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Genetic analyses of meat, fat and carcass quality traits measured by rapid methods

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Abstract

The overall aim of this thesis was to analyse meat and fat quality traits using quantitative genetic methods. This study has demonstrated that it is possible to establish simple laboratory practices and high quality rapid analyses of meat and fat quality traits at a research abattoir. Developed multivariate calibration methods and Near-infrared spectroscopy (NIRS) data were used to predict many of the traits studied, and large-scale recordings provided the basis for the genetic analysis of the meat and fat quality traits. In addition, estimated genetic parameters of body composition traits from large-scale Computed tomography (CT) scan of live boars yielded new information about the growth of various body tissues.

The results show that low labour, large-scale measuring methods can provide high heritabilities for several traits. The meat quality traits: pH in *M. gluteus medius*, *M. gluteus profundus* and *M. longissimus dorsi* and drip loss, meat colour and meat composition traits of *M. longissimus dorsi* showed heritabilities from 0.12 to 0.50 in Landrace and from 0.22 to 0.62 in Duroc. The fat quality traits: fatty acid composition, fat moisture content, and fat colour in subcutaneous fat showed heritabilities from 0.06 to 0.67 in Landrace and from 0.01 to 0.57 in Duroc. The CT traits: growth of muscle, fat, bone and internal organs showed heritabilities from 0.19 to 0.53 in Landrace and from 0.43 to 0.59 in Duroc. On the basis of the parameters estimated here, breeding for a higher lean meat percentage and lower feed convention ratio is expected to result in deterioration of meat and fat quality traits.

In view of the genetic parameters and size of the heritabilities and genetic correlations, some new traits for meat, fat and carcass quality are recommended in the breeding programme for Norwegian Landrace and Duroc. Among the traits investigated, the traits of greatest importance are NIRS-predicted intramuscular fat, drip loss, a* value in meat and NIRS-predicted moisture and fatty acids composition in subcutaneous fat. The percentage of oleic acid, C18:1n-9, from the NIRS analysis is highly heritable and may improve technological quality, sensory properties and human health. A selection for L* value or reflectance in meat is discouraged due to the undesirable influence of the IMF in the measuring. CT scanning makes it possible to select directly for the growth rate of muscle, fat, bones and internal organs of live boars. Pig meat has many qualities important for human nutrition, and is a good source for essential minerals and nutrients, e.g. heme iron, protein with a good amino acid profile and good fatty acids. This study has demonstrated the possibilities of selecting for some of these component traits.

Norsk sammendrag

Hovedmålet med denne avhandlingen var å analysere kjøtt- og fettkvalitetsegenskaper med kvantitative genetiske analyser. Dette studiet har vist at det er mulig å etablere enkle laboratorierutiner og hurtige målemetoder av høy kvalitet for kjøtt- og fettkvalitetsegenskaper ved et forskningsslakteri. Utviklede multivariate kalibreringsmetoder og Near-infrared spektroskopi (NIRS) data ble brukt til å predikere mange av de studerte egenskapene. Storskala datafangst gir grunnlaget for genetisk analyse av kjøtt- og fettkvalitetsegenskaper. I tillegg er det estimert genetiske parametere for kroppssammensetning fra storskala skanning med datatomografi (CT) av levende råner, som gir ny informasjon om veksten av ulike vev.

Resultatene viser at lite arbeidskrevende, storskala målemetoder kan gi høye arvegrader for flere av egenskapene. Kjøttkvalitetsegenskaper som pH i *M. gluteus medius*, *M. gluteus profundu* og *M. longissimus dorsi* og drypptap, kjøttfarge og kjøttsammensetningsegenskaper av *M. longissimus dorsi* hadde arvegrader fra 0,12 til 0,50 hos landsvin og fra 0,22 til 0,62 hos duroc. Fettkvalitetsegenskaper som fettsyresammensetning, vannprosent i fett og fettfarge i subkutant fett hadde arvegrader fra 0,06 til 0,67 hos landsvin og fra 0,01 til 0,57 hos duroc. CT-egenskaper som vekst av muskel, fett, bein og indre organer hadde arvegrader fra 0,19 til 0,53 hos landsvin og fra 0,43 til 0,59 hos duroc. På bakgrunn av parameterne estimert her er det forventet at avl for en høyere kjøttprosent og fôrutnyttelse vil resultere i forringelse av kjøtt- og fettkvalitetsegenskapene.

I lys av de genetiske parametere, størrelsen på arvegradene og de genetiske korrelasjonene, anbefales Norsvin å ta med noen nye kvalitetsegenskaper for kjøtt-, fett- og slaktekvalitet i avlsarbeidet for norsk landsvin og duroc. De viktigste egenskapene er NIRS-predikert intramuskulært fett, drypptap, a* verdi i kjøtt og NIRS-predikert vanninnhold og fettsyresammensetning i subkutant fett. Andelen oljesyre, C18:1n-9, målt med NIRS-analyse er høyt arvelig, og kan bedre den teknologiske og sensoriske kvaliteten samt human helse. Grunnet uønsket påvirkning av IMF, frarådes seleksjon for L* verdi og refleksjon i kjøtt. CT-skanning gjør det mulig å selektere direkte for tilvekst av muskel, fett, bein og indre organer. Svinekjøtt har mange positive kvaliteter for human ernæring og er en god kilde for viktige mineraler og næringsstoffer, f. eks heme jern, høykvalitets protein og gunstige fettsyrer. Denne studien har vist at det er mulig å selektere for noen av disse komponentegenskapene.

Preface

This study was performed between 2005 and 2010 as part of a project covering both genetics and nutrition for the improvement of pig meat quality. The project included the Department of Animal and Aquacultural Sciences at the Norwegian University of Life Sciences (UMB), Animalia - Norwegian Meat and Poultry Research, Felleskjøpet Fôrutvikling (FKF) and Norsvin. Thanks to the Foundation for Research Levy on Agricultural Products, the Research Council of Norway and Norsvin for their financial support.

First and foremost, I want to thank my supervisors, Odd Vangen, Laila Aass and Jørgen Ødegård for their wonderful support and feedback with this research. Thanks as well to Jørgen Kongsro as a co-author analysing the CT images and for being a good discussion partner in multivariate statistics. Thanks to Jakob Jøns, FOSS for help with the NIRS technology. Thanks to Animalia and Biobank AS for their goodwill and help with the meat and fat quality analyses introduced in this study and to Norsvin Delta for running the CT scanning. Thanks to Terje Frøystein, Animalia and Daniel Schwörer, Suisag and Anna Haug, UMB for their valuable suggestions in meat science. Thanks as well to Odd Vangen, Hallgeir Sterten, Eli Bryhni and Olav Eik-Nes, who initiated this project, and especially to Olav for persuading me to start this work and for believing in my idea of buying expensive NIRS instruments. Norsvin, my chief Bjarne Holm and my dear colleagues are also gratefully acknowledged for making my workplace the best it could be.

Finally, thanks to my family and friends for their support and patience. Thanks to Ole Bjarne for being my beloved husband and to our wonderful kids, Oleane 4 years and Johan 2 years.

List of papers

1. Gjerlaug-Enger E, Aass L, Ødegård J and Vangen O 2010. Genetic parameters of meat quality traits in two pig breeds measured by rapid methods. Animal, 4:11, 1832-1843.

2. Gjerlaug-Enger E, Kongsro J, Aass L, Ødegård J and Vangen O 2010. Prediction of fat quality in pig carcasses by near-infrared spectroscopy. Submitted to Animal.

3. Gjerlaug-Enger E, Aass L, Ødegård J, Kongsro J and Vangen O 2011. Genetic parameters of fat quality in pig measured by near-infrared spectroscopy. Animal, accepted on January 2, 2011.

4. Gjerlaug-Enger E, Kongsro J, Ødegård J, Aass L and Vangen O 2010. Genetic parameters between slaughter pig efficiency and growth rate of different body tissues estimated by computed tomography in live boars of Landrace and Duroc. Submitted to Animal.

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Abbreviations

ADG	Average daily gain
ADG1	Average daily gain from birth to 25 kg
ADG2	Average daily gain from 25 kg to 100 kg
AI	Artificial insemination
BF	Backfat thickness
BG	Bone growth
C16:0	Palmitic acid
C16:1n-7	Palmitoleic acid
C18:0	Steric acid
C18:1n-9	Oleic acid
C18:2n-6	Linoleic acid
C18:3n-3	α-linolenic acid
C20:4n-6	Arachidonic acid - AA
C20:5n-3	Eicosapentaenoic acid - EPA
C22:6n-3	Docosahexaenoic acid - DHA
CT	Computed tomography
CT-LMP	LMP calculated from CT data
DFD	Dark, firm, dry
EBV	Estimated breeding value
EZ-DripLoss	Drip loss measured with the EZ-DripLoss method
FCR	Feed convention ratio
FG	Carcass fat growth
FK	Felleskjøpet
FOM	Fat-o-meater
GM	*M. gluteus medius*
GMO	Genetically-modified organisms
GP	*M. gluteus profundus*
HGP	Hennessy Grading Probe
ID	Identification
IMF	Intramuscular fat

LD	*M. longissimus dorsi*
LMG	Lean meat growth
LMP	Lean meat percentage
MG	Muscle growth
MUFA	Mono-unsaturated fatty acid
n-3	Omega 3
n-6	Omega 6
NCG	Non-carcass tissue growth
NIR	Near-infrared
NIRS	Near-infrared spectroscopy
NIRS-IMF	NIRS-predicted IMF
NKF	Norges Kjøtt- og Fleskesentral
NOK	Norwegian kroner
NSA	Norsk Svineavlslag
P:S	Polyunsaturated/saturated fatty acid
PhD	Doctor of Philosophy
PLS	Partial least square
PSE	Pale, soft, exudative
PUFA	Polyunsaturated fatty acids
R^2	Explained variation/total variation
R^2_{Val}	Explained variation/total variation in validation test set
SBR	Schmid, Bondzynski, and Ratzalaff
SD	Standard deviation
SF	Subcutaneous fat
SQL	Structured Query Language
TBV	Total breeding value
UMB	Norwegian University of Life Sciences
WHC	Water holding capacity

General Introduction

Meat quality

Modern pig breeds are raised for the human consumption of meat, and meat quality is of particular importance for the entire industry. After several decades with the selection of leanness and efficiency in pig production, carcass quality has changed dramatically in the desired direction, but the selection has also resulted in negative responses in meat quality traits. In the 1980s, the work with meat quality thrived in several countries and questions of interest were sensorially related in terms of quality of SF and level of IMF, consumer perception of quality, genetic correlation between meat, fat and carcass quality, economic impact of the meat quality and measurement methods for the detection of meat quality traits (Frøystein, 1985).

Many studies found an unfavourable relationship between the pigs' leanness and the quality of the meat (reviewed by Hovenier, 1993; Sellier, 1998), and sensory panels were utilised for the description of a large spectrum of attributes important to pig meat, e.g. appearance, colour, flavour, odour and texture (Risvik, 1994). These sensory characteristics of the meat could be associated with some technological quality traits, including intramuscular fat, drip loss, cooking loss, firmness, electrical conductivity, glycolytic potential, pH and colour (Cameron, 1990; Sellier, 1998; Huff-Lonergan *et al*., 2002). The connection between sensory and instrument measured traits was not straightforward (Cameron, 1993; Moller and Iversen, 1993), but technological quality traits became accepted as important parameters in the work on meat quality (Sellier, 1998). A large number of scientific studies have been conducted in this field (Cameron, 1990; Hermesch *et al*., 2000; Suzuki *et al*., 2005), and some breeding companies have made the economic commitment to breed for these meat quality traits.

Much of the recent research on meat quality has applied new technology with a higher accuracy and rapid methods which replace more established laboratory methods (e.g. NIRS analyses, FT-IR analyses, Raman, Electronic nose and Fluoresence spectroscopy) or completely new properties of the meat (e.g. enzyme activity, antioxidants and fatty acids). In this thesis, genetic parameters are estimated for meat quality traits, analysed with rapid measurement methods, and presented in Paper 1.

Fat quality

Fat quality is an important topic, and in recent years, research on fat quality traits has been a popular field in relation to porcine product quality. In the same way as for meat quality, fat quality has changed in accordance with the rising lean meat content in pig carcasses. It is well known that the nutritional value of pig meat depends on the fat content and fatty acid composition of the carcass, among others, as both factors influence human health. Appropriate quality traits for breeding purposes, should be favourable for both the fat quality and healthiness of pig meat.

The amount of PUFA consumed have to cover the requirement for essential fatty acids, but too high intakes of PUFA may be harmful as because if can be transformed to peroxidation products (Cicero *et al*., 2008; Givens, 2008; Haug, 2010), and a high n-6 to n-3 ratio has an unfavourable effect on the incidence of several human diseases (Simopoulos, 2000). The ratio of n-6 to n-3 is completely related to the pigs' food (Øverland *et al*., 1996; Enser *et al*., 2000; Bryhni *et al*., 2002), but the C18:1n-9 is the key fatty acid that can improve the technological quality, sensory properties and factors important for human nutrition (Cicero *et al*., 2008; Givens, 2008; Egelandsdal and Haug, 2010; Haug, 2010).

Due to the breeding for a high protein to fat ratio in the carcass, the content of PUFA is high in modern pigs (Sellier, 1998). Firmness of SF exhibited a strong genetic relationship with fatty acid composition and moisture content in fat in a study by Cameron (1990), and several studies present high heritabilities for fatty acids (Schwörer *et al*., 1988; Bout *et al*., 1989; Sellier *et al*., 2010). High costs regarding the laboratory analysis of fatty acids, such as GC, may be the primary reason for the small amount of selection for fatty acid composition in practical breeding. A rapid method such as NIRS is a non-destructive, environmentally friendly and has demonstrated prediction of fatty acid composition (Schwörer *et al*., 1999; Garrido-Varo *et al*., 2008; Perez-Marin *et al*., 2009). Except for one paper (Fernandez *et al*., 2003), genetic parameters for fatty acids in pig SF which was predicted by NIRS is not available in recent literature. The combination of NIRS technology, the calibration work and the subsequent prediction of large-scale data material for the estimation of genetic parameters are dealt with in Papers 2 and 3.

Carcass quality and efficient lean meat growth

The breeding for a higher lean to fat content in pig carcasses has been very successful for several breeds around the world. Historically, a typical selection programme for pigs has included postweaning growth and BF at market weight measured on carcasses with a ruler or an ultrasound probe in live animals. A study comparing these methods and model development was carried out by Lynch (1967). A few breeding programmes also included feed intake and efficiency and carcass information from relatives (Clutter and Brascamp, 1998).

Efficient lean meat growth combines the growth traits and leanness of the pigs, and is defined by several researchers as the optimal trait for market pig production (Clutter and Brascamp, 1998; Fowler *et al*., 1976). A few studies have estimated the genetic parameters for lean meat growth (Mrode and Kennedy, 1993; Chen *et al*., 2002). Genetic parameters for this trait, as well as the growth of other body tissues, are discussed in more detail in Paper 4.

New technologies have great importance for carcass quality, and some breeding programmes include data from the abattoirs. Modern instruments for pig classification at the abattoirs, i.e. online pork carcass grading with the AutoFOM ultrasound system, which is a new version of the FOM (SFK Technology A/S, Herlev, Denmark), and several versions of HGP instruments (Hennessy Grading Systems, Auckland, New Zealand). The CT technology ordinarily used in hospitals has excellent measuring ability for body composition and was exploited early on farm animals (Afonso, 1992; Vangen and Thompson, 1992; Kolstad, 2001; Kvame and Vangen, 2007). This technology has recently been applied to carcasses as a supplement to dissection to update the equations for pig classification at abattoirs in several European countries. In 2008, Norsvin (Norwegian Pig Breeders' Association) started to use CT technology for large-scale measurements for breeding purposes. The large advantage with this technology is discussed in Paper 4, in which CT data are used for the estimation of genetic parameters in two Norwegian pig breeds.

The development of carcass and pig meat quality in Norway over the last 60 years

First breeding goal: BF, ADG and FCR

The NSA (later named Norsvin) was established as a cooperative organization in 1958, although local organizations were established even earlier than that. In the 1950s, ultrasound technology was already an important tool for measuring BF in live pigs. This research was established in cooperation with researchers at the Agricultural University in Ås (later named UMB), who were among the first to start with ultrasound technology on animals (Lynch, 1967). An average BF of 34 mm was measured with ultrasound on Norwegian Landrace in 1953 (Haug, 1993). The ultrasound BF records were combined with growth measured as the age at market size (first 90 kg, then 100 kg), and these observations were used for the phenotypic selection of gilts in the nucleus. Specific nucleus herds were established in 1963 and Standal (1977) developed the testing scheme used for the selection of pigs in these herds. The development of BF in Norwegian Landrace boars at the test station is presented in Figure 1.

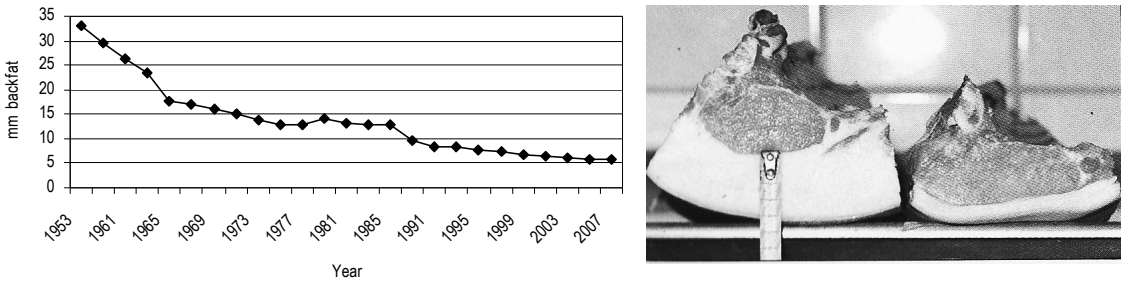

Figure 1 - Selection for decreased backfat changed the phenotype of the pigs dramatically. Phenotypic data from boar test station. Photo: Wiik and Vangen, 1978.

Artificial insemination, using semen from boars selected from the boar test station, was an important early breeding tool in Norsvin. As early as 1959, a boar testing station, featuring individual feed recordings, ultrasound BF measurements, and the growth and exterior evaluation of boars, was established in the southeastern part of Norway (Storhove from 1959-1965, and later Bjørke, both in Hedmark country). In the breeding plan from 1977, the second boar test station at Bjørke was started and the annual testing capacity increased from 400 to 850 boars (Jensen, 2008).

Starting from the 1950s, the breeding values for Norwegian pig breeds were estimated at the Department of Animal Breeding at UMB (Standal, 1981). The research work which resulted from the calculations of selection indices taken from the offspring testing of boars was published by Skjervold and Ødegård (1959a). In 1969, the first simple computer (electronic calculator) was used for the index calculation, and the carcass quality was included in the index (Moen, 1969). The very first breeding values for boars was an F index, which consisted of an ultrasound of the BF, growth and FCR from the boar's own performance and his tested full-sibs, together with the % of premium cuts at dissection of the two full-sibs (Hemma *et al*., 1979), and more information was later included in the index.

Early selection for carcass and meat quality

By the 1960s, research on IMF in pig meat was conducted at UMB, and Vold (1969) found a variation from 1.5 to 2.5% IMF in LD in Norwegian pigs. He discussed the connection between IMF and sensory traits, and pointed out the importance of IMF for the juiciness and taste of the pig meat. At this time, the knowledge about the unfavourable relationship between IMF and leanness was nonexistent.

Offspring testing of the boars was established in 1933 in Norway, and in 1958 the first test station was built in southeast Norway (Hellerud, Akershus). In 1977, the offspring was changed to a full-sibs test for the tested boars (Hemma *et al*., 1979). Two full-sibs, a female and a castrate, were tested. There were three different sib test stations, with one in the southeast (Hellerud, Akershus), one in the west (Særheim, Rogaland) and one in the middle (Skatval, Nord-Trøndelag) of Norway. The carcass and meat quality traits were measured using carcasses from these animals at NKF's meat research facility named "Norges Slakteri Laboratorium" (Norway's Slaughterhouse Laboratory) (Løren, Oslo).

The first routines for carcass and meat quality recording were carried out by Vold (1965) and Erling Løvseth (Lundsvold pers. comm.), and the experimental dissection for selection purposes in Norsvin was similar to commercial cutting, but also included the weight of the whole ham, neck and loin, and was only trimmed for fat, several BF measurements, subjective bacon quality score, subjective Japan colour score and reflectance (photoelectric instrument, Figure 2). The loin eye area was measured manually in combination with a planimeter, which is (a real instrument). PSE was previously a problem for meat quality (Frøystein, 1985), and by 1972, colour was included in the breeding programme. Offspring information about a pale colour could put a quick end to an AI boar's life (Hemma *et al*., 1979). With a total genetic change of NOK 12/slaughter pig/year in the period from 1970 to 1976, the genetic trend of colour was slightly unfavourable with 0.03 colour points per year, despite the selection for colour in offspring test (Standal, 1981).

Up until 1987, the changes in meat quality had been negative, but after a large revision of the breeding programme and the breeding plan that year (Hvamstad *et al*., 1987) in which meat quality was thoroughly discussed by Frøystein (1987), the meat colour and IMF were intended to be weighted to avoid a further reduction in meat quality traits. In 1987, the breeding goal included FCR, ADG, % of premium cuts, bacon quality, meat colour, IMF and reproduction (weaned piglets at three weeks and sperm quality) in addition to phenotypic selection for exterior and semen quality (Figure 4).

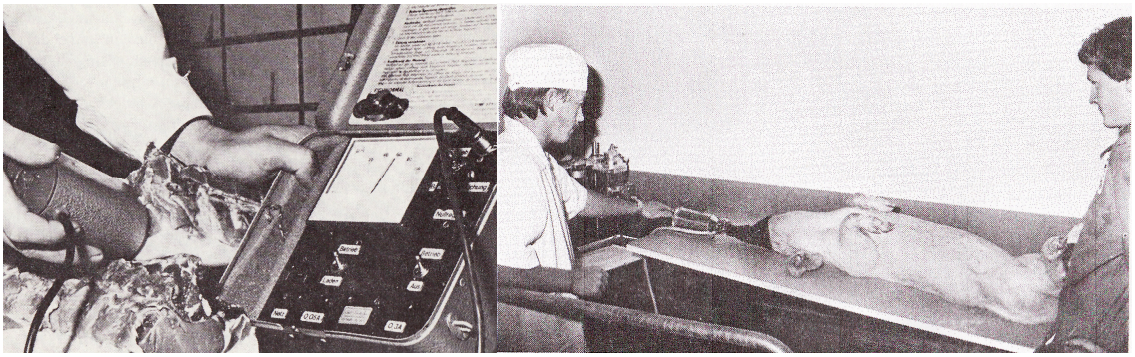

Figure 2 - (1) Reflectance measurement with photoelectric instrument. (2) Stress testing of boars with halothane gas. Photos: Hemma *et al*., 1979.

The "porcine stress syndrome" in Landrace and other pig breeds selected for muscling is caused by a defect in the ryanodine receptors. The mutated gene is called the halothane gene because the pigs carrying two copies of this allele do not tolerate Halothane anaesthesia gas. This mutation gives stress sensitive pigs and PSE meat at slaughter. Stress testing of boars started in 1978 with halothane gas, and offspring testing of all semen boars started at Norsvin in 1982 (Hvamstad *et al*., 1987; Sehested *et al*., 1988). A small population homozygous for the halothane-gene was served with semen from the Norsvin boars. The offspring were tested with halothane gas, and halothane-susceptible piglets were detected. If the boar passed this test (6 halothane-negative pigs in a litter), it could produce semen for the entire population. In 1989, a molecular genetic test (HAL-1843) was available, and the rest of the carriers were detected within a few years (Grindflek pers. comm.).

In 1992, an Oracle database was ready for use, and the entire pedigree and data records were stored in this database. A large adjustment in the breeding programme in that same year (Sehested and Blichfeldt, 1992) included the real LMP from the dissection of the shoulder, loin and ham into different tissues. The new LMP by Røe and Sehested (only documented by the SQL-equation) was made to optimize this parameter to the maximum advantage for the industrial cutting of pig. This equation was used until 2008, and more details about the equation of LMP are given in Paper 3.

To avoid genotype x environment interactions, the sib test stations were replaced with half-sib testing in commercial slaughter pig herds in 1993, including a change in the feeding regime from norm to *ad libitum*. During the same year, the sib test station at Skatval was converted to a boar test station, and the boar test capacity was increased to 1700 boars per year (Jensen, 2008). The capacity at the half-sib stations was increased from 1250 to 2800 animals in the period from 1993 to 2008, mainly due to an increased herd size in the two half-sib herds. Increased capacity at Bjørke boar test station gave on average 2400 boars tested annually from 1996 to 2008.

In 1982, the first CT scanner was installed at UMB, and Vangen (1988) gave a review of the first research on CT technology. In the 1980s, CT was evaluated for breeding purposes in pigs (Skjervold and Vangen 1981; Skjervold, 1982; Hvamstad *et al*., 1985; Vangen, 1991). In a two-year period in the 1990s, a CT scanner at the Agricultural University at Ås was used for the calculation of body composition traits in live boars from the Norsvin boar test stations. The travelling distance and workload made it too expensive to continue CT scanning at that time, but in 2008 Norsvin closed down two of its half-sib herds and three boar test stations, and started a new boar test station equipped with a modern CT scanner. The CT scanning of the boars is part of a test programme that includes 3500 Landrace and Duroc boars every year (Figure 3). More information about this technology is presented in Paper 4.

