
Intensity	6.30	6.31	6.11	6.13	6.20	6.37	0.13	ns
Meat	3.03	3.20	3.27	3.01	2.99	2.75	0.20	ns
Pork	3.42	3.41	3.63	3.10	3.14	3.19	0.30	ns
Metal	3.94[ab]	3.77[a]	4.18[b]	3.77[a]	3.87[ab]	3.90[ab]	0.12	*
Fish oil	1.12[a]	1.54[ab]	1.25[ab]	1.29[ab]	1.80[ab]	2.12[b]	0.29	*
Rancid	1.67	1.54	1.41	1.37	1.90	1.77	0.27	ns
WOF	2.39	2.62	2.10	2.28	2.27	2.17	0.32	ns
Flavour								
Intensity	6.01	6.14	6.09	6.03	6.17	6.36	0.13	ns
Meat	3.60	3.59	3.58	3.58	3.21	2.99	0.23	*
Pork	3.00	3.25	3.01	2.98	3.20	3.27	0.22	ns
Sour	2.85[ab]	2.72[ab]	2.93[ab]	3.02[b]	2.50[ab]	2.29[a]	0.24	*
Sweet	2.90[ab]	3.15[b]	3.00[ab]	3.00[ab]	2.87[a]	2.87[ab]	0.09	*
Metal	4.26[ab]	4.05[a]	4.43[b]	4.25[ab]	4.13[ab]	4.20[ab]	0.11	*
Fish oil	1.10[a]	1.61[a]	1.17[a]	1.29[a]	1.92[ab]	2.67[b]	0.28	***
Rancid	1.82[ab]	1.97[ab]	1.56[a]	1.69[a]	2.33[ab]	2.79[b]	0.35	*
WOF	2.66	3.37	2.66	2.96	2.99	2.61	0.42	ns
Texture								
Tenderness	5.53[a]	6.01[ab]	5.77[ab]	6.11[b]	6.13[b]	5.99[ab]	0.17	**
Juiciness	5.17	5.54	5.49	5.56	5.60	5.52	0.16	ns

LF: Low fat, LFF2: Low fat + 4.8 g fish oil, PK1: 41.2 g palm kernel oil, PK2F1: 38.7 g palm kernel oil + 2.5 g fish oil, PK3F2: 36.1 g palm kernel oil + 5.2 g fish oil, PK4F3: 34.0 g palm kernel oil + 7.2 g fish oil.
[a] Standard error of difference.
[b] Significance of differences between samples in each row. ns: $p > 0.05$; t: $p < 0.1$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

creased inclusion of fish oil further suggests a tendency of saturation of this activity.

This experiment show that feeding pigs even low levels of VLC $n-3$ fatty acids improves fatty acid composition of pork with an attendant benefit for human health.

Pigs fed low-fat diets seemed to have an extended de novo synthesis compared to the pigs fed the medium-fat PK diets, resulting in a high proportion of oleic acid (C18:1) and thereby more MUFA in the muscle. This is consistent with other studies (Busboom, Rule, Colin, Heald, & Mazhar, 1991; Kloareg et al., 2005), who found more than 45% of C18:1 deposited when using diets without added fat.

Interestingly, the low-fat group LFF2 having the same inclusion of fish oil/MJ as the PK3F2 group showed decreased VLC $n-3$ fatty acids content (Fig. 1) and had significantly lower deposition of C20:5$n-3$ and a similar tendency for C22:5$n-3$ and C22:6$n-3$.

These results suggest that the VLC $n-3$ fatty acids may be better conserved in pork by using them in diets containing other, possibly easier oxidized fatty acids.

Sex affected the fatty acids composition. The review by Wood et al. (2008) concluded that entire males have a higher proportion of PUFA compared to females due to their thinner backfat level. In the present experiment, no difference in leanness between entire males and females was found. The females had a higher content of C18:1 and total MUFA content, and the males a higher content of C18:0. This may suggest that females have a higher delta-9-desaturase activity. This result is consistent with experiments on male and female rats, where the males had more C18:0 and less C18:1 suggesting that female sex steroids increased delta-9-desaturase activity (Thorling & Hansen, 1995). Male pigs had higher PUFA levels in agreement with a review by Lebret and Mourot (1998) suggesting male pigs to be healthier for humans compared to females and most probably castrated males since they normally have more SFA than both female and entire male pigs (Nurnberg et al., 1998).

4.3. Other quality parameters of M. longissimus dorsi

The entire males had 2.3 g less protein than females, which agrees with earlier results (Poltarsky & Palanska, 1991). However, the males in the current experiment had 2.9 g more moisture in the muscle than females, which conflicts with earlier findings (Poltarsky & Palanska, 1991). Meat from entire males appeared to be more red and yellow expressed in higher a^* and b^* values. Only a slight and statistically not significant reduction in lightness L^* values between the sexes were, however, found. This may somehow conflict with the research of Channon, Kerr, and Walker (2004), who reported entire males to have darker meat colour. Calculated values for Hue and Chroma resulted in higher levels in males compared to females, further indicating a more intense colour.

No significant effect of diet on other quality parameters was found in this experiment.

4.4. Sensory quality

The sensory tests were conducted with a trained panel to give an indication of product acceptability. The first sensory analysis was performed after short-term storage (at −80 °C), often referred to as "fresh", of neck and belly. The taste panel detected no major differences among the dietary treatments, even for the highest dietary inclusion of fish oil. This agrees with earlier experiments with 0.4% dietary fish oil, (Bryhni et al., 2002), and with an even higher inclusion of 1% dietary fish oil (Leskanich, Matthews, Warkup, Noble, & Hazzledine, 1997). This lack of differences between samples of "fresh" neck and belly, even with the highest inclusion of fish oil is further in agreement with Hertzman et al. (1988) who found no difference in fresh meat between groups fed different levels of fishmeal. Our experiment showed that even with a 4.5-fold higher percentage of C22:5$n-3$ and C22:6$n-3$ in meat, no adverse sensory quality was detected.

After long-term storage, a new sensory profiling was conducted on bellies. The statistics according to the pairwise Tukey test did not reveal differences in sensory quality among dietary groups even though the model showed significant difference among the groups for the attributes meat odour and fish oil flavour. Increasing levels of fish oil led to a slight numerical increase in fish oil odour and flavour and a decrease in meat odour. There were some indications of a dose-dependent effect of increasing dietary fish oil leading to increasing fish odour and flavour and camouflaging meat odour and flavour. Feeding fish oil increased the level of VLC $n-3$ fatty acids and other PUFAs in the pork, leading to greater susceptibility of the pork to oxidation without a more pronounced rancid odour or flavour, even with the highest inclusion of fish oil. There was no effect of additional dietary fat on sensory properties despite differences in fatty acid composition by increased VLC $n-3$ fatty acids content in medium-fat level compared to low fat. Reheating to produce warmed-over flavour (WOF) gave, however, higher rancid and fish oil flavour of meat with the highest inclusion of fish oil. The attributes warmed-over flavour and odour were not significantly different among the dietary groups. This could have been expected, because WOF is highly related to oxidation and meat containing more polyunsaturated fatty acids is more likely to oxidize and develop WOF (Jensen, Flensted-Jensen, Skibsted, & Bertelsen, 1998).

5. Conclusion

Feeding entire male and female pigs increasing dietary fish oil levels led to a dose-dependent increase in the VLC $n-3$ fatty acids, C20:5$n-3$ (EPA), C22:6$n-3$ (DHA) and especially C22:5$n-3$ (DPA) in the *M. longissimus dorsi*. After short-term storage, no differences in sensory quality among the dietary groups were detected in neck and belly. After long-term storage, a tendency of increased fish oil flavour in neck and belly was observed in the highest fish oil group. Reheating of the long-term stored belly caused higher fish oil flavour and odour scores and higher metal flavour and odour scores for the highest fish oil group. Increased efficiency in retaining VLC $n-3$ fatty acids was obtained when feeding diets with medium-fat levels. Both male and female pigs seemed to have high elongation of EPA to DPA. Entire male pigs had higher levels of PUFA, VLC $n-3$ fatty acids and lower levels of C18:1, indicating a lower delta-9-desaturase activity than in female pigs. This experiment confirms dietary fish oil as an effective means of altering the fatty acid composition of pork in order to provide human consumers with a healthy product without off-flavours and off-odours when limited fish oil amounts are used.

Acknowledgements

The authors acknowledge the financial support of Norwegian Research Council and Levy on Agricultural Products. The assistance and cooperation of The Animal Production Experimental Centre staff at the Norwegian University of Life Sciences, Ås and Animalia, Norwegian Meat and Poultry Research Centre staff is gratefully acknowledged. Finally we thank colleagues for their help and advices.

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Paper III

Changes in texture, colour and fatty acid composition of inner and outer layers of pig shoulder fat due to different dietary fat sources

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Keywords: Pig, fat quality, fatty acids, sex, texture, colour

Abstract

Two experiments with 72 slaughter pigs in each were conducted. Entire males and females were individually fed restricted. Palm kernel-, soybean- and fish-oil were used in varying combinations, giving different dietary fat levels (29 – 80 g/kg) and iodine values ranging from 50 to 131. Shoulder fat was analyzed for fatty acid composition (inner and outer layer), firmness and colour. A clear dose-response relationship was seen between fatty acids in diets and in shoulder fat. Interestingly, the very long chain n-3 fatty acids seemed to be deposited more efficiently when additional fat was included in the diet. Both high and low dietary iodine values changed towards less extreme iodine values in fat. Low-fat diets enhanced *de novo* synthesis of fatty acids. Males revealed a higher percentage of PUFA and a lower percentage of C18:1 and MUFA. Fat firmness, but not colour, was influenced by sex and dietary fat source.

1. Introduction

Fat is an important part of the pork carcass for the processing industry and the consumers. Fat of good technological and sensory quality has been defined as white and firm, while poor quality fat has been described as soft, oily, wet, grey and floppy (Wood, 1984). Hugo & Roodt (2007) reviewed the significance of fat quality and concluded that colour and consistency were the main criteria, and the most important measures were iodine value, C18:2*n*-6 content, C18:0/C18:2*n*-6 ratio and the double bond index. The best fat quality for further processing is, however, often found to be in contrast to the high nutritional quality of the product.

The composition of the modern pig has changed during the last decades towards leaner genotypes (Lebret & Mourot, 1998). This has influenced the fat distribution and the lean meat percentage in the pig, and the technological and sensory fat quality has according to Lebret & Mourot (1998) become reduced due to leaner pork carcasses. It has been observed that pigs generally deposit dietary fatty acids in a dose-response manner in subcutaneous fat (Wood et al., 2008), while low-fat diets lead to increased *de novo* synthesis of C16:0, C18:0 and C18:1 fatty acids (Kloareg, Bellego, Mourot, Noblet & van Milgen, 2005). All details in the regulation of fatty acid deposition versus *de novo* synthesis are, however, far from clear.

Pork production has in most countries been based on castrate and female pigs but several countries aim to abandon the castration procedure in near future. Production of entire males may be more common and the influence on lean meat percentage, fat distribution and fatty acid composition of primary cuts are of interest. Wood, Enser, Whittington, Moncrieff & Kempster (1989) observed differences in fatty acid composition between entire male and female pigs and concluded that backfat thickness was the main reason. A positive correlation

between backfat thickness and percentage of saturated fatty acids has been described by Fiego, Santoro, Macchioni & De Leonibus (2005). But fatty acid composition may also be influenced by steroid hormones. Thorling & Hansen (1995) showed that castrated rats given female hormone oestrogen developed the same fatty acid composition as females. Hallenstvedt, Kjos, Rehnberg, Øverland & Thomassen (2010) however, reported that in pigs with the same backfat thickness and lean percentage, female pigs had more MUFA and less SFA and PUFA in meat than entire male pigs.

Two experiments were conducted to evaluate the effects of different dietary fat levels, fatty acid compositions and sex (entire male and female) on the body fat distribution and the fat quality in terms of colour, texture and fatty acid composition.

2. Materials and methods

2.1 Experimental diets

Two experiments (Exp. 1 and Exp. 2) with growing-finishing pigs were carried out under similar conditions, but with different levels and composition of dietary fat. Information about ingredients and experimental diets have been reported elsewhere (Hallenstvedt, Øverland, Rehnberg, Kjos & Thomassen, Submitted; Hallenstvedt et al., 2010). The iodine values of the experimental diets were calculated using the AOAC method Cd 1c-85, and the iodine value product (IVP) was calculated as: IVP = percentage of fat x iodine value x 0.1

Exp. 1

Three experimental diets were formulated to contain mainly barley, oat, soybean meal and different blends of soybean oil and palm kernel oil. The diets were designed to give a wide variation in iodine values/iodine value product: 60/48.3 (LowIVP), 97/76.8 (MedIVP) and

120/98.6 (HighIVP). All diets contained 210 mg vitamin E as *dl*-α-tocopheryl acetat per kg and the same quantity and composition of amino acids per MJ. The fatty acid composition and calculated content of the experimental diets are presented in Table 1.

Exp. 2

Six experimental diets, two with low fat and four with medium fat levels, were formulated with different blends of palm kernel oil and fish oil. The two low fat diets were: a basal diet primarily consisting of barley and soybean meal, and no added fat (LF), and the basal diet added 4.8g/kg fish oil (LFF2). The medium fat diets contained (in g/kg) palm kernel oil and fish oil in the following ratios: 41:0 (PK1), 39:2.5 (PK2F1), 36:5.2 (PK3F2) and 34:7.2 (PK4F3). The LFF2 and PK3F2 diets had the same amount of fish oil inclusion per energy unit. The IV ranged from 50 to131 and the calculated IVP from 33.5 to 48.6. All diets had 212 mg vitamin E as *dl*-α-tocopheryl acetat per kg and the same quantity and composition of amino acids per MJ. The fatty acid composition and calculated parameters of the experimental diets are presented in Table 1.

2.2 Animals and housing

The two experiments followed similar procedures. Seventy two crossbred [(Norwegian Landrace x Yorkshire) x Duroc] pigs weighing approximately 25 kg live weight, 36 entire males and 36 females descending from two sires were used. The pigs were allotted by litter, live weight and sex in a randomized block design. In Exp. 1, 24 animals per treatment and in Exp. 2, 12 animals per treatment were used. All pigs were individually fed according to a restrictive feeding scale described by Øverland (1997). The pigs were fed twice daily and remaining feed was collected after each feeding and weighted to record the feed intake of each pig.