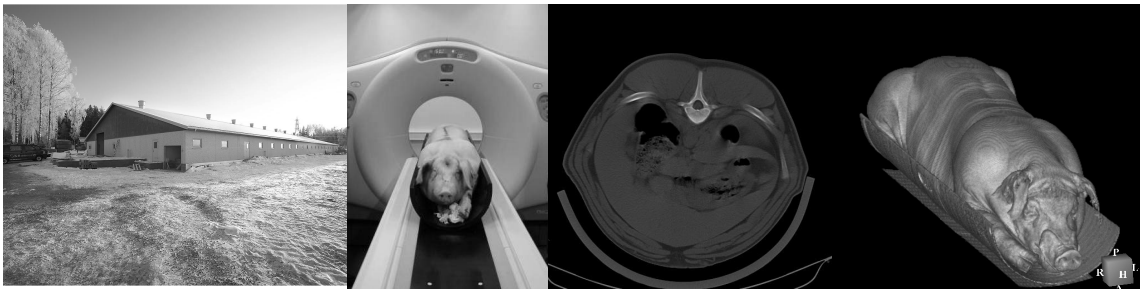

Figure 3 - (1) Test station Norsvin Delta. (2) The CT machine. (3) Cross-sectional image (tomogram). (4) Spiral scan of a boar, 1100 slices. Photos: (1) Støldal, 2008, (2) Mælumsæter, 2008, (3-4) Kongsro, 2009.

Despite a reduced PSE problem due to elimination of the halothane gene and selection for meat quality (colour and IMF), a selection for increased leanness yielded a reduced meat quality, with the pig meat being characterised as dry and less tender. The pig production was effective and the consumers were happy with the reduced fat content, but started to miss the juicy pig meat quality they had previously become accustomed to. The idea of a third breed was launched to solve this problem. In 1984, the Duroc and Hampshire sire breeds were tested, and the Duroc distinguished itself with an excellent meat quality (Ianssen pers. comm.). In 1986, Nortura (Norway's largest slaughter organisation, previously

named NKF) and Norsvin imported Duroc from Denmark and the first Duroc herd was established (Stolpestad, Hedmark). A new importing of pregnant Duroc gilts from Canada was done in 1992. The Duroc population increased from 400 sows and then up to 700 sows in the period from 1996 to 2006 (Maurud pers. comm.; Olsen pers. comm.). In 1994, the crossbred slaughter pig was presented to the consumer market with the brand name "Gilde edelgris" to meet the consumers' request for a better pig meat quality.

Later applied research work for meat quality

Chemically determined IMF was included from 1996 and only Duroc boars above 95 in TBV were selected. This corresponds to a half genetic SD under the mean, and was a strong regulation in view of the unfavourable correlations to leanness. In 1999, the trait was instead given an economic weight in the TBV (Figure 4). For the Landrace, the IMF analysis was put to an end. The reason for this was that the method had a high cost, and did not have enough accuracy to create a variation due to the smaller variation in this breed. In 1996, a PhD study was started in cooperation between UMB and Norsvin with the aim of finding QTLs for meat and fat quality traits (Grindflek, 2000). Significant QTLs for IMF and some fatty acids were detected.

The dissection procedure in the half-sib test was extended in 1998 to include several meat quality traits such as: ultimate pH in LD, GM and GP, HGP2-reflectance and Japan colour score in LD. In a study by Holm (2000), the genetic parameters were estimated for these traits and tested for their importance to sensory properties. His recommendation was to use ultimate pH, HGP2-reflectance in the LD muscle for the breeding of Landrace and Duroc. In 2001, these traits were implemented in the breeding programme and given high economic weights in the calculations of TBV, in order to prevent further decrease in meat quality.

The weighting of traits in the breeding goal for Norwegian breeds

From 1958 Norwegian Landrace and Yorkshire were both bred as all round breeds, with high economic weight for slaughter pig efficiency and carcass quality traits. Compared to many other countries, the feed costs have been high in Norway for many decades. From 1958 to 1992, FCR was the most important trait in the breeding programme for Norwegian Landrace (Figure 4). Important maternal traits, number weaned and litter weight at three weeks was suggested as new traits in the breeding in 1959 (Skjervold and Ødegård 1959b). The first maternal trait, number born alive, was included in the total EBV in 1992 (Sehested and Blichfeldt, 1992). Norsvin ended the breeding work for Yorkshire in 1998, and later imported Yorkshire from other Nordic countries, first from Finland in 1999 and later from

Sweden in 2006 (Maurud pers. comm.). From 1999, the Landrace has been bred as a pure dam line, with increased weight on reproductive and maternal traits. The most important new maternal traits include litter weight in 2004 and piglet survival in 2010 (Olsen, 2011). Until 1999 selection for exterior trait was based on independent threshold selection. From 1999 different exterior traits have also been included in the EBV (Figure 4).

The Duroc has been bred as a sire line for its entire selection history in Norsvin. The Duroc pigs imported to Norway were rather fat, but a well-organised selection has changed the population. The current Norwegian Duroc population is now efficient with respect to lean meat growth. Notwithstanding, the pH and IMF has been given a high economic value in the breeding goal to help keep the meat quality at its original level for these traits.

The adjustment in the breeding programme in 2010 (Olsen, 2011) is noteworthy. The economic weighting of the traits in Landrace aims at no genetic change in carcass and meat quality. The level of the number of live born piglets is considered high enough and this trait therefore has a low weight in the new breeding goal. The Landrace is now bred towards better FCR, ADG, mothering ability and health traits (exterior and disease) (Figure 4). It is still a goal for the Duroc to increase their LMP, ADG, FCR and health traits, and to keep the IMF and WHC at a high level (Figure 4). In general, the genetic improvement is approximately NOK 31/slaughter pig/year for both Landrace and Duroc, despite very different breeding goals (Norsvin, 2009).

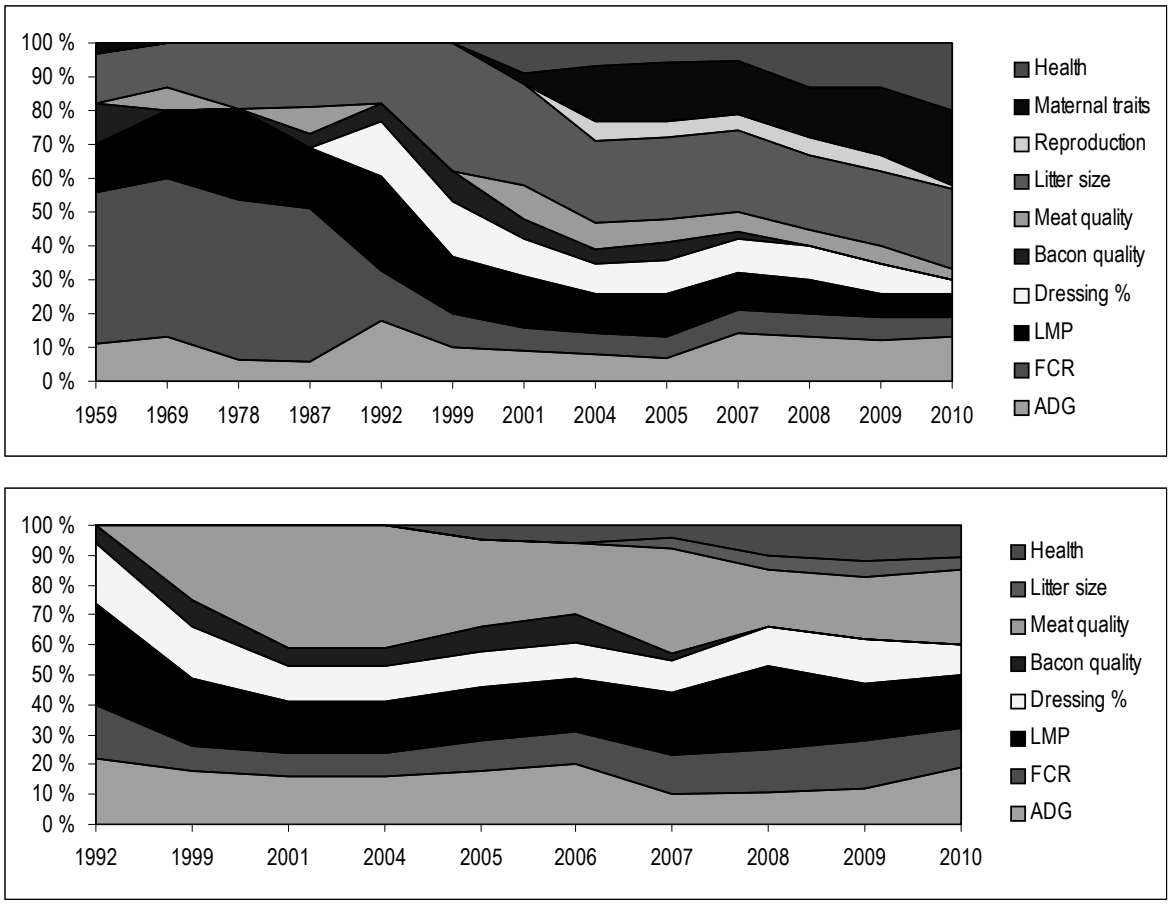

Figure 4 - Trends in the breeding goal for (1) Landrace and (2) Duroc (Olsen, 2011).

In addition to meat and carcass quality, the research in other areas that are the basis for this great diversity of traits in Figure 4 (Tajet and Olsen, 2000; Olsen, 2011) are conformation (Tomter, 1984; Grindflek 1995; Kolstad and Sehested, 1991) reproduction, maternal traits, longevity (Skjervold and Ødegård 1959b; Holm, 2004; Thingnes, 2010; Grindflek and Sehested, 1996), piglet survival (Zumbach *et al*., 2009; Olsen, 2011) vitality (Hvamstad *et al*., 1987; Holm, 2004), appetite, feed intake (Standal and Vangen, 1985; Lundgren *et al*., 2010), defects, disease (Grindflek *el al*., 2006; Andersen-Ranberg 2009; Andersen-Ranberg 2011) and teat quantity and quality (Wold, 2009; Long, 2010). Among other studies, this research has created a large diversity in the types of traits for the breeding goal (Figure 4), and 26 and 19 different traits in the breeding programmes for Landrace and Duroc, respectively. This large diversity in traits has made the breeding robust and sustainable (Vangen, 2006; Olsen, 2011), which is of vital importance for success in future global markets (Vangen, 2009).

Aims of the study

In 2005, a new research programme in meat and fat quality was started in Norway as a cooperation between feed industry (FKF), meat research industry (Animalia), pig breeders (Norsvin) and UMB (Department of Animal and Aquacultural Sciences). The aim of this study was to improve pig meat quality through nutrition and genetics.

The aims for the quantitative genetic part of the project were to find new meat and fat quality parameters and then to estimate the genetic variation for these traits. The meat and fat characteristics of particular interest were: pH, IMF, WHC, meat colour, reflection and fatty acids.

Subsequently, the study had the following sub-goals:

1. To find rapid methods for large-scale testing of animals for meat and fat quality.
2. To investigate the genetic variation for: pH, IMF, WHC, meat colour and reflection in Norwegian pig breeds.
3. To analyse the genetic relationships between the various meat quality traits.
4. To investigate the heritability for fatty acid composition in SF.
5. To analyse the genetic relationships between fat and meat quality traits.
6. To study the fatty acid composition in pig fat in relation to human health.

In addition, CT technology was of particular interest for the project. However, the CT scanning at the boar test station was not in place when the project was started. This element was implemented at a later stage.

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animal

Genetic parameters of meat quality traits in two pig breeds measured by rapid methods

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To study genetic variation in meat quality traits measured by rapid methods, data were recorded between 2005 and 2008 on samples of M. longissimus dorsi *(LD) in Landrace (*n *= 3838) and Duroc (*n *= 2250) pigs included in the Norwegian pig breeding scheme. In addition, ultimate pH levels in the glycolytic LD (loin muscle) and M. gluteus medius (GM, ham muscle), and in the oxidative* m. gluteus profundus *(GP, ham muscle) were recorded as an extended data set (*n *= 16 732 and* n *= 7456 for Landrace and Duroc, respectively) from 1998 to 2008. Data were analysed with a multi-trait animal model using AI-REML methodology. Meat from Duroc had considerably more intramuscular fat (IMF), less moisture and protein, appeared darker with higher colour intensity and had lower drip loss than meat from Landrace. The heritability estimates (s.e. 0.01 to 0.07) for pH in LD (0.19 and 0.27 for Landrace and Duroc, respectively), GM (0.12 and 0.22) and GP (0.19 and 0.38), drip loss (0.23 and 0.33), colour values: L* (lightness) (0.41 and 0.28), a* (redness) (0.46 and 0.43), b* (yellowness) (0.31 and 0.33), IMF (0.50 and 0.62), muscle moisture (0.31 and 0.50) and muscle protein content (0.40 and 0.54) in LD all demonstrated moderate-to-high genetic variation for these traits in both breeds. Near infrared spectroscopy and EZ-DripLoss are modern technologies used in this study for the determination of chemical components and drip loss in meat. These methods gave higher heritabilities than more traditional methods used to measure these traits. The estimated genetic correlations between moisture and IMF in Duroc, and pH and drip loss in Duroc were both −0.89. Interesting differences between the two breeds in numerical value of some genetic correlations were observed, probably reflecting the differences in physiology and selection history between Landrace and Duroc. The estimated genetic correlation between drip loss and pH was much stronger in Duroc than in Landrace (−0.89 and −0.63, respectively). This might be due to the high pH in Duroc, whereas Landrace had a lower pH closer to the iso-electric point for muscle proteins. The positive genetic correlation between the L* value in meat and IMF in Duroc (0.50) was an effect of differences in visible marbling, rather than meat colour. For Landrace, this correlation was negative (−0.20). IMF content showed favourable genetic correlations to drip loss (−0.36 and −0.35 for Landrace and Duroc, respectively).*

Keywords: quantitative genetic, drip loss, ultimate pH, Minolta colour, near infrared spectroscopy

Implications

The results from this study support genetic selection for several pig meat quality traits. The choice of rapid methods makes it possible to test a large number of animals and accurately estimate genetic parameters at an acceptable cost. This is likely to reduce the cost of existing performance testing programmes. This work also shows that it is possible to establish simple routines, and to use preparation methods and instruments that are safe, user- and environmentally friendly, and that do not require chemical solvents.

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Introduction

For decades, pig-breeding programmes have focused mainly on the reduction of production costs of pig meat. Selection has been aimed at increased litter size and lean meat percentage in addition to weight gain and improved feed conversion. Currently, to meet consumer expectations, breeding goals are changing their focus towards meat quality traits because of the high economic value of these traits.

The Norwegian Pig Breeders Association (Norsvin, Norway) operates the national recording scheme and breeding programmes. The Norwegian Landrace is bred as a dam line, and its breeding goal in 2008 consisted of production efficiency, carcass quality, meat quality, litter size, reproduction, maternal

efficiency, health and defects. The Norwegian Duroc is bred as a sire line, and its breeding goal (2008) consisted of production efficiency, carcass quality, meat quality, health and defects. Selection for meat quality is based on pH and intramuscular fat (IMF). The two breeds differ with regard to meat quality, and complement each other in crossbreeding programmes for slaughter pig production, which in Norway is based on Landrace × Yorkshire dams crossed to Landrace × Duroc sires. Hence, the end product contains 50% Landrace, 25% Duroc and 25% Yorkshire genes.

Drip loss, pH and IMF content have been reported to be favourably and significantly correlated with traits such as sensory tenderness, flavour and firmness scores (Huff-Lonergan *et al*., 2002). In addition, IMF content and pH have been found to be positively correlated, while colour lightness and moisture content were negatively correlated with flavour appreciation, tenderness and acceptability (Cameron, 1990). Consumer preference for meat seems to be strongly affected by changes in colour, appearance and texture (Risvik, 1994).

This study was designed to estimate genetic parameters for known meat quality traits measured by novel, low labour-intensive equipment that automates parts of the recording process. The costs of measuring meat quality traits are generally very high. Methods for measuring traits such as drip loss and texture are time consuming, and methods for chemical determination of components like fat, moisture and protein are expensive and time consuming and require the use of chemical solvents. It was important to find methods that are inexpensive, rapid, environmentally friendly, safe and user-friendly. Since the number of animals that had to be tested was large, relatively high equipment prices were nonetheless acceptable.

Material and methods

Animals

In the nucleus populations of the two breeds, approximately 50 Landrace and 50 Duroc average information (AI) elite boars are mated annually to 2200 Landrace and 700 Duroc sows, respectively. The proportion of first parity litters is 55% for Landrace and 60% for Duroc. Boars are used in AI for a period of 12 weeks. The average generation interval is 1.2 years in both populations.

The elite boars from the two breeds are selected based on BLUP breeding value estimates, including individual performance and performance of relatives; 3500 boars are tested annually for growth rate, feed intake, backfat thickness and exterior score. All boars used for breeding are tested to be negative for the halothane allele.

Annually, 2800 female and castrate littermates of the above boars are performance tested and slaughtered for carcass evaluation. This takes place in two testing stations (one in central and one in south-eastern Norway), each of which is connected to a different commercial abattoir. Both stations test both breeds with no environmental differences before or after slaughtering. Pigs are kept in mixed-sex single-breed groups of 12 pigs per pen, and fed *ad libitum* on conventional concentrates containing 14.5% to 15.8% protein and 9.33 MJ net energy/kg. Major feedstuff compounds are barley (48%), oats (22%), peas (5%), soy meal extract (16%) and rendering (animal) fat (2.4%). Data for the present experiment were collected over a period of 3 years on these sib-test animals. In this data set, full-sib group size was two (25% females and 75% castrates); average half-sib group size was 28. The average start and end weights were 30 and 113 kg live weight. Slaughter was performed in weekly batches; pigs were stunned in an atmosphere with 90% CO_2. The carcasses were ex-sanguinated, scalded and split within 30 min *post mortem*. After 45 min the carcasses were carried through a cooling tunnel with a temperature of −22°C and an air velocity of 8 to 10 m/s. After 5 min in a temperate area with 15°C, the carcasses were chilled at 1°C to 3°C for 20 h until a core temperature of 7°C in the ham was reached. The carcasses were transported from the abattoirs to a partial dissection line at Animalia, the Norwegian Meat and Poultry Research Centre.

Meat quality methods

Comprehensive meat quality evaluations at the partial dissection line were performed on approximately 60 pigs/week from 2005 to 2008, totally 6088 animals. Meat quality measurements were carried out on samples from the glycolytic loin muscle LD on the day of carcass dissection, 2 to 9 days *post mortem*. In addition, ultimate pH was measured in the glycolytic ham muscle *m. gluteus medius* (GM) and the oxidative ham muscle *m. gluteus profundus* (GP) on an extended data set. This data set consisted of animals tested in the same sib test from 1998 to 2005. The glycolytic LD and GM muscles were chosen because of the high commercial value of the loin and ham primary cuts. The oxidative GP muscle was chosen because of the importance of pH in this muscle for smoked-cured ham production. The quality traits were measured as follows:

(a) pH: Ultimate pH of the LD at the level of the last rib curvature, and ultimate pH of the GM and GP, were measured 2 to 9 days *post mortem*, using an insertion pH electrode (WTW 82362, pH 330i, Welheim, Germany). The pH electrode was calibrated daily to pH 4.01 and 7.00 Hamilton Duracal pH buffers (Hamilton Bonaduz AG, Switzerland) at 5.0°C. Some outliers with pH values of more than four standard deviations below or above the mean pH were excluded from the data files.
(b) Colour: The meat colour of bloomed (1 h at 2°C) pork chops was measured using a Minolta Chroma Meter CR-400 (measurement area 8 mm), with a D65 illuminant calibrated against a white tile. The tristimulus parameters L*, a* and b* values (also referred to as the CIELAB color space), representing lightness (L* = 0 is completely black, and L* = 100 is completely white), redness (positive a* values mean red colours and negative a* values mean green colours) and yellowness (positive b* values mean yellow colours and negative b*

values mean blue colours), respectively, were measured in duplicate on three fixed sites of each chop surface of the loin, in the *dorsal ventral* direction. The position was 2 cm *anterior* to 3 cm *posterior to* the last rib curvature. The average of six measurements on each chop was used. The Minolta instrument was connected to a computer, and a barcode scanner gave quick and accurate identification. Operator identification and recording time were also automatically stored.

(c) IMF, moisture and protein: The FOSS FoodScan Tm near-infrared spectrophotometer (FOSS, Denmark) with an artificial neural network calibration model was used for the determination of fat, moisture and protein in the same loin chops as used for meat colour measurements. The instrument used was the near infrared spectroscopy (NIR) transmission, with a moving grating monochromator, scanning the region from 850 to 1050 nm. Loin chops were trimmed for fat and homogenised by grinding for 30 s using a mixer (Robot Coupe r5a+, W 1100, Robot Coupe, USA, Inc.). Approximately 180 g, ground samples were placed in a 140 mm round sample dish, and the dish was placed in the FoodScan equipment, taking 16 scans of each of the sample tested. Operator identification was entered, the barcode number for the animal was scanned and the meat product profile within the software was selected. The NIR scanning process took about 1 min. The calibration used in this study gave the results in percentage of (g/100 g) fat, moisture and protein. The FoodScan instrument was calibrated against chemical analyses for these ingredients. The standard error of prediction (SEP) for our NIR analysis predicting IMF was 0.17 for Landrace and 0.23 for Duroc. The correlations between chemically analysed IMF and NIR analysed IMF were 0.87 for Landrace and 0.95 for Duroc.

(d) Drip loss: The EZ-DripLoss method, developed at the Danish Meat Research Institute (Rasmussen and Andersson, 1996), was carried out with two samples of approximately 10 g from a slice posterior to the chops used for colour measurements, 3 to 5 cm *posterior* to the last rib curvature. A circular knife with a 2.5 cm diameter and the special containers ensured equal treatment for all samples. The samples were always taken from the *dorsal* part and from the *ventral* part of the slice. The samples were placed in pre-weighed drip loss containers (C. Christensen ApS, Denmark), and stored in a refrigerator at 5°C. Twenty-four hours after sampling, each container was weighed, including meat and drip loss and once again for drip loss. All drip loss measurements were expressed as a percentage of the initial sample weight (Rasmussen and Andersson, 1996). The method was automated by using a digital scale (Mettler-Toledo AS, Norway, model XS603SDR) and a barcode scanner connected to a computer. In addition to identification and sample weights, the records also included the time of recording, which makes the routine more flexible for variation around the 24-h period for dripping. This time effect was expected to be a covariate in the models for drip loss. The EZ-DripLoss method gives relatively high drip loss levels because of the high ratio between sample area and volume. The temperature in the refrigerator was also relatively high to ensure larger variation for this trait.

Statistical analyses

Initial computations were performed using SAS Proc GLM (SAS Inst., Inc., Cary, NC, USA) to evaluate non-genetic factors, that is, fixed effects to be included in the model. Various sub-models of the full model, which included the fixed effects of sex, herd, abattoir × slaughter day, calibration, storage day, slaughter weight and dripping time, were tested. The herd was defined as the animals' herd of origin until transfer to the test station. Because of the fixed connection between test station and abattoir, there was no need for the effect of station in the models. The effect of calibration was only relevant for the colour measurements. The storage time before carcass dissection ranged from 2 to 9 days. This was due to the large number of pigs tested every week, in combination with the time needed for the partial dissection and meat quality measurements (a team of five people spent approximately 4 days of work on a batch of 60 half carcasses). Slaughter weight was included in all models for the multi-trait analysis, independent of the significance level. Average carcass weights were 83 (Landrace) and 81 kg (Duroc), with standard deviations of 5.5 kg. Dripping time was only relevant for the trait drip loss; average dripping time was 24 h to 20 min with a standard deviation of 1 h to 50 min. Records with times >48 h were excluded from the analyses.

Estimation of (co)variance components was performed using multi-trait animal models, analysed with restricted maximum likelihood (REML) methodology. The DMU 6.7 software package (Madsen and Jensen, 2008) and the AI algorithm were used in the estimation. Asymptotic standard errors of (co)variance components were computed from the inverse average information matrix. For Landrace, the multi-trait model with 10 traits reached convergence without any problems. However, due to computational constraints, six multi-trait animal models with four to six traits each were used to cover all trait combinations for Duroc. The final multi-trait (co)variance matrices for the additive genetic and residual effects were constructed from these six multi-trait analyses by an expectation maximisation algorithm producing positive definite matrices (Mäntysaari, 1999). The following general mixed model (the full model) was applied in the multi-trait analysis within each breed:

$$\begin{aligned} Y_{ijklmn} = {} & \mu_i + \text{Sex}_{ij} + \text{Herd}_{ik} + \text{Abattoir} \times \text{Slaughter day}_{il} \\ & + \text{Calibration}_{im} + \beta_{i1} \times \text{Storage}_n + \beta_{i2} \\ & \times \text{Storage}_n^2 + \beta_{i3} \times \text{Storage}_n^3 + \gamma_i \times \text{Weight}_n \\ & + \delta_i \times \text{Dripping time}_n + a_{in} + e_{in} \end{aligned}$$

where Y_{ijklmn} is the observed trait i on pig n, of sex j, from herd k, slaughtered at day l and measured within calibration m; μ_i is the overall mean of trait i; Sex_{ij} is the fixed effect of

sex j (1, 2), within trait i; Herd_{ik} is the fixed effect of herd k (1 to 45 for Landrace and 1 to 10 for Duroc) within trait i; *Abattoir* × slaughter day_{il} is the fixed effect of abattoir-day l (1 to 247 for Landrace and 1 to 213 for Duroc), within trait i; Calibration_{im} is the fixed effect of the calibration m (1 to 301 for Landrace and 1 to 246 for Duroc), within trait i; Storage_n is the storage time before dissection of carcass in days (ranging 2 to 9) of pig n; β_{i1} is the fixed first-order regression coefficient of storage time for trait i; β_{i2} is the fixed second-order regression coefficient of storage time for trait i; β_{i3} is the fixed third-order regression coefficient of storage time for trait i; Weight_n is the slaughter weight of pig n; γ_l is the fixed first-order regression coefficient of slaughter weight for trait i; Dripping time_n is the dripping time of pig n; δ_i is the fixed first-order regression coefficient of dripping time for trait i; a_{in} is the random additive genetic effect of pig n for trait i; e_{in} is the random residual effect of pig n for trait i.

The additive genetic effects were assumed to be $\sim N(\mathbf{0}, \mathbf{G}\otimes\mathbf{A})$ and the residual effect was assumed $N(\mathbf{0}, \mathbf{R}\otimes\mathbf{I})$, where **A** is the additive relationship matrix, **G** is the additive genetic (co)variance matrix, **I** is an identity matrix of dimension equal to number of animals with data and **R** is the residual (co)variance matrix.

In addition, the same model, with the additional fixed effect of breed, was applied to the combined Landrace and Duroc data sets. SAS Proc GLM was used to estimate the least square means for breed effects.

Pedigree files

The pedigree file contained all tested animals and their ancestors traced back seven generations. The final pedigree files for Landrace and Duroc included 29 979 and 12 468 individuals (17 013 and 7469 individuals with data), respectively. The average coefficient of inbreeding was 0.06 for Landrace and 0.07 for Duroc.

Results

Breed differences

The basic statistics of the traits studied are presented in Table 1; least square means for the breeds are presented in Table 2. The models with their fixed effects are presented in Table 3. Landrace and Duroc were significantly different ($P < 0.0001$) for all meat quality traits. Landrace had considerably more drip loss than Duroc. For all three muscles included in this study, Landrace had lower pH than Duroc. Meat from Duroc appeared darker (lower L* values) with

Table 1 *Numbers of animals per trait, mean, s.d. of means, minimum and maximum for Landrace and Duroc*

Trait	*n*	Mean	s.d.	Minimum	Maximum
EZ-DripLoss (%)[a]					
Landrace	3838	6.77	1.97	0.13	15.44
Duroc	2250	3.70	1.82	0.14	11.99
Ultimate pH in *Longissimus dorsi*					
Landrace	16,732	5.51	0.10	5.06	5.98
Duroc	7456	5.62	0.12	5.13	6.13
Ultimate pH in *Gluteus medius*					
Landrace	16,604	5.54	0.12	5.10	6.04
Duroc	7461	5.63	0.12	5.13	6.14
Ultimate pH in *Gluteus profundus*					
Landrace	16,735	5.90	0.21	5.04	6.76
Duroc	7460	5.95	0.21	5.07	6.82
L* value[a]					
Landrace	3429	48.21	2.63	41.16	61.20
Duroc	1989	47.69	2.55	40.40	59.10
a* value[a]					
Landrace	3429	6.88	1.17	3.08	13.28
Duroc	1989	7.87	1.23	3.76	12.62
b* value[a]					
Landrace	3429	2.84	1.20	-1.38	7.81
Duroc	1989	3.38	1.35	-0.75	8.55
Intramuscular fat content (%)[a]					
Landrace	3775	1.34	0.32	0.49	5.60
Duroc	2201	3.17	0.90	0.98	8.85
Muscle moisture content (%)[a]					
Landrace	3785	74.79	0.53	70.77	77.04
Duroc	2205	73.52	0.79	68.90	76.28
Muscle protein content (%)[a]					
Landrace	3785	23.15	0.42	21.10	25.15
Duroc	2205	22.65	0.48	20.26	24.04

[a]Measurement done in *L. dorsi*.

Table 2 *Least square means for effect of breed, Landrace and Duroc. Sub-models as described in Table 3*

Trait	Landrace	Duroc	Difference	Significance
EZ-DripLoss (%)[a]	6.93	3.62	3.31	***
Ultimate pH in *Longissimus dorsi*	5.51	5.62	−0.11	***
Ultimate pH in *Gluteus medius*	5.54	5.63	−0.09	***
Ultimate pH in *Gluteus profundus*	5.90	5.96	−0.06	***
L* value[a]	48.18	47.54	−0.96	***
a* value[a]	6.86	7.82	−0.54	***
b* value[a]	2.75	3.29	0.64	***
Intramuscular fat content (%)[a]	1.25	3.22	−1.97	***
Muscle moisture content (%)[a]	74.84	73.44	1.40	***
Muscle protein content (%)[a]	23.18	22.66	0.52	***

[a]Measurement done in *L. dorsi*.
***$P < 0.001$.

higher colour intensity (higher a* and b* values), compared to Landrace. Duroc had more IMF, and thus less moisture and protein, than Landrace.

Fixed effects

The fixed effects included in the models (sex, herd, abattoir × slaughter day, calibration, storage day, slaughter weight and dripping time) had various influences on the traits in this study, independent of breed (Table 3). The effect of abattoir × slaughter day (which includes the effects of test station) was significant for all meat quality traits.

Sex effects. Least square means for the effect of sex on the traits in both breeds are presented in Table 4. For each trait, the contrast between sexes had the same sign for both breeds. However, the contrasts between sexes were more pronounced for Duroc than for Landrace. In general, females had more drip loss and tended to have lower pH than castrates. There was no difference between the sexes for pH in LD for Landrace, and for pH in GP for either of the breeds. The drip loss difference between the sexes may be related to the different levels of IMF and moisture content in the LD muscle, with females showing more moisture and less fat. Castrates showed higher L*, a* and b* values. These differences between sexes are interesting from a biological point of view, but they are small and both sexes can be treated equally during meat processing.

Storage effects. Storage (the number of days of storage from slaughter to dissection) significantly increased pH in both breeds (Table 3). From day 2 to day 9, pH increased from 5.47 to 5.56 in Landrace and from 5.60 to 5.70 in Duroc. The increase in pH influenced muscle water holding capacity (WHC), and there was thus relatively less drip from the carcasses with longer storage time. Two to 9 days of storage from slaughter to dissection increased WHC by 2.0% in Landrace and 1.5% in Duroc (data not shown in tables).

Heritability estimates for meat quality

The meat quality evaluation methods used in this study produced moderate-to-high heritability estimates (Table 5). These tended to be higher in Duroc than in Landrace, except for L* and a* values measured on chops from the LD muscle. Correspondingly, Duroc also showed higher genetic variance for most traits (Table 5). The standard errors for the heritability estimates were relatively low due to the large volume of data in this study.

Relationships among meat quality traits

Genetic and phenotypic correlations between meat quality traits are shown in Table 6. Drip loss had negative genetic and phenotypic correlations with pH in LD, GM and GP. Genetic and phenotypic correlations among pH measured in the different muscles were all positive, with a wide range (from 0.10 to 0.84). The correlations for pH in the GM ham muscle were stronger towards the LD loin muscle (measured at a 30 cm distance) than towards the GP ham muscle (measured at a 5 cm distance), possibly due to the glycolytic nature of GM and LD and the oxidative nature of GP, with a higher pH level. In Landrace, the genetic correlation between pH in GM and GP was much higher than in Duroc.

For meat colour in LD, both Landrace and Duroc had medium-to-high positive genetic and phenotypic correlations between L* and b* values, and between a* and b* values. At the same time there were small negative genetic correlations between L* and a* values, and the phenotypic correlations between L* and a* values were almost zero for the two breeds.

The spectroscopic analyses for percentages of fat, moisture and protein in LD showed negative genetic and phenotypic correlations between IMF and moisture, especially for Duroc. The phenotypic relationship between moisture and protein was different for the two breeds, with Landrace having strong negative genetic and phenotypic correlations, while the same correlations were slightly positive for Duroc. The two breeds were rather different with regard to the composition of fat, moisture and protein, with Duroc showing a much larger variation for these traits (both phenotypic and genetic) than Landrace (Tables 1 and 5).

Genetic and phenotypic correlations of pH measured in LD with the L* and b* values were moderate to high for both breeds, indicating that muscles with low pH also were paler

Table 3 *Fixed effects, significance and* R^2 *of the models for each trait in Landrace and Duroc*

	Fixed effects				Regression coefficients					R^2 Landrace	R^2 Duroc
	Sex	Herd	Abattoir × slaughter day	Calibration	β_1 Storage	β_2 Storage	β_3 Storage	γ Weight	δ Dripping time		
EZ-DripLoss (%)[a]	x z		x z	~	x z	x z	x z	x z	x z	0.27	0.30
Ultimate pH in *Longissimus dorsi*	z	x	x z	~	x z			x z	~	0.38	0.40
Ultimate pH in *Gluteus medius*	x z	x z	x z	~	x z			x	~	0.33	0.39
Ultimate pH in *Gluteus profundus*		x	x z	~	x			x z	~	0.30	0.28
L* value[a]	x z	x z	x z	x z				z	~	0.68	0.84
a* value[a]	x z		x z	x z	z			x z	~	0.48	0.59
b* value[a]	x z	x	x z	x z					~	0.60	0.65
Intramuscular fat content (%)[a]	x z	z	x z	~				x z	~	0.28	0.30
Muscle moisture content (%)[a]	x z	z	x z	~				x z	~	0.31	0.34
Muscle protein content (%)[a]	z	x	x z	~				x	~	0.25	0.27

[a]Measurement done in *L. dorsi*.
x = significant effect ($P < 0.05$) for Landrace.
z = significant effect ($P < 0.05$) for Duroc.
~ = not tested.
Empty cells = non-significant ($P > 0.05$).

and yellower. The correlation of pH with the a* value was small for Landrace and moderate for Duroc. The genetic and phenotypic correlations between the L* value and IMF were negative for Landrace and positive for Duroc. Genetic and phenotypic correlations between pH and IMF in LD were small-to-moderately positive for both breeds.

Discussion

Breed differences

The Norwegian Landrace and Duroc breeds were quite different with regard to meat quality traits. This may be explained by both the origin of the breeds and the selection they have been exposed to. During the past few decades, the Norwegian Landrace has mainly been selected for growth efficiency and sow fertility as a dam line, whereas the Norwegian Duroc has been selected for growth efficiency and meat quality as a sire line.

The small amount of IMF in Landrace has become a breed characteristic due to many generations of selection for lean meat. Still, small variation and a low level of IMF in Landrace give similar CV for both breeds and thus a scaling effect. Further, the low level of IMF in Landrace reduces the variation of moisture and protein in the muscle and results in more homogeneous meat. Duroc had a very good WHC with small drip loss. One of the advantages of the EZ-DripLoss method is increased variation and a high level of drip (Otto *et al*., 2004), which increases measurement accuracy and produces a more symmetric frequency distribution of drip loss. In spite of a large difference in WHC between Landrace and Duroc in this study, the variation in drip loss was almost the same in the two breeds, leading to a larger CV for Duroc than for Landrace.

In our study, Duroc LD muscle was darker, had a more intense, red colour and contained more fat and less moisture than the Landrace muscle. This was also found by Cameron *et al*. (1990) studying the LD muscle in Duroc and British Landrace pigs. Young *et al*. (2005) observed higher WHC and ultimate pH, lower colour determinants; a*, b* and L* values in LD muscle from Duroc, compared with Landrace, and Duroc had juicier meat than Landrace. This is in agreement with our observations of drip loss, pH and L* value, but opposite to our observations of a* and b* values. Berg *et al*. (2003) studied several breeds and found higher WHC, IMF content and ultimate pH, and lower L* value in LD muscle for Duroc, compared to American Landrace. This is in agreement with our results.

Other fixed effects

The day of slaughter was an important fixed effect in the models for drip loss, the L* value and pH. These traits are affected by variation over time from last feeding to slaughter, and by animal treatment before slaughter, both influencing the glycogen content in the muscle. In addition, carcass handling and temperature management influence the temperature–pH interaction *post mortem*.

Table 4 *Least square means for effect of sex in Landrace and Duroc. Sub-models as described in Table 3*

Trait	Female	Castrates	Difference	Significance
EZ-DripLoss (%)[a]				
Landrace	6.95	6.67	0.28	***
Duroc	3.99	3.59	0.40	***
Ultimate pH in Longissimus dorsi				
Landrace	5.51	5.51	0.00	ns
Duroc	5.61	5.62	−0.01	***
Ultimate pH in *Gluteus medius*				
Landrace	5.54	5.55	−0.01	*
Duroc	5.62	5.64	−0.02	***
Ultimate pH in *Gluteus profundus*				
Landrace	5.90	5.90	0.00	ns
Duroc	5.96	5.95	0.01	ns
L* value[a]				
Landrace	48.16	48.36	−0.20	*
Duroc	47.06	47.73	−0.67	***
a* value[a]				
Landrace	6.74	6.93	−0.19	***
Duroc	7.66	7.88	−0.22	***
b* value[a]				
Landrace	2.64	2.95	−0.31	***
Duroc	3.03	3.41	−0.38	***
Intramuscular fat content (%)[a]				
Landrace	1.21	1.40	−0.19	***
Duroc	2.85	3.41	−0.56	***
Muscle moisture content (%)[a]				
Landrace	74.86	74.74	0.12	***
Duroc	73.65	73.34	0.31	***
Muscle protein content (%)[a]				
Landrace	23.19	23.15	0.04	ns
Duroc	22.81	22.55	0.26	***

[a]Measurement done in *L. dorsi*.
* = $P < 0.05$, *** = $P < 0.01$, ns = non-significant ($P > 0.05$).

Sex effects. The larger difference in meat quality traits between the two sexes in Duroc, compared to Landrace, is supported by Jelenikova *et al*. (2008). They estimated the differences between Landrace and Duroc (among other breeds) and between females and castrates, for traits related to tenderness and IMF, and also observed a better eating quality (tenderness and juiciness) for females than for castrates. Contrary to most studies, they found that females had higher IMF content than castrates. In our study, castrates had higher IMF content than females; this was supported by Larzul *et al*. (1997) and Bahelka *et al*. (2007), who found significantly higher lean meat percentage and lower levels of IMF in females than in castrates. Latorre *et al*. (2003) found significantly higher IMF content, and less moisture and protein in castrates than in females. This was in agreement with our results.

Castrates had lighter meat with higher colour intensity than females. This finding was supported by Lloveras *et al*. (2008), who reported higher L*, b* (significant) and a* (not significant) values in castrates than in females.

In our study, females had significantly higher drip loss than castrates. This was also found in some commercial crossbreeds with a significant effect of lower pH and a tendency to higher drip loss in females (Lloveras *et al*., 2008). It is generally accepted that the eating quality of pig meat (measured as tenderness, juiciness and flavour) is similar for castrates and females (Cisneros *et al*., 1996; Ellis *et al*., 1996; Leach *et al*., 1996).

Storage effects. All muscles, independent of species, show an initial *post-mortem* reduction in pH from 7 to around 5.5 due to anaerobic metabolism producing lactate in the first hours after killing. The increase in pH that occurred from days 2 to 9 in our study influenced the muscle WHC, and we observed less drip loss from carcasses having a longer storage time. No significant effect of storage time was observed on muscle moisture content (Table 3), so that the effect of evaporation or drip from the LD muscle was most likely limited. The increase in pH was probably an effect of denaturation and enzymatic hydrolysis of the muscle proteins, giving an increased concentration of nitrogen compounds, which had a buffering effect on the pH. The effect of drip loss after storage was studied by van Moeseke and de Smet (1999), who found a reduction of drip loss after storage.

Table 5 *Heritabilities (h^2) with s.e., genetic and phenotypic s.d. (σ_a, σ_p) in Landrace and Duroc*

Trait	h^2	s.e.	σ_a	σ_p
EZ-DripLoss (%)[a]				
Landrace	0.23	0.04	0.85	1.78
Duroc	0.33	0.05	0.92	1.60
Ultimate pH in *Longissimus dorsi*				
Landrace	0.19	0.02	0.037	0.086
Duroc	0.27	0.03	0.052	0.100
Ultimate pH in *Gluteus medius*				
Landrace	0.12	0.01	0.033	0.098
Duroc	0.22	0.03	0.047	0.098
Ultimate pH in *Gluteus profundus*				
Landrace	0.19	0.02	0.077	0.179
Duroc	0.38	0.03	0.117	0.191
L* value[a]				
Landrace	0.41	0.05	1.36	2.11
Duroc	0.28	0.06	1.02	1.95
a* value[a]				
Landrace	0.46	0.05	0.70	1.03
Duroc	0.43	0.07	0.72	1.10
b* value[a]				
Landrace	0.31	0.04	0.51	0.92
Duroc	0.33	0.06	0.61	1.05
Intramuscular fat content (%)[a]				
Landrace	0.50	0.05	0.21	0.29
Duroc	0.62	0.07	0.65	0.82
Muscle moisture content (%)[a]				
Landrace	0.31	0.04	0.25	0.46
Duroc	0.50	0.06	0.49	0.69
Muscle protein content (%)[a]				
Landrace	0.40	0.05	0.25	0.39
Duroc	0.54	0.07	0.32	0.44

However, these authors did not measure weight and moisture content of individual muscles, only weight loss from half carcasses and without considering pH. They concluded that the reduction of drip loss measured 5 days *post mortem* was an effect of carcass drip, whereas in our understanding reduction of drip loss was shown to be an effect of pH. In our study, there was also a reduction of carcass weight after storage, but the LD was not affected, probably because this muscle was left intact when splitting the carcasses.

Heritabilities

For the meat quality traits examined in this study, the estimated heritabilities are moderate to high, and with relatively small standard errors (Table 5). Our results are in agreement with heritability estimates for drip loss published by Hovenier *et al*. (1992), who used a filter paper method, and also with those of de Vries *et al*. (1994) and Hermesch *et al*. (2000a), who both used a bag method. Lower heritability estimates were found by Suzuki *et al*. (2005), who used loin chops hanging from a wire in specimen cases, and by van Wijk *et al*. (2005), who used a method similar to the EZ-DripLoss method. All methods for the determination of drip loss are relatively sensitive to variations in operator and procedure. The amount of drip loss is affected by the direction of muscle fibres, contact between muscle and bag/case, handling of the meat and temperature variation. In our study, pincers were used to minimise meat handling. The special containers ensured equal direction of the muscle fibres and contact between muscle and container wall for all samples. A refrigerator, rather than a refrigeration room, was chosen to ensure a stable temperature. The connection of a digital scale to a computer reduced the risk of data errors via miskeying. These factors potentially increased the accuracy of the method, and thus contributed to the high heritability estimate for drip loss in our study.

For pH measurements in different muscles, the estimated heritabilities ranged from low to moderately high. Heritability estimates for ultimate pH in LD in the literature were also low (Hermesch *et al*., 2000a; Kadarmideen *et al*., 2004; van Wijk *et al*., 2005). These literature estimates were for Landrace and Large White and compare, as expected, better than our estimates for Landrace than for Duroc.

pH IS, in most cases, was used as an indirect selection trait for drip loss. The results from this study showed that direct selection for drip loss could be more efficient than indirect selection for pH. The genetic correlation between drip loss and pH limits the effect of indirect selection, and especially for Landrace this correlation was far <1. In this study, the estimated heritabilities for drip loss were higher than for pH (both measurements were taken at last rib in the LD), thus indicating a better accuracy for drip loss than for pH. Drip loss is relatively costly to measure. The bag method described by Honikel (1987) is an accepted method for measuring drip loss, but it is time consuming and requires much care. According to Rasmussen and Andersson (1996) who developed the EZ-DripLoss method, it is easier to use in an abattoir setting in a reproducible way. This was supported by Otto *et al*. (2004), who confirmed that the method has high sensitivity and estimated a correlation of 0.86 between the two drip loss methods.

The estimated heritabilities for meat L* value in LD were moderately high for Landrace and somewhat lower for Duroc. The Duroc estimate was similar to the highest values reported in earlier studies (Hermesch *et al*., 2000a; Suzuki *et al*., 2005; van Wijk *et al*., 2005), while the Landrace estimate was much higher. Common to the study reporting the highest heritability (Hermesch *et al*., 2000a) and our study was a fixed effect for all the animals tested at the same time, which had a large effect on the heritability estimate in our study (in spite of good routines for calibration). The estimated heritabilities for the a* and b* values in LD were moderately high, between the estimates of Sonesson *et al*. (1998) and van Wijk *et al*. (2005).

The heritabilities estimated for IMF were in good agreement with previously reported estimates published by Hovenier *et al*. (1992), de Vries *et al*. (1994) and Suzuki *et al*. (2005), who used chemical analyses, and Hermesch *et al*. (2000a) and Kadarmideen *et al*. (2004), who used NIR for the IMF measurements, as in this study. In addition, heritabilities estimated for marbling were lower in two studies

Table 6 *Phenotypic correlations (below diagonal) and genetic correlations (above diagonal; s.e. between brackets) for meat quality traits in Landrace and Duroc*

	EZ_Drip	pHuLD	pHuGM	pHuGP	L_Meat	a_Meat	b_Meat	IMF	Moisture	Protein
Landrace										
EZ_Drip		−0.63	−0.54	−0.14	0.44	0.05	0.38	−0.36	0.39	−0.26
		(−0.09)	(0.11)	(0.13)	(0.10)	(0.12)	(0.11)	(0.10)	(0.11)	(0.11)
pHuLD	−0.32		0.84	0.31	−0.79	−0.10	−0.69	0.42	−0.11	−0.10
			(0.04)	(0.07)	(0.06)	(0.11)	(0.07)	(0.09)	(0.12)	(0.11)
pHuGM	−0.25	0.34		0.55	−0.59	−0.22	−0.62	0.21	0.19	−0.10
				(0.06)	(0.08)	(0.12)	(0.09)	(0.11)	(0.12)	(0.12)
pHuGP	−0.15	0.23	0.34		−0.39	−0.09	−0.35	0.11	0.05	0.09
					(0.09)	(0.12)	(0.11)	(0.11)	(0.12)	(0.12)
L_Meat	0.30	−0.44	−0.26	−0.18		−0.29	0.55	−0.20	0.15	−0.05
						(0.09)	(0.07)	(0.09)	(0.11)	(0.10)
a_Meat	0.04	−0.14	−0.08	−0.09	−0.02		0.57	0.17	0.06	−0.19
							(0.07)	(0.09)	(0.11)	(0.10)
b_Meat	0.26	−0.42	−0.25	−0.23	0.63	0.63		0.03	0.13	−0.23
								(0.10)	(0.12)	(0.11)
IMF	−0.23	0.22	0.14	0.07	−0.13	0.16	0.07		−0.37	−0.36
									(0.08)	(0.08)
Moisture	0.20	0.06	0.11	0.16	0.06	−0.09	−0.10	−0.41		−0.65
										(0.06)
Protein	−0.20	−0.15	−0.09	−0.06	0.00	0.01	0.02	−0.22	−0.63	
Duroc										
EZ_Drip		−0.89	−0.62	−0.49	0.27	0.25	0.55	−0.35	0.08	0.36
		(0.04)	(0.08)	(0.09)	(0.14)	(0.12)	(0.10)	(0.10)	(0.12)	(0.11)
pHuLD	−0.59		0.82	0.23	−0.42	−0.40	−0.74	0.11	0.12	−0.38
			(0.04)	(0.08)	(0.12)	(0.11)	(0.07)	(0.11)	(0.12)	(0.10)
pHuGM	−0.42	0.50		0.10	−0.20	−0.29	−0.51	0.08	0.12	−0.54
				(0.08)	(0.14)	(0.12)	(0.10)	(0.12)	(0.12)	(0.10)
pHuGP	−0.29	0.23	0.26		−0.21	−0.12	−0.33	0.18	−0.01	0.12
					(0.13)	(0.12)	(0.11)	(0.11)	(0.11)	(0.11)
L_Meat	0.32	−0.37	−0.28	−0.13		−0.13	0.62	0.50	−0.65	−0.14
						(0.14)	(0.09)	(0.10)	(0.10)	(0.13)
a_Meat	0.15	−0.24	−0.11	−0.13	−0.06		0.63	0.19	−0.11	−0.22
							(0.08)	(0.11)	(0.12)	(0.11)
b_Meat	0.42	−0.51	−0.34	−0.23	0.64	0.62		0.38	−0.53	−0.09
								(0.10)	(0.09)	(0.12)
IMF	−0.31	0.11	0.09	0.08	0.37	0.23	0.36		−0.89	−0.59
									(0.02)	(0.07)
Moisture	0.15	0.09	0.06	0.08	−0.34	−0.24	−0.42	−0.83		0.28
										(0.11)
Protein	0.22	−0.32	−0.32	0.03	−0.18	−0.11	−0.07	−0.51	0.11	

EZ_Drip = drip loss in *L. dorsi*, method EZ-DripLoss; pHuLD = ultimate pH in *L. dorsi*; pHuGM = ultimate pH in *Gluteus medius*; pHuGP = ultimate pH in *Gluteus profundus*; L_Meat = Minolta L* value in *L. dorsi*; a_Meat = Minolta a* value in *L. dorsi*; b_Meat = Minolta b* value in *L. dorsi*; IMF = intramuscular fat content (mg/g) in *L. dorsi*; moisture = muscle moisture content (mg/g) in *L. dorsi*; protein = muscle protein content (mg/g) in *L. dorsi*.

using marbling as a method for measuring IMF (Sonesson *et al*., 1998; van Wijk *et al*., 2005). In a preliminary analysis in this study, IMF predicted by NIR was found to have higher heritability estimates, compared with IMF predicted by chemical analysis. In a small data set of 365 Duroc pigs, the heritability for IMF from chemical analysis was estimated at 0.40 ± 0.11. For the same animals, and the same model, the heritability for IMF predicted by NIR was 0.61 ± 0.11. One advantage of NIR is that a larger volume of meat can be tested. With NIR, 180 g of meat were tested in 16 replications. For chemical analysis, only 5 g of meat were tested, and the sampling error could therefore be rather high. However, the SEP for our NIR analysis was not much higher than the SEPs for chemical analysis. The heritability estimates for IMF measured as a marbling score in the literature were lower than those based on chemical analysis or NIR, which might be due to reduced precision.