90 At a live weight of approximately 95 kg the pigs were delivered to a commercial abattoir for
91 slaughter. Three slaughter days were needed to reach the adequate target live weight for each
92 experiment. All treatments were equally distributed between each slaughter day.
93
94 **2.3 Collection of samples and lean meat percentages**
95 Two hours after slaughter shoulder fat was sampled between the 5^{th} and 7^{th} dorsal vertebra
96 from the right side of the carcass. The skin was removed and the fat sample was trimmed into
97 two equal sized samples of 3x4 cm, and wrapped in coded plastic bags. The samples were
98 then stored in a refrigerator at 4 °C for one or 15 days for colour and texture analysis. The fat
99 samples stored for one day were after the analyses of colour and texture divided into outer
100 and inner fat layers and each layer vacuum-packed and frozen in -80 ºC for fatty acid
101 analysis.
102
103 Lean meat percentage (Lean_GP2) was determined at the slaughter line with a commercially
104 GP2Q pistol (Hennessey System) as described by Kjos, Overland, Bryhni & Sorheim (2000).
105 The left sides of the one night chilled carcasses were separated into primal cuts. Three primal
106 cuts were weighted and dissected: ham, loin and shoulder. Each primal cut was dissected into
107 lean meat, fat and bone. Lean meat percentage was calculated for ham (Lean ham round), loin
108 (Lean loin) and shoulder (Lean shoulder) and as mean for the dissected cuts (Overall lean) for
109 each animal.
110
111 **2.4 Fat firmness and thickness**
112 Subjective measurements of fat firmness (judged by handling) were taken in the neck region
113 using a scale from 1 to 15 indicating increasing firmness (Overland, Skrede & Matre, 2001).
114 The shoulder fat samples were analyzed for texture using TA-XT2 texture analyzer (Stable

115 Micro Systems Ltd., Surrey, UK). In Exp. 1 a flat- ended cylindrical probe (SMS P/0.5) was
116 used. In Exp. 2 a needle (SMS p/2N) and a knob (SMS P/0.25 S) were used. The TA-XT2
117 was placed at 17 °C during the testing period, and calibrated using a 2 kg check weight on
118 each test day. The shoulder fat sample, with an average temperature of 7 °C, was placed on
119 the platform with the outer layer turned down. A test speed of 1.0 mm/s and compression
120 until 90 % was used. The peak force (in Newton, N) was recorded from the time-force graph
121 and used as the measure of firmness. This response variable has previously been found to
122 correlate well with sensory assessment of salmon fillet firmness (Mørkøre & Einen, 2003)
123
124 Measurements of backfat thickness were conducted with a ruler during separation into primal
125 cuts. Each animal was measured on five spots. Shoulder fat thickness in mm (Shoulder) was
126 measured below the shoulder blade (*scapula*), 5 cm backwards from the cranial end of primal
127 cut. Two dorsal fat thickness measurements of belly were taken; one 6 cm from the cranial
128 end (Belly 1) and one behind the last rib (Belly 2). Backfat thickness of the loin (Loin) was
129 measured at the cut surface of the last rib, above the rib vertebrae. Ham fat thickness (Ham)
130 was measured at the cut surface lateral to the aitchbone (*Ischium).*
131
132 **2.5 Fatty acid composition**
133 Fatty acid analyses were carried out separately on outer and inner layers of shoulder fat. The
134 samples were homogenized, extracted with HCl:petrol ether and methylated in H_2SO_4 in
135 methanol before analyses by gas chromatography (Hallenstvedt et al., 2010). Each fatty acid
136 was expressed as g/100g of total fatty acids.
137
138
139

2.6 Colour measurements by Minolta Chromameter and PhotoBox

Colour of the shoulder fat samples was measured using a Minolta CR200 instrument (Osaka, Japan) to obtain the CIELAB L*, a*, b* and to further calculate Hue and Chroma (saturation) values. The Hue was calculated as Hue = $\tan^{-1}$ (b*/a*) and Chroma as Chroma = $\sqrt{(a^{*2} + b^{*2})}$. The standard instrumental procedure for Minolta Chromameter was followed before use. The fat sample was placed on a light grey plate with the outer layer turned down, displaying the inner layer. All samples were cooled to 7 °C core temperature. Three colour measurements were taken of each sample and mean values were calculated.

Digital photographs were taken of the same fat samples using the PhotoBox. This is a light proof aluminium box (800 mm x 830 mm x 955 mm) equipped with black inside walls and standardized illumination (Folkestad et al., 2008). On top of the box a camera (Dolphin F145C, Allied Vision Technologies, Stadtroda, Germany) is located and controlled by a computer. The camera was calibrated with a QPcard 101, and a black plate with fat samples was placed in the box and photographed. L*, a* and b* values were given for each fat sample and Hue and Chroma were calculated.

2.7 Statistics

In each experiment the results were statistically analyzed using a general linear model (GLM) of analysis of variance (ANOVA) in Minitab 15 software (Minitab Inc, PA, USA). The factors used in the model were sex (entire male and female) and diet (LowIVP, MedIVP and HighIVP in Exp. 1 and LF, LFF2, PK1, PK2F1, PK3F2 and PK4F3 in Exp. 2). A GLM was also used to find the effect of layer on fatty acid composition and storage on colour parameters, both were included in the model. Tukey test was used to show differences among groups.

3. Results

There were no sex x diet interactions (P>0.05) for any of the parameters measured in both experiments; therefore, only the main effects (sex, diet, layer and storage) expressed as lsmeans values are reported in the tables for both experiments.

3.1 Production and carcass composition. Exp.1

There were no significant differences among diets (*P*>0.05) on growth rate, feed intake or the calculated feed conversion ratio (FCR, kg/weight gain). Entire males, however, had lower feed intake (*P*<0.01) than the female pigs. For more details see Hallenstvedt et al., (Submitted).

(Hallenstvedt et al.,)Sex had a slight influence on the carcass composition where entire males had less backfat thickness in ham and less lean meat percentage in shoulder (*P*<0.05) (Table 2).

The group fed diet LowIVP had highest backfat thickness at the Loin (*P*<0.001) and Belly1 (*P*<0.05).

3.2 Properties of shoulder fat. Exp.1

The subjectively measured backfat firmness was significantly higher in the LowIVP group compared to the MedIVP and HighIVP groups (*P*<0.001) and the same tendency was found using the TA-XT2.

Entire male and female pigs showed differences in fatty acid composition of outer and inner layers of shoulder fat (Table 4 and 5). The percentage of the medium long chain fatty acids

C12:0 and C14:0 (P<0.001), and of C18:2n-6 and C18:3n-3 (P<0.01) were higher in entire males. This resulted in significantly higher level of total PUFAs and lower level of MUFAs in males compared to female pigs. Female pigs had higher content of MUFA (P<0.01), especially C18:1 (P<0.001).

The fatty acid profile in both layers changed according to the dietary fatty acid composition in a clear dose-dependent manner. As the PUFA content of the diet increased, there was an increase in PUFA and C18:2n-6 content of the shoulder fat (P<0.001). A significant difference in iodine values of approximately 25 was observed between the LowIVP and HighIVP group. Pigs fed the LowIVP-diet with a high palm kernel oil content had high percentages of total SFA and especially the main fatty acids in palm kernel oil C12:0, C14:0 and C16:0 (P<0.001). Higher percentages of C16:1n-7, C18:1n-9 and MUFA were observed in pigs fed the LowIVP-diet (P<0.001) than pigs fed MedIVP and HighIVP diets.
The fatty acid composition differed between the two fat layers (Table 8). The outer shoulder fat layer had in general a lower content of C18:0 and SFA (P<0.01) and a higher content of C16:1n-7 and C18:1n-9 (P<0.001) and MUFA (P<0.001).

Sex influenced shoulder fat colour parameters after one and to less extent after 15 days of storage (Table 9). One day stored shoulder fat from females revealed higher a*-values by both digital photography (P<0.001) and Minolta (P<0.01). The L* value (M1_L*) was significantly higher in male than female pigs (P<0.001). The LowIVP-group showed higher L* values (P<0.05) than the HighIVP group for both storage times when Minolta was used, while using DP no significant differences due to feeding treatment was found.

214 The effect of storage on fat colour is presented in Table 11. Fat colour changed upon longer
215 storage time, clearly showing higher values of L*, b*, Hue- and Chroma using both DP and
216 Minolta. Only DP showed a significant increase in L* value during storage (P<0.001).
217
218 **3.1. Production and carcass composition. Exp. 2**
219 There were no significant differences among diets with respect to growth rate, feed intake or
220 the calculated feed conversion ratio (FCR, MJ/weight gain) (P>0.05). Entire males, however,
221 had a lower FCR than females (P<0.001). For more details see Hallenstvedt et al., (2010).
222
223 Carcass composition and fat firmness are presented in Table 3. Entire male and female pigs
224 had the same lean meat percentages using the GP2 and primal cuts methods. Entire males had
225 higher belly 2 thickness measurement (P<0.001), lower fat thickness of loin (P<0.001) and
226 lower fat thickness of the ham (P<0.01) than female pigs. No significant differences were
227 found among the dietary groups in lean content. The LF diet gave, however, significantly
228 lower fat thickness in Belly 1, located cranially, than the PK4F3 diet with the highest fish oil
229 inclusion (P<0.001).
230
231 **3.2 Properties of shoulder fat. Exp.2**
232 The subjective measurement of fat firmness (Table 3) revealed differences among the groups
233 due to both sex and diet, while no such differences were detected using the TA-XT2
234 instrument. Fat from male pigs was firmest (P<0.05) and the pigs fed the LF diet were
235 significantly more firm than pigs in the PK4F3 group who were given the highest dietary fish
236 oil inclusion (P<0.001).
237

238 The fatty acid profiles of the outer and inner layers of the shoulder fat are presented in Table
239 6 and 7. Sex influenced the fatty acid profile in which entire males had lower percentages of
240 MUFA and C18:1 and higher percentages of PUFA and C18:2*n*-6 ($P<0.01$) than the female
241 pigs.
242
243 The fatty acid profiles were highly affected by dietary treatments. The percentages of C12:0,
244 C14:0, C16:1 were significantly higher in animals fed the four PK diets ($P<0.001$) than those
245 fed the LF diets. Significantly lower content of C16:0 was observed in the LFF2 than in the
246 PK groups ($P<0.001$). The MUFA content was highest in LF fed pigs ($P<0.01$) while the
247 percentage of C18:1 was considerably higher ($P<0.001$) in both LF groups among the PK
248 groups.
249
250 The percentages of the essential fatty acids C18:2*n*-6 and C18:3*n*-3 were highest in the low
251 fat fed pigs ($P<0.001$). In general a dose-response between dietary and deposited n-3 fatty
252 acids was observed. The percentages of C20:5*n*-3 and C22:5*n*-3 were significantly lower in
253 the PK1 and LF groups than in the other dietary groups ($P<0.01$). Both F2 groups with the
254 same fish oil inclusion had in outer layer higher C20:5*n*-3 and C22:5*n*-3 percentages than
255 PK2F1 and lower than PK4F3 which had the highest inclusion of fish oil ($P<0.001$). C22:6*n*-
256 3 was highest in the PK4F3 group ($P<0.001$). Total PUFA was significantly higher in the
257 LFF2 group than in the PK groups ($P<0.001$).
258
259 There were significant differences in some fatty acids between the shoulder fat layers. The
260 outer shoulder fat layer had a significantly higher percentage of MUFA and the fatty acids
261 C16:1 and C18:1 ($P<0.001$). Conversely, C18:0 and C18:2*n*-6 were lower in outer shoulder
262 fat layer compared to the inner layer ($P<0.001$).

263 The fat colour results are presented in Table 10. Digital photographs (DP) of the fat samples
264 showed no sex difference in colour of one day stored fat (P>0.05). Minolta measured,
265 however, significantly higher b* value (P<0.01) along with a higher Chroma value (P<0.05)
266 in males than in female pigs. After 15 days of storage, male fat had significantly higher b*
267 and Hue values with both types of measurements. Using digital photography or Minolta gave
268 no difference between the dietary groups when measuring one day old fat (P>0.05). After 15
269 days of storage observations with both DP and Minolta showed that the LF group had
270 significantly higher Hue value than the PK1 and PK2F1 groups.
271
272 During storage, the fat colour changed as determined by the LAB-values from both DP and
273 Minolta (Table 11). Storage for 15 days gave significantly higher b* and Chroma values with
274 both DP and Minolta. In addition, Minolta measured higher L* and Hue values in long-stored
275 fat (P<0.001).
276
277 **4. Discussion**
278 **4.1 Carcass composition**
279 Sex effects on carcass composition have been widely described, but mainly castrates and
280 females have been compared (Serrano, Valencia, Nieto, Lázaro & Mateos, 2008; Wagner,
281 Schinckel, Chen, Forrest & Coe, 1999). In general, the gilts have been shown to have higher
282 lean meat percentage and lower total fat in several primal cuts. In current experiments, female
283 pigs were found to have higher ham backfat thickness compared to entire males. Fat
284 thickness behind the last rib of the belly (Belly 2) was higher and the fat thickness at the loin
285 was lower in entire males. In total this did not affect the GP2 lean meat percentage and the
286 calculated lean meat percentage based on the primal cuts. This agrees with results seen in
287 lambs (Barone, Colatruglio, Girolami, Matassino & Zullo, 2007) and with Beattie,

288 Weatherup, Moss & Walker (1999) who found no difference in lean meat in entire male and
289 female pigs. In the first experiment, entire males had less shoulder lean meat percentage than
290 female pigs, while this was not observed in Exp. 2. The sex effect on carcass composition is
291 not consistent, but it has been concluded that when modelling body composition, sex should
292 be included (Doeschl-Wilson, Green, Fisher, Carroll, Schofield & Whittemore, 2005)
293
294 In both experiments, fat depths in Belly 1 were affected by dietary treatments giving higher
295 fat depth with more dietary saturated fatty acids. The same was seen in the loin. This is partly
296 in agreement with Dugan, Aalhus, Robertson, Rolland & Larsen (2004) who found an
297 increase in subcutaneous fat levels when feeding tallow in comparison to canola oil to
298 barrows, but not to gilts. Felton & Kerley (2004), on the other hand, studied the effect of
299 different fat sources on steer carcasses and found no difference in backfat thickness due to the
300 ratio of PUFA:SFA.
301
302 **4.2 Firmness**
303 Female pigs had in Exp. 2 firmer backfat scores than entire males. When using the TA-XT2
304 instrument, the same result was shown in Exp. 1 as well. The difference in firmness between
305 entire males and females may be explained by the higher levels of MUFA, especially C18:1,
306 combined with a lower level of PUFA, giving higher firmness score in agreement with
307 Nishioka & Irie (2006).
308
309 Our study confirms previous findings suggesting that backfat firmness increases with reduced
310 dietary PUFA (Teye, 2009; Warnants, Van Oeckel & Boucque, 1996; Wood, Buxton,
311 Whittington & Enser, 1986). In general, high inclusion of dietary palm kernel oil gave the
312 highest firmness scores in agreement with earlier findings (Teye, Wood, Whittington, Stewart

313 & Sheard, 2006b). In Exp. 1, the medium and high dietary iodine values gave similar
314 firmness scores but softer fat than pigs given the low dietary iodine value. In both
315 experiments, the degree of fat firmness increased with increasing dietary SFA content as
316 found by Piedrafita, Christian & Lonergan (2001). Lopez-Bote, Isabel & Daza (2002) partly
317 replaced dietary PUFA with MUFA and indicated the increase in fat firmness observed to be
318 due to a reduced melting point. In Exp. 2, we observed a softer fat in pigs given low-fat diets,
319 this may be explained by the higher PUFA and higher MUFA content.
320
321 **4.3 Fatty acid composition**
322 The sex (entire male and female) influenced the fatty acid composition of both outer and
323 inner layer of shoulder fat. In both experiments, entire males had higher percentages of
324 C14:0, C18:2*n*-6, C18:3*n*-3 and PUFA, while females had higher percentage of MUFA,
325 especially C18:1. These results agrees with the findings of Wood et al., (1989) who found
326 higher percentage of C18:1*n*-9 and lower percentage of C18:2*n*-6 and C18:3*n*-3 in backfat
327 from above *M. longissimus dorsi* at the last rib. The authors concluded that this difference
328 was due to differences in backfat thickness where the females had higher fat thickness and,
329 therefore, more C18:1 and less C18:2*n*-6 and C18:3*n*-3. In the current experiments, the
330 differences in C18 fatty acids in the shoulder fat can not entirely be ascribed to differences in
331 backfat thickness. We observed the same difference in meat (Hallenstvedt et al.,2010), and
332 the results suggested that a higher delta-9-desaturase activity are found in female pigs. The
333 female steroid hormones have been reported to increase C18:1*n*-9 in rats (Thorling &
334 Hansen, 1995).
335
336 Dietary fat composition had a great influence on fatty acid composition in shoulder fat, as
337 expected. A dose-dependent relationship between dietary fatty acid content and level of fatty

acids in shoulder fat was observed, except for the diets with low fat content. Using the SFA, and especially C12:0 and C14:0 rich palm kernel oil alone or in combinations with other dietary fatty acids gave rather low levels of these fatty acids in the shoulder fat. It seems that the deposition of these fatty acids is low and most likely the pig oxidizes or elongates and desaturates these towards other stored fatty acids such as C16:0 and C16:1. Low levels of C12:0 and C14:0 has been shown in both muscle and fat tissues when 28 g/kg of dietary palm kernel oil was used (Teye, Sheard, Whittington, Nute, Stewart & Wood, 2006a; Teye et al., 2006b). In addition, the palm kernel oil rich diets led to a higher iodine value compared to the IV in the diets. High levels of the unsaturated soybean oil, high in C18:2*n*-6, deposited less C18:2*n*-6 compared to the more saturated diets in Exp. 1 and gave lower IV than originally in the diet.