The estimated heritabilities for muscle moisture and protein content were moderately high, and higher than the heritabilities of moisture content reported by Cameron (1990) and Lo *et al*. (1992). No heritability estimates were found in the literature for muscle protein content. The different

magnitude for heritabilities estimated for IMF, moisture and protein content for the two breeds in our study can partly be explained by a greater variation for these traits in Duroc than in Landrace (Table 1). Larger variation resulted in less effect of the SEP, and better estimates.

Genetic correlations among meat quality traits

The large difference between the breeds in the magnitude of the genetic correlation between pH in GM and GP may be due to a different composition of muscles fibres in Landrace and Duroc. If Landrace pigs have higher amounts of glycolytic muscle fibres in the oxidative GP muscle than Duroc, the genetic correlation to the glycolytic GM muscle would be higher in Landrace than in Duroc.

Negative genetic correlations between drip loss and pH were in agreement with drip loss measured with a filter paper method by Hovenier *et al*. (1992), a bag method by Sonesson *et al*. (1998) and a method similar to EZ-DripLoss method by van Wijk *et al*. (2005). The iso-electric point of meat occurs at a pH of about 5.4 to 5.6. After normal rigor mortis, meat has a pH of about 5.5, and thus the lowest WHC possible. In this study, pH in LD averaged 5.51 and 5.62 for Landrace and Duroc, respectively. Landrace was thus at a point where a pH change in both directions would lead to increased WHC, while in Duroc a lower pH is expected to decrease WHC and higher pH is expected to increase it. This may explain the higher correlation between pH and drip loss in Duroc than in Landrace. However, no significant effect of any pigs having low drip loss in combination with low pH was observed in these data. This was not expected, in view of the theory of pH and iso-electric point.

The genetic correlations among L*, a* and b* values in our study were similar for the two breeds, and had the same sign as correlations presented by van Wijk *et al*. (2005). The low negative correlations between L* and a* values showed that darker meat tended to be redder. The high genetic correlations between L* and b* values indicate that lighter meat was also yellower. For the meat industry, pale pig meat is not desirable, and there has been considerable focus on a reduction of PSE-meat incidence. Improving the quality of pork redness, which also affects the appearance of the meat, is a new challenge (Risvik, 1994). In this regard, the a* value is correlated to the content of pigment and myoglobin in the muscle. Pigments contain iron, and thus make meat nutritionally important to humans. Lindahl *et al*. (2001) found that 90% of the variation in the a* value of LD and *M. biceps femoris* was explained by the content of pigment and the size of the fractions of myoglobin oxidised from blooming.

Reduction of IMF in pork leads to increased water content in the meat. The results from this study also indicate that pork with higher water content and a lower level of IMF tended to have more drip loss. A negative genetic correlation between IMF and water content in the meat was also reported by Cameron (1990). The relationships between water content in the meat and drip loss were not found in the literature, but genetic correlations between IMF and drip loss were reported by Hovenier *et al*. (1992), de Vries *et al*. (1994) and van Wijk *et al*. (2005). In our study, this genetic correlation between IMF and drip loss was a little stronger.

In Landrace, there was a negative genetic correlation between L* value and IMF, whereas this correlation was positive in Duroc. A negative genetic correlation between lightness and IMF was reported by Hovenier *et al*. (1992), and positive genetic correlations were reported by Hermesch *et al*. (2000b) and Suzuki *et al*. (2005). Suzuki *et al*. (2005) studied a Duroc population with a high level of IMF (4.25%), and found that meat with higher IMF was lighter in colour. Muscles with high levels of IMF are usually more oxidative than muscles with low levels, since oxidative muscles use more fat in metabolism (Essén-Gustavsson and Fjelkner-Modig, 1985). Essén-Gustavsson *et al*. (1994) showed that lipids are stored mainly in type I fibres and in some type IIA fibres. Oxidative muscle has more mitochondria and a higher myoglobin content, and a higher pH *post mortem*. All these factors give oxidative muscles a darker colour than glycolytic muscles. The positive genetic correlation between L* value and IMF for Duroc in this study most likely comes from visible fat cells detected when colour was measured, and not from the meat colour. There was almost no marbling in Landrace, and darker meat (lower L* value) also had more IMF in this breed.

Both breeds showed low-to-moderately positive genetic correlations between pH and IMF in LD. Correlations of the same magnitude were estimated by Cameron (1990) and Sonesson *et al*. (1998). An explanation for this positive relationship may be that oxidative muscles with more IMF have higher pH *post mortem* because their metabolism is more based on fat and less on glycogen, as fat metabolism does not produce lactic acid *post mortem*.

In most meat quality studies, only a small part of the muscle can be used for analysis due to high measurement costs. Many studies took their measurements in the LD at the position of the last rib, but this position seems to be linked to better meat quality and is not necessarily representative of the quality at other positions of that same muscle. Lundstrom and Malmfors (1985) studied the whole LD muscle and found the most stable and best meat quality with minimum drip loss near the last rib, and higher light scattering in the anterior and posterior parts of LD. In our study, a difference from the *dorsal* to the *ventral* part was observed. The *ventral* part of the muscle had a lighter colour, higher L* value, and a larger drip loss, compared to the *dorsal* part. This contrast was visible even to the human eye under normal indoor lighting. The difference in L* value was about 2.5 units, and the difference in drip loss was approximately 0.7%. The effect was similar for Landrace and Duroc (data not shown in tables). This physiological relationship has been found earlier, both in pigs (Christensen, 2003; Otto *et al*., 2004) and in cattle (Hoset, 2008).

Conclusions

These results show that it is possible to obtain high heritabilities for meat quality traits measured by rapid, low labour-intensive methods. All the methods are safe, user-friendly and robust with regard to operator effects. It is equally important that

the methods are environmentally friendly and do not require chemical solvents.

Our results enabled Norsvin to include new meat quality traits in their breeding goal and to reduce the costs of measuring meat quality traits. Among the meat quality traits studied, IMF and drip loss might be the most important traits for selection. The methods chosen for these traits were suitable for rapid measurement, which make it possible to test large numbers of animals each year. The estimated heritability for IMF analysed with NIR was higher than the heritability for chemically analysed IMF. In addition, NIR analysis gave estimates for water and protein content in meat, which can be important for future work. For drip loss, the EZ-DripLoss method gave heritabilities of moderate magnitude, and correlations to pH suggest that selection for drip loss may add valuable information to the breeding programme. The L* value was highly related to pH and drip loss; however, the L* value was affected by the content of IMF, thus making this trait unsuitable for selection programmes. The a* value was less correlated with other traits, and added new and valuable information. As the a* value had a high heritability and additive variance, and only a few unfavourable correlations to other traits, a large response is expected from breeding for this trait.

Meat quality traits are important for the pig meat industry. It is still desirable to increase carcass leanness, but a sustainable breeding programme should also more rapidly improve meat quality traits for breeders, for the industry and for consumers.

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Paper 2

Prediction of fat quality in pig carcasses by near-infrared spectroscopy

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Abstract

This study was conducted to evaluate the potential of near-infrared spectroscopy (NIRS) technology for prediction of the chemical composition (moisture content and fatty acid composition) of fat from fast-growing, lean slaughter pigs samples coming from breeding programs. First (I) NIRS method: A total of 77 samples of intact subcutaneous fat from pigs were analysed with the FOSS FoodScan near-infrared spectrophotometer (850-1050 nm) and then used to predict the moisture content by using partial least squares (PLS) regression methods. The best equation obtained has a coefficient of determination for cross-validation (R^2_{CV}*) and a root mean square error of a cross-validation (RMSECV) of 0.88 and 1.18%, respectively. The equation was further validated with (*n = *15) providing values of 0.83 and 0.42 for the coefficient for determination and validation of the (*R^2_{val}*) and root mean square error of prediction (RMSEP), respectively. Second (II) NIRS method: In this case, samples of melted subcutaneous fat were analysed in a FOSS XDS near-infrared rapid content analyser (400-2500 nm) using a FOSS XDS near-infrared rapid content analyser, spectra (400-2500 nm). Equations based on modified partial least squares (MPLS) regression methods demonstrated that NIRS technology could predict the fatty acid groups, the main fatty acids and the iodine value accurately with* $R^2_{CV,}$ *RMSECV,* R^2_{val} *and RMSEP of 0.98, 0.38%, 0.95 and 0.49% (SFA), 0.94, 0.45%, 0.97 and 0.65% (MUFA), 0.97, 0.28%, 0.99 and 0.34% (PUFA), 0.76, 0.61%, 0.84 and 0.87% (Palmitic acid, C16:0), 0.75, 0.16%, 0.89 and 0.10%*

(Palmitoleic acid, C16:1n-7), 0.93, 0.41%, 0.96 and 0.64% (Steric acid, C18:0), 0.90, 0.51%, 0.94 and 0.44% (Oleic acid, C18:1n-9), 0.97, 0.25%, 0.98 and 0.29% (Linoleic acid, C18:2n-6), 0.68, 0.09%, 0.57 and 0.16% (α-linolenic acid, C18:3n-3) and 0.97, 0.57%, 0.97 and 1.22% (iodine value, calculated). The magnitude of this error demonstrated quite good accuracy using these rapid methods in prediction of the moisture and fatty acid composition of carcass from animals involved in a breeding scheme.

Keywords: fat moisture, fatty acids composition, near-infrared spectroscopy, gold reflector, repeatability file

Implications

This work shows that it is possible to establish simple routine methods for measuring pig fat quality, as well as using preparation methods and instruments that are safe, user and environmentally friendly and do not require chemical solvents. The results reveal that several parameters for fat quality can be predicted by NIRS technology, and a continuation of this work documents the use of NIRS predicted values in the estimation of genetic parameters for breeding purposes. The high heritabilities obtained in the follow-up study clearly demonstrate the power of NIRS and the high prediction ability of the calibrations developed in this study.

Introduction

Information in relation to fat moisture content and fatty acid composition in pig fat is important when evaluating fat quality in view of the technological quality and fatty acid profile with regard to human health. Water (moisture) is expected to yield several peaks in the near-infrared (NIR) band width, and the water content can be predicted using near-infrared spectroscopy (NIRS) (Isaksson *et al.*, 1992; Buning-Pfaue, 2003; Anderson 2007). Fatty acid composition is usually analysed with labour-intensive methods such as gas chromatography (GC), which are expensive when large sample sets are to be analysed. For that reason, using methods without any harmful effects on the environment for rapid determination of the composition of pig fat is of great interest. Fat quality predicted by NIRS shows spectral information related to fatty acids (Schwörer *et al.*, 1999; Garrido-Varo *et al.*, 2008). For instance, NIRS absorption bands at approximately 1600-1800 nm and 2100-2200 nm are due to the straight carbon chain and cis double bonds, respectively (Sato *et al.*, 1991). NIRS has many

advantages, and using NIRS methods and their known performance enables non-professional personnel to conduct routine analyses of large sample series at a relatively low cost. Several studies have reported that NIRS technology permits prediction of the composition of Palmitic, C16:0; Steric, C18:0; Oleic, C18:1n-9 and Linoleic, C18:2n-6 acids of subcutaneous fat from the Iberian pig breed (de Pedro *et al*., 1992; Garcia-Olmo *et al*., 2001; Fernandez *et al.*, 2003). However, Garrido-Varo *et al*., (2004) on a review of the NIRS analysis of fats and oils analysis concluded, that although it is possible, from a methodological standpoint, to minimize the sources of variation affecting NIR analysis of fats and oils, there are still some unresolved "routine" issues (Perez-Marin *et al*., 2007). The Iberian breed is very different from Norwegian commercial breeds (Landrace and Duroc). The Norwegian breeds are leaner, more feed efficient and faster growing, and there are also large differences in fatty acid composition. This work was a calibration study using cross-validation and test set validation in order to investigate the possibilities of NIRS technology in predicting the moisture content and fatty acid composition of subcutaneous fat from fast-growing, lean slaughter pigs samples, for its routine use in the prediction of moisture content and fatty acid composition of animal carcasses involved in breeding schemes.

Material and methods

Animals

Fat samples were collected between 2005 and 2007 from Norwegian Landrace and Duroc pigs, which were all animals included in the national breeding scheme. The carcass records made available for these analyses came from half-sib tested females and castrated males from two different test stations. The pigs were fed conventional concentrates *ad libitum* during the test period (30 - 113 kg live weight), and more detailed information about the test and slaughtering is described by Gjerlaug-Enger *et al*. (2010b).

The fat samples

The determination of fat moisture content, fatty acid groups, several fatty acids and iodine value were carried out on samples of subcutaneous fat collected from the area between the loin and ham in the coxal region of the carcasses. The fat samples obtained from these carcasses included the hide/skin, as well as the fat between the hide and the lean and some lean. The samples were randomly chosen from both breeds and both stations from all seasons of the year in order to cover the variation within this population (test station with purebred pigs).

Two NIRS methods, using different instruments and sample preparations, were used in this study. Figures 1a and 1b show the working routine for the two NIRS methods: I) Moisture content in subcutaneous fat, and II) Fatty acids of subcutaneous fat. There was no overlap between the methods, so none of the animals (or samples) had both NIRS analyses for fat moisture content and fatty acid composition. All of the samples analysed were from different pigs, although some samples were analysed in duplicate, thereby yielding more than one spectrum per sample. See the experimental design for the two methods used in Tables 1a and 1b.

First (I) NIRS method: Moisture content in subcutaneous fat

Sample preparation - Seventy seven samples (FSC, Figure 1a and Table 1a) were prepared for NIRS analysis. The sample containing all the fat layers was cut into small pieces (brick size: 3-5 mm), with no further homogenisation performed. Approximately 12 g of this tissue was placed in a 50 mm round plastic petri dish and the dish was placed in the NIR instrument. The sample preparation took approximately one minute.

The NIRS analysis - Transmission spectra from the FoodScan near-infrared spectrophotometer (FOSS NIRSystems, Hillerød, Denmark) were used to develop calibrations for the prediction of the fat moisture content in the FSC samples. This instrument uses NIRS transmission with a moving grating monochromator, scanning the region from 850 to 1050 nm with data collection every 2 nm, taking a total of 16 scans in one minute from each sample tested and recorded as the logarithm of the inverse of the reflectance (log(1/R)).

Reference values - The moisture content (%) was measured by drying 6 g tissue samples mixed with sand at 105 °C until a constant weight was obtained (anonymous, 1974).

Second (II) NIRS method: Fatty acids of subcutaneous fat

Sample preparation - A microwave fat melting technique (de Pedro *et al*., 1997) was used to prepare the samples to obtain the total lipid content from 112 subcutaneous fat samples (XDS - Figure 1b and Table 1b). The samples were cut into pieces in the same way as the analysis of the fat moisture content, although none of the samples were analysed for both fat moisture and fatty acids. In the microwave fat melting technique, 6-8 g of fat were placed in a glass vial, which was then placed in a microwave oven with a rotating turntable to ensure a homogeneous sample heating. To obtain melted fat, the oven was set at 160 W for 4 minutes, simultaneously placing four vials of the sample inside it. The resulting samples were added to 1.5 ml Eppendorf conical polypropylene tubes, using snap caps

with a pipette. These were then centrifuged for 1 minute in an Eppendorf centrifuge, and the moisture in the bottom of the tubes was removed using a 200 µl pipette. The extracted fat samples were stored in Eppendorf tubes under refrigeration at -20 ^{o}C. Two parallel tubes were made from each melted fat sample, with one used for the reference values of fatty acid composition in the fat and the other for a NIRS analysis. Approximately three minutes for sample preparation was used on each sample.

Reference values - Laboratory values for the fatty acid composition were determined by gas chromatography (GC).

PCA analysis of fatty acid composition - Fatty acid profiles from the reference data were investigated by principal component analysis (PCA) using the Unscrambler version 8.0 (Camo ASA, Olso, Norway). The principal components (PC) are uncorrelated (orthogonal), and express much of the total variability in the data set through comparison of only a few PCs. In this study, a score plot shows how the different samples relate to each other with respect to the PCs, and the correlation loading plot depicts the identification the various fatty acids. The fat moisture was not included in the PCA analysis because none of the animals had both their fat moisture content and fatty acid composition analysed.

The NIRS analysis - Transflectance spectra (*n* = 200 spectra, some of the 112 samples were analysed with different gold reflectors (GR), yielding a total of 200 spectra, for details see Table 1b) of the total lipids from the subcutaneous fat samples were obtained using an XDS near-infrared rapid content analyser (FOSS NIRSystems, Hillerød, Denmark). Spectra were collected in the visible and near-infrared range from 400 to 2500 nm, with data collection every 2 nm. Each sample was scanned once using a circular quartz cuvette and a gold plated reflector that provides optical path lengths of 0.2 mm. This combination of transmission and reflection is known as Transflectance, which can greatly enhance the sensitivity of thin samples. Thirty µl of the fat, thermostated at 45 ºC, was added to the cuvettes with a pipette, and the GR was placed without any air bubbles in the thin measurement layer. The GRs and cuvettes were washed in warm water with an ordinary household detergent and dried with paper tissue. Afterwards, they were heated to 45 ºC, together with the fat samples. Approximately 10 cuvettes were used, leaving some clean, warm cuvettes in the incubator at all times. The spectrum of each sample was an average of 25 sub-scans and was recorded as the log(1/R). The NIRS scanning process took one minute, and the total amount of time used for each sample was 2 to 3 minutes for the NIRS analysis.

Repeatability file algorithm for minimising the effects of different GRs - For efficient routine laboratory work, it was important that more than one GR be used for routine analyses. Different spectra shapes with different GRs made it necessary to include a repeatability (rep) file with different GRs in the models. A rep file serves to minimise the influence of such unwanted effects on the results of NIRS calibrations (Tillmann and Paul, 1998) and that it is specially important when working with liquid fat samples (Perez-Marin *et al*., 2007; Perez-Marin *et al*., 2009). The rep file in this study contains only spectra of three samples measured repeatedly with four different GRs. The variation in the spectra of any of the respective single samples is related to the different GRs used. The four GRs were rotated to reduce the confounding factor of GRs and temperature in the rep file. During the development of calibration, this rep file was added to the calculations. The goal of the rep file is to make the calibrations insensitive to the change in GRs, without reducing the accuracy of the calibration.

Spectra pre-processing for both NIRS methods

WinISI software (version 1.61, FOSS NIRSystems/Tecator Infrasoft International, LLC, Silver Spring, MD, USA) was used for the spectra collection and chemometric analysis of NIRS data. For the FSC samples (method I), the entire wavelength range from 850 to 1050 nm was used. The XDS samples (method II) were scanned in the Vis and NIR regions (400 to 2500 nm), while the best calibration models were found when the wavelength range was limited to the NIR region (1100 to 2500 nm).

The scatter correction - Scatter is a nonlinear function that can distort the relationship between the NIR spectrum and the reference value. Only Inverse Multiplicative Scatter Correction (MSC) or no scatter correction was tested for the FSC samples, as recommended by the WinISI III Manual (2005). For the XDS samples, five scatter corrections were tested to improve calibration accuracy: SNV, Detrend, SNV and Detrend combined, Standard MSC and Weighted MSC (WinISI III Manual, 2005). SNV scales each spectrum to have a standard deviation of 1.0. Detrend removes the linear and quadratic curvature of each spectrum. The standard MSC is the normal multiplicative scatter correction using the mean of the file. The weighted MSC corrects for the mean and standard deviation at each wavelength.

The mathematical pre-processing - The FSC samples were tested with 0,0,1,1 and 1,1,1,1, in which the first digit is the number of the derivative, the second is the gap over which the derivative is calculated, the third is the number of data points in a running average or smoothing and the fourth is the second smoothing (Shenk and Westerhaus, 1995). In the work performed to optimise the calibration accuracy, several other combinations of mathematical treatments were tested. For the XDS samples,

several more complex pretreatments with derivatives were tested: 1,4,4,1; 2,4,4,1; 2,8,4,1; 2,8,6,1; 3,10,10,1 and 4,10,10,1, according to the instructions in the WinISI III manual (2005).

Extended calibration set on the basis of the Global H levels

In seeking to create a robust calibration, the data set used in the calibration was extended to better predict future samples with similar spectrum. The Mahalanobis distance (H statistic) was calculated from the PCA scores of the spectra (X-data). This H value indicates how different a sample spectrum is from a mean sample set. Some samples with high Global H (GH) values (GH>3) were sent to a laboratory in order to obtain reference data, and the samples were then added to the calibration set to make the calibration a better fit with the population (WinISI III manual, 2005). We ended up with the number of samples which are outlined in the experimental design in Tables 1a and 1b.

Multivariate calibration with cross-validation for both NIRS methods

In the calibration sets, each sample consists of both reference values (Y) and NIRS spectra (X). The FSC spectra were related to fat moisture content, and the XDS spectra were connected to the fatty acid profiles. The regression methods were performed by partial least squares (PLS) and modified partial least squares (MPLS) to develop the best calibration equations (Martens and Naes, 1989), for FSC and XDS respectively. The MPLS method is similar to PLS regression, which uses both the X and Y values to form the factors useful to make the fitting. In MPLS, the residuals at each wavelength, obtained after each factor is calculated are standardised before calculation the next factor (Garrido-Varo *et al*., 2008).

The cross-validation - A k-fold cross-validation was performed on the calibration set, which was divided into 6 and 5 groups (k = 6 or k = 5) for the FSC and XDS samples, respectively. This was the default number from the WinISI software and is dependent on the amount of samples in the calibration. A smaller data set needs a larger k. Each group is validated using a calibration developed from the other samples, with the validation errors then combined in a root mean square of cross-validation (RMSECV). This procedure was performed until all the samples were used for both model development and prediction.

The optimal number of PLS factors - The number of PCs in the PLS/MPLS models (PLS factors) yielding a significant change in RMSECV, and not the lowest RMSECV, determined the optimal number of PLS factors to be used for the calibration. With this procedure an overfitting due to too many PLS factors, thereby reducing the validation performance, was avoided (Esbensen, 2000). The accuracy of the calibration models was assessed based on RMSECV and R^2_{cv}.

Validation test

A validation with new pigs was carried out to check the performance of the models. The validation test of the FSC samples followed the recommendations of Esbensen (2000), and the prediction accuracy was evaluated by a validation test set of 15 FSC samples (Table 1a). A more complex validation test was performed for the XDS samples. In terms of the XDS samples, the aim of this validation was to check the prediction ability and robustness of the models in handling various GRs. The validation test set consisted of 20 x 4 XDS samples (Table 1b). The same cuvettes were used for all four GRs on each fat sample. Due to the time needed to run all GRs the use of GRs was rotated, thus eliminating the systematic effect between GRs and temperature.

The statistics used for this evaluation were: the R^2 of the reference data with the predicted data in the test set (R^2_{val}). The slope of the regression line related the NIRS predicted values to the reference values. The bias calculated as the simple difference between the average of the reference values and the predicted values: The root mean square error of prediction (RMSEP), calculated by taking the square root of the average squared prediction error. The standard error of prediction (SEP), expresses the accuracy of NIR results, corrected for the bias. The RMSEP value includes the SEP and bias, and the relationship between them is given by $RMSEP^2 \approx SEP^2 + bias^2$, so when the bias is small, the RMSEP tends towards the SEP (Esbensen, 2000).

Repeatability and reproducibility of predicted data

The model repeatability and reproducibility was tested on different test sets. In both cases, calculations were made by taking the covariance between the repeated samples over the variance of all samples. For the FSC samples, analysing the same samples in rapid succession, with two analyses of each sample, tested the instrument's repeatability. The repeatability is the ability of an instrument to yield consistent measurement readings on multiple runs of the same sample.