The fat from pigs fed the LowIVP diet in Exp. 1 and nearly all diets in Exp. 2, was of good quality since IV was below the critical point of 65 (Lea, Swaboda & Gatherum, 1970) or 70 (Barton-Gade, 1987). This is an indication that the product can be suitable for further processing since excess PUFA (high iodine value) may result in oxidative breakdown leading to rancidity and higher incidences of off-flavour, abnormal colour and firmness (Russo & Nanni Costa, 1995 as cited by Lo Fiego, Santoro, Macchioni & De Leonibus, 2005).

In Exp. 2, LFF2 and PK3F2 diets contributed with the same amount of fish oil but the percentage of VLC *n*-3 fatty acids were higher when another dietary fat was added (Fig. 1). It seems that deposition of the nutritionally favourable fatty acids is higher, ie. less is metabolically oxidized when given together with other, more easily oxidized fatty acids in high-fat diets. This is similar to the findings observed in the meat from the same animals, but there the difference in deposition of fatty acids was even higher (Hallenstvedt et al.,2010).

363 The low-fat diets seemed to enhance the *de novo* synthesis of fatty acids, as indicated by the
364 higher percentage of C18:0 and C18:1 in the shoulder fat of these pigs. Leat, Cuthberson,
365 Howard and Gresham (1964) fed a semi-synthetic, nearly no-fat diet to pigs and found that
366 depot fat contained approximately 25% of C16:0, and more than 50% of C18:1. Others have
367 found similar results when feeding low-fat diets to pigs (Ding, Lapillonne, Heird &
368 Mersmann, 2003; Kloareg et al., 2005). The reason can be that high starch levels in the diets
369 lead to high insulin levels. Insulin has been reported to increase the *de novo* fatty acid
370 synthesis as reviewed by Hillgartner, Salati & Goodridge (1995) and further shown by Jump,
371 Botolin, Wang, Xu, Christian & Demeure (2005) in hepatic tissue. In Exp. 1 the classical *de*
372 *novo* synthesized fatty acids were highest in the low-fat diet group and decreased linearly in
373 the MedIVP and HighIVP diet groups. This is most probably due to the PUFAs reducing
374 effect on the novo synthesis of fatty acids as described by others (Hillgartner et al., 1995;
375 Jump et al., 2005; Sessler & Ntambi, 1998).
376
377 The backfat and shoulder fat can theoretically be divided into three separate layers, but due to
378 the live weight at slaughter and the site used for sampling in the present experiments, the
379 inner layer was negligible, implying that the inner layer refers to the middle layer as well
380 (Fortin, 1986). Differences in fatty acid composition between the outer and inner shoulder fat
381 layer were found, demonstrating that the outer layer contains more C16:1, C18:1 and total
382 MUFA, and less SFA, especially C18:0, in agreement with earlier results by Irie & Sakimoto
383 (1992), Apple, Maxwell, Galloway, Hamilton & Yancey (2008) and as indicated by Wiseman
384 & Agunbiade (1998). Furthermore, it is notable that PUFA was highest in the inner layer in
385 Exp. 2 which conflicts with findings of Apple et al. (2008) but agrees with Wiseman and
386 Agunbiade (1998) who reported higher levels of C18:2*n*-6 and C18:3*n*-3 in the inner layer.
387

388 **4.4 Fat colour**

389 Fat colour showed few consistent changes due to sex or diet. Maw, Fowler, Hamilton &
390 Petchey (2003) suggested that higher content of C18:2*n*-6 and C18:3*n*-3 acid giving higher
391 yellowness scores was mainly due to the pigments associated with these fatty acids since the
392 fatty acids themselves are colourless (O'Connor, 1960 as cited by Maw et al. 2003). Even if
393 the range in C18:2*n*-6 and C18:3*n*-3 acids were wide in our experiments, especially in Exp. 1,
394 no difference in shoulder fat colour was found. This concur most other studies, finding no
395 correlation between fatty acid composition and fat colour (Leskanich, Matthews, Warkup,
396 Noble & Hazzledine, 1997; Teye et al., 2006b). The colour change of fat during rather long
397 storage times indicating a more yellow fat and as well higher Hue values.
398
399 Measurements of fat colour were in our experiments conducted with two different methods.
400 The Minolta method is quite often used, but measurements have to be done manually on each
401 sample. The digital photography (PhotoBox) has been successfully used to evaluate colour of
402 fish, especially salmon. This instrument has, according to our knowledge, not been used on
403 pig fat samples earlier. The PhotoBox may speed up the time used for analysis, since several
404 (20-40) samples can be measured simultaneously. The correlations between the results
405 obtained with the two methods (Minolta and PhotoBox) were in our experiments not very
406 high, and further studies of using the PhotoBox instrument are consequently needed.
407
408 **5. Conclusion**

409 To conclude, the entire male and female pigs used in our studies revealed similar lean meat
410 percentage, but females had more stored fat in hind parts compared to entire males. The range
411 of dietary fat levels and fatty acid composition used had little influence on fat distribution.
412 Feeding diets low in PUFA, and especially diets high in palm kernel oil, led to increased fat

413 firmness. Male pigs had higher PUFA and lower MUFA levels, in particular C18:1, in
414 shoulder fat than female pigs. We suggest that the lower content of C18:1 and more 18:0 is
415 due to lower activity of the delta-9-desaturase activity in entire male pigs. For several of the
416 dietary fatty acids an almost linear deposition in pig fat was found, especially when
417 supplementary fat was added to the diet, while the medium length fatty acids C12:0 and
418 C14:0 seemed to be mainly oxidized or metabolized to longer chain fatty acids. Importantly,
419 the nutritionally positive VLC n-3 fatty acids seem to be conserved and deposited to a higher
420 extent when pigs were fed high-fat diets. Iodine value or iodine value product seemed to be a
421 good feed quality parameter for fat firmness.
422
423 **Acknowledgement**
424 The authors acknowledge the financial support by The Foundation for Research Levy on
425 Agricultural Products, The Research Council of Norway, Animalia, Norwegian Meat and
426 Poultry research Centre and Felleskjøpet Fôrutvikling. The assistance and cooperation of The
427 Animal Production Experimental Centre staff at the Norwegian University of Life Sciences,
428 Ås and Animalia, Norwegian Meat and Poultry Research Centre staff is gratefully
429 acknowledged.
430

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559
560

561 Table 1

562 Fatty acid composition (g/100g of total fatty acids) and fat content of the experimental diets, Exp. 1 and Exp. 2

	Exp. 1			Exp. 2					
	LowIVP	MedIVP	HighIVP	LF	LFF2	PK1	PK2F1	PK3F2	PK4F3
C10:0	1.7	0.8	0.2	<0.1	0.1	2.3	2.2	2.1	2.0
C12:0	26.4	11.6	2.2	0.1	1.2	33.5	31.3	29.5	28.0
C14:0	9.5	4.1	0.9	0.3	1.8	11.9	11.3	10.9	10.6
C16:0	13.4	13.5	14.1	22.3	20.0	12.4	12.6	12.8	12.9
C16:1*n*-7	0.1	0.1	0.1	0.2	1.6	0.1	0.4	0.8	1.0
C18:0	2.2	2.4	2.5	1.6	2.0	2.3	2.3	2.4	2.4
C18:1[A]	20.9	23.1	23.6	13.2	14	15.7	15.7	15.5	15.5
C18:2*n*-6	21.4	38.5	49.6	53.4	42.1	16.7	16.7	16.4	16.2
C18:3*n*-3	1.7	3.8	5.1	5.7	4.8	1.7	1.8	1.8	1.9
C20:1*n*-9	0.4	0.4	0.4	0.9	1.1	0.3	0.4	0.5	0.5
C20:4*n*-6	<0.1	<0.1	<0.1	<0.1	0.2	<0.1	<0.1	0.1	0.1
C20:5*n*-3	<0.1	<0.1	<0.1	0.1	3.5	<0.1	0.9	1.6	2.2
C22:5*n*-3	<0.1	<0.1	<0.1	<0.1	0.3	<0.1	0.1	0.2	0.2
C22:6*n*-3	<0.1	<0.1	<0.1	<0.1	2.7	<0.1	0.5	1.4	1.6
SFA	53.2	32.4	19.9	24.3	25.1	62.4	59.7	57.7	55.9
MUFA	21.4	23.6	24.1	14.3	16.7	16.1	16.5	16.8	17.0
PUFA	23.1	42.3	54.7	59.2	53.6	18.4	20.0	21.5	22.2
n-6	21.4	38.5	49.6	53.0	42.3	16.7	16.7	16.5	16.3
n-3	1.7	3.8	5.1	5.8	11.3	1.7	3.3	5.0	5.9
n-6/*n*-3	12.6	10.1	9.7	9.2	3.7	9.8	5.1	3.3	2.8
P/S[B]	0.5	1.4	3.2	2.6	2.3	0.3	0.4	0.4	0.4
Iodine value (IV)	60	97	120	120	131	50	54	62	67
Iodine value product (IVP)	48.3	76.8	98.6	35.8	44.7	33.5	39.2	45.0	48.6
Crude fat g/kg DM	80.3	78.2	80.7	29.2	33.6	67.0	72.5	72.6	72.6

563 [A]Sum of *n*-7 and *n*-9

564 [B] P=C18:2, C18:3, C20:4, C22:5 and C22:6, S=C12:0, C14:0, C16:0

Table 2

Influence of sex and diet on backfat thickness, fat firmness and lean meat percentages, Exp. 1

	Sex				Diet				
	Entire male	Female	Sed[A]	Sign[B]	LowIVP	MedIVP	HighIVP	Sed[A]	Sign[B]
Fat thickness, mm									
Shoulder	11.9	12.4	0.53	ns	12.7	11.8	11.9	0.67	ns
Belly 1	14.8	14.0	0.54	ns	15.5[b]	14.0[ab]	13.8[a]	0.68	*
Belly 2	15.2	14.7	0.82	ns	16.1	14.6	14.1	1.04	ns
Loin	10.9	12.0	0.54	ns	13.5[b]	10.5[a]	10.3[a]	0.69	***
Ham	9.79	11.0	0.57	*	11.2	9.93	10.1	0.72	ns
Fat fimness									
Backfat firmness (0-15)	11.2	11.1	0.14	ns	11.7[b]	11.0[a]	10.8[a]	0.18	***
Shoulder fat firmness (TA-TX2), N	155	179	11.7	*	170	169	162	14.7	ns
Lean meat percentages, %									
Lean_GP2	56.6	56.9	0.49	ns	57.0	56.6	56.6	0.63	ns
Lean ham round	70.8	70.1	0.46	ns	70.8	70.6	70.0	0.58	ns
Lean shoulder	62.5	63.6	0.43	*	63.4	63.1	62.7	0.54	ns
Lean loin	70.6	70.7	0.75	ns	70.6	71.3	69.9	0.99	ns
Overall lean	68.0	67.9	0.37	ns	68.2	68.3	67.3	0.47	ns

[A]Standard error of difference

[B]Significance of differences between samples in each row. ns: $p>0.05$; *: $p<0.05$; **: $p<0.01$; ***: $p<0001$.

Table 3

Influence of sex and diet on backfat thickness, fat firmness and lean meat percentages, Exp. 2

	Sex				Diet							
	Entire male	Female	Sed[A]	Sign[B]	LF	LFF2	PK1	PK2F1	PK3F2	PK4F3	Sed[A]	Sign[B]
Fat thickness, mm												
Shoulder	11.4	11.5	0.58	ns	10.9	10.6	12.0	12.6	11.8	10.8	1.03	ns
Belly 1	14.4	14.1	0.46	ns	12.8^{a}	13.2^{ab}	15.2^{ab}	14.4^{ab}	14.7^{ab}	15.3^{b}	0.81	*
Belly 2	14.4	11.9	0.65	***	12.5	12.8	12.8	14.7	12.5	13.9	1.14	ns
Loin	9.7	11.8	0.48	***	9.55	10.6	10.6	11.8	10.8	11.2	0.84	ns
Ham	9.60	11.0	0.47	**	9.55	9.33	10.3	11.6	10.3	10.7	0.82	ns
Fat fimness												
Backfat firmness (0-15)	11.0	11.2	0.11	*	10.6^{a}	10.9^{ab}	11.3^{b}	11.3^{b}	11.1^{b}	11.3^{b}	0.20	***
Shoulder fat fimrnessTA-TX2_knob, N	140	128	8.52	ns	137	132	134	128	130	141	15.23	ns
Shoulder fat fimrnessTA-TX2_needle, N	8.15	7.85	0.47	ns	8.17	7.14	8.05	7.85	8.32	8.45	0.82	ns
Lean meat percentages, %												
Lean_GP2	57.0	57.2	0.43	ns	57.3	57.0	57.2	56.7	57.3	57.1	0.78	ns
Lean ham round	71.5	70.9	0.36	ns	72.0	71.3	70.7	70.6	71.4	71.1	0.63	ns
Lean shoulder	64.9	64.7	0.30	ns	64.8	64.5	65.0	64.2	65.3	64.9	0.54	ns
Lean loin	69.5	70.6	0.59	ns	70.3	70.0	70.3	69.2	69.9	70.5	1.03	ns
Overall lean	68.6	68.7	0.34	ns	69.0	68.6	68.7	68.0	68.8	68.8	0.60	ns

[A]Standard error of difference

[B]Significance of differences between samples in each row. ns: $p>0.05$; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

Table 4

The fatty acid composition (g/100g of total fatty acids) in outer shoulder fat layer from entire males and females fed different IV levels, Exp. 1

	Sex				Diet				
	Entire male	Female	Sed[A]	Sign[B]	LowIVP	MedIVP	HighIVP	Sed[A]	Sign[B]
C10:0	0.10	0.10	0.01	ns	0.10	0.10	0.09	0.01	ns
C12:0	1.19	0.99	0.04	***	1.97[c]	1.03[b]	0.27[a]	0.05	***
C14:0	3.55	3.25	0.08	***	5.50[c]	3.22[b]	1.48[a]	0.10	***
C16:0	21.8	21.7	0.27	ns	23.8[c]	21.6[b]	19.8[a]	0.34	***
C16:1*n*-7	2.35	2.34	0.09	ns	3.16[c]	2.16[b]	1.71[a]	0.11	***
C18:0	10.8	10.7	0.27	ns	10.9	10.6	10.8	0.35	ns
C18:1[C]	33.9	36.2	0.44	***	36.9[b]	34.5[a]	33.7[a]	0.55	***
C18:2*n*-6	21.0	19.5	0.45	**	13.2[a]	21.4[b]	26.1[c]	0.57	***
C18:3*n*-3	1.73	1.60	0.04	**	0.97[a]	1.77[b]	2.26[c]	0.05	***
C20:1*n*-9	0.63	0.66	0.02	ns	0.66	0.64	0.63	0.03	ns
C20:4*n*-6	0.27	0.25	0.01	ns	0.23[a]	0.27[ab]	0.28[b]	0.02	*
C20:5*n*-3	<0.1	<0.1			<0.1	<0.1	<0.1		
C22:5*n*-3	0.11	0.11	0.01	ns	0.10	0.11	0.11	0.01	ns
C22:6*n*-3	0.09	0.09	0.01	ns	0.09	0.09	0.09	0.01	ns
∑ SFA	37.4	36.7	0.48	ns	42.3[c]	36.5[b]	32.4[a]	0.61	***
∑ MUFA	36.8	39.2	0.49	***	40.7[b]	37.3[a]	36.0[a]	0.62	***
∑ PUFA	22.7	21.1	0.49	**	14.2[a]	23.2[b]	28.4[c]	0.62	***
n-6/*n*-3 ratio	11.1	11.1	0.10	ns	11.6[c]	11.0[b]	10.7[a]	0.13	***
P/S[D] ratio	0.94	0.88	0.03	ns	0.45[a]	0.90[b]	1.32[c]	0.04	***
Iodine Value	76.7	75.9	0.76	ns	63.6[a]	78.1[b]	86.8[c]	0.96	***

[A]Standard error of difference

[B]Significance of differences between samples in each row. ns: $p>0.05$; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$

[C]Sum of *n*-7 and *n*-9

[D]P=C18:2, C18:3, C20:4, C22:5 and C22:6, S=C12:0, C14:0, C16:0

Table 5

The fatty acid composition (g/100g of total fatty acids) in inner shoulder fat layer from entire males and females fed different IV levels, Exp. 1

	Sex				Diet				
	Entire male	Female	Sed[A]	Sign[B]	LowIVP	MedIVP	HighIVP	Sed[A]	Sign[B]
C10:0	0.10	0.09	0.01	ns	0.10	0.10	0.08	0.01	ns
C12:0	1.31	1.08	0.06	***	2.26[c]	1.05[b]	0.27[a]	0.07	***
C14:0	3.73	3.36	0.10	***	6.02[c]	3.22[b]	1.40[a]	0.13	***
C16:0	22.2	22.2	0.28	ns	25.0[c]	21.8[b]	19.8[a]	0.36	***
C16:1*n*-7	1.72	1.71	0.08	ns	2.34[c]	1.56[b]	1.24[a]	0.10	***
C18:0	12.6	12.8	0.32	ns	13.3	12.4	12.4	0.41	ns
C18:1[C]	31.2	33.1	0.32	***	33.4[c]	32.1[b]	31.0[a]	0.41	***
C18:2*n*-6	21.8	20.5	0.46	**	13.5[a]	22.5[b]	27.6[c]	0.58	***
C18:3*n*-3	1.78	1.66	0.04	**	0.96[a]	1.84[b]	2.36[c]	0.05	***
C20:1*n*-9	0.61	0.66	0.02	*	0.64	0.64	0.62	0.03	ns
C20:4*n*-6	0.23	0.22	0.02	ns	0.20[a]	0.25[b]	0.23[ab]	0.02	*
C20:5*n*-3	<0.1	<0.1			<0.1	<0.1	<0.1		
C22:5*n*-3	0.10	0.08	0.01	*	0.07[a]	0.10[b]	0.10[b]	0.01	***
C22:6*n*-3	0.08	0.05	0.01	ns	0.05	0.08	0.07	0.02	ns
∑ SFA	40.0	39.5	0.53	ns	46.6[c]	38.5[b]	34.0[a]	0.68	***
∑ MUFA	33.5	35.5	0.37	***	36.4[c]	34.3[b]	32.9[a]	0.47	***
∑ PUFA	24.0	22.5	0.50	**	14.7[a]	24.7[b]	30.4[c]	0.63	***
n-6/*n*-3 ratio	11.4	11.9	0.15	**	12.6[b]	11.2[a]	11.1[a]	0.19	***
P/S[D] ratio	0.96	0.92	0.04	ns	0.44[a]	0.95[b]	1.42[c]	0.04	***
Iodine Value	75.0	73.9	0.86	ns	59.8[a]	77.0[b]	86.6[c]	1.09	***

[A]Standard error of difference

[B]Significance of differences between samples in each row. ns: $p>0.05$; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$

[C]Sum of *n*-7 and *n*-9

[D]P=C18:2, C18:3, C20:4, C22:5 and C22:6, S=C12:0, C14:0, C16:0

Table 6

The fatty acid composition (g/100g of total fatty acids) in outer shoulder fat layer from entire males and females fed different palm kernel and fish oil levels, Exp. 2

	Sex				Diet							
	Entire male	Female	Sed[A]	Sign[B]	LF	LFF2	PK1	PK2F1	PK3F2	PK4F3	Sed[A]	Sign[B]
C10:0	0.10	0.11	0.01	ns	0.10	0.10	0.10	0.12	0.11	0.11	0.01	ns
C12:0	1.55	1.38	0.07	*	0.10^{a}	0.18^{a}	2.18	2.21^{b}	2.15^{b}	1.97^{b}	0,.1	***
C14:0	4.37	4.11	0.10	*	1.37^{a}	1.53^{a}	5.72^{b}	5.75^{b}	5.72^{b}	5.33^{b}	0.18	***
C16:0	24.5	24.5	0.18	ns	24.0^{ab}	23.6^{a}	24.8^{b}	24.9^{b}	24.9^{b}	24.7^{b}	0.32	***
C16:1*n*-7	3.78	3.79	0.08	ns	3.37^{a}	3.34^{a}	4.12^{b}	3.88^{b}	3.97^{b}	4.00^{b}	0.14	***
C18:0	10.8	10.5	0.21	ns	11.4^{bc}	11.8^{c}	9.60^{a}	10.4^{ab}	10.3^{ab}	10.4^{ab}	0.37	***
C18:1[C]	38.1	39.4	0.45	**	42.3^{b}	41.5^{b}	38.5^{a}	36.9^{a}	36.4^{a}	36.8^{a}	0.80	***
C18:2*n*-6	11.7	11.1	0.18	**	12.5^{b}	12.4^{b}	10.6^{a}	11.0^{a}	11.1^{a}	10.9^{a}	0.32	***
C18:3*n*-3	1.08	1.03	0.02	*	1.15^{b}	1.17^{b}	0.97^{a}	1.01^{a}	1.01^{a}	1.04^{a}	0.04	***
C20:1*n*-9	0.64	0.66	0.02	ns	0.75^{c}	0.70^{bc}	0.64^{ab}	0.61^{a}	0.58^{a}	0.62^{ab}	0.32	***
C20:4*n*-6	0.21	0.21	0.01	ns	0.28^{c}	0.23^{bc}	0.22^{ab}	0.18^{a}	0.19^{ab}	0.19^{ab}	0.02	***
C20:5*n*-3	0.15	0.14	0.01	ns	0.00^{a}	0.21^{c}	0.00^{a}	0.10^{b}	0.24^{c}	0.32^{d}	0.01	***
C22:5*n*-3	0.23	0.24	0.01	ns	0.09^{a}	0.31^{c}	0.08^{a}	0.19^{b}	0.33^{c}	0.41^{d}	0.02	***
C22:6*n*-3	0.25	0.26	0.01	ns	0.08^{a}	0.30^{c}	0.06^{a}	0.21^{b}	0.39^{d}	0.48^{e}	0.02	***
∑ SFA	41.3	40.6	0.41	ns	37.0^{a}	37.2^{a}	42.4^{b}	43.4^{b}	43.2^{b}	42.5^{b}	0.73	***
∑ MUFA	42.5	43.8	0.49	**	46.4^{c}	45.6^{bc}	43.2^{ab}	41.4^{a}	41.0^{a}	41.4^{a}	0.87	***
∑ PUFA	13.6	13.0	0.21	**	14.1^{cd}	14.6^{d}	11.9^{a}	12.6^{ab}	13.2^{bc}	13.3^{bc}	0.37	***
n-6/*n*-3 ratio	7.40	7.29	0.15	ns	9.75^{d}	6.39^{b}	9.83^{d}	7.39^{c}	5.74^{b}	4.85^{a}	0.27	***
P/S[D] ratio	0.45	0.44	0.01	ns	0.55^{c}	0.57^{c}	0.36^{a}	0.38^{ab}	0.40^{ab}	0.41^{b}	0.02	***
Iodine Value	65.0	65.1	0.43	ns	68.5^{d}	70.2^{d}	61.3^{a}	61.9^{ab}	63.7^{bc}	64.9^{c}	0.77	***

[A]Standard error of difference

[B]Significance of differences between samples in each row. ns: $p>0.05$; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$

[C]Sum of *n*-7 and *n*-9 form

[D]P=C18:2, C18:3, C20:4, C22:5 and C22:6, S=C12:0, C14:0, C16:0

Table 7

The fatty acid composition (g/100g of total fatty acids) in inner shoulder fat layer from entire males and females fed different palm kernel and fish oil levels, Exp. 2

	Sex				Diet							
	Entire male	Female	Sed[A]	Sign[B]	LF	LFF2	PK1	PK2F1	PK3F2	PK4F3	Sed[A]	Sign[B]
C10:0	0.09	0.10	0.01	ns	0.10^{ab}	0.08^{a}	0.10^{ab}	0.11^{b}	0.10^{ab}	0.11^{b}	0.01	*
C12:0	1.43	1.36	0.09	ns	0.10^{a}	0.12^{a}	2.10^{b}	2.20^{b}	1.97^{b}	1.88^{b}	0.16	***
C14:0	4.07	3.98	0.14	ns	1.14^{a}	1.23^{a}	5.59^{b}	5.76^{b}	5.34^{b}	5.11^{b}	0.25	***
C16:0	24.3	24.4	0.28	ns	23.9^{ab}	22.7^{a}	24.9^{bc}	25.4^{c}	24.7^{bc}	24.6^{bc}	0.49	***
C16:1 n-*7*	2.73	2.90	0.08	*	2.49^{a}	2.41^{a}	3.08^{b}	2.90^{b}	3.01^{b}	3.04^{b}	0.14	***
C18:0	14.5	14.0	0.27	*	15.4^{a}	16.0^{a}	13.1^{b}	13.8^{b}	13.7^{b}	13.7^{b}	0.47	***
C18:1[C]	35.0	36.2	0.40	**	39.1^{c}	38.3^{c}	35.3^{b}	33.2^{a}	33.8^{ab}	33.9^{ab}	0.71	***
C18:2 n-6	12.5	11.9	0.19	**	13.0^{b}	13.2^{b}	11.5^{a}	11.9^{a}	11.9^{a}	11.7^{a}	0.34	***
C18:3 n-3	1.14	1.10	0.02	ns	1.12^{b}	1.20^{b}	1.04^{a}	1.08^{a}	1.08^{a}	1.08^{a}	0.04	***
C20:1 n-9	0.72	0.71	0.03	ns	0.85^{b}	0.77^{ab}	0.69^{a}	0.64^{a}	0.67^{a}	0.66^{a}	0.04	***
C20:4 n-6	0.22	0.21	0.01	ns	0.26^{c}	0.24^{bc}	0.23^{abc}	0.18^{a}	0.19^{ab}	0.19^{ab}	0.02	***
C20:5 n-3	0.18	0.16	0.01	*	0.02^{a}	0.25^{c}	0.00^{a}	0.12^{b}	0.26^{c}	0.36^{d}	0.02	***
C22:5 n-3	0.28	0.26	0.02	ns	0.09^{a}	0.38^{c}	0.08^{a}	0.23^{b}	0.37^{c}	0.47^{c}	0.04	***
C22:6 n-3	0.30	0.27	0.02	ns	0.06^{a}	0.38^{c}	0.04^{a}	0.24^{b}	0.45^{cd}	0.53^{d}	0.04	***
∑ SFA	44.4	43.8	0.46	ns	40.6^{a}	40.1^{a}	45.7^{b}	47.2^{b}	45.8^{b}	45.3^{b}	0.81	***
∑ MUFA	38.4	39.8	0.42	**	42.4^{c}	41.5^{c}	39.0^{b}	36.7^{a}	37.5^{ab}	37.6^{ab}	0.74	***
∑ PUFA	14.6	13.9	0.24	**	14.6^{bc}	15.6^{c}	12.9^{a}	13.7^{ab}	14.2^{b}	14.4^{b}	0.42	***
n-6/*n*-3 ratio	7.25	7.35	0.17	ns	9.67^{d}	6.01^{b}	10.16^{d}	7.40^{c}	5.61^{ab}	4.97^{a}	0.30	***
P/S[D] ratio	0.50	0.47	0.01	ns	0.57^{b}	0.65^{c}	0.39^{a}	0.41^{a}	0.44^{a}	0.45^{a}	0.03	***
Iodine Value	63.7	63.3	0.62	ns	65.8^{d}	69.0^{e}	59.3^{a}	60.0^{ab}	62.9^{bc}	63.7^{cd}	1.09	***

[A]Standard error of difference

[B]Significance of differences between samples in each row. ns: $p>0.05$; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

[C]Sum of n-7 and n-9

[D]P=C18:2, C18:3, C20:4, C20:5, C22:5 and C22:6, S=C12:0, C14:0, C16:0

Table 8

Differences in fatty acid composition, inner and outer layer of shoulder fat, Exp. 1and Exp. 2

	Exp. 1				Exp. 2			
	Outer layer	Inner layer	Sed[A]	Sign[B]	Outer layer	Inner layer	Sed[A]	Sign[B]
C10	0.10	0.10	0.00	ns	0.11	0.10	0.00	ns
C12	1.05	1.15	0.15	ns	1.47	1.39	0.17	ns
C14	3.27	3.43	0.34	ns	4.26	4.02	0.35	ns
C16	21.6	22.0	0.39	ns	24.5	24.3	0.20	ns
C16:1	2.28	1.68	0.11	***	3.78	2.82	0.07	***
C18	10.7	12.7	0.21	***	10.7	14.3	0.23	***
C18:1[C]	34.7	31.9	0.39	***	38.7	35.6	0.50	***
C18:2*n*-6	20.9	21.8	1.08	ns	11.4	12.2	0.18	***
C18:3*n*-3	1.73	1.78	0.11	ns	1.06	1.12	0.02	***
C20:1	0.64	0.63	0.01	ns	0.65	0.71	0.02	***
C20:4*n*-6	0.26	0.23	0.01	**	0.21	0.21	0.01	ns
C20:5*n*-3	<0.1	<0.1			0.14	0.17	0.02	ns
C22:5*n*-3	0.11	0.09	0.00	**	0.24	0.27	0.03	ns
C22:6*n*-3	0.09	0.07	0.01	***	0.25	0.28	0.03	ns
SFA	36.8	39.4	0.93	**	41.0	44.1	0.56	***
MUFA	37.6	34.2	0.47	***	43.1	39.2	0.49	***
PUFA	23.1	24.1	1.19	ns	13.3	14.3	0.22	***
n-6/*n*-3ratio	11.1	11.5	0.13	***	7.33	7.32	0.35	ns
P/S[D]ratio	0.95	0.98	0.07	ns	0.45	0.49	0.02	*
IV	77.2	75.4	1.97	ns	65.0	63.5	0.67	*