For the XDS samples, a reproducibility model was preformed to investigate the accuracy of the models with the different GRs, and the data set was the same as for the validation test set. The reproducibility is the ability of the same instrument to yield consistent measurement readings regardless of who performs the measurements, for measurements analysed with different instruments, or in this case, the repeated analysis with different GRs. Therefore, the evaluation of the XDS instrument's reproducibility requires a measurement of the same XDS sample by different GRs under the same conditions. The reproducibility (reprod) was calculated between each combination of GR 1 to 4 ($reprod_{1-2}$, $reprod_{1-3}$, $reprod_{1-4}$, $reprod_{2-3}$, $reprod_{2-4}$ and $reprod_{3-4}$) and for each fatty acid, fatty acid group and iodine number.

Results

The PCA analysis of fatty acids, fatty acids groups and iodine value demonstrated a high degree of correlation between the variables, and 96% of the variation could be explained with two PCs only. The correlation loadings (Figure 2) give the correlations between each fatty acid and the selected PCs, with concentric circles of radii corresponding to 50% and 100% of the explained variance. The squared distance between the point of a fatty acid and its origin equals the fraction of the variance of the total variable explained by the two PCs in the plot. The correlation loading plot for the 10 fatty acid variables reveals high loadings of the iodine value and SFA in PC1 located along the horizontal axis with negative correlations to each other, while the vertical axis (PC2) in the plot represents more of the MUFA and PUFA groups with negative correlations to each other. In addition, this plot shows the high influence of C18:1n-9 to the MUFA, as well as C18:2n-6 being the main fatty acid for the PUFA. The Pearson correlation coefficients between the same parameters for the same samples (Table 2) reveal the same picture as the correlation loadings, with correlations close to one between C18:1n-9 and MUFA, and between C18:2n-6 and PUFA for the subcutaneous fat in these pigs. The correlation loadings are standardised, and this plot demonstrates that a relatively high degree of the variation of the minor fatty acids, such as C18:3n-3 and C16:1n-7, are explained by these two PCs.

For the FSC samples used in the prediction of fat moisture content, there was only one broad absorption band in the corrected spectra at the wavelength area of 930 nm, as shown in Figure 3. The shapes of the XDS samples used for the prediction of fatty acids show different peaks for wavelengths in the region of 1208-1210 nm, 1390-1394 nm, 1414-1416 nm, 1720-1726 nm, 1758-1762 nm, 1758-1762 nm, 1900 nm, 1926-1928 nm, 2142-2146 nm, 2306-2310 nm, 2348 nm and 2384 nm, as shown in Figure 4.

The NIR spectra from the XDS samples (Figure 4) come from the validation set with 20 samples analysed using four different GRs, and the various shapes exhibit large differences between the GRs, which is also shown in Figure 5 with the differences in GH values. The average GH value of spectra from GR2, GR3 and GR4 was 18.6 before these GRs were implemented in the calibration, and the average GH value was 1.0 for the same spectra in the final calibration.

The Inverse MSC method was the best scatter correction of the FSC samples, whereas the Standard MSC method is the best scatter correction of the XDS samples (Isaksson and Naes, 1988). The best mathematical pre-processing was 0,0,1,1 and 2,4,4,1 treatments for FSC and XDS samples,

respectively. The MPLS equation was slightly better than the PLS equation for the XDS models, while the PLS equation is recommended for the FSC samples (Jøns, pers. comm.).

The statistics of the regression and cross-validation results for the NIRS prediction of fat moisture content, fatty acid groups, several fatty acids and iodine value in subcutaneous fat are presented in Table 3. The R^2 of the reference data compared to the equation-predicted data varied from 0.99 for C18:2n-6 to 0.80 for C16:0, and the R^2 from cross-validation varied from 0.98 to 0.68 for SFA and C18:3n-3, with the R^2*'s* fat moisture content being between the latter two R^2. The standard error of prediction for the calibration (SEC) and cross-validation (RMSECV) was 1.05 and 1.18 for fat moisture content, respectively, and varied from 0.06 and 0.09 for C18:3n-3 to 0.55 and 0.61 for C16:0. The relationship between the standard deviation of the reference data and the standard error of the cross-validation (RPD) ranged from 7.1 to 1.9 for SFA and C16:1n-7, respectively, while the relationship between the range of the reference data and the standard error of cross-validation (RER) was 42.4 and 9.1 for the same two fat parameters.

The number of PLS factors used for several of the fat parameters was approximately half the number of PLS factors giving the minimum RMSECV, since an increased number of PLS factors resulted in little improvement in the RMSECV and R^2_{CV} (Figure 6 and Table 4). Nevertheless, a relatively high number of PLS factors were needed to make the distinction between the C18:2n-6 and C18:3n-3 fatty acids (Figure 2 and Table 2). With five PLS factors, the correlation between C18:2n-6 and C18:3n-3 was 0.89, whereas with nine PLS factors the correlation was 0.74, which was similar to the correlations between C18:2n-6 and C18:3n-3 for the reference values in the calibration set. These correlations were from an extended data set not included in this calibration set (Gjerlaug-Enger *et al*., 2010a).

For the FSC samples used to predict fat moisture content, three outliers were eliminated. A relatively conservative criterion based on a T value (residual/RMSECV) of 3.0 was used for the FSC samples. No outlier elimination passes were conducted for the XDS samples, though one sample was removed for all fatty acids parameters. This sample had a T value > 5 for several of the fatty acids as well as some strange reference values for fatty acids composition, which indicated that something was wrong with the GC analysis. T outliers are characterised by a large difference between reference and predicted values, and a T>2.5 is often used for the removal of outliers (Shenk and Westerhaus, 1995). In our calibrations, a few samples had T-values between 2.5 and 5, but they were not removed.

GH outliers are samples whose spectra differ notably from the mean sample spectrum, with a GH>3 often being used for the removal of outliers (Shenk and Westerhaus, 1995; WinISI III Manual 2005). The maximum GH value for the FSC and XDS samples was 5.10 and 6.44, respectively, and it was generally small problems with the variation of the spectra used in the final models.

Validation results for fat moisture (Table 5a) and fatty acids (Table 5b) show the NIRS's prediction ability for new samples. Figures 7, 8a and 8b show the NIRS values versus reference values for the fat moisture content of C18:1n-9 and C18:2n-6, respectively, from validation. No large outliers were detected, and the plots demonstrate good prediction ability for the calibrations. The three selected fat parameters are presented here due to the importance of these characteristics in determining porcine fat quality and human nutrition.

The repeatability of predicted fat moisture (Table 5a) was 0.99 for the FSC samples analysed in replicate, and the reproducibility for fatty acid groups, fatty acids and iodine value (Table 5b) analysed with various GRs ranged from 0.95 to 0.97 for SFA, MUFA, PUFA, C18:0, C18:1n-9, C18:2n-6 and iodine value, while C16:0, C16:1n-7 ranged from 0.90 to 0.97 and C18:3n-3 ranged from 0.74 to 0.89.

Discussion

The two NIRS methods used in this study are different from each other with regard to wavelength range and scanning method, i.e. transmission vs. transflection. The different wavelength ranges are chosen because of the expectations of different absorption features for the subcutaneous fat components of moisture and fatty acids composition. It is well known, and previous experiments have demonstrated (Gjerlaug-Enger *et al*., 2010b), that homogenisation improve the accuracy with NIRS technology. The best results with the XDS instrument can be achieved with transflection through a thin homogeneous layer, although other methods, i.e. fibre optics, are also available on the market. An analysis using fibre optics and a probe requires simple sample preparation. For example, a reflectance probe placed directly on the raw material could also yield fat moisture with the XDS instrument. Several absorption bands of OH groups in water are in the range of the XDS instrument. Rossel and McBratney (1998) found strong absorption bands of OH groups in soil water at approximately 1450, 1950 and 2500 nm. Unfortunately, a probe analysis would go only a few millimetres into the tissue, thus making it much more sensitive to the nature of a food sample. The advantage with the FoodScan is the multi-point scan through the entire fat layer. This instrument is specially developed to predict fat, moisture and protein in meat (Anderson,

2007); for that reason, it was expected to be suitable for the prediction of moisture in fat samples as well.

The use of the NIRS methods (I and II) in this study required different sample preparations. The preparation of the XDS samples with the melting technique was necessary to acquire the thin fat layer (thickness: 0.2 mm) needed for the transflection method used for the detection of fatty acids. As a consequence of this, the preparation of the XDS samples with the melting technique yielded a separation of moisture and fat, though the fat moisture content could not be predicted from this method. In contrast, the FSC samples were intact raw samples in regard to their subcutaneous fat composition. The low near-infrared wavelengths (850-1050 nm) can penetrate through the fat pieces (thickness: 8 mm), while the wavelengths with smaller waves (1100-2500 nm) in the XDS instrument is not able to penetrate this thickness.

The preparation of the FSC samples for the fat moisture analysis was simple since the fat pieces were intact, and had a low degree of homogeneity. Fat is difficult to homogenise with a mixer since a change in composition and tissue components sticking to the equipment create errors. With method I (Figure 1a), different levels of fat in the petri dishes may create errors if these factors are not controlled for. The result may be a spectral outlier for the fat moisture analysis in the FoodScan instrument, and no studies using a similar method were found in the literature. Method II required homogeneous fat to optimise accuracy, and our study used a microwave melting method to obtain the total lipids. Studies comparing fat extraction with solvents and microwave melting revealed similar results for fatty acids with both methods, although the melting method is much simpler to perform, requires less time and avoids the use of solvents that are difficult to manage and handle (Gonzalez-Martin *et al*., 2002; Garcia-Olmo *et al*., 2002).

The amount of time used for the XDS analysis was reduced by 50% when two GRs were used for a routine analysis. It was possible for one person to run 26 NIRS analyses in one hour; this was the maximum found under normal working conditions when we checked the time recorded for the analyses. Hence, a calibration that was robust for several GRs was performed. This was a challenging task, and our study demonstrates a large sensitivity towards the use of different GRs (Figures 4 and 5). The surface of the GRs probably exerted an influence on the spectra for the transflection method used for the XDS samples. To the best of the authors' knowledge, the problem with the GRs was not documented in the literature and was therefore quite surprising. The experimental design for the XDS samples is complicated due to the change in GRs used during the study (Table 1b). The first GR we

started to use (GR1) was the one that had the largest degree of difference from the other GRs. In Figure 5, we see some examples of GH values (first data series, average GH values of 18.6) for new samples analysed with a calibration (Standard MSC and mathematical pre-processing: 2,4,4,1) consisting only of GR1. The rep file used in the final calibration increased the value of the first 78 samples analysed with GR1, but a rep file does not exert a direct influence on the GH values for new samples. The acceptable level of the GH values (second data series, average GH values of 1.0) for GR2, GR3 and GR4 in Figure 5 was an effect of the 14 samples analysed with GR2, GR3 and GR4 (point b, Table 1b) that were included in the calibration. With concern to the validation test, the rep file had a larger effect than these 14 x 3 samples in the calibration's prediction ability (not presented in any tables). This experiment was a success for this type of calibration work, which aimed to make robust calibrations that were suitable for all GRs. The successful use of the repeatability file algorithm in NIRS calibrations to correct for various error sources, e.g. NIR instrument differences, transfer purposes, residual moisture and temperature variation in other studies and day-to-day variation in their own study, is thoroughly described by Perez-Marin *et al*. (2007).

The monitoring of a "check-fat" sample is recommended for method II in order to ensure a reliable application of the equations, and GR4 was used for this purpose for routine analysis (Table 1b). In general, for both this "check-fat" sample and other fat samples analysed with the XDS instrument, we see a change in the spectra with increased GH values over time. We also saw a tendency for the GH values to drift. When carrying out our work, this problem disappeared when we cut out the spectrum from 400 to 1100 nm. In a follow-up study using these calibrations on 5006 new samples, there were no major problems with high GH values or prediction ability. Perez-Marin *et al*. (2007) have reported similar problems. NIR calibrations on fats and oils can have a high precision. However, despite the high degree of accuracy afforded by the equations obtained, considerable deviations from expected values were detected when these equations were applied to new samples. This particularly occurred when spectra were recorded at a later period than those from the calibration samples, which may prove to be a challenge when using NIRS for the routine analysis of fats (Perez-Marin *et al*., 2007). Our calibration for the melted fat has been performed with samples collected over a long period of time and the validation set was done on samples taken 3 to 9 months after the calibration samples were analysed (Table 1b). Taken together, the long sampling time, the GC analysis of new samples with high GH values (GH outliers) and the use of the rep file could be the reason why the calibrations were relatively robust in the prediction of new samples.

The correlation of the loading plots from PCA analysis exhibited a high degree of correlation between the fatty acids. This relationship is a result of some of the fatty acids being converted to other fatty acids and in vivo uptake mechanisms. It is important to be aware of the reference data (Y) when analysing the parameters with NIRS. A large absorption of wavelengths for molecules in large quantities makes NIRS the method best suited for the prediction of fatty acids with a large content. Consequently, the correlations between the fatty acids make this method less robust for predictions with respect to a low composition of fatty acids, thus causing fatty acids with less than 1% to be left out of this study.

The NIR spectra of subcutaneous fat (FSC samples) for the calibration of fat moisture content (method I) are shown in Figure 3. Similar studies are not found in the literature, but moisture is expected to yield an absorption line at 930 nm (Duarte, 1995). This is in agreement with our results, which also finds the highest absorbance values, with a large variation in the area around 930 nm. In FSC samples with one fat (CH) and one moisture (OH) absorption band, this information is already the largest spectral variation. A model with no derivation (Inverse MSC and 0,0,1,1) gave the best calibration. A complicated scatter correction and mathematical pre-processing may amplify noise rather than reinforce the signal for the NIRS method I. The biggest variation between the spectra from the FSC samples in Figure 3 was caused by different samples, although the largest diversity of XDS samples in Figure 4 was due to the four GRs.

The spectra from the XDS samples with sharp, well-defined peaks (Figure 4) are similar to the spectra in the review by Garrido-Varo *et al*. (2004), which also shows the transflectance spectra of fat and oils with absorptions from 1100 to 2500 nm. The various peaks are associated with cis double bounds, CH bond vibration and a number of bonds giving the chain lengths. The mathematical pre-processing with second-derivative spectra used in our study is also commonly used in other studies, and the advantage was a better discrimination between peaks that overlap in the original spectra. The mathematical pre-processing (Standard MSC and 2,4,4,2) highlights the spectral information that distinguishes the different fatty acids in the NIRS method II.

A relatively low number of PLS factors in the PLS/MPLS models was used to make a more robust and global calibration, thereby avoiding an overfitting due to too many factors. This strategy worked well for large fatty acids groups (SFA, MUFA and PUFA), for C18:1n-9, for iodine value and for fat moisture content, though the other fatty acids required an increased number of PLS factors. Some evidence for this was the unfavourable increase in correlation between C18:2n-6 and C18:3n-3, with too low numbers for PLS factors. The acids C18:2n-6 and C18:3n-3 were similar in chemical structure and

showed a high correlation in the fat from pigs (Figure 2 and Table 2), although a too low number of PLS factors did not make a distinction between the fatty acids. Still, the number of PLS factors in the calibrations (Table 3) was below the number of PLS factors yielding the lowest RMSECV. On average, the R^2_{val} was two percentage points higher for the minimum RMSECV. In Figure 6, the minimum RMSECV for C18:1n-9 was at 11 PLS factors (4 PLS factors were used), and more PLS factors increased the RMSECV again due to overfitting.

There are repeated measurements with different GRs for 14 samples (14 animals) in the calibration (Table 1b). The variation between GRs was greater than the variation between the XDS samples for the raw spectra (Figure 4). However, the rep file, scatter correction and mathematical pre-processing will reduce these differences, so therefore the cross-validation results are slightly overestimated for R^2_{val} and RMSECV.

The R^2 and R^2_{CV} could likewise be improved if an increased number of outliers were removed, as the largest effect had the removal of outliers on fatty acids with a low content and low variation. Our choice of removing few outliers was done in order to make the calibration more global and the prediction ability better. The outliers can often contain important information if they do not significantly affect the RMSECV. Nonetheless, the removal of outliers may increase the risk of overfitting data to the limited data set, which will not increase the reliability of the prediction.

The calibrations were made for pigs grown in two test stations only with almost identical feed. Despite this, a relatively large variation was obtained. It is important that this variation covers the population in which the calibrations will be used for later predictions. A larger variation in fatty acids could be obtained if pigs were picked randomly from different herds, although the calibrations in our work were designed for the prediction of values in terms of breeding value estimations on purebred animals (Landrace and Duroc pigs) tested for carcass and meat quality parameters in a breeding programme. Even so, the variation of fatty acids for pigs in this study were similar to those used to make calibrations for fatty acids in other studies (Fernandez *et al*., 2003; Garcia-Olmo *et al*., 2005; Perez-Marin *et al*., 2009). These studies were conducted on Iberian pigs in Spain fed several diets based on extensive feeding programmes that were different from Norwegian conventional feedstuffs. The Spanish feed is generally high in C18:1n-9 and low in C18:2n-6 and these pigs, which are used for dry ham production (Fernandez *et al*., 2003), have a lower LMP and higher age than the Norwegian pigs in our study. The standard errors of prediction and R^2 for the calibration and cross-validation were of the same magnitude

in both our study and the Spanish studies (Fernandez *et al*., 2003; Garcia-Olmo *et al*., 2005; Perez-Marin *et al*., 2009).

Minimum values for RPD and RER of 3 and 10, respectively, are recommended by Williams and Sobering (1996), and the results of our work demonstrated a limited predictive ability for fatty acids with low concentrations (C16:1n-7 and C18:3n-3) in addition to some problems with C16:0 (Table 3). NIRS has the best predictive ability for organic components with large volumes, and the C16:1n-7 and C18:3n-3 fatty acids are often left out of similar publications (Fernandez *et al*., 2003; Perez-Marin *et al*., 2009) or have an R^2 similar to our work for a study that also uses a microwave melting technique (Gonzalez-Martin *et al*., 2002). Shenk and Westerhaus (1996) indicated that NIRS equations with R^2 values higher than 0.9 may have an excellent precision, while those with R^2 values between 0.5 and 0.9 have values with good precision, meaning that from this all of our equations from both NIRS methods have a good future potential.

The test set validation was performed to check the quality of the calibrations for fat moisture and fatty acids made by the FSC and XDS samples, respectively. Shenk *et al*. (2001) assume the following control limits for this test: the SEP should not exceed 1.30 times the SEC. Our validation of fat moisture, C18:3n-3 and iodine value does not meet this requirement, but this limit worked fine for the other parameters. The predicted values for fat moisture are plotted against the reference values in Figure 7. The SEP values are slightly too large, though the bias and slope are satisfactory, resulting in a reliable R^2_{val} at 0.82 for method I. For the fat moisture, we see a larger SEP than RMSEP, which is probably caused by the limited amount of samples and the small SEP in comparison to the size of the bias (Table 5a).

The predicted values for C18:1n-9 and C18:2n-6 are plotted against the reference values in Figures 8a and 8b, respectively, and the validations worked well for these two fatty acids. For all fatty acid parameters in general (Table 5b), the bias was too large for MUFA, C16:0, C18:0 and the iodine value, whereas the slope was good for all parameters, except for C16:0 and C18:3n-3. The R^2_{val} was above 0.90 for all parameters despite C16:0, C16:1n-7 and C18:3n-3. The $RMSEP^2 \approx SEP^2 + bias^2$ works for all equations made for the XDS samples. The prediction ability of the models adjusted for bias (i.e. SEP) shows excellent results for all parameters from method II, except for C18:3n-3 and C16:0.

The repeatability of predicted fat moisture (Table 5a) and the reproducibility for fatty acid parameters (Table 5b) demonstrate a small error variance of these NIRS methods. The transmission and

transflection, with multiple scans through the sample, makes the prediction similar for repeated analyses. For the FSC samples, we see repeatability close to one and a coincidentally high SEP value. This allows for a legitimate question to be asked as to whether the NIRS method is more accurate than the reference method. In a similar study on meat quality, the FoodScan instrument gave a better estimated heritability than the reference method (Büchi Caviezel) in determining the fat percentage of meat (Gjerlaug-Enger *et al*., 2010b). The heritability is as much of a corresponding parameter as the repeatability; the difference is that a permanent environment is left out of the model, so only a genetic covariance between animals remains.

The reproducibility for the fatty acid parameters (Table 5b) documents the robustness of the models in handling four GRs. With the predicted values for C18:1n-9 and C18:2n-6 in Figures 8a and 8b, we see a repeated analysis with different GRs. The large similarity in predicted values for each sample yielding a good reproducibility was significantly improved when the rep file was implemented in the calibration. Problems with the use of multiple GR were not discussed in previous studies, so it was important to emphasise the issue in this study.

The calibrations presented here are intended for the Norwegian breeding programme for pigs, with the aim being to breed a better fat quality. Approximately 2000 animals are tested annually for several meat quality parameters for breeding purposes, and in our opinion the methods presented here are rapid enough to be used for this number of animals. A respective total of 5278 and 5006 pigs have already been tested with the NIRS methods I and II presented here, and they exhibited high accuracy in a study on genetic parameters (Gjerlaug-Enger *et al*., 2010a).

Conclusions

This work has shown that it is possible to use NIRS technology for the prediction of fat moisture content and several fatty acid composition of large number of samples coming from Norwegian pigs breeding programme.