[A]Standard error of difference

[B]Significance of differences between samples in each row. ns: $p>0.05$; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$ within experiment

[C]Sum of n-7 and n-9

[D]P=C18:2, C18:3, C20:4, C20:5, C22:5 and C22:6, S=C12:0, C14:0, C16:0

Table 9

Colour of shoulder fat, inner layer (at 7th vertebra), for entire males and females and different dietary treatments after one and 15 days of storage, Exp.1

		Sex				Diet				
		Entire male	Female	Sed[A]	Sign[B]	LowIVP	MedIVP	HighIVP	Sed[A]	Sign[B]
PhotoBox										
1 day storage	DP1_L*	69.4	69.5	0.57	ns	70.5	69.1	68.9	0.71	ns
	DP1_a*	8.67	10.3	0.38	***	9.25	9.80	9.45	0.48	ns
	DP1_b*	2.71	2.10	0.19	**	2.12	2.41	2.66	0.24	ns
	DP1_Hue	17.8	12.0	1.49	***	13.7	14.7	16.4	1.88	ns
	DP1_Chroma	9.13	10.6	0.35	***	9.55	10.2	9.67	0.44	ns
15 days storage	DP2_L*	70.5	69.1	0.41	***	70.5	69.8	69.2	0.52	ns
	DP2_a*	7.26	7.09	0.46	ns	6.73	7.15	7.64	0.58	ns
	DP2_b*	4.64	4.32	0.41	ns	4.76	4.04	4.64	0.51	ns
	DP2_Hue	33.4	31.4	3.48	ns	35.6	29.7	31.9	4.39	ns
	DP2_Chroma	8.76	8.44	0.25	ns	8.51	8.37	8.92	0.32	ns
Minolta										
1 day storage	M1_L*	80.9	79.5	0.32	***	80.9^{b}	79.8^{a}	79.9^{a}	0.41	*
	M1_a*	4.35	5.02	0.21	**	5.00	4.50	4.55	0.27	ns
	M1_b*	4.96	4.86	0.29	ns	5.01	4.77	4.95	0.37	ns
	M1_Hue	48.2	44.2	1.47	**	45.0	46.2	47.3	1.84	ns
	M1_Chroma	6.63	7.01	0.32	ns	7.12	6.60	6.74	0.40	ns
15 days storage	M2_L*	81.6	80.9	0.25	**	81.7^{b}	81.3^{ab}	80.8^{a}	0.32	*
	M2_a*	3.54	3.00	0.37	ns	3.43	3.11	3.27	0.47	ns
	M2_ b*	7.35	6.94	0.50	ns	8.00^{b}	6.35^{a}	7.09^{ab}	0.64	*
	M2_Hue	63.5	64.4	0.31	ns	64.4	63.0	64.4	3.89	ns
	M2_Chroma	8.09	7.79	0.40	ns	8.72^{b}	7.18^{a}	7.93^{ab}	0.50	*

[A]Standard error of difference

[B]Significance of differences between samples in each row. ns: $p>0.05$; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$

Table 10

Colour of shoulder fat, inner layer (at 7th vertebra), for entire males and females and different dietary treatments after one and 15 days of storage, Exp. 2

		Sex				Diet							
		Entire male	Female	Sed[A]	Sign[B]	LF	LFF2	PK1	PK2F1	PK3F2	PK4F3	Sed[A]	Sign[B]
PhotoBox													
1 day storage	DP1_L*	70.2	69.5	0.90	ns	68.8	69.6	70.4	70.7	68.9	70.7	1.58	ns
	DP1_a*	9.71	9.01	0.48	ns	9.42	9.71	9.17	9.41	9.40	9.04	0.84	ns
	DP1_b*	2.34	2.53	0.23	ns	2.69	2.80	1.91	2.37	2.44	2.39	0.40	ns
	DP1_Hue	14.2	17.2	2.17	ns	17.3	16.8	12.1	14.8	17.1	16.1	3.82	ns
	DP1_Chroma	10.1	9.47	0.40	ns	9.92	10.2	9.40	9.77	9.88	9.46	0.71	ns
15 days	DP2_L*	69.7	69.4	0.36	ns	69.4	69.0	70.4	69.7	69.2	69.7	0.64	ns
	DP2_a*	8.71	9.16	0.30	ns	8.62	8.71	8.93	9.33	9.16	8.84	0.53	ns
	DP2_b*	3.37	2.70	0.16	***	3.68^{b}	3.35^{ab}	2.59^{a}	2.83^{a}	2.88^{ab}	2.87^{ab}	0.29	**
	DP2_Hue	21.5	16.7	1.27	***	23.8^{b}	21.6^{ab}	16.2^{a}	16.9^{a}	17.6^{ab}	18.4^{ab}	2.27	**
	DP2_Chroma	9.40	9.58	0.27	ns	9.47	9.38	9.31	9.80	9.63	9.32	0.47	ns
Minolta													
1 day	M1_L*	81.4	81.1	0.26	ns	80.7	80.6	81.8	81.9	81.2	81.3	0.46	ns
	M1_a*	5.26	4.87	0.21	ns	5.09	5.06	4.71	5.02	5.43	5.06	0.04	ns
	M1_b*	4.80	4.35	0.16	**	5.00	4.70	4.28	4.60	4.60	4.28	0.28	ns
	M1_Hue	42.6	41.9	1.18	ns	44.7	43.3	42.5	42.3	40.3	40.5	2.08	ns
	M1_Chroma	7.14	6.55	0.23	*	7.15	6.92	6.39	6.83	7.14	6.65	0.40	ns
15 days	M2_L*	82.4	81.7	0.29	*	82.8	82.2	82.1	81.9	81.5	81.7	0.51	ns
	M2_a*	5.24	5.26	0.22	ns	4.70	4.79	5.29	5.66	5.73	5.33	0.39	*
	M2_ b*	5.85	5.20	0.24	**	6.00	5.73	4.99	5.37	5.74	5.33	0.42	ns
	M2_Hue	48.0	44.8	1.21	*	51.6^{b}	50.4^{b}	43.3^{a}	43.0^{a}	45.0^{ab}	45.0^{ab}	2.13	***
	M2_Chroma	7.90	7.44	0.28	ns	7.69	7.50	7.28	7.84	8.13	7.56	0.49	ns

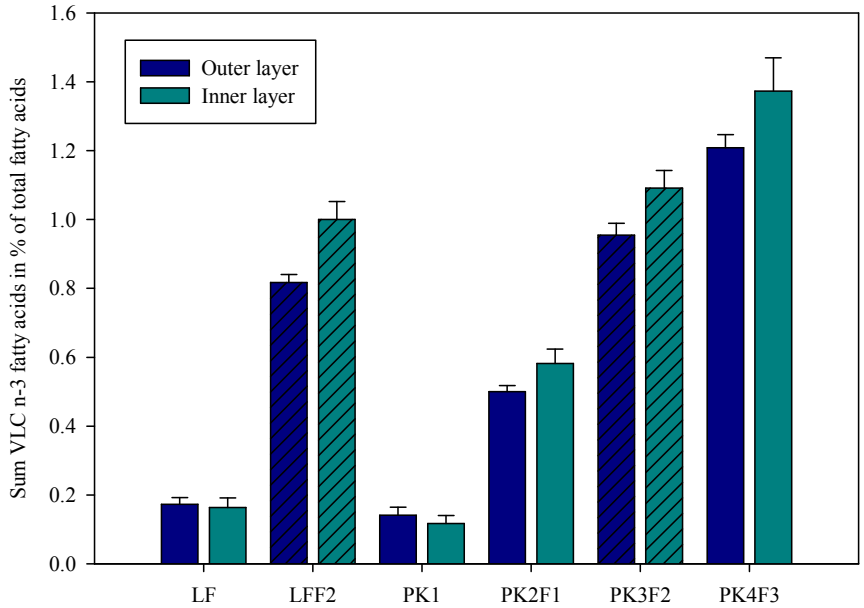
[A]Standard error of difference

[B]Significance of differences between samples in each row. ns: $p>0.05$; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$

631 Table 11
632 Influence of storage on shoulder fat colour, Exp. 1and Exp. 2

	Exp. 1				Exp. 2			
	1 day storage	15 days storage	Sed[a]	Sign[b]	1 day storage	15 days storage	Sed[A]	Sign[B]
M_L*	80.3	81.3	0.24	***	81.3	82.0	0.20	***
M_a*	4.60	3.30	0.21	***	5.06	5.26	0.15	ns
M_b*	4.91	7.11	0.29	***	4.56	5.45	0.14	***
M_Hue	46.7	63.8	1.61	***	42,2	46.3	0.18	***
M_Chroma	6.76	7.89	0.26	***	6.84	7.47	0.89	***
DP_L*	69.4	69.9	0.37	ns	69.8	69.5	0.47	ns
DP_a*	9.38	7.22	0.31	***	9.35	8.94	0.27	ns
DP_b*	2.48	4.48	0.22	***	2.4	3.02	0.15	***
DP_Hue	15.5	32.3	1.86	***	15.7	19.0	0.24	*
DP_Chroma	9.76	8.62	0.24	***	9.76	9.49	1.29	ns

633 [A]Standard error of difference
634 [B]Significance of differences between samples in each row. ns: $p>0.05$; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$ within experiment



635

636 Fig. 1 Percentage of VLC n-3 fatty acids in outer and inner layers of shoulder

637 fat in pigs fed diets containing different dietary oils (Exp. 2).

Paper IV

Production and deposition of very long chain n-6 and n-3 fatty acids in pigs as affected by dietary fat level and composition

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Keywords: pig, n-3 fatty acids, fish oil, fatty acid biosynthesis, elongase, desaturase

Abstract

The main objective of the study was to investigate the pigs ability to metabolize dietary C18 n-3 and n-6 fatty acids to longer chain fatty acids. Experimental animals were not exposed to any animal protein and only low levels of VLC n-3 in uterus during the farrowing period and during the weaning prior to the feeding experiment. At start of the experiment 12 piglets were sacrificed, and backfat, and neutral lipids and phospholipids in M. longissimus dorsi (LD) were analyzed for fatty acid composition. A total of 72 entire male and female pigs were restricted fed a low-fat (LF) diet, or diets containing either soybean oil (SBO) or soybean oil-fish oil (SBO-FO). Initial and final live weights were 33.6 and 105 kg, respectively. Outer and inner backfat layers, and neutral lipids and phospholipids in LD were analyzed for fatty acid composition. In pigs fed the LF and SBO diets, very low percentages of n-6 (0.2-0.5%) and n-3 (0.2-0.3%) VLC fatty acids were found in backfat and in neutral fat of LD. In the SBO-FO fed pigs the percentages of n-3 VLC fatty acids were significantly increased (1.3-1.8%) while n-6 VLC fatty acids remained low (0.2-0.3%), indicating a higher nutritional quality. When comparing backfat layers, the outer layer had less SFA and more MUFA and PUFA, while the iodine value and the n-6/n-3 ratio were similar. Significantly more PUFA and higher iodine value of fat from males than females were found. In the LD phospholipids of the LF group a high percentage of C20:4n-6 and a low level (3.2%) of n-3 VLC fatty acids with C22:5n-3 dominating was observed. Feeding SBO resulted in a similar pattern. When comparing to the composition of phospholipids at start of the experiment the percentage C22:6n-3 seemed to be reduced to about 50% in both LF and SBO groups. These results suggest that pigs have a limited ability to produce C22:6n-3 from dietary C18:3n-3. In LD phospholipids from SBO-FO fed pigs less C20:4n-6 and significantly more VLC n-

3 fatty acids (8.0%) were found, now with C20:5n-3 and C22:6n-3 dominating. This indicates that C20:5n-3 and C22:6n-3 are preferably included in phospholipids, and that less of the C20:5n-3 is elongated to C22:5n-3. In LD phospholipids few differences related to sex were seen.

Implications

In recent years the nutritional quality of meat products has become increasingly important. Feeding different fat sources change the fatty acids in meat products for human consumption. Dietary fat sources with a healthier fatty acid composition will consequently have important implications for human health. Especially when using PUFAs in the diet, several modifications of these fatty acids may occur in the animal body (Duran-Montge *et al.*, 2008). The objectives of the present work were to study the deposition of PUFAs in growing-finishing pigs and their ability to elongate and desaturate the fatty acids within the *n*-3 and *n*-6 families.

Introduction

Fatty acids of the *n*-6 and *n*-3 families are considered to be essential for pigs. An optimal balance among the very long chain (VLC) fatty acids is important for good health and well being in humans (Simopoulos, 2002) and similar effects for pigs can be anticipated. Innis (2008) further emphasize the importance of providing sufficient amounts of docosahexaenoic acid (DHA, C22:6*n*-3) to ensure normal growth and development of brain and nervous tissue in piglets as a model for humans. In addition, the contents of *n*-3 fatty acids in pork is important when the production of more healthy meat for human consumption have been investigated (Wood *et al.*, 2008, Hallenstvedt *et al.*, 2010, Realini *et al.*, 2010).

The monogastric pig highly reflect the fatty acid composition provided by the diet and meat products fortified with n-3 fatty acids have been shown to be a substantial contributor of these fatty acids in human diets (Bourre, 2005). Feeding linseeds to pigs has led to higher content of *n*-3 fatty acids, particularly C18:3*n*-3 (Enser *et al.*, 2000). Linoleic acid (C18:2*n*-6) serves as a precursor to arachidonic acid (ARA, C20:4*n*-6), while α-linolenic acid (LNA, C18:3*n*-3) can be elongated and desaturated into eicosapentaenoic acid (EPA, C20:5*n*-3), docosapentaenoic acid (DPA, C22:5*n*-3) and DHA. The ability of pigs to make these transformations is still debated. Enser *et al.* (2000) found an increase in both EPA and DHA in pigs when they were fed on linseeds while Raes *et al.* (2004) concluded in a review that a limited increase in DHA is obtained by feeding pigs C18:3*n*-3 rich diets. To increase the VLC *n*-3 fatty acids including DHA in pork, fish products can be used (Haak *et al.*, 2008).

A dietary increase of C18:2*n*-6 has led to raised tissue content of C18:2*n*-6, and an increase in C20:4*n*-6 was further seen when diets low in *n*-3 fatty acids were used (Lu *et al.*, 2008). High intakes of C20:4*n*-6 has recently attracted new interest as a negative factor in relation to human health (Hyde and Missailidis, 2009). Because fatty acids of the *n*-3 and *n*-6 families are metabolized by the same enzyme systems (Cook and McMaster, 2002) it is of interest to examine what happens when both are provided by the diet. Nguyen (2003) presented a mathematical relationship between dietary intake and deposition of fatty acids in pigs, but Hallenstvedt *et al.* (2010) however, found that dietary fat level may influence the balance between the oxidation and deposition of *n*-3 fatty acids. The intermediary metabolism of fatty acids in pigs, with special emphasis to the *n*-6 and *n*-3 fatty acids are, consequently still somewhat unclear, and is the main topic of this study.