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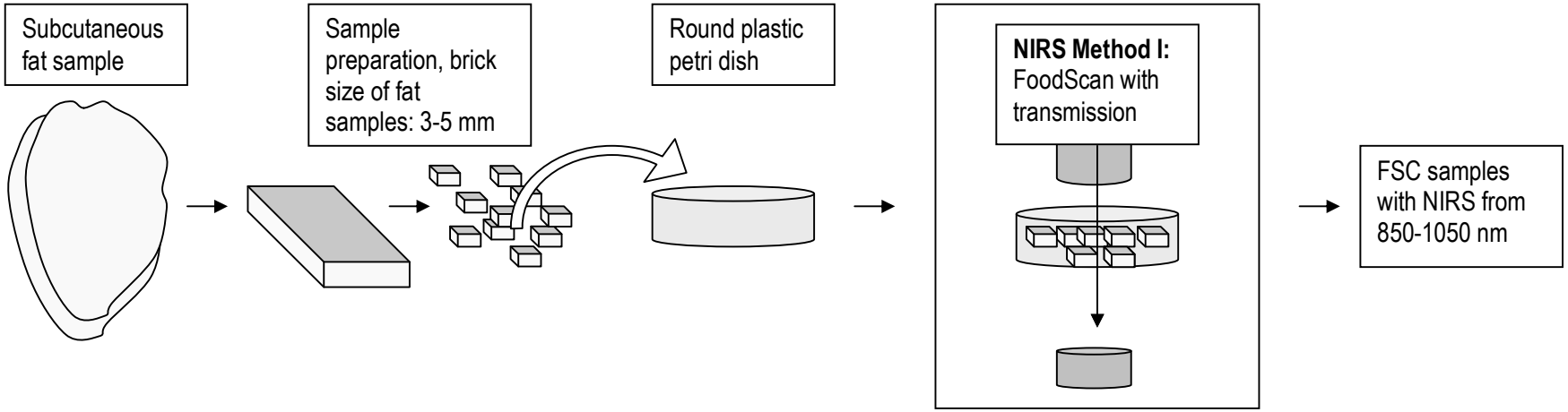

Figure 1a *Sample preparation of subcutaneous pig fat for NIRS prediction of fat moisture content in FSC samples*

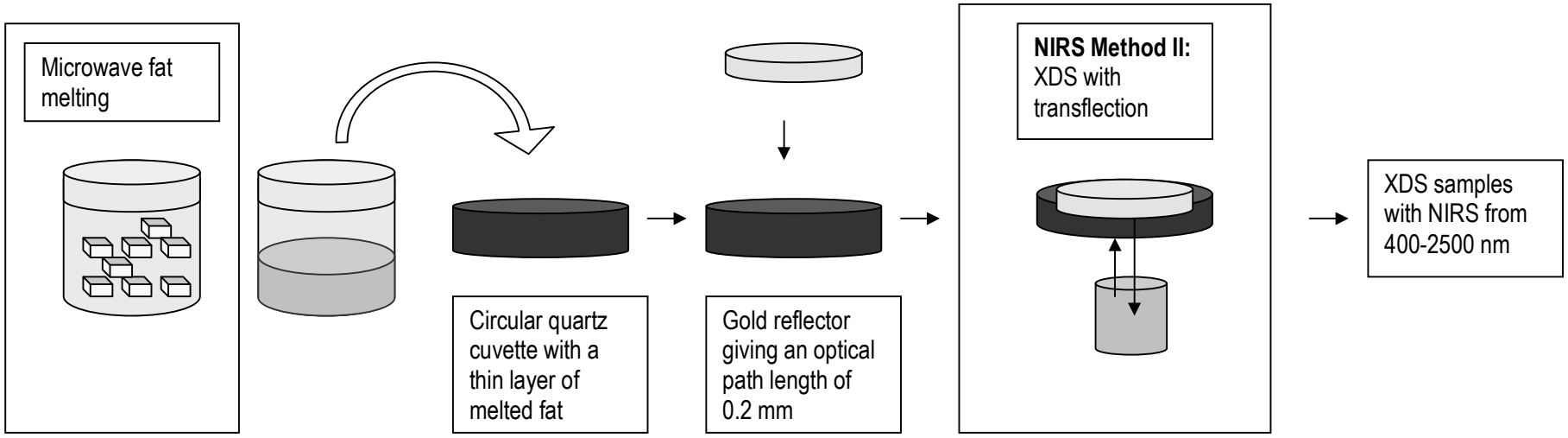

Figure 1b *Sample preparation of subcutaneous pig fat for NIRS prediction of fatty acid composition in XDS samples*

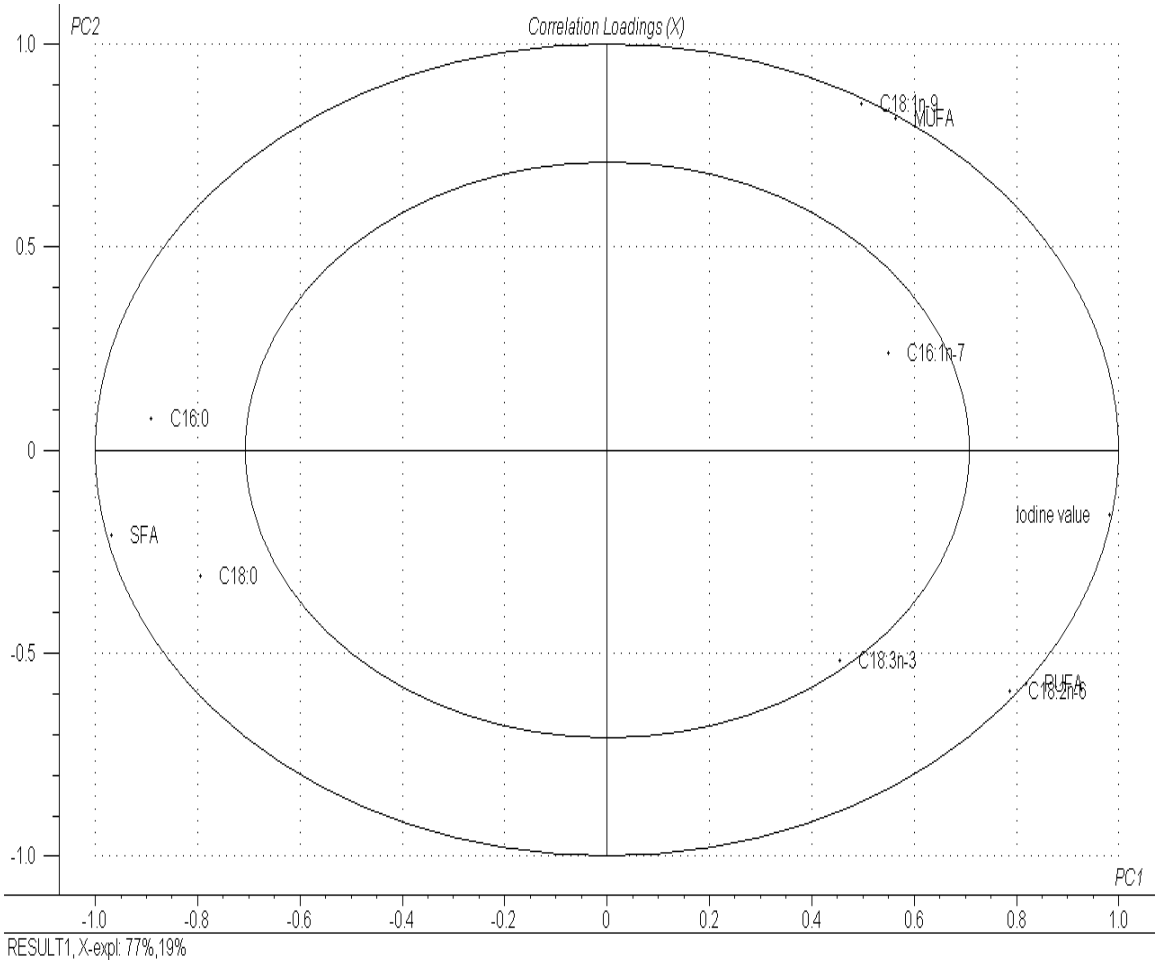

Figure 2 *Correlation loading plot of PC1-PC2 for main fatty acids in subcutaneous fat. Due to the high correlation between MUFA and C18:1n-9 and between PUFA and C18:2n-6, the labels are overlapping*

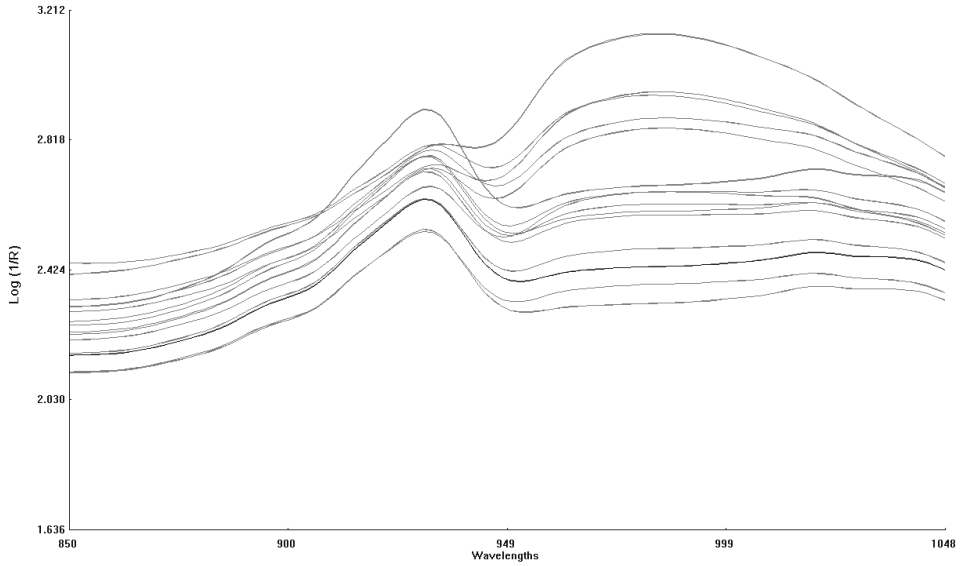

Figure 3 *NIR spectrum from FoodScan near infrared spectrophotometer (Foss). The figure shows the test set for repeatability, and the two replicates are so similar that it is hard to see that each line is two different spectra. Repeatability for this analysis was 0.99, and these FSC samples are used for the prediction of fat moisture composition*

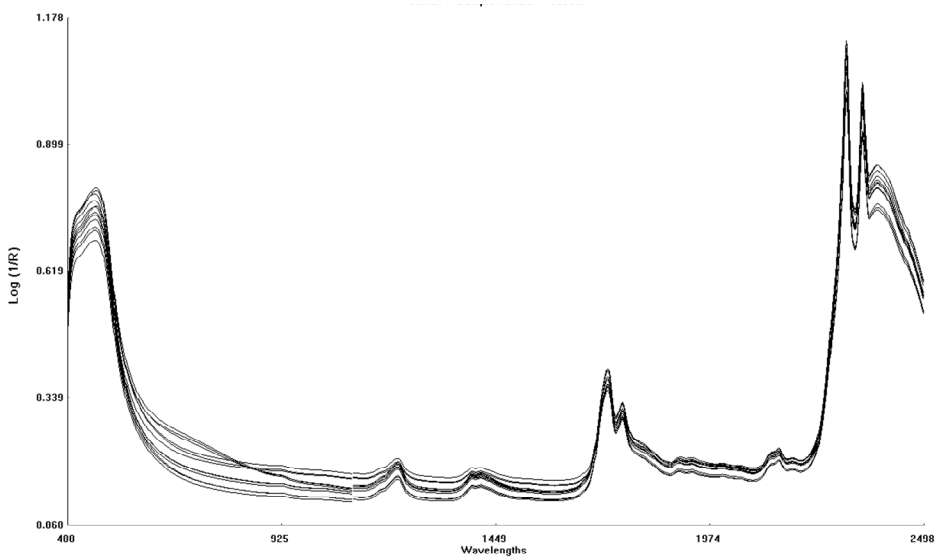

Figure 4 *NIR spectrum from XDS near infrared rapid content analyser (Foss). The 12 samples comprise the repeatability file used in calibration. The various shapes show the differences between the four different gold reflectors. The XDS analysis was used for the prediction of fatty acid composition in the XDS samples*

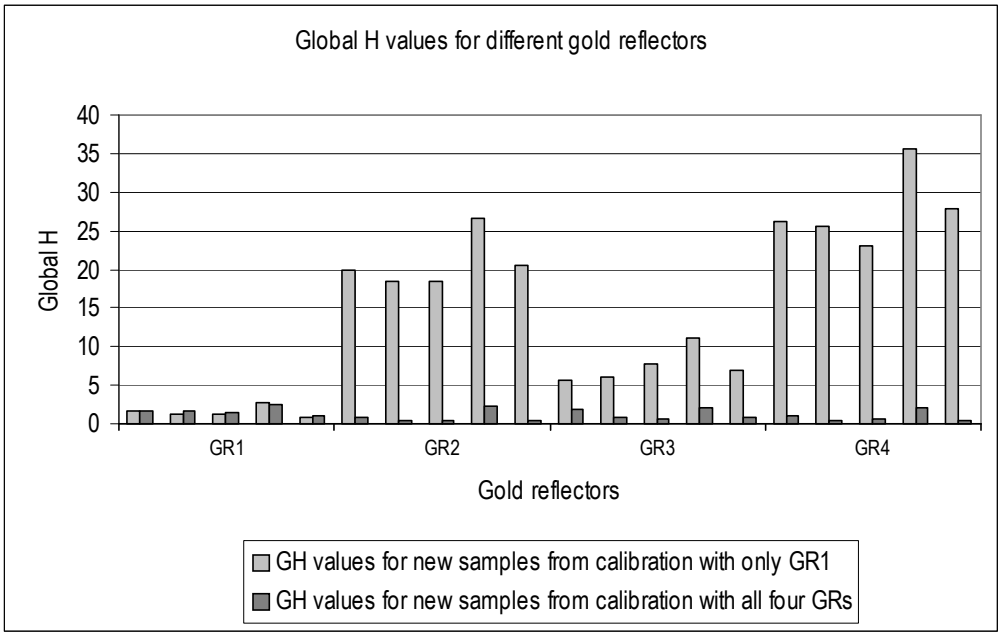

Figure 5 *Global H (GH) values for XDS samples before and after, including all gold reflectors (GR) in the calibration (Table 1b). The first data series (light grey dot/axes) shows the GH values for new samples analysed with all four GRs with an equation containing only samples from GR1, while the other data series (dark grey dots/axes) shows GH values for new samples analysed with a model that contains all four GRs*

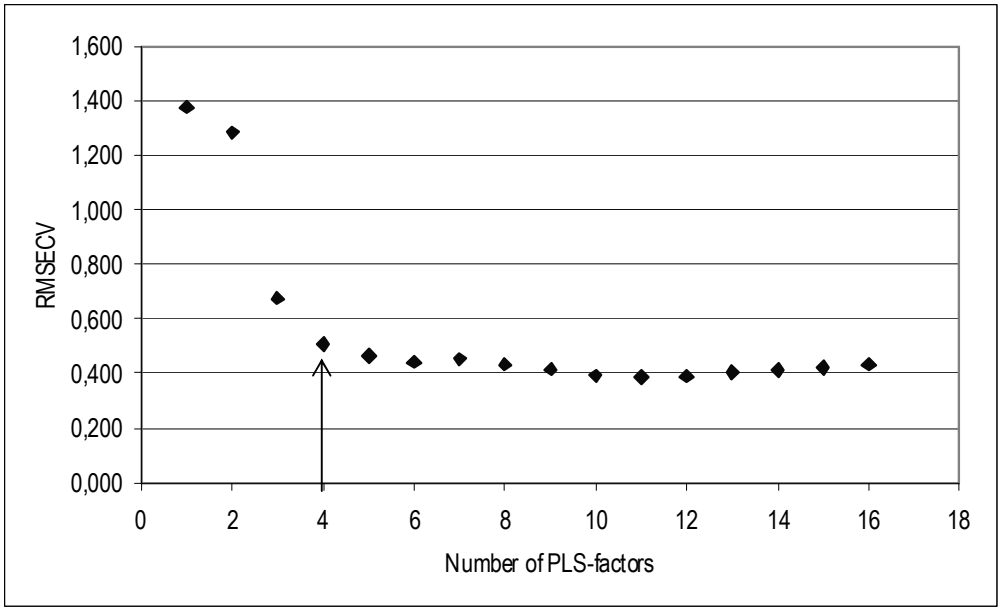

Figure 6 *Standard error of cross-validation (RMSECV) as a function of the number of PLS factors from the calibration model for C18:1n-9 based on a standard multiplicative scatter correction (Standard MSC) method and a mathematical pre-processing of: 2,4,4,1. The arrow indicates the number of PLS factors used in the final calibration results presented in Table 4*

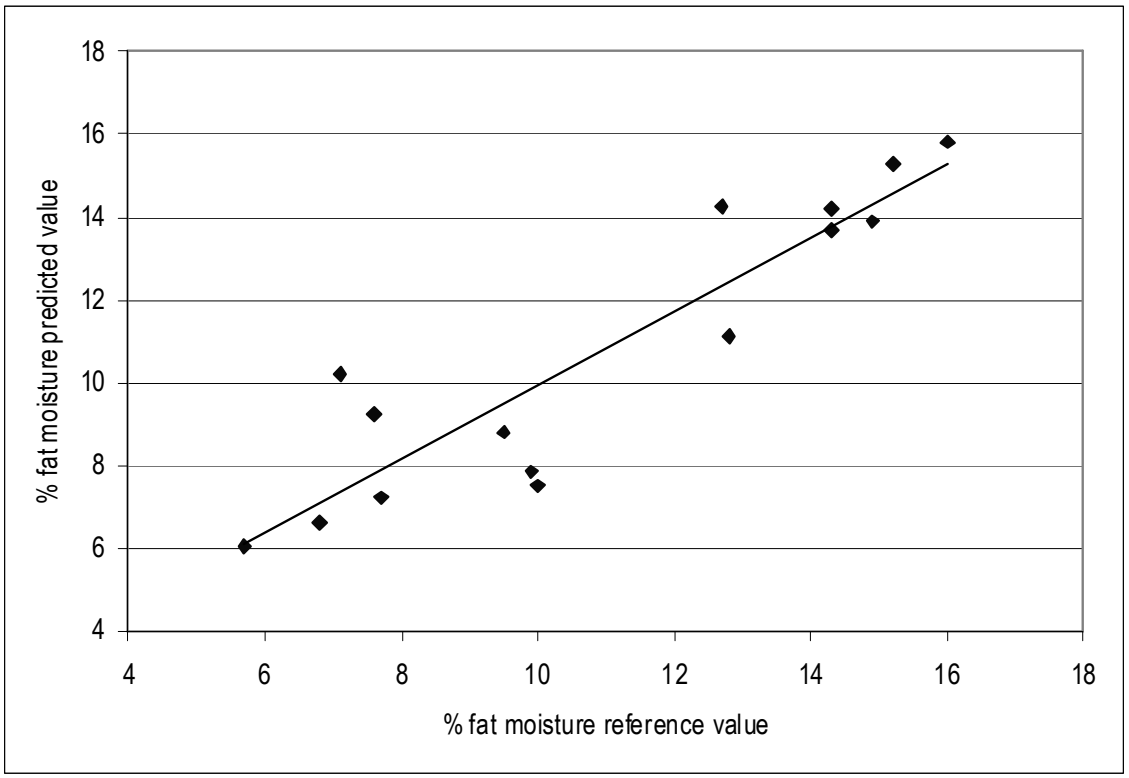

Figure 7 *NIRS predicted values versus reference values for fat moisture content from the validation of 15 samples*

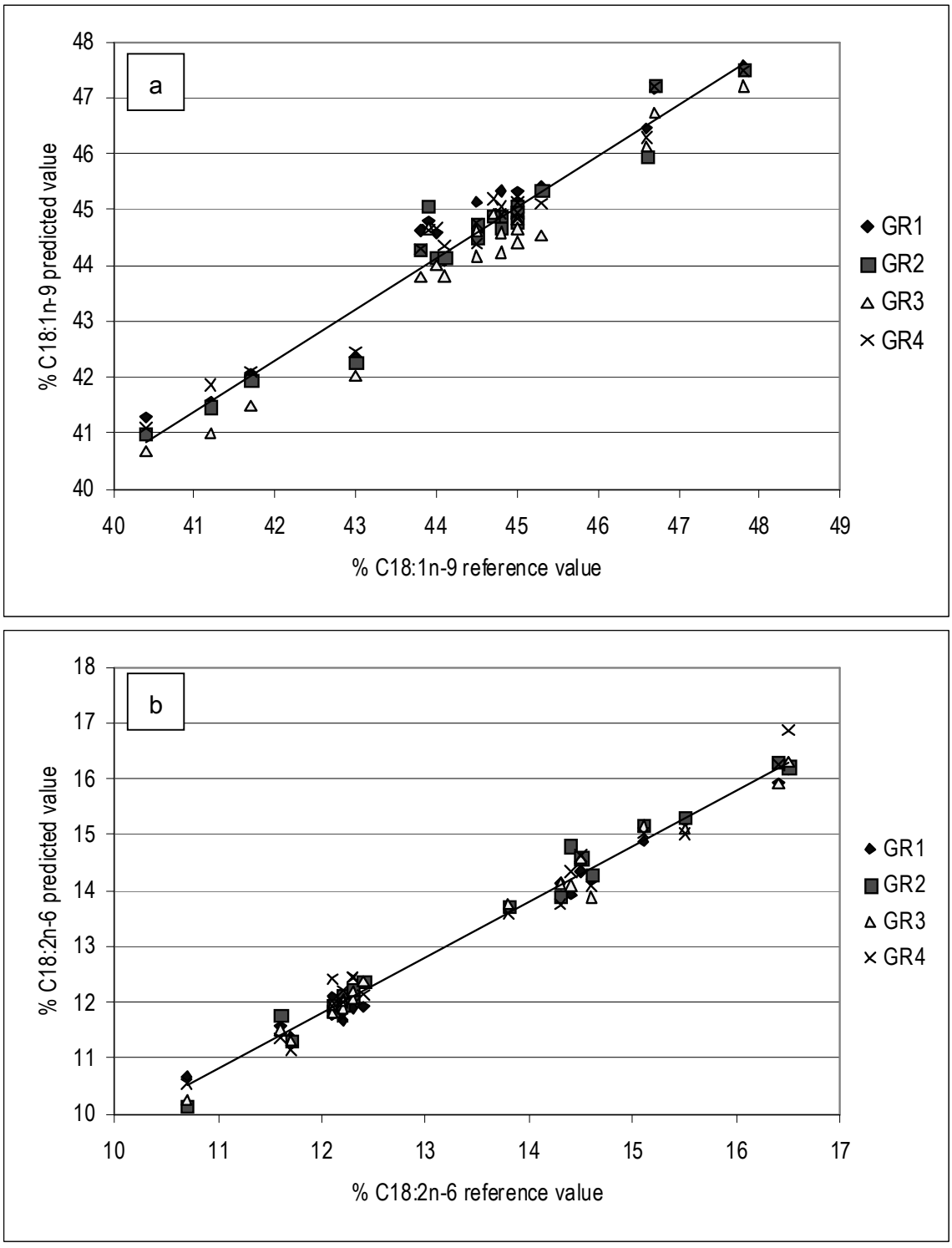

Figure 8 *NIRS predicted values versus reference values for C18:1n-9 (a) and C18:2n-6 (b) from validation of 20 samples measured with 4 different gold reflectors (GR)*

Table 1a *The experimental design for the FSC samples*

Experimental design for the FSC samples	Number of animals = number of spectra
a) Calibration work (Table 3)	62
b) Outlier eliminated	-3
c) Validation (Table 5a and Figure 7)	15
d) Calibration work (Gjerlaug-Enger *et al.,* 2010a)	77

a) Best calibration, models with Inverse MSC and mathematical pre-processing: 0,0,1,1.

b) Three outliers were eliminated from the model. A relatively conservative criterion based on a T-value of 3.0 was used.

c) Validation with 15 spectra from FSC samples in test set. The 15 spectra are 15 new pigs in a validation set to test the calibration (validation presented in Table 5a).

d) Last calibration set (sum of the calibrations and validation set) with Inverse MSC and mathematical pre-processing: 0,0,1,1. This calibration is not presented in this study, but the result was very similar, with only a slight improvement. This model is used for the prediction of fatty acids for 5278 pigs in a subsequent work by Gjerlaug-Enger *et al*., 2010a.

Table 1b *The experimental design for the XDS samples with an overview of the samples analysed with the different gold reflectors (GR)*

Experimental design for the XDS samples	Number of animals	GR1	GR2	GR3	GR4	Total number of spectra
a) Calibration work	78	78				78
	GR2, GR3 and GR4 were introduced					
b) Three new GRs was tested	14	0	14	14	14	42
		Large differences in spectra between GR1, GR2, GR3 and GR4 (Figure 4 and 5)				
c) Calibration work (Table 3) (a+b)	92	78	14	14	14	120
d) Rep file (Figure 4)	3	3	3	3	3	12
e) Outlier eliminated	-1	-1	0	0	0	-1
f) Validation (Table 5b and Figure 8a and 8b)	20	20	20	20	20	80
g) Calibration work (Gjerlaug-Enger *et al.,* 2010a) (a+b+f)	112 + 3	98 + 3	34 + 3	34 + 3	34 + 3	200 + 12
h) Recommended GRs for routine analysis		x	x	x		
i) Daily analysis of a “check-fat” sample					x	

a) First calibration work with only GR1 (not presented), analysed in the period from 10 - 27 July 2006.

b) Calibrations with GR1 were tested on 14 new samples analysed with three new GRs (these calibrations are not presented). Large GH values reveal large differences between GRs, ranging from 5.6 to 35.7 for the three new GRs in models with Standard MSC and mathematical pre-processing: 2,4,4,1 (Figure 5). Analysed in the period from 22 January-5 February 2007.

c) Best calibrations were calculated (presented in Table 3), and a repeatability (rep) file was used in this calibration work. The best models for all fatty acids were Standard MSC and mathematical pre-processing: 2,4,4,1.

d) The rep file contains three samples analysed with all four GRs (shown in Figure 4), yielding a total of 12 spectra. Analysed in the period from 22 January-5 February 2007.

e) One outlier from GR1 was eliminated for all models (Table 3); this sample had T values > 5 for four fatty acids. No further limit based on T-values was used.

f) Validation with 80 spectra in the test set (Table 5b). The 80 spectra are 20 new XDS samples analysed with four GRs on 4 May 2007.

g) The last calibration set (sum of the calibrations and validation set) with Standard MSC and mathematical pre-processing: 2,4,4,1. These calibrations are not presented in this study, but the results were very similar, with only a slight improvement. The models are used for the prediction of fatty acids for 5006 pigs in a subsequent work by Gjerlaug-Enger *et al*., 2010a.

h) After successful implementation of the rep file, all GRs are usable for routine analysis.

i) For routine calibrations, a “check-fat” sample is recommended for daily analysis; GR4 was used for that purpose.

Table 2 *Pearson correlation coefficients for samples with reference values from GC in the calibration set*

	SFA	MUFA	PUFA	C16:0	C16:1n-7	C18:0	C18:1n-9	C18:2n-6	C18:3n-3	Iodine value
SFA (%)	~									
MUFA (%)	-0.70 ***	~								
PUFA (%)	-0.67 ***	-0.01 ns	~							
C16:0 (%)	0.85 ***	-0.46 ***	-0.77 ***	~						
C16:1n-7 (%)	-0.54 ***	0.53 ***	0.30 ***	-0.30 ***	~					
C18:0 (%)	0.86 ***	-0.65 ***	-0.48 ***	0.50 ***	-0.67 ***	~				
C18:1n-9 (%)	-0.65 ***	0.98 ***	-0.08 ns	-0.42 ***	0.39 ***	-0.59 ***	~			
C18:2n-6 (%)	-0.65 ***	-0.05 ns	0.99 ***	-0.72 ***	0.27 ***	-0.49 ***	-0.12 *	~		
C18:3n-3 (%)	-0.32 ***	-0.16 *	0.67 ***	-0.44 ***	0.11 *	-0.17 *	-0.21 **	0.62 ***	~	
Iodine value	-0.91 ***	0.44 ***	0.89 ***	-0.89 ***	0.52 ***	-0.70 ***	0.36 ***	0.85 ***	0.54 ***	~

Same data set is used for the correlation loading plot of PC1 - PC2 in Figure 2.

* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$ and ns = non-significant ($P > 0.05$).

Table 3 *Calibration statistics for the NIRS equations and cross-validation for moisture content and main fatty acids in subcutaneous fat*

	n	Mean	Range	SD	Min	Max	SEC	R^2	RMSECV	R^2_{CV}	N PLS	RPD	RER
Moisture (%)	59	9.7	17.4 - 3.5	3.50	4.41	19.76	1.05	0.90	1.18	0.88	4	3.0	11.8
Saturated fatty acids (%)	119	35.2	44.9 - 28.8	2.7	27.07	43.36	0.35	0.98	0.38	0.98	5	7.1	42.4
Monounsaturated fatty acids (%)	119	47.75	52.3 - 40.0	1.86	41.91	53.53	0.41	0.95	0.45	0.94	5	4.2	27.6
Polyunsaturated fatty acids (%)	119	15.99	22.4 - 11.4	1.84	10.58	21.32	0.25	0.98	0.28	0.97	5	6.5	39.1
Palmitic acid, C16:0 (%)	119	21.06	24.4 - 17.8	1.27	17.23	24.88	0.55	0.80	0.61	0.76	5	2.1	10.8
Palmitoleic acid, C16:1n-7 (%)	119	2.26	3.0 - 1.5	0.31	1.34	3.18	0.12	0.86	0.16	0.75	8	1.9	9.1
Steric acid, C18:0 (%)	119	11.98	15.4 - 8.7	1.72	6.81	17.15	0.30	0.96	0.41	0.93	8	4.2	16.4
Oleic acid, C18:1n-9 (%)	119	44.05	47.8 - 36.3	1.6	39.25	48.86	0.45	0.92	0.51	0.90	4	3.2	22.7
Linoleic acid, C18:2n-6 (%)	119	13.12	18.8 - 9.4	1.54	8.5	17.75	0.16	0.99	0.25	0.97	9	6.1	37.0
α-linolenic acid, C18:3n-3 (%)	119	1.27	1.8 - 0.9	0.2	0.68	1.86	0.06	0.84	0.09	0.68	9	2.2	9.9
Iodine value	119	71.16	82.0 - 61.2	3.88	59.3	82.91	0.51	0.98	0.57	0.97	5	6.8	36.4

N = the number of samples used for regression development. Mean = the average of the reference data. Range = the range of reference data. SD = the standard deviation of reference data. Min = the minimum value of predicted data. Max = the maximum value of predicted data. SEC = the standard error of the calibration (the average difference between reference values and the equation predicted values in the calibration data set). R^2 = the R^2 of the data with the predicted data. RMSECV = the root mean squared error of cross-validation, which is calculated by taking the square root of the average squared prediction error. R^2_{CV} = the R^2 of the data with the cross-validation results. N PLS = the number of principal components in the PLS/MPLS models. RPD = the relationship between the standard deviation of the reference data and the standard error of cross-validation. RER = the relationship between the range of the reference data and the standard error of cross-validation.

Table 4 *Regression results of NIRS equations for C18:1n-9 from subcutaneous fat*

N PLS	SEC	R^2	RMSECV	R^2_{CV}
1	1.322	0.336	1.377	0.290
2	1.227	0.428	1.284	0.382
3	0.562	0.880	0.674	0.830
4	**0.450**	**0.923**	**0.507**	**0.903**
5	0.401	0.939	0.464	0.919
6	0.379	0.945	0.442	0.927
7	0.376	0.946	0.453	0.923
8	0.314	0.963	0.432	0.930
9	0.290	0.968	0.417	0.935
10	0.271	0.972	0.392	0.942
11	**0.249**	**0.976**	**0.386**	**0.944**
12	0.227	0.980	0.391	0.943
13	0.210	0.983	0.406	0.938
14	0.202	0.985	0.413	0.936
15	0.186	0.987	0.421	0.934
16	0.168	0.989	0.434	0.929

See Table 3 for abbreviation definitions.

The number of principal components (N PLS) and RMSECV are plotted in Figure 6.

Table 5 *Validation results for (a) fat moisture and (b) fatty acids*

(a)	n animals	n spectra	Mean reference	Mean predicted	Bias	SD reference	SD predicted	Slope	R^2_{val}	RMSEP	SEP	Repeatability
Fat moisture (%)	15	15	10.967	10.792	0.175	3.521	3.44	0.93	0.83	1.42	1.46	0.99

(b)	n animals	n spectra	Mean reference	Mean predicted	Bias	SD reference	SD predicted	Slope	R^2_{val}	RMSEP	SEP	Reproducibility range between four GRs
Saturated fatty acids (%)	20	80	48.16	48.29	-0.14	2.02	1.98	0.99	0.95	0.49	0.48	0.96 - 0.97
Monounsaturated fatty acids (%)	20	80	16.48	15.92	0.56	1.97	1.95	1.00	0.97	0.65	0.33	0.96 - 0.97
Polyunsaturated fatty acids (%)	20	80	34.70	34.79	-0.09	2.83	2.94	0.96	0.99	0.34	0.33	0.97 - 0.97
Palmitic acid, C16:0 (%)	20	80	20.41	21.12	-0.71	1.06	1.23	0.79	0.84	0.87	0.51	0.94 - 0.97
Palmitoleic acid, C16:1n-7 (%)	20	80	2.30	2.31	-0.02	0.27	0.28	0.93	0.89	0.10	0.09	0.90 - 0.96
Steric acid, C18:0 (%)	20	80	12.32	11.82	0.50	1.90	1.70	1.10	0.96	0.64	0.40	0.95 - 0.97
Oleic acid, C18:1n-9 (%)	20	80	44.34	44.43	-0.09	1.75	1.65	1.02	0.94	0.44	0.44	0.95 - 0.97
Linoleic acid, C18:2n-6 (%)	20	80	13.35	13.16	0.19	1.67	1.68	0.98	0.98	0.29	0.22	0.96 - 0.97
α-linolenic acid, C18:3n-3 (%)	20	80	1.32	1.29	0.03	0.23	0.15	1.19	0.57	0.16	0.16	0.74 - 0.89
Iodine value	20	80	72.60	71.65	0.95	4.07	3.96	1.01	0.97	1.22	0.76	0.96 - 0.97

Bias = the simple difference between the average of the reference values and the predicted values in the test set. Slope = the slope of the regression line relating the NIRS predicted values to the reference values should be close to 1. R^2_{val} = the R^2 of the reference data with the predicted data in the test validation set. RMSEP = the root mean square error of prediction, which is calculated by taking the square root of the average squared prediction error. SEP = the standard error of prediction, which expresses the accuracy of NIR results corrected for the bias. Repeatability = the covariance between the repeated samples over the variance of all samples. Reproducibility = the covariance between the repeated samples analysed with different GRs over the variance of all samples.

Paper 3

Genetic parameters of fat quality in pigs measured by near-infrared spectroscopy

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Abstract

Subcutaneous fat from Norwegian Landrace (n=3230) and Duroc (n=1769) was sampled to investigate the sources of variation and genetic parameters of various fatty acids, fat moisture percentage and fat colour, with the lean meat percentage (LMP) also included as a trait representing the leanness of the pig. The pigs were from half-sib groups of station-tested boars included in the Norwegian pig breeding scheme. They were fed ad libitum *to obtain an average of 113 kg live weight. Near-infrared spectroscopy (NIRS) was applied for prediction of the fatty acids and fat moisture percentage, and Minolta was used for the fat colour measurements. Heritabilities and genetic correlations were estimated with a multi-trait animal model using AI-REML methodology. Fat from Landrace had considerably more monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and fat moisture, as well as less saturated fatty acids (SFA) than fat from Duroc. The heritability estimates (SE 0.03-0.08) for the various fatty acids were as follows: Palmitic, C16:0 (0.39 and 0.51 for Landrace and Duroc, respectively); Palmitoleic, C16:1n-7 (0.41 and 0.50); Steric, C18:0 (0.46 and 0.54); Oleic, C18:1n-9 (0.67 and 0.57); Linoleic, C18:2n-6 (0.44 and 0.46); α-linolenic, C18:3n-3 (0.37 and 0.25) and n-6/n-3 ratio (0.06 and 0.01). The other fat quality traits revealed the following heritabilities: fat moisture (0.28 and 0.33), colour values in subcutaneous fat: L* (whiteness) (0.22 and 0.21), a* (redness) (0.13 and 0.24) and b* (yellowness) (0.07 and 0.17) and LMP (0.46 and 0.47). LMP showed high positive genetic correlations to PUFA (C18:2n-6 and C18:3n-3), which implies that selecting leaner pigs changes the*

fatty acid composition and deteriorates fat quality. Higher concentrations of PUFA are not beneficial as the ratio of n-6 and n-3 fatty acids becomes unfavourably high. Due to the high genetic correlation between C18:2n-6 and C18:3n-3 and a low heritability for this ratio, the latter is difficult to change through selection. However, a small reduction in the ratio should be expected if selection aims at reducing the level of C18:2n-6. Selection for more C18:1n-9 is possible in view of the genetic parameters, which is favourable for the eating quality, the technological quality and the human nutrition. The NIRS technology and the high heritabilities found in this study make it possible to implement fat quality traits to achieve the breeding goal in the selection of a lean pig with better fat quality.

Keywords: quantitative genetics, fatty acids, fat colour, fat moisture, near-infrared spectroscopy

Implications

Results from this study support the genetic selection of several pig fat quality traits. The choice of rapid methods makes it possible to test a large number of animals and calculate genetic parameters with a high accuracy at an acceptable cost.

Introduction

A subcutaneous fat of good quality in meat products is defined as firm and white, while a poor fat quality yields problems with a soft texture, insufficient drying, an oily appearance, the development of rancidness and a separation between muscle and adipose tissue (e.g. belly and ham) when cutting. The quality of pig fat is influenced by its lipid content and fatty acid composition, with fatty acid composition being of importance in relation to human health. Currently, Western diets have an unfavourably high ratio between n-6 and n-3 fatty acids, which is typical for modern pig fat. N-6 and n-3 fatty acids compete with each other for incorporation into membrane lipids, attachment to several enzymes and in the production of eicosanoids. Furthermore, n-6 and n-3 fatty acids have the opposite effect on inflammation in the human body. N-6 fatty acids have an unfavourable effect on the incidence of several important diseases in the general human population (e.g. coronary heart disease, hypertension, type 2 diabetes, arthritis and other inflammatory diseases, autoimmune diseases, and cancer) (Simopoulos, 1999). Saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) are synthesized de novo in

the pig. However, important polyunsaturated fatty acids (PUFA) in pigs such as linoleic acid (C18:2n-6 is the n-6 fatty acid with the greatest volume) and α-linolenic acid (C18:3n-3 is the n-3 fatty acid with the greatest volume) cannot be synthesized de novo. As a result, levels of these fatty acids in pig fat will reflect dietary changes (Enser *et al.*, 2000). Uncertainties exist as to what extent selection can change the ratio between n-6 and n-3 fatty acids. The degree of fatty acid unsaturation in mitochondrial membrane lipids has been documented as one of the biochemical parameters most strongly correlated with longevity. Oleic acid (C18:1n-9 is the n-9 fatty acid with the greatest volume) competes for incorporation into membrane lipids and triglycerides at the same positions as several n-6 and n-3 fatty acids, and oleic acid is a stable fatty acid that does not trigger oxidative stress. This relationship between C18:1n-9 and n-6 fatty acids is thoroughly discussed in a paper by Haug *et al.* (2010), who study human nutrition in relation to fatty acids in meat. Pig fat is rich in C18:1n-9, which is important for both good eating (Cameron *et al.*, 2000) and the technological quality of fat (Suzuki *et al.*, 2006). In contrast, 18:2n-6 is the primary reason for high iodine value and technological quality problems (Carnier *et al.*, 2003).

Few estimates of the genetic parameters in terms of the percentage of fatty acids in subcutaneous fat analysed by chemical methods are reported in the literature, and actual studies all demonstrate high heritabilities for several fatty acids (Schwörer *et al.*, 1988; Bout *et al.*, 1989; Cameron, 1990). The study by Clop *et al.* (2003) was the first study of a genome scan detection of QTL affecting fatty acid composition in Iberian x Landrace crossbred pigs, and they found several significant QTL's. Traditionally, fatty acids are analysed using labour-intensive methods such as gas chromatography (GC), which is both expensive and time-consuming. Near-infrared spectroscopy (NIRS) patterns of liquid fat contain information on fatty acid composition (Schwörer *et al.*, 1999; Garrido-Varo *et al.*, 2008). NIRS has the advantage of being used for the analysis of a high number of samples at a relatively low cost. Rapid methods make it possible for a large number of animals to be tested, and genetic parameters can be calculated at an acceptable price for the fat quality traits recorded. Several Spanish studies have reported that NIRS technology is able to accurately predict the percentage of oleic, linoleic, palmitic and stearic acids in subcutaneous fat from the Iberian breed (de Pedro *et al.*, 1992; Garcia-Olmo *et al.*, 2001; Fernandez *et al.*, 2003), and one study has previously estimated the heritabilities for fatty acids predicted from NIRS equations (Fernandez *et al.*, 2003). Medium high heritabilities were estimated for four fatty acids with a large impact on fat quality. In Spain, there is much ongoing research in this field, and some breeding organisations uses the fatty acid in the breeding goals for pig lines specialized in the cured ham production. Suisag in Switzerland did previously use breeding values for iodine value estimated by NIRS, and they have also much experience on NIRS and fat quality. In the

literature there was not found any other works on the NIRS technology for fat quality in practical breeding programs.

The composition of fatty acids is correlated to other traits such as the fat moisture and fat colour, and this is a part of the technological fat quality problem. In this study, fat moisture percentage in intact subcutaneous fat was predicted by NIRS using a new method designed by Gjerlaug-Enger *et al.* (2010a), and fat colour was also measured with a spectroscopic method.

The aim of this study was to estimate the genetic parameters for new fat quality traits in pigs by the use of novel low labour-intensive methods and NIRS (developed by Gjerlaug-Enger *et al.,* 2010a). The NIRS methods and their known performance enable untrained personnel to conduct routine analyses of numerous and extensive sample series. If these methods can detect significant genetic variations of a certain size it would be possible to utilize them in the selection program for pigs to improve both fat quality and fatty acid profile to the benefit of human health.

Material and methods

Animals

This study is an expansion of an earlier work on meat quality traits presented by Gjerlaug-Enger *et al.* (2010b). Fat quality data were recorded between 2005 and 2008 in Norwegian Landrace (*n*=3230 for fat quality, *n*=4797 for lean meat percentage) and Duroc (*n*=1769 for fat quality, *n*=2819 for lean meat percentage) pigs, with all animals included in the national pig breeding scheme. The carcass records available for these analyses originated from half-sib tested females and castrated males. In the two half-sib testing stations (one in central and one in southeastern Norway), 2800 females and castrates were tested annually. The animals were half-sib groups of station-tested boars and were kept in mixed-sex groups with 12 as the size of pen. The breeds were kept in separate pens. The pigs started the test at an average of 29.8 (s.d.: 3.3) kg and 77.4 (s.d.: 7.4) days old, and finished at an average of 113.1 (s.d.: 6.7) kg live weight and 162.4 (s.d.: 10.7) days old. The pigs were slaughtered at two different abattoirs and all carcasses were transported to a partial dissection line at the Norwegian Meat and Poultry Research Centre (Animalia). More detailed information about the tests and slaughtering has been described by Gjerlaug-Enger *et al.* (2010a).

All pigs were fed *ad libitum* on conventional concentrates containing 14.5 to 15.8% total protein and 9.33 MJ net energy/kg. The major feedstuff compounds were barley (48%), oats (22%), peas (5%), soy meal extract (16%) and rendering (animal) fat (2.4%). No fatty acid analysis of the feed was performed, although some changes in fat quality from different feed batches may have occurred. The model used for statistical analysis is robust for environmental changes such as this.

Carcass composition traits

One carcass composition trait was included in this study in order to obtain a measurement of the leanness of the pigs. The lean meat percentage (LMP) was chosen because it is an important trait in the breeding goal and has been so for many decades when breeding for leaner pigs. Carcasses were split into ham, shoulder and loin, and each part was dissected into lean meat, fat, bone and skin. The trim (mix of lean and fat) from between the primary cuts was analysed for fat percentage (Scanalyser, model S2, Scanbio, Aalborg, Denmark). The belly and neck were not dissected because of the time required for the dissection of those cuts, and a predicted fat percentage for those cuts was calculated from the fat percentage of the trim. These equations were: fat percentage neck = 0.48273 + (0.64847 * fat percentage of the trim) and fat percentage belly = 6.09748 + (1.2539 * fat percentage in the trim). In total, 114 registrations were made for each carcass. Finally, an equation using the weight of the different components with the percentage of fat and lean was used to predict an LMP for the entire carcass. This equation was based on a dissection study in 1987 of 120 pigs in which all cuts of the half carcass were dissected into lean meat, fat, bone and skin (Roe, pers. comm. 2010). All animals with fat quality traits recorded also had an LMP.

Fat quality traits

The determination of the various fatty acids, iodine value, fat moisture percentage and fat colour was carried out on samples of subcutaneous fat taken from the area between the loin and ham in the coxal region of the carcasses. The samples obtained from the carcass included the hide/skin, the fat between the hide and the lean, and some lean. The latter was carefully removed from the sample before the colour measurements were taken.

Colour of subcutaneous fat

The fat colour was measured using a Minolta Chroma Meter CR-400 (measurement area with an aperture of 8 mm), with a D65 illuminant calibrated against a white tile. The tristimulus parameters L*, a* and b* (also referred to as the CIELAB color space), representing whiteness (L* = 0 is completely black, and L* = 100 is completely white), redness (positive a* values mean red colours and negative a* values

mean green colours) and yellowness (positive b* values mean yellow colours and negative b* values mean blue colours), were measured on three random spots of each fat sample surface in the inner fat layer free from any contamination of the lean. The average of three measurements for each sample was used for statistical analysis. With the Minolta instrument connected to a computer, a barcode scanner gave quick and accurate sample identification, and the operator identification and recording time were also automatically stored.

Moisture percentage of subcutaneous fat

The FoodScan near-infrared spectrophotometer (FOSS NIRSystems, Hillerød, Denmark) was used for determining the moisture percentage, with the same fat sample used for the fat colour measurements. The samples with all fat layers represented were cut into small pieces (brick size: 3-5 mm) with without further homogenisation. Approximately 12 g of these samples were placed in a 50 mm round plastic petri dish, and the NIRS scanning process took one minute. The method and calibration development were described by Gjerlaug-Enger *et al.* (2010a).

Fatty acids of subcutaneous fat

A microwave fat melting technique was used to prepare the samples to obtain the total lipids from the subcutaneous fat sample, previously cut into pieces for the analysis of the fat moisture percentage. Transflection spectra of the total lipids from the subcutaneous fat samples were obtained using a XDS near-infrared rapid content analyser (FOSS NIRSystems, Hillerød, Denmark). Each sample was scanned once for one minute using a circular quartz cuvette and gold-plated reflectors that provided optical path lengths of 0.2 mm. This method and the calibration development are described in the previous paper of the present study (Gjerlaug-Enger *et al*., 2010a).

Prediction of fatty acids and fat moisture percentage

The regression results of NIRS equations for several fatty acids and the moisture percentage of subcutaneous fat were presented by Gjerlaug-Enger *et al*. (2010a). In total, samples from 5006 and 5278 pigs were analysed in the FOSS XDS near-infrared rapid content analyser for fatty acid composition and in the FOSS FoodScan near-infrared spectrophotometer for the moisture percentage in subcutaneous fat. Six fatty acids and fat moisture demonstrated sufficiently satisfactory results to be used as traits in this study. These were the Palmitic, C16:0; Palmitoleic, C16:1n-7; Steric, C18:0; Oleic, C18:1n-9; Linoleic, C18:2n-6 and α-linolenic, C18:3n-3 fatty acids, which accounted for 95% of the total amount of fatty acids found by chemical methods (GC). The other fatty acids in the subcutaneous fat of the pigs in this study had a concentration lower than 1% in the fat tissue. The ratio between C18:2n-6

and C18:3n-3 used in our study corresponds to the n-6 to n-3 ratio, which is usually calculated as the sum of all n-6 and n-3 fatty acids, although the long-chain PUFA's were too low in concentration for the NIRS equations used here.

A few samples with strange spectra were eliminated for the genetic analyses. H outliers (GH) are samples whose spectra differed notably from the mean sample spectrum. Samples with a GH value higher than 3 were eliminated from the data set for the prediction of fatty acids and moisture percentage.

Quantitative genetic analyses

In preliminary analyses, the fixed effects of sex, herd, abattoir x slaughter day, calibration, storage day and carcass weight were tested with SAS Proc GLM (SAS Inst., Inc., Cary, NC). A herd was defined as the animals' herd of origin until transfer to the test station. Animals from the two test stations were slaughtered at two different abattoirs, thus the abattoir x slaughter day effect was confounded with the station. The effect of calibration was the effect for each time the Minolta instrument was started and calibrated. Every series of samples has one calibration, and some large shifts between series were detected without any explanation other than the error from the calibration. This effect was only relevant for the fat colour measurements. The calibration was confounded with abattoir x slaughter day; therefore only the calibration number was used. The storage time from slaughter to dissection ranged from two to nine days, and the average carcass weights were 83 kg (Landrace) and 81 kg (Duroc) with standard deviations of 5.5 kg for both breeds.

The breeds were analysed separately, but the same method was used. The different traits (C16:0, C16:1n-7, C18:0, C18:1n-9, C18:2n-6, C18:3n-3, n-6/n-3 ratio, fat moisture percentage, L*, a* and b* values in fat and LMP) were analysed in different analyses and a general multi-trait animal model were used for the genetic analyses:

$$\mathbf{Y} = \mathbf{X\beta} + \mathbf{Zu} + \mathbf{e}$$

where: **Y** is a matrix of observations, **β**, **u** and **e** are vectors of fixed, additive genetic and residual effects, respectively, and **X** and **Z** are known incidence matrices. An estimation of (co)variance components was performed using multi-trait animal models and analysed with restricted maximum likelihood (REML) methodology. The DMU 6.7 software package (Madsen and Jensen, 2008) and the

average information (AI) algorithm were used in the estimation. Asymptotic standard errors of (co)variance components were computed from the inverse average information matrix.

Due to computational constraints because of linear dependency and high correlations between the fatty acids, all traits could not be analysed in the multi-trait analysis simultaneously. Two matrices of genetic parameters are presented for each breed. The first matrix with 7 traits (C16:0, C16:1n-7, C18:0, C18:1n-9, C18:2n-6, C18:3n-3 in fat and LMP) was calculated from several analyses of three traits: the LMP plus two fatty acids together, to cover all the combinations of fatty acids. In addition, the ratio between C18:2n-6 and C18:3n-3 was analysed and presented for Landrace, while the correlation between this ratio and the other fatty acids could not be estimated for Duroc, probably due to the low genetic variation for this trait. The average correlations are presented and the heritabilities are estimated from single trait analyses.

Subsequent analyses with three of the most important fatty acids for human health (C16:0, C18:1n-9, C18:2n-6), fat moisture percentage, L*, a* and b* values in fat and LMP were calculated simultaneously as one multi-trait model and reached convergence without any computational problems for both breeds. The three fatty acids in these analyses additionally represented the three fatty acids groups: SFA, MUFA and PUFA.

For traits containing estimates of heritabilities, standard errors and standard derivations from both sets of matrices (first and second), average estimates were presented. None of the estimates going into an average differed by more than two-tenths.

The pedigree file contained all tested animals and their ancestors born between January 2002 and February 2008. In total, 5 generations of animals were included, and the first generation was the base population. The base populations were 478 and 254 Landrace and Duroc pigs, respectively, born in the period from 1999 to 2001. Average coefficient of inbreeding was 0.06 for Landrace and 0.07 for Duroc. The final pedigree files for Landrace and Duroc included 9140 and 5405 individuals (4797 and 2819 individuals with data), respectively.

Additionally, the same model, with the additional breed x sex effect replacing the sex effect, was applied to the combined Landrace and Duroc data sets. A SAS Proc GLM was used to estimate least square means for the breed x sex effects. If an effect was significant for one of the breeds, it was included in the analysis for the breed x sex effects.

Results

Breed and sex differences

Basic statistics of the traits studied are in Table 1 and the models for multi-trait analysis with their fixed effects are in Table 2. The least square means for the breed x sex effects are in Table 3 in which the models from Table 2 were used, except for the breed x sex effect replacing the sex effect.

Landrace and Duroc were significantly different for almost all fat quality traits measured. Females and castrates from the two breeds were also significantly different for the majority of the traits, and the difference between the two sexes had the same sign for significant differences. For the SFAs (C16:0 and C18:0), the Duroc had a higher percentage than Landrace, and castrates had a higher percentage than females. For the MUFAs (C16:1n-7 and C18:1n-9), Landrace had a higher percentage than Duroc and castrates had a higher percentage of C18:1n-9 than females, although no sex differences were found for C16:1n-7. For the PUFAs (C18:2n-6 and C18:3n-3), Landrace had a higher percentage than Duroc, and females had a higher percentage than castrates. Landrace had more moisture in subcutaneous fat than Duroc, and females had higher levels than castrates. There were no differences in L* value (whiteness) between castrates for the two breeds, and the castrates had generally whiter fat than the females. For a* and b* values (redness and yellowness, respectively) the differences between the sexes were small, while Landrace had a lower a* value in the fat than Duroc. Landrace had a higher LMP than Duroc and females were leaner than castrates.