Pigs fed low-fat diets show an increased endogenous fatty acid synthesis (Kloareg *et al.*, 2005). The effect of sex; entire male and female, is further included in the present study. Sex has been shown to have an impact on fatty acid composition and may consequently influence the meat quality (Barton-Gade, 1987). A study showed that the fatty acid deposition pattern in different tissues varies (Romans *et al.*, 1995). This is still of interest and also included in the current work.

Materials and methods

Pre experimental period

The animals used in the present experiment were not exposed to any animal protein sources from gestation throughout the piglet period until weaning. Sows were fed special gestation and lactation diets and piglets were provided with a diet with plant protein and fat sources to reduce the potential deposition of *n*-3 fatty acids from animal sources. At approximately day the 30 of lactation period a milk sample was taken from each sow. The samples were mixed and analyzed for fatty acid composition. The aim of this treatment was to reduce the *n*-3 fatty acid content in the experimental pigs, if possible, enhance the activities of delta 5 and 6 desaturases and elongases. The pigs were crossbred [(Norwegian Landrace x Yorkshire) x Duroc] and bred from two boars and nine sows. At start of the feeding experiment 12 siblings were slaughtered. Backfat, neutral lipids and phospholipids of LD were analysed for fatty acid composition and used for a base-line lipid composition.

Animals and treatments

A total of 36 entire male and 36 female pigs with an average live weight of 33.6 kg were distributed in 12 pens according to sex, litter and live weight. The animals were individually and restricted fed in accordance with a feeding scale for growing-finishing pigs (Øverland, 1997). To obtain less variation in live weight at slaughter, three different slaughter days were used based on the targeted live weight of 105 kg. The pigs were slaughtered at a commercial abattoir. Animals were well treated and cared for according to the animal law (the Animal Protection Act of December 20th, 1974).

Diets

Three experimental diets were formulated and produced by pelleting technology. Chemical contents of the feed ingredients and diets, are presented in Table 3. The basal diet consisted of barley, oat and soybean meal (low fat, LF). The other diets consisted of LF with soybean oil (SBO) or soybean oil and fish oil (SBO-FO) added. All three diets were prepared with 110 mg/kg vitamin E as *dl*-α-tocopherol acetate.

Carcass and sample collection

At the slaughter line lean meat percentage was measured with a commercially GP2Q instrument (Hennessy System Ltd, Auckland, New Zealand). The instrument determines two backfat thicknesses between the 10th and 11th rib, 6 cm from the midline (Fat GP1) and behind the last rib, 8 cm from the midline (Fat GP2). During division in primal cuts backfat thickness in the cutting edge between the 10th and 11th rib was measured with a ruler (Fat R2).

The day after slaughter meat samples of approximately 200g for fatty acid analysis were collected from the P2 area of *M. longissimus dorsi* (LD) (Overland *et al.*, 1999). Backfat at the same location was taken out as a 4x4 cm piece, divided in outer and inner layers and analyzed separately. The young siblings had not developed two visible backfat layers, so only a total backfat analyze was performed.

Intra-muscular fat and fatty acid composition

Lipid was extracted from backfat and LD using methanol:chloroform 1:2 as described by Folch, Lees & Stanley (1957). A silicic column (Sep-Pak® Plus Silica cartridge, Waters), hexane diethyl ether and methanol was used to separate the total lipids in neutral lipids and phospholipids. Methyl esters of fatty acids from muscle and backfat were analyzed by gas chromatography (Cp-wax 52CB 25 m capillary column) identified and expressed as percentage of total fatty acids. Iodine value was calculated according to the procedure based on fatty acid composition (AOCS, 1998).

Statistical analysis

Results are presented as least means squares with standard error of difference (SED). The general linear model procedure in Minitab 15 software (Minitab Inc., PA, USA) was used. Factors included in the model were sex (entire male and female) and diet (LF, SBO and SBO-FO) for all the parameters analyzed. Initial live weight was included as covariate for the growth and feed data. Differences between pair-wise combinations of the least square means were tested using Tukey test.

Results

No significant interactions between diet and sex were found when included in the model for the measured parameters. Only least mean squares for the main effects are presented.

Fatty acid composition of the diets and sow milk during piglet production

Fatty acid composition of the diets used during gestation, lactation and piglet period are presented in Table 1. Fatty acid profile of the sow milk is also presented. All diets were high in *n*-6 fatty acids, especially C18:2*n*-6, and low in *n*-3 fatty acids. Only traces of C20:5*n*-3 was found in the gestation and piglet diets. The sow milk had high percentage of SFA, low percentages of *n*-3 fatty acids.

Fatty acid composition of backfat and M. longissimus dorsi of young siblings

Backfat showed a high percentage of MUFA, mainly C18:1*n*-9, a high percentage of C18:2*n*-6 and little C20:4*n*-6. The major *n*-3 fatty acid was C18:3*n*-3 while the percentage of VLC *n*-3 fatty acids was minor. The neutral lipids from LD revealed similarities to backfat in fatty acid composition, however, a higher MUFA content, in particular C18:1*n*-9, and less PUFA and C18:2*n*-6 were found. The phospholipids had low percentage of MUFA, especially C18:1*n*-9, but high percentage of PUFA, with a high percentage of C18:2*n*-6 and a considerable content of C20:4*n*-6 (Fig. 1). The fatty acid analysis showed relatively low percentage of C18:3*n*-3 and of VLC *n*-3 fatty acids, here with C22:5*n*-3 as the most prominent (Fig. 2).

Animal performance and carcass quality

The entire males had a significantly lower feed intake and a lower feed conversion ratio than female pigs (Table 5). In addition males tended to have a higher average

daily gain. Females had a higher intra muscular fat percentage (*P*<0.001). No significant difference between males and females in backfat thickness was seen. The LF fed pigs had a slightly higher average daily feed intake than the SBO and SBO-FO diet groups during the experimental period. Feed conversion ratio as FU/kg weight gain was significantly lower in these groups as compared to the LF group. Pigs fed SBO or SBO-FO diets had highest Fat GP1 thickness.

Fatty acid composition as affected by sex

Sex influenced the percentages of several fatty acids in outer and inner backfat layer, and in neutral lipids and phospholipids of LD (Table 6, 7, 8 and 9). Males had significantly lower percentages of C16:0, total SFA, C18:1*n*-9 and total MUFA in both backfat layers and in neutral fat of LD. In addition, males had the highest iodine value (P<0.001) and the highest percentage of total PUFA (P<0.001), mainly due to the high percentages of C18:2*n*-6 and C18:3*n*-3 (P<0.001) in both backfat layers and neutral fat in LD. Interestingly, few differences due to sex were found in phospholipids of LD*,* only C16:1*n*-7 was lower and C18:0 were higher in males than in females.

Fatty acid composition in backfat and neutral lipids as affected by diet

As expected, both dietary fat level and fatty acid composition influenced the fatty acid composition in the backfat and in the meat. Pigs fed the LF diet had significantly higher percentages of SFA, C16:0 and C18:0 in neutral lipids of muscle, and in outer and inner layer of backfat as compared to the SBO and SBO-FO fed pigs. Further the LF fed pigs had the highest MUFA, C18:1*n*-7 and C18:1*n*-9 (P<0.001) percentages in all tissues. Sum of *n*-6 and *n*-3, total PUFA and iodine value were substantially lower

200 in LF compared to the high fat dietary groups. Feeding pigs the SBO diet increased
201 PUFA and iodine values (P<0.001), compared to both LF and SBO-FO fed pigs, in
202 LD neutral lipids as well as in the outer and inner backfat layers. The percentages of
203 the C18:2*n*-6 and C20:4*n*-6 were significantly higher in the SBO fed pigs. Also the
204 C18:3*n*-3 was highest in the SBO group. The SBO-FO diet gave significantly higher
205 percentages of the VLC *n*-3 fatty acids C20:5*n*-3, C22:5*n*-3 and C22:6*n*-3 in neutral
206 lipids of LD and both backfat layers. The percentage of C20:4*n*-6 was lower in the
207 SBO-FO group than the SBO, but similar to what was found in the LF fed pigs.
208 C18:3*n*-3 was also found to be less in SBO-FO but still higher than in LF fed pigs.
209
210 *Fatty acid composition in LD phospholipids as affected by diet*
211 Phospholipids in pigs fed the LF diet had less SFA and PUFA, while the MUFA
212 percentage was substantially higher than in the pigs given the two diets containing
213 added fat. In particular, a major increase in C16:1, but also higher percentages of
214 C18:1*n*-7 and C18:1*n*-9 were observed as compared to the SBO or the SBO-FO fed
215 pigs. Low percentages of the VLC *n*-3 fatty acids were found in the LF group with the
216 C22:5*n*-3 as the dominating. When comparing to the composition of the
217 phospholipids in young siblings (Fig. 1 and 2), the percentages of C18:2*n*-6 and
218 C20:4*n*-6 were similar. The percentages of 20:5*n*-3 and 22:5*n*-3 were also kept at a
219 similar level, while the percentage of 22:6*n*-3 seemed to be reduced.
220
221 When SBO was added to the diet, significantly higher percentages of both C18:2*n*-6
222 and C18:3*n*-3 than in the LF pigs were observed, while the percentage of C20:4*n*-6
223 and C22:5*n*-3 both were significantly reduced. When comparing to the young siblings
224 the percentage of C22:6*n*-3 was now reduced by nearly 50%. The increase in

C18:2*n*-6 was also seen in the SBO-FO group, while the 18:3*n*-3 was similar to the pigs fed the LF diets. A further significant reduction in C20:4*n*-6 was found, and as expected, the percentages of C20:5*n*-3 and C22:6*n*-3 were substantially increased in SBO-FO fed pigs. Interestingly, the percentage of C22:5*n*-3 was similar to that found in the LF group, and only slightly increased as compared to the SBO fed pigs (Fig.2).

Fatty acid composition in different tissues

The outer backfat layer had generally higher percentages of MUFA and C18:1*n*-7 and lower percentages of C16:0, C18:0 and SFA than the inner layer. The neutral fat of LD had a substantial higher content of MUFA combined with a lower percentage of PUFA compared to backfat. Low contents of *n*-6 and *n*-3 fatty acids were observed in neutral fat in LD compared to backfat. The percentages of *n*-6 and *n*-3 fatty acids seemed to be higher in the outer backfat layer as compared to inner layer, but the *n*-6/*n*-3 ratio was similar.

Discussion

Fatty acid composition of the sow milk, the piglet diet and of the backfat and M. longissimus dorsi in young siblings

Sows used for experimental piglet production were fed a diet without any animal ingredients from insemination through the lactation period because this has been shown to have an impact on the sow milk fatty acid composition (Amusquivar *et al.*, 2010). Analyses of sow milk showed slightly lower *n*-3 fatty acid percentage compared to the study by Bazinet *et al.* (2003) where total *n*-3 fatty acids were reported to be 2.8%. In a study by Arbuckle and Innis (1993) sows were fed diets added vegetable oil or fish oil. The milk from sows fed fish oil clearly showed an

250 increased content of C18:3*n*-3 and the VLC *n*-3 fatty acids C20:5*n*-3 and C22:6*n*-3.
251 In our current study C18:3*n*-3 was the major *n*-3 fatty acid in sow milk. Similarly to the
252 sow milk the piglet diet contained hardly any detectable levels of VLC *n*-3 fatty acids.
253 Still, in the young siblings the C22:5*n*-3 was the dominating VLC *n*-3 fatty acid in both
254 backfat, neutral lipids and phospholipids of LD. This may be taken as an indication
255 that young pigs are capable to desaturate and elongate the C18:3*n*-3 to C22:5*n*-3. To
256 what extent a capacity to further metabolize C22:5*n*-3 to C22:6*n*-3 is present, is more
257 uncertain.
258
259 There is a common knowledge that the fatty acid composition changes during growth
260 and differ among tissues. The fatty acid composition in different tissues of young
261 siblings showed similarities between backfat and neutral lipids of LD with high MUFA
262 and the low PUFA content. The phospholipids in LD were as expected high in PUFA
263 and had the lowest saturation as found earlier (Wood, 1984). Interestingly, the
264 phospholipids showed a preference towards C18:2*n*-6 compared to the C18:3*n*-3
265 fatty acid when substantial amounts of both fatty acids were present in the piglet diet.
266 In phospholipids a higher C20:4*n*-6 percentage than provided by the piglet diet was
267 found and may indicate a synthesis from C18:2*n*-6.
268
269 *Animal growth, feed intake and carcass measurements*
270 Sex had an impact on the performance parameters as reported earlier (Högberg *et*
271 *al.*, 2004, Lundström *et al.*, 2009). Entire males had a higher feed intake than female
272 pigs, and this agrees with results reported earlier (Hallenstvedt *et al.*, Submitted).
273 Further, the males had a lower feed conversion ratio than females. Males have
274 previously been reported to have an increased feed conversion ratio than castrated

males . This lower feed conversion ratio in entire males can partly be ascribed to a slightly higher daily growth rate. Lean meat percentage has been reported to be higher in males than females, mainly because of a higher fat content in females (Babol and Squires, 1995). This was not observed in the present experiment. Female pigs had, however, a higher intra-muscular fat content as previously reported , indicating a higher ability for lipid synthesis as reported by Eguinoa *et al.* (2003) but in conflict with Högberg *et al.* (2004).

Different dietary composition did influence the feed intake. LF fed pigs had a higher feed intake than the other groups, mainly to compensate for the lower energy content in the diets (Chiba *et al.* 1985). The pigs fed the low fat diet also converted the feed less efficiently (kg feed/kg gain).This is most probably due to a lower energy content and a lower digestibility of this diet compared to the high fat diets (Noblet *et al.*, 1993). When calculating feed units by use of digestibility coefficients no such difference was seen.

Fatty acid composition as influenced by sex

Backfat and neutral lipids in LD were less saturated and lower in MUFA in males than in females. This has been explained by the lower backfat thickness in males than females . We have suggested that the substantial higher MUFA in females is due to a higher delta-9-desaturase activity (Hallenstvedt *et al.,* Submitted). Significantly more PUFA and higher iodine value of fat in males versus females were further observed. Males had higher percentages of both *n*-6 and *n*-3 fatty acids which agree with results by Riley *et al.* (2000) and Högberg *et al.* (2004).

300 *Fatty acid composition in backfat and neutral lipids as affected by diet*

301 The LF fed pigs had in outer and inner backfat layer and in neutral lipids of LD high
302 percentages of SFA, C16:0, C18:0, in addition to high MUFA, especially C18:1*n*-7
303 and C18:1*n*-9. This clearly indicates an extended *de novo* synthesis. The LF diet
304 provided most of the energy as starch. The *de novo* synthesis of fatty acids has most
305 probably been up regulated by the higher insulin secretion due to the high starch
306 level (Hillgartner *et al.*, 1995). The resulting fatty acid composition obtained in the
307 current experiment with high C18:1, C18:0, C16:1 and C16:0 is in agreement with
308 earlier experiments (Ding *et al.*, 2003). Of the *n*-3 fatty acids, the LF diet contained
309 mostly C18:3*n*-3, and in backfat layers and neutral fat of LD C18:3*n*-3 was still the
310 prominent fatty acid. VLC *n*-3 fatty acids, especially C22:5*n*-3 were, however, found.
311 This is a strong indication of metabolism of C18:3*n*-3 towards the VLC *n*-3 and
312 especially C22:5*n*-3 as reported by Juárez *et al.* (2010) studying backfat and by Riley
313 *et al.* (2000) studying muscle.

314

315 A high soybean oil inclusion gave as expected high PUFA and an increased iodine
316 value in both backfat layers and neutral lipids of LD. The main fatty acid in soybean
317 oil is C18:2*n*-6, and this was highly deposited in backfat layers and neutral lipids of
318 LD. This has been shown earlier when Lu *et al.* found a significant percentage of
319 dietary C18:2*n*-3 in neutral lipids of LD. In a study by D'Arrigo *et al.* (2002) feeding
320 the pigs sunflower oil, also high in C18:2*n*-6, an increased percentage of both
321 C18:2*n*-6 and C20:4*n*-6 was found in backfat, this further supports the presence of
322 elongation and desaturation of C18:2*n*-6. When compared to the fatty acid
323 composition at start of the experiment, an increase in C18:2*n*-6 and C18:3*n*-3 was

observed in the SBO fed animals, while low percentages of the VLC *n*-3 fatty acids were found.

Dietary fish oil is a contributor of the VLC *n*-3 fatty acids C20:5*n*-3 and C22:6 *n*-3 and pigs fed SBO-FO had markedly raised percentages of C20:5*n*-3, C22:5*n*-3 and C22:6*n*-3 in all tissues. The rather high content of C22:5*n*-3 compared to the dietary supply suggests that an elongation of C20:5*n*-3 had occurred. The ratio between C22:5*n*-3 and C20:5*n*-3 was reduced when feeding fish oil, it can be anticipated that the enzyme system has been saturated or that C22:6*n*-3 work as a feedback regulator. Interestingly in backfat and neutral lipids of LD from SBO-FO fed pigs a reduced percentage of C20:4*n*-6 was observed. This may be due to the competition between the *n*-6 and *n*-3 fatty acids for the same enzyme systems. C18:3*n*-3 is found as a better substrate for delta-6 desaturase (Cook and McMaster, 2002). Similar results was reported by Cherian & Sim (1995) with a concomitant reduction in C20:4*n*-6 in muscle and backfat with a high dietary C18:3*n*-3 level.

Fatty acid composition in phospholipids in LD as affected by diet

A rather low SFA and PUFA content was observed in phospholipids when pigs were fed the LF diet. It seems that the *de novo* synthesis of fatty acids has had an impact on the fatty acid composition, even if such an effect has been reported to be relatively low (Riley *et al.*, 2000). Pigs fed LF also showed rather low VLC *n*-3 fatty acid content, however, C22:5*n*-3 was the most significant and suggests an elongation from C18:3*n*-3 as found in total lipids of liver by Lauridsen, Hojsgaard & Sorensen (1999) when feeding pigs low fat diet.

In general, phospholipids maintained a relatively high C20:4*n*-6 content when comparing the slaughter pigs with young siblings. This rather high C20:4*n*-6 content during the feeding experiment suggests elongation and desaturation of C18:2*n*-6 because the diets nearly lack C20:4*n*-6.

In LD phospholipids similar percentages of C20:5*n*-3 and C22:5*n*-3 were seen in the slaughter pigs fed LF and SBO diets compared to the young siblings. The C22:6*n*-3 seemed to be reduced when feeding LF or SBO diets during the growing-finishing period. The results suggests that a conversion from C18:3*n*-3 to C20:5*n*-3 and C22:5*n*-3 occurs, while the further metabolism from C22:5*n*-3 to C22:6*n*-3 is, at the best very limited. Similar conclusions were drawn by Lu *et al*. (2008). Enser *et al.* (2000), on the other hand, also found an increase in C22:6*n*-3. So even when the content of VLC *n*-3 fatty acids had been kept very low in the piglets, no indication of a significant 22:6*n*-3 production was seen. When feeding fish oil (SBO-FO) VLC*n*-3 increased significantly but now the C20:5*n*-3 was the most prominent fatty acid. This may be explained by a saturation in the conversion towards C22:5*n*-3 or a inhibiting effect of C22:6*n*-3 as seen earlier . Along with the high VLC *n*-3 fatty acids when feeding fish oil a reduced percentage of C20:4*n*-6 was seen. This has also been observed by Calder (2003). Calder (2003) emphasised the benefits of exchanging the C20:4*n*-6 in membranes by C20:5*n*-3 fatty acid, because it can be anticipated that the pigs then, due to the anti-inflammatory effects of the VLC *n*-3 fatty acids, will be less susceptible to autoimmune diseases and have a better immune system.

Conclusion

Our experiment clearly show that when feeding LF or SBO diets very low percentages of VLC *n*-3 are found in outer and inner backfat layer and neutral lipids of LD. Introducing dietary fish oil substantially increased the VLC *n*-3 fatty acids and improve the nutritional quality of pork. The outer backfat layer seemed to have more MUFA and PUFA combined with less SFA while the iodine value and *n*-6/*n*-3 ratio were similar. Male pigs had significantly higher PUFA and iodine value and lower MUFA, in particular C18:1*n*-9 in backfat and in neutral lipids of LD than female pigs. The LD phospholipids of pigs fed the low fat diets showed high C20:4*n*-6 and low VLC *n*-3 percentages, mostly C22:5*n*-3. When compared to the young siblings, pigs fed the SBO and LF diets seemed to have about a 50 percentage reduction in the percentage of *n*-3 fatty acids. This suggests a very restricted ability in these pigs to produce C22:6*n*-3 from dietary C18:3*n*-3. The fatty acid composition in LD phospholipids was less affected by sex than neutral lipids and backfat.

Acknowledgement

This study was funded by The Research Council of Norway, The Foundation for Research Levy on Agricultural Products and Felleskjøpet Fôrutvikling. The authors highly appreciate the help by The Animal Production Experimental centre staff at the Norwegian University of Life Sciences, Ås and the cooperation by Nortura Hed-Opp slaughterhouse staff, Rudshøgda.

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519
520

521 Table 1. Fatty acid composition (% of total fatty acids) of gestation diet, lactation diet, sows milk and
522 piglet diet

Fatty acid	Gestation diet	Lactation diet	Sow milk	Piglet diet
C10:0	<0.1	<0.1	0.2	<0.1
C12:0	0.1	<0.1	0.3	<0.1
C14:0	0.3	0.3	3.2	0.2
C16:0	18.1	14.8	35.1	15.5
C16:1*n*-7	0.3	0.3	8.8	0.2
C18:0	2.5	3.4	3.1	3.5
C18:1[a]	32.3	26.4	23.9	22.2
C18:2*n*-6	41.2	46.5	21.5	50.6
C18:3*n*-3	2.9	5.8	2.0	5.5
C20:1*n*-9	0.8	0.5	0.2	0.4
C20:4*n*-6	<0.1	<0.1	0.2	<0.1
C20:5*n*-3	0.1	<0.1	0.1	0.1
C22:5*n*-3	<0.1	<0.1	0.1	<0.1
C22:6*n*-3	<0.1	<0.1	<0.1	<0.1
SFA	21.0	18.5	41.9	19.2
MUFA	33.4	27.2	32.9	22.8
PUFA	44.2	52.3	23.9	56.2
n-6	41.2	46.5	21.7	50.6
n-3	3.0	5.8	2.2	5.6
n-6/*n*-3	13.7	8.02	9.86	9.04
Iodine value	108	120	74	122

523 [a]Sum of *n*-7 and *n*-9
524
525
526 Table 2. Fatty acid composition (% of total fatty acids) of backfat and of neutral lipids and
527 phospholipids of M. *longissimus dorsi* in young siblings

			M. *longissimus dorsi*			
	Backfat	SD	Neutral lipids	SD	Phospholipids	SD
C10:0	0.06	0.01	0.13	0.03	0.05	0.03
C12:0	0.07	0.07	0.11	0.05	0.02	0.01
C14:0	1.21	0.08	1.37	0.12	0.23	0.04
C16:0	21.8	1.16	22.4	1.03	18.9	0.65
C16:1*n*-7	2.58	0.43	3.20	0.46	0.71	0.13
C18:0	10.6	1.24	10.6	1.00	13.0	0.86
C18:1*n*-7	2.48	0.18	3.32	0.23	3.06	0.16
C18:1*n*-9	29.7	1.67	34.9	1.94	10.9	1.31
C18:2*n*-6	25.9	1.99	18.2	2.08	33.5	1.43
C18:3*n*-3	2.10	0.16	1.54	0.15	0.87	0.08
C20:1*n*-9	0.53	0.05	0.65	0.05	0.20	0.05
C20:4*n*-6	0.35	0.03	0.62	0.19	10.7	0.69
C20:5*n*-3	0.11	0.04	0.08	0.05	0.66	0.07
C22:5*n*-3	0.14	0.02	0.19	0.08	1.49	0.11
C22:6*n*-3	0.12	0.02	0.10	0.08	0.92	0.13
SFA	33.7	1.24	34.6	1.71	32.2	0.72
MUFA	35.2	1.87	42.0	2.14	14.9	1.52
PUFA	28.8	2.17	20.7	2.27	48.1	1.62
n-6	26.3	2.01	18.8	2.10	44.2	0.46
n-3	2.47	0.05	1.90	0.06	3.93	0.06
n-6/*n*-3	10.6	0.27	9.88	0.63	11.3	0.79
Iodine value	86.6	3.57	79.0	3.59	130	2.51

528

529 Table 3. Ingredient composition, analyzed and calculated contents of the diets used in the
530 feeding experiment

	Diet		
	LF	SBO	SBO-FO
Barley	666.0	584.0	585.0
Oats	80.0	80.0	80.0
Soybean meal	209.0	221.0	221.0
Soy oil	0	61.0	30.0
Fish oil	0	0	31.0
Limestone	12.6	13.4	13.0
Monocalcium phosphate	13.0	17.5	17.1
Sodium chloride	4.8	5.4	5.4
Aminoacids	2.6	5.6	5.2
Premix[a]	1.3	1.3	1.3
Cholin chloride	0.5	0.5	0.5
Analyzed chemical composition			
Dry matter g/kg	869	880	878
Crude protein g/kg DM	199	196	202
Crude fat g/kg DM	33	96	96
Crude fibre g/kg DM	56	59	60
Total ash g/kg DM	60	64	64
Calculated content			
Metabolizable energy (MJ/kg)	12.4	13.7	13.6
Feed units (FU/kg)[b]	1.00	1.13	1.12

531 [a] Vitamin-mineral premix provided the following amounts per kg of feed: 134 mg Fe, 125 mg Zn, 75 mg
532 Mn, 18 mg Cu, 1 mg I, 0.3 mg Se, 9000 I.U. vitamin A, 1125 I.U. vitamin D_3, 5.6 mg B_2, 19 mg
533 pantothenic acid and 25µg B_{12}.
534 [b] Net energy estimated from CVB (2003) tables.
535
536 Table 4. Fatty acid composition (% of total fatty acids) of the soybean oil, fish oil and the diets used in
537 the feeding experiment

	Fat source		Diet		
	Soybean oil	Fish oil	LF	SBO	SBO-FO
C10:0	<0.1	<0.1	<0.1	<0.1	<0.1
C12:0	<0.1	<0.1	0.1	0.1	0.1
C14:0	0.1	8.4	0.3	0.2	3.7
C16:0	11.1	12.4	23.9	15.3	15.1
C16:1*n*-7	0.1	4.4	0.2	0.1	1.9
C18:0	3.1	1.1	2.0	3.0	2.1
C18:1[a]	23.0	11.2	18.6	22.5	18.0
C18:2*n*-6	54.9	1.5	47.1	50.8	32.4
C18:3*n*-3	6.2	1	4.9	5.7	4.0
C20:1*n*-9	0.2	13.2	0.7	0.3	5.0
C20:4*n*-6	<0.1	0.4	0.0	0.0	0.1
C20:5*n*-3	<0.1	4.7	0.2	0.0	1.7
C22:1	<0.1	23.4	0.3	0.2	8.2
C22:5*n*-3	<0.1	0.7	0.0	0.0	0.2
C22:6*n*-3	<0.1	6.0	0.0	0.0	2.1
SFA	14.3	22.0	26.3	18.6	21.0
MUFA	23.3	52.2	19.5	22.9	24.9
PUFA	61.1	14.3	52.2	56.5	39.0
n-6	54.9	1.9	47.1	50.8	32.5
n-3	6.2	12.4	5.1	5.7	8
n-6/*n*-3	8.9	0.2	9.2	8.9	5.0
Iodine value	131	108	112	123	115
Iodine value product	.	.	37	118	110

538 [a]Sum of *n*-7 and *n*-9
539

Table 5. Performance in the feeding experiment

	Sex				Diet				
	Male	Female	Sed[a]	*P*-value	LF	SBO	SBO-FO	Sed[a]	*P*-value
Number of pigs	35	36			23	24	24		
Daily weight gain (g/day)	918	883	19.78	0.075	891	917	892	24.80	0.459
Avarage daily feed intake (kg)	2.04	2.13	0.03	0.006	2.18[b]	2.04[a]	2.04[a]	0.04	0.001
Average daily feed intake (FU)	2.22	2.31	0.04	0.01	2.18	2.30	2.30	0.04	0.012
FCR (kg/weight gain)	2.22	2.42	0.04	<0.001	2.46[b]	2.23[a]	2.28[a]	0.05	<0.001
FCR (FU/kg weight gain)	2.41	2.63	0.04	<0.001	2.46	2.52	2.57	0.05	0.090
Lean meat GP2 (%)	56.0	55.5	0.41	0.214	56.5[b]	55.1[a]	55.8[ab]	0.51	0.021
Fat GP1 (mm)	11.3	11.9	0.41	0.158	10.6[a]	11.9[b]	12.2[b]	0.51	0.006
Fat GP2 (mm)	13.0	13.5	0.54	0.385	12.6	13.9	13.3	0.68	0.128
Fat R2 (mm)	14.6	15.7	0.81	0.165	15.5	15.0	14.8	1.00	0.764
Intra-muscular fat (%)	2.55	3.08	0.15	0.001	2.82	2.74	2.87	0.18	0.781

[a]Standard error of difference

Table 6. Fatty acid composition (% of total fatty acids) of the outer backfat layer

	Sex				Diet				
	Male	Female	Sed[a]	*P*-value	LF	SBO	SBO-FO	Sed[a]	*P*-value
C10:0	0.06	0.07	0.00	0.201	0.08[b]	0.06[a]	0.06[a]	0.00	<0.001
C12:0	0.08	0.07	0.00	0.064	0.07[a]	0.07[a]	0.08[b]	0.00	0.002
C14:0	1.34	1.31	0.02	0.206	1.23[b]	0.99[a]	1.76[c]	0.02	<0.001
C16:0	21.0	21.5	0.22	0.039	23.8[c]	18.8[a]	21.1[b]	0.26	<0.001
C16:1*n*-7	1.77	1.73	0.05	0.404	2.05[b]	1.19[a]	2.00[b]	0.06	<0.001
C18:0	10.8	11.4	0.26	0.015	13.8[b]	9.82[a]	9.62[a]	0.32	<0.001
C18:1n-7	2.34	2.23	0.03	0.575	2.72[c]	1.96[a]	2.31[b]	0.04	<0.001
C18:1n-9	30.6	32.3	0.37	<0.001	36.4[b]	28.6[a]	29.3[a]	0.45	<0.001
C18:2*n*-6	25.1	22.7	0.48	<0.001	15.7[a]	32.4[c]	23.7[b]	0.59	<0.001
C18:3*n*-3	2.15	1.99	0.04	<0.001	1.20[a]	2.79[c]	2.21[b]	0.05	<0.001
C20:1*n*-9	1.31	1.26	0.03	0.075	0.82[b]	0.56[a]	2.47[c]	0.04	<0.001
C20:4*n*-6	0.23	0.23	0.01	0.463	0.22[a]	0.27[b]	0.20[a]	0.01	<0.001
C20:5*n*-3	0.18	0.19	0.01	0.200	0.06[a]	0.06[a]	0.44[b]	0.01	<0.001
C22:5*n*-3	0.28	0.30	0.01	0.114	0.12[a]	0.15[b]	0.60[c]	0.01	<0.001
C22:6*n*-3	0.29	0.30	0.01	0.505	0.04[a]	0.05[a]	0.80[b]	0.01	<0.001
SFA	33.2	34.3	0.45	0.018	39.0[c]	29.7[a]	32.6[b]	0.55	<0.001
MUFA	36.0	37.6	0.42	<0.001	42.0[c]	32.3[a]	36.1[b]	0.52	<0.001
PUFA	28.2	25.7	0.52	<0.001	17.3[a]	35.7[c]	27.9[b]	0.64	<0.001
n-6	25.2	22.9	0.48	<0.001	15.9[a]	32.6[c]	23.9[b]	0.59	<0.001
n-3	2.90	2.77	0.04	0.010	1.41[a]	3.05[b]	4.05[c]	0.06	<0.001
n-6/*n*-3	9.56	9.08	0.15	0.002	11.4[c]	10.7[b]	5.89[a]	0.18	<0.001
Iodine value	78.9	83.6	0.87	<0.001	64.9[a]	92.6[c]	86.4[b]	1.07	<0.001

[a]Standard error of difference

547 Table 7. Fatty acid composition (% of total fatty acids) of the inner backfat layer

	Sex				Diet				
	Male	Female	Sed[a]	*P*-value	LF	SBO	SBO-FO	Sed[a]	*P*-value
C10:0	0.07	0.07	0.00	0.126	0.09[b]	0.06[a]	0.07[a]	0.00	<0.001
C12:0	0.08	0.08	0.00	0.271	0.08[ab]	0.07[a]	0.08[b]	0.00	0.006
C14:0	1.35	1.30	0.02	0.021	1.24[b]	0.99[a]	1.74[c]	0.03	<0.001
C16:0	21.9	22.6	0.23	0.005	24.9[c]	19.8[a]	22.1[b]	0.28	<0.001
C16:1*n*-7	1.44	1.46	0.04	0.534	1.72[b]	0.91[a]	1.72[b]	0.05	<0.001
C18:0	12.9	13.7	0.26	0.004	16.4[b]	11.9[a]	11.5[a]	0.32	<0.001
C18:1*n*-7	2.07	2.10	0.03	0.236	2.42[c]	1.73[a]	2.12[b]	0.03	<0.001
C18:1*n*-9	29.2	31.7	0.34	<0.001	35.6[b]	27.4[a]	28.3[a]	0.42	<0.001
C18:2*n*-6	24.2	20.7	0.49	<0.001	13.7[a]	31.3[c]	22.4[b]	0.61	<0.001
C18:3*n*-3	2.06	1.82	0.04	<0.001	1.04[a]	2.68[c]	2.10[b]	0.05	<0.001
C20:1*n*-9	1.34	1.27	0.03	0.074	0.83[b]	0.56[a]	2.53[c]	0.04	<0.001
C20:4*n*-6	0.21	0.20	0.01	0.189	0.20[a]	0.24[b]	0.19[a]	0.01	<0.001
C20:5*n*-3	0.17	0.17	0.01	0.800	0.05[a]	0.04[a]	0.42[b]	0.01	<0.001
C22:5*n*-3	0.29	0.28	0.01	0.350	0.10[a]	0.14[b]	0.60[c]	0.01	<0.001
C22:6*n*-3	0.31	0.30	0.01	0.209	0.03[a]	0.04[a]	0.85[b]	0.01	<0.001
SFA	36.3	37.7	0.45	0.003	42.7[c]	32.8[a]	35.5[b]	0.55	<0.001
MUFA	34.1	36.5	0.38	<0.001	40.6[c]	30.6[a]	34.7[b]	0.47	<0.001
PUFA	27.2	23.5	0.54	<0.001	15.1[a]	34.5[c]	26.6[b]	0.66	<0.001
n-6	24.4	20.9	0.50	<0.001	13.9[a]	31.5[c]	22.6[b]	0.61	<0.001
n-3	2.83	2.57	0.05	<0.001	1.22[a]	2.91[b]	3.97[c]	0.06	<0.001
n-6/*n*-3	9.51	9.09	0.13	0.001	11.4[c]	10.8[b]	5.68[a]	0.15	<0.001
Iodine value	83.6	78.9	0.87	<0.001	64.9[a]	92.6[c]	86.4[b]	1.07	<0.001

548 [a]Standard error of difference

549

550 Table 8. Fatty acid composition (% of total fatty acids) of the neutral lipids in M. *longissimus dorsi*

	Sex				Diet				
	Male	Female	Sed[a]	*P*-value	LF	SBO	SBO-FO	Sed[a]	*P*-value
C10:0	0.16	0.17	0.01	0.839	0.18[b]	0.16[ab]	0.15[a]	0.01	0.036
C12:0	0.15	0.14	0.01	0.387	0.14	0.14	0.15	0.01	0.871
C14:0	1.63	1.67	0.03	0.278	1.60[b]	1.47[a]	1.87[c]	0.04	<0.001
C16:0	23.1	24.3	0.24	<0.001	25.1[c]	22.3[a]	23.6[b]	0.30	<0.001
C16:1*n*-7	2.54	2.92	0.08	<0.001	3.24[c]	2.18[a]	2.77[b]	0.10	<0.001
C18:0	12.6	13.0	0.25	0.125	14.2[b]	12.2[a]	12.0[a]	0.31	<0.001
C18:1n-7	3.08	3.36	0.06	<0.001	3.86[c]	2.75[a]	3.06[b]	0.08	<0.001
C18:1n-9	33.9	37.5	0.42	<0.001	40.6[b]	33.0[a]	33.5[a]	0.52	<0.001
C18:2*n*-6	16.7	11.9	0.57	<0.001	7.28[a]	20.2[c]	15.4[b]	0.70	<0.001
C18:3*n*-3	1.49	1.09	0.05	<0.001	0.59[a]	1.80[c]	1.48[b]	0.06	<0.001
C20:1*n*-9	1.02	0.95	0.05	0.165	0.74[b]	0.59[a]	1.62[c]	0.06	<0.001
C20:4*n*-6	0.41	0.30	0.05	0.039	0.28[a]	0.48[b]	0.31[a]	0.07	0.008
C20:5*n*-3	0.16	0.14	0.02	0.189	0.07[a]	0.06[a]	0.31[b]	0.02	<0.001
C22:5*n*-3	0.24	0.21	0.02	0.108	0.07[a]	0.16[b]	0.45[c]	0.02	<0.001
C22:6*n*-3	0.25	0.23	0.02	0.348	0.08[a]	0.07[a]	0.57[b]	0.03	<0.001
SFA	37.6	39.3	0.45	0.001	41.2[c]	36.4[a]	37.8[b]	0.56	<0.001
MUFA	40.6	44.7	0.53	<0.001	48.5[c]	38.5[a]	41.0[b]	0.65	<0.001
PUFA	19.2	13.9	0.65	<0.001	8.37[a]	22.7[c]	18.5[b]	0.80	<0.001
n-6	17.0	12.2	0.59	<0.001	7.56[a]	20.6[c]	15.6[b]	0.73	<0.001
n-3	2.13	1.67	0.07	<0.001	0.81[a]	2.09[b]	2.80[c]	0.09	<0.001
n-6/*n*-3	8.51	8.13	0.26	0.155	9.46[b]	9.88[b]	5.63[a]	0.33	<0.001
Iodine value	74.9	67.9	0.98	<0.001	59.9[a]	78.9[c]	75.5[b]	1.20	<0.001

551 [a]Standard error of difference

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554 Table 9. Fatty acid composition (% of total fatty acids) of the phospholipids in M. *longissimus dorsi*

	Sex				Diet				
	Male	Female	Sed[a]	*P*-value	LF	SBO	SBO-FO	Sed[a]	*P*-value
C10:0	0.18	0.23	0.04	0.255	0.26	0.19	0.16	0.04	0.073
C12:0	0.08	0.09	0.02	0.397	0.11	0.08	0.07	0.02	0.338
C14:0	0.34	0.36	0.03	0.576	0.32	0.34	0.39	0.03	0.085
C16:0	20.1	20.6	0.28	0.075	19.5^{a}	20.6^{b}	20.8^{b}	0.34	<0.001
C16:1*n*-7	0.87	1.07	0.07	0.005	1.47^{b}	0.67^{a}	0.78^{a}	0.08	<0.001
C18:0	10.4	9.81	0.20	0.002	9.68^{a}	10.5^{b}	10.2^{ab}	0.24	0.003
C18:1*n*-7	2.35	2.32	0.05	0.511	2.78^{c}	2.03^{a}	2.19^{b}	0.06	<0.001
C18:1*n*-9	11.9	12.1	0.44	0.563	14.8^{b}	10.6^{a}	10.6^{a}	0.54	<0.001
C18:2*n*-6	34.8	34.1	0.50	0.126	30.9^{a}	37.9^{c}	34.5^{b}	0.62	<0.001
C18:3*n*-3	0.86	0.81	0.03	0.105	0.81^{a}	0.90^{b}	0.78^{a}	0.03	0.003
C20:1*n*-9	0.37	0.33	0.03	0.135	0.20^{a}	0.16^{a}	0.69^{b}	0.04	<0.001
C20:4*n*-6	8.48	8.71	0.26	0.389	10.1^{c}	8.88^{b}	6.84^{a}	0.32	<0.001
C20:5*n*-3	1.71	1.72	0.12	0.954	0.86^{a}	0.61^{a}	3.68^{b}	0.15	<0.001
C22:5*n*-3	1.64	1.73	0.05	0.071	1.71^{b}	1.52^{a}	1.82^{b}	0.06	<0.001
C22:6*n*-3	1.20	1.24	0.08	0.660	0.62^{a}	0.50^{a}	2.54^{b}	0.10	<0.001
SFA	31.1	31.1	0.25	0.837	29.9^{a}	31.7^{b}	31.6^{b}	0.31	<0.001
MUFA	15.5	15.8	0.52	0.473	19.2^{b}	13.5^{a}	14.3^{a}	0.64	<0.001
PUFA	48.7	48.3	0.60	0.437	45.0^{a}	50.3^{b}	50.2^{b}	0.74	<0.001
n-6	43.3	42.8	0.60	0.363	41.0^{a}	46.8^{b}	41.3^{a}	0.75	<0.001
n-3	5.41	5.49	0.20	0.679	4.01^{a}	3.53^{a}	8.83^{b}	0.25	<0.001
n-6/*n*-3	9.75	9.37	0.38	0.311	10.4^{b}	13.4^{c}	4.91^{a}	0.47	<0.001
Iodine value	131	131	1.17	0.676	129^{a}	127^{a}	136^{b}	1.44	<0.001

555 [a]Standard error of difference

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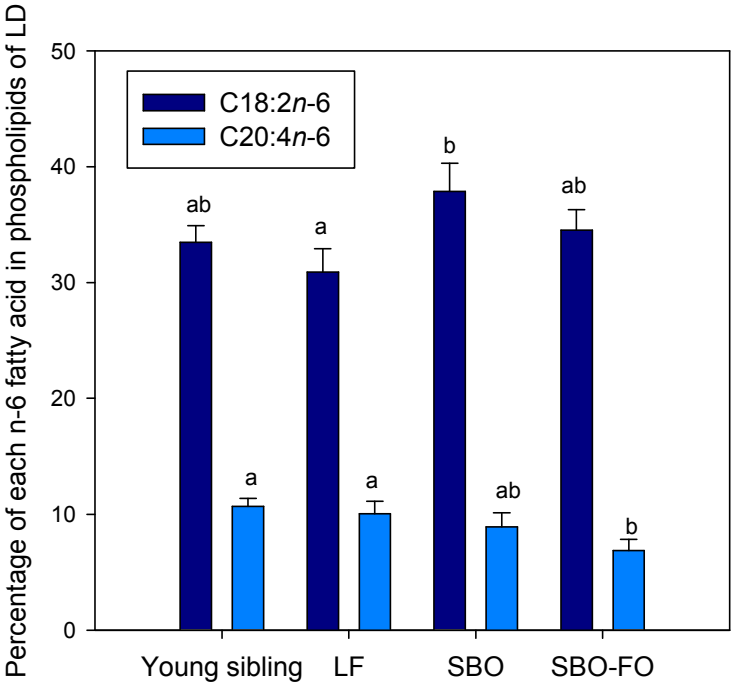

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559 Fig. 1 Percentage of C18:2*n*-6 and C20:4*n*-6 in phospholipids of LD in young siblings and
560 pigs fed the different dietary treatments; low fat (LF), soybean oil added diet (SBO) or
561 soybean oil and fish oil added diet (SBO-FO).

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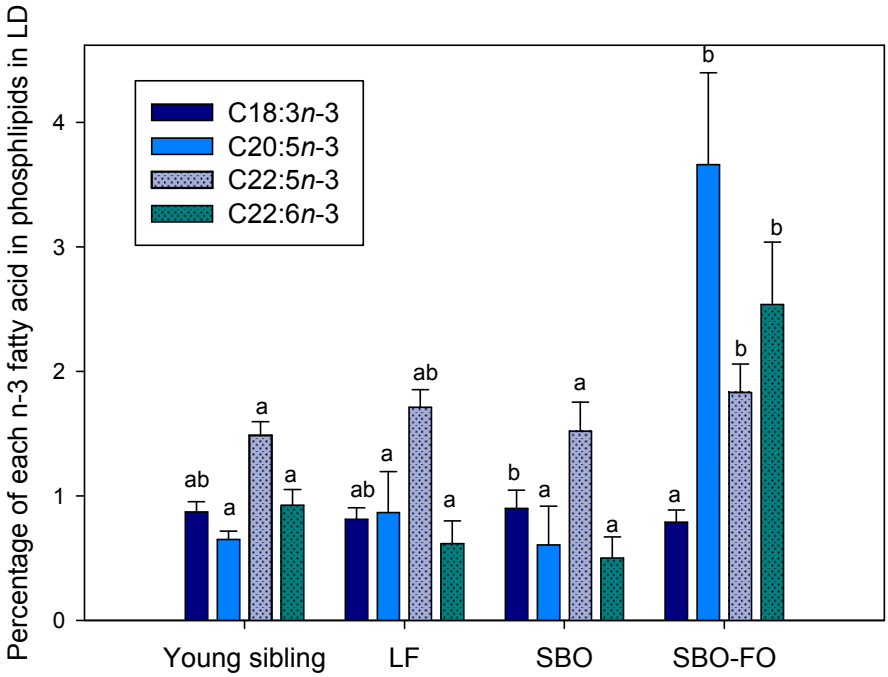

Fig. 2. Percentage of C18:3*n*-3, C20:5*n*-3, C22:5*n*-3 and C22:6*n*-3 in phospholipids of LD in young siblings and pigs fed the different dietary treatments; low fat (LF), soybean oil added diet (SBO) or soybean oil and fish oil added diet (SBO-FO).