Fixed effects

The fixed effects included in the models (sex, herd, abattoir x slaughter day, calibration, storage day and carcass weight) had various influences on the traits in this study, independent of breed (Table 2). The effect of abattoir x slaughter day (which includes the effects of the test station) was significant for all fat quality traits. Storage (the number of days of storage from slaughter to dissection) significantly decreased the moisture percentage in subcutaneous fat for both breeds (Table 2), but was not significant for any of the other traits. From days three to nine, the moisture percentage in subcutaneous fat decreased from 10.7% to 8.5% in Landrace, and from days two to eight the reduction was from 9.7% to 7.4% in Duroc (data not shown in tables).

Heritability estimates for fat quality

The fat quality evaluation methods used in this study gave moderate to high heritability estimates for several fatty acids and moisture percentage in subcutaneous fat predicted by NIRS and low heritability

estimates for fat colour measured with Minolta (Tables 4a and 4b). On average, the heritabilities were the same size in Landrace and Duroc, though Duroc had larger phenotypic variation on average for these traits (Tables 4a and 4b). The heritabilities of C18:1n-9 were highest in both breeds, ranging from 0.57 in Duroc to 0.67 in Landrace. The estimated heritabilities of the ratio between C18:2n-6 and C18:3n-3, corresponding to the n-6 to n-3 ratio, were low and not significantly different from zero in Duroc. The standard errors for the heritability estimates were relatively low due to the large volume of data in this study.

Relationships among fatty acids and LMP

The genetic and phenotypic correlations among primary fatty acids and LMP are shown in Tables 5a and 5b. The sign and size of the parameters were similar for both breeds with only a few exceptions. The LMP revealed negative genetic and phenotypic correlations to C16:0, C18:0 and C18:1n-9 and positive genetic and phenotypic correlations to C16:1n-7 (not significant for Duroc), C18:2n-6 and C18:3n-3.

The genetic and phenotypic correlations between C16:0 and C18:0 were positive, while the correlations between C16:0 and MUFAs and PUFAs were all negative (not significant for C18:1n-9 in Landrace). C16:1n-7 and C18:0 were strongly negatively, genetically and phenotypically correlated, while C16:1n-7 was positively correlated to C18:1n-9 (not significant for Landrace) and C18:0 was negatively correlated to C18:1n-9. The PUFAs (C18:2n-6 and C18:3n-3) showed high phenotypic correlations to each other and genetic correlations close to one.

The genetic correlations between the ratios C18:2n-6 and C18:3n-3 and SFA were high and positive, while the correlations were medium to low and negative between this ratio and the MUFA and PUFA. A positive genetic correlation was estimated between LMP and the C18:2n-6 to C18:3n-3 ratio.

Relationships between fatty acids, fat quality traits and LMP

Genetic and phenotypic correlations between fat quality traits and LMP are shown in Tables 6a and 6b. The parameters were similar for both breeds with only small exceptions. The moisture percentage in subcutaneous fat was negatively correlated to C16:0 and C18:1n-9 and positively correlated to C18.2n-6. Strong relationships were observed between the L* value of subcutaneous fat and the fatty acids, with positive genetic and phenotypic correlations to C16:0 and C18:1n-9 and negative correlations to C18.2n-6. Lower correlations were estimated between the fatty acids and a* and b* values. The moisture percentage in subcutaneous fat was negatively correlated to the L* value and positively

correlated to a* and b* values (not significant in Landrace). The L* value was negatively correlated to a* and b* values, while a* and b* values were positively correlated with each other. The LMP was positively correlated to the fat moisture percentage and a* and b* values, and negatively correlated to the L* value.

Discussion

The inner and outer fat layers were mixed together when preparing samples for analyses of fat moisture and fatty acids. This was done in order to make the sample preparation quick, but previous studies have found different concentrations of fatty acids in the inner and outer fat layer, with more SFA in the inner layer (Schwörer *et al*., 1988; Suzuki *et al*., 2006) and higher iodine value in the outer layer (Schwörer *et al*., 1994). It is well documented that the degree of saturation of fatty acids increases from the outside to the inside of the body. The temperature differences in different fat depots may affect the activity of the delta-9 desaturase enzyme (Sellier *et al*., 2010). For both the meat industry and consumers, subcutaneous fat is handled as one piece.

Breed and sex differences

No previous studies using NIRS for the prediction of fatty acids have looked at differences between the sexes. Analysing fatty acids with chemical methods, other studies found significantly higher concentrations of PUFA and 18:3n-3 in backfat from females than from castrates (Suzuki *et al*., 2006; Tikk *et al*., 2007), in addition to higher female concentrations of 18:1n-9 and lower concentrations of C16:0 and C18:0 (Suzuki *et al*., 2006). This is in agreement with our studies. As reported by de Smet *et al*. (2004), differences in fatty acid composition between the sexes are found in several studies. Schwörer *et al*. (1988) found significant differences between fatty acids from GC for Landrace and Yorkshire, and Feránderz *et al*. (2003) reported breed differences between fatty acids predicted by NIRS for some Iberian breeds. In a study including Belgian Landrace and Duroc of equal leanness, the Duroc had a significantly higher percentage of C16:0 and PUFA, C18:2n-6 and C18:3n-3, and a lower content of C18:1n-9 in backfat than Landrace (Raj *et al*., 2010). Sellier *et al*. (2010) found more 18:1n-9 in Landrace than in Large White pigs. For C16:0 and 18:1n-9 we observed similar differences, but for PUFAs this was not in agreement with our results. In our study Duroc had more SFA, while Landrace had more MUFA and PUFA. For the de novo synthesised fatty acids, Duroc store more fatty acids as SFA, while to a larger degree Landrace transform the SFA to MUFA. C16:0 is the first finite product from fatty acid de novo synthesis, and Duroc store more of this acid than Landrace. A larger activity of delta-

9 desaturase enzyme in Landrace could have caused the breed difference observed in C16:1n-7 and C18:1n-9 concentrations (both C16:1n-7 (c9) and C18:1n-9 (c9) have the double bound in the ninth position for the carboxyl end of the fatty acid). The higher percentage of PUFA in Landrace is probably an effect of the lower level of backfat in this breed than in Duroc.

Differences in the moisture percentage of subcutaneous fat for different sexes or breeds are not reported in the literature, but our results were as expected in view of the higher level of fatness and lower percentage of PUFA in castrates and in Duroc. Fat from females had lower L* and a* values, but was comparable with castrates in b* values (Winzig and Beutling, 2006). In our study, the females had lower L* and b* values (not for Duroc) and equal a* values. For C18:2n-6, fat moisture and L* value, the differences between the sexes were much larger for Landrace than for Duroc in spite of similar differences in LMP for the two breeds. Duroc had both lower fat moisture and whiter fat, although the higher value for redness might be a breed characteristic, despite expectations.

Heritability estimates of the fat quality traits

Few studies have reported estimated heritabilities for fatty acids. Sellier (1998) reported pooled average values of 0.51 for C18:0 and 0.58 for C18:2n-6 in subcutaneous fat. Schwörer *et al.* (1988) and Suzuki *et al.* (2006) reported heritability estimates of fatty acids in the inner and outer layer of subcutaneous fat. In view of the standard errors, the size of the heritabilities was similar for the pair of parameters for a particular fatty acid, and the heritabilities were very high for some of the highest quantity fatty acids and low for fatty acids with low concentrations (e.g. C14:0 and C16:1). For the chemical methods used in the studies referred to above, measuring fatty acid composition on a large number of animals for breeding value estimation is, at present, not possible at a reasonable cost (de Smet *et al.*, 2004). Fernandez *et al.* (2003) presented one of the first studies reporting heritabilities for fatty acids measured by NIRS. An optic fibre probe was applied on intact subcutaneous fat using a FOSS-NIRSystem 6500 instrument. The estimated heritabilities in their study were 0.31 (C16:0), 0.41 (C18:0), 0.30 (C18:1) and 0.29 (C18:2). The estimated heritabilities for fatty acids in our study were intermediate to the values from chemical and NIRS methods, except for the high estimates for C18:1n-9 in both breeds. In the study by Fernandez *et al.* (2003), the use of an optic fibre probe might be the reason for the decreased accuracy of NIRS equations and lower heritabilities than in our study, though the use of an optic fibre probe has the advantage of needing very little sample preparation. The XDS instrument in our study is also a newer NIRS instrument from FOSS than the NIRSystem 6500 used by Fernandez *et al.* (2003), but with similar properties. The Iberian pigs used in their study were different from the pigs in our study with regard to feeding, carcass weight, fatness and age.

The low estimated heritabilities of the ratio between C18:2n-6 and C18:3n-3 was low and not significantly different from zero in Duroc. This shows that very few, if any, enzymes or uptake mechanisms in a pig's body can distinguish between these fatty acids.

Cameron (1990) reported a heritability estimate of moisture percentage in subcutaneous fat of 0.27, which is the same size as in our work, while Sellier *et al.* (2010) found a higher value (0.59). As expected, the moisture percentage in pig fat measured by NIRS was not found in the literature, as this method was designed for our study.

Heritability estimates for the fat colour of subcutaneous fat was also not reported in the literature, and the heritabilities estimated in our study were quite low. The method was not laboratory intensive, and the estimated heritabilities could possibly be increased if the sample preparation was improved. In addition, measurement errors could be large if the lean was not properly removed for the fat sample, with another problem possibly being the thickness of the fat layer. Since the lights from the Minolta instrument penetrate several millimetres of fat, the chopping board under the samples needs to be white. The outer fat layer has more PUFA than the inner layer, thus it may have been better to measure the outer layer. However, this would increase problems with the backfat thickness. Suisag (a Swiss pig breeding company) also has some experience with this method in the Swiss pigs performance testing station (Schwörer, pers comm. 2006).

Relationships among fatty acids and LMP

In pigs and other one-stomached animals, glucose is broken down to pyrovate, and in the de novo synthesis the pyrovate is converted into Acetyl-CoA which is used to make C16:0. The concentrate used in the current study is a conventional feed used for slaughtered pigs in Norway. This feed is high in carbohydrates and low in fat (4-5%), which stimulates a relatively high de novo synthesis of fatty acids from carbohydrates. This situation affects the relationships among the fatty acids.

The genetic and phenotypic correlations between various fatty acids demonstrated that the concentration of the dominant C18:1n-9 fatty acid in the pig's subcutaneous fat (43-44% of the total fatty acid content) was positively correlated to the monounsaturated fatty acid 16:1n-7 (not significant for Landrace). These are all fatty acids produced by desaturation through the delta-9 desaturase enzyme. Additionally, a negative association between C18:1n-9 and C18:0, and between C16:1n-7 and C16:0 was observed. This could be explained by the fact that C16:0 and C18:0 are converted to C16:1n-7 and

C18:1n-9 by the delta-9 desaturase enzyme. Since C16:0 goes either to C16:1n-7 or C18:0, this gives a strong negative correlation between those fatty acids.

The essential fatty acids, C18:2n-6 and C18:3n-3, are not synthesised by de novo synthesis and reflect dietary composition. These and longer chain n-6 and n-3 fatty acids compete with each other for incorporation into membrane lipids, as well as binding to several enzymes and eicosanoid production. As shown in Tables 5a and 5b, the genetic correlations between C18:2n-6 and C18:3n-3 were 0.96 to 0.97, meaning they are almost the same trait. The phenotypic correlations between C18:2n-6 and C18:3n-3 were 0.67 and 0.77 in Landrace and Duroc, respectively, which was similar to the ratio between these two fatty acids in animals having the desired fatty acid composition (used for NIRS calibration). This is positive since NIRS technology can give unreliable estimates of the correlations because of the correlations between fatty acids and between spectral characteristics of the fatty acids (Gjerlaug-Enger *et al*., 2010a).

Genetic correlations between 18:2n-6 and the C18:2n-6 to C18:3n-3 ratios were positive. The selection for a lower C18:2n-6 percentage can decrease the ratio between C18:2n-6 and C18:3n-3. The selection for a better ratio is not efficient in view of the low heritability for this ratio, but the selection of a lower C18:2n-6 can be favourable for improved eating quality, improved human nutrition (Haug *et al*., 2010) and technological quality (Suzuki *et al*., 2006).

Only one study has previously presented genetic correlations between primary fatty acids analysed with NIRS, and reported a very large positive correlation between C18:1 and C18:2 in Iberian pigs (Fernandez *et al*., 2003). This is not in agreement with our work, which estimated a negative correlation in both breeds. The explanation for this difference might be the feed given to the Iberian pigs because one of the highest quantity components of the fattening diet was acorns, which have a high concentration (66%) of C18:1n-9. In our work, the pigs were fed a typical modern pig diet rich in cereals, containing a relatively high percentage of C18:2n-6 and low percentage of C18:1n-9.

The concentrations of dietary fatty acids (C18:2n-6 and C18:3n-3) is larger in pigs that have a high LMP with a smaller contribution of de novo fatty acid synthesis. This could be an explanation for the association between leaner carcasses and a higher C18:2 percentage of subcutaneous fat, also described by Wood *et al*. (1994). Cameron (1990) and Suzuki *et al*. (2006) reported that C16:0, C18:0 and C18:1n-9 were positively correlated to backfat, while C18:2n-6 was negatively correlated (both phenotypically and genetically) to backfat. These findings were confirmed in our study.

Fatty acids from feed can yield a better ratio between the fatty acids, and with regard to the ratio between C18:2n-6 and C18:3n-3, this must be solved through dietary changes in view of the genetic parameters. There are several oils with a more favourable n-6 and n-3 ratio than those traditionally used for pig concentrates, and there does not need to be a large amount of additives to make a favourable ratio (Enser *et al*., 2000). For the n-9 and n-6 ratio, breeding could be a better strategy since the concentration of C18:1n-9 in the feed has to be very high before the uptake mechanism for this fatty acid becomes more efficient than de novo synthesis, as well as the fact that this concentrate would be too expensive for the commercial production of pigs for slaughter.

Relationships between fatty acids, fat quality traits and LMP

Cameron (1990) reported negative phenotypic and genetic correlations between the moisture percentage in subcutaneous fat and C16:0, C18:0 and C18:1n-9, and positive correlations to C18.2n-6. This is in agreement with our work, although their method for measuring fat moisture percentage was probably different from ours since the average fat moisture percentage in their study was about twice the level as the one in our work.

A study of pigs that were fed fish oil did not reveal any significant relationship between the colour of fat and the fatty acid composition, despite significant changes in the hardness of the fat and the iodine value (Irie and Sakimoto, 1992). Another study reported noticeable differences in fat colour (L* and a* values) among pigs with different fatty acid profiles (Jaturasitha *et al.*, 2008), which is in agreement with our results. SFA, MUFA and PUFA have very different structures and melting temperatures, and the fat colour is related to fat firmness. SFA, and to some degree MUFA, give a firm white fat, while PUFA gives a softer fat with more colour (a lower L* value and higher a* and b* values). The fat moisture is not clean water, but probably more like the water inside the other cells, containing organic molecules as well as common organelles, which is a part of all cells. The genetic parameters for these relationships are not found in the literature.

A lean weight and carcass lean percentage were positively correlated (both phenotypically and genetically) to the fat moisture percentage (Cameron, 1990; Sellier *et al.,* 2010). Schwörer (pers comm. 2006) found negative correlations between the moisture percentage in subcutaneous fat and backfat thickness, and positive correlations between fat moisture percentage and iodine value. Correlations between fat colour and LMP are not found in the literature, but the correlations in our study show that selection of a higher LMP gives subcutaneous fat with more colour, which is to be expected since the PUFA and fat moisture percentage increase with increased leanness.

Several of the fat parameters, particularly the fatty acids, showed a large genetic variation. These fatty acids can be seen as a direct trait of fat quality, while the fat moisture and fat colour are more like indirect traits. The high heritabilities for SFA and MUFA show that it is a genetic variation in the de novo synthesis of fatty acids, since most of these fatty acids are probably synthesised from carbohydrates with the feed used in our study. C18:2n-6 and C18:3n-3 are essential fatty acids and the genetic variation in these fatty acids indicate that genetic differences may exist in the uptake mechanisms of these PUFAs.

Conclusion

When aiming at measuring fat quality traits on a larger scale, it is important to find methods that are inexpensive, rapid, environmentally friendly, safe and user-friendly. The NIRS technologies used in our study facilitate the large-scale recording of fat quality at an acceptable cost. The high heritabilities for C18:1n-9 and C18:2n-6 and other fatty acids in the subcutaneous fat estimated in our study suggest the possibility for the genetic improvement of fatty acid composition. The direct selection of fatty acids makes it possible for favourable changes in terms of eating quality, in addition to improved human nutrition and technological quality. Increased concentrations of C18:1n-9 and decreased concentrations of C18:2n-6 will meet this requirement. A high heritability was estimated for the fat moisture percentage, and heritability estimates for this trait measured with NIRS have not been previously reported. Our study estimates the correlations between the fatty acids and fat moisture percentage, and clarifies in which direction the selection for a reduced fat moisture percentage will affect the fatty acids. For all fat quality traits in this study, it is clear that a selection for an increased LMP will result in a decreased fat quality. With rapid methods, it is possible to select for both LMP and fat quality. The correlations are unfavourable, but improvement in both traits is possible if the selection is efficient. The perfectly slaughtered pig of the future should have the leanness of modern pigs and a good fat quality high in 18:1n-9 and low in 18:2n-6 fatty acids.

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Table 1 *Numbers of animals per trait, mean, standard deviations of means, minimum and maximum for Landrace and Duroc*

TRAIT		N	Mean	SD	Min.	Max.
C16:0	Landrace	3230	20.45	1.29	16.22	25.05
	Duroc	1769	21.22	1.17	17.43	25.40
C16:1n-7	Landrace	3230	2.38	0.21	1.36	3.04
	Duroc	1769	2.15	0.26	1.15	3.35
C18:0	Landrace	3230	11.71	1.24	6.99	16.44
	Duroc	1769	13.45	1.50	7.83	17.90
C18:1n-9	Landrace	3230	44.31	1.44	39.08	49.39
	Duroc	1769	43.46	1.71	38.67	48.90
C18:2n-6	Landrace	3230	14.26	1.71	10.28	20.46
	Duroc	1769	13.01	1.35	9.10	18.46
C18:3n-3	Landrace	3230	1.45	0.19	0.84	2.31
	Duroc	1769	1.36	0.17	0.78	2.03
n-6/n-3	Landrace	3230	9.87	0.91	7.27	15.04
	Duroc	1769	9.60	0.85	7.07	13.34
Moisture	Landrace	3372	9.75	2.68	3.23	20.21
	Duroc	1906	8.73	2.12	3.29	17.84
L_Fat	Landrace	3254	76.04	2.38	67.75	84.35
	Duroc	1860	75.76	1.83	69.75	82.67
a_Fat	Landrace	3254	3.9	1.18	0.48	12.15
	Duroc	1860	4.43	1.07	0.86	10.19
b_Fat	Landrace	3254	5.64	0.87	2.17	9.57
	Duroc	1860	5.79	0.81	2.94	9.24
LMP	Landrace	4797	63.42	2.64	48.28	71.45
	Duroc	2819	57.85	2.87	46.07	68.97

[a] Abbreviation definitions for traits: C16:0 = palmitic; C16:1n-7 = palmitoleic; C18:0 = steric; C18:1n-9 = oleic; C18:2n-6 = linoleic; C18:3n-3 = α-linolenic; n-6/n-3 = ratio of C18:2n-6 and C18:3n-3; Moisture = fat moisture percentage; L_Fat = L* value; a_Fat = a* value; b_Fat = b* value. All fat traits are measured in subcutaneous fat. LMP = lean meat percentage.

Table 2 *Fixed effects of the models for the analysed traits[a] in Landrace and Duroc*

	Fixed effects				Regression coefficients	
	Sex	Herd	Abattoir x slaughter day	Calibration	Storage	Carcass weight
C16:0	x z	x z	x z	~		x z
C16:1n-7		x z	x z	~		x z
C18:0	x z	x z	x z	~		x z
C18:1n-9	x z	x z	x z	~		x z
C18:2n-6	x z	x z	x z	~		x z
C18:3n-3	x z	x z	x z	~		x z
n-6/n-3	x	x z	x z	~		x z
Moisture	x z	x z	x z	~	x z	x z
L_Fat	x z	x z	~	x z		x z
a_Fat		x z	~	x z		x z
b_Fat	x	x z	~	x z		x z
LMP	x z	x z	x z	~		x z

[a] See Table 1 for trait abbreviation definitions.
x = significant effect (P <0.05) for Landrace.
z = significant effect (P <0.05) for Duroc.
~ = not tested.
Empty cells = non-significant (P >0.05).

Table 3 *Least square means for effect of breed x sex for the analysed traits[a] in Landrace and Duroc*

TRAIT		Female	Castrates	Difference between sex
C16:0	Landrace	19.52[a]	20.65[b]	-1.13
	Duroc	20.73[b]	21.45[c]	-0.72
C16:1n-7	Landrace	2.41[a]	2.38[a]	0.03
	Duroc	2.12[b]	2.15[b]	-0.03
C18:0	Landrace	11.09[a]	11.83[b]	-0.74
	Duroc	13.37[c]	13.61[d]	-0.24
C18:1n-9	Landrace	44.16[a]	44.41[b]	-0.25
	Duroc	43.14[c]	43.43[d]	-0.29
C18:2n-6	Landrace	15.63[a]	13.89[b]	1.74
	Duroc	14.03[b]	12.94[c]	1.09
C18:3n-3	Landrace	1.56[a]	1.42[b]	0.14
	Duroc	1.44[b]	1.34[c]	0.10
n-6/n-3	Landrace	10.06[a]	9.84[b]	0.22
	Duroc	9.81[b]	9.69[b]	0.12
Moisture	Landrace	11.30a	9.25b	2.06
	Duroc	9.34[b]	8.38[c]	0.96
L_Fat	Landrace	77.26[a]	78.73[b]	-1.47
	Duroc	78.09[c]	78.64[b]	-0.55
a_Fat	Landrace	4.33[a]	4.31[a]	0.02
	Duroc	4.87[b]	4.76[b]	0.10
b_Fat	Landrace	6.32[a]	6.44[b]	-0.13
	Duroc	6.47[b]	6.45[b]	0.02
LMP	Landrace	65.33[a]	62.85[b]	2.48
	Duroc	59.55[c]	57.18[d]	2.37

[a] See Table 1 for trait abbreviation definitions.
Probability of significance for each trait: different letters = P <0.001, equal letters = non-significant (P >0.001).

Table 4a *Heritabilities (h^2) for the analysed traits[a] with standard errors, genetic and phenotypic standard deviations (σ_a, σ_p) in Landrace*

	h^2	SE	σ_a	σ_p
C16:0[b]	0.39	0.05	0.55	0.89
C16:1n-7	0.41	0.06	0.11	0.17
C18:0	0.46	0.06	0.64	0.94
C18:1n-9[b]	0.67	0.06	0.86	1.05
C18:2n-6[b]	0.44	0.06	0.95	1.43
C18:3n-3	0.37	0.05	0.09	0.15
n-6/n-3	0.06	0.03	0.14	0.58
Moisture	0.28	0.05	1.22	2.32
L_Fat	0.22	0.04	0.76	1.64
a_Fat	0.13	0.03	0.32	0.89
b_Fat	0.07	0.03	0.20	0.74
LMP[b]	0.47	0.05	1.50	2.20

[a] See Table 1 for trait abbreviation definitions.
[b] Traits included in more than one analyses, average values are presented.

Table 4b *Heritabilities (h^2) for the analysed traits[a] with standard errors, genetic and phenotypic standard deviations (σ_a, σ_p) in Duroc*

	h^2	SE	σ_a	σ_p
C16:0[b]	0.51	0.07	0.60	0.84
C16:1n-7	0.50	0.08	0.17	0.24
C18:0	0.54	0.08	0.96	1.31
C18:1n-9[b]	0.57	0.08	1.05	1.39
C18:2n-6[b]	0.46	0.07	0.77	1.14
C18:3n-3	0.25	0.06	0.06	0.13
n-6/n-3	0.01	0.03	0.07	0.63
Moisture	0.33	0.06	1.08	1.88
L_Fat	0.21	0.06	0.59	1.28
a_Fat	0.24	0.06	0.40	0.83
b_Fat	0.17	0.05	0.26	0.63
LMP[b]	0.47	0.06	1.67	2.45

[a] See Table 1 for trait abbreviation definitions.
[b] Traits included in more than one analyses, average values are presented.

Table 5a *Phenotypic correlations (below diagonal) and genetic correlations (above diagonal; standard errors between brackets) for main fatty acids and LMP[a] in Landrace*

	C16:0	C16:1n-7	C18:0	C18:1n-9	C18:2n-6	C18:3n-3	n-6/n-3	LMP
C16:0		-0.21	0.71	-0.06	-0.80	-0.68	-0.63	-0.61
		(0.11)	(0.06)	(0.10)	(0.04)	(0.07)	(0.16)	(0.07)
C16:1n-7	-0.17		-0.78	0.06	0.27	0.11	0.47	0.43
			(0.05)	(0.10)	(0.10)	(0.11)	(0.17)	(0.09)
C18:0	0.48	-0.72		-0.33	-0.51	-0.31	-0.74	-0.52
				(0.09)	(0.08)	(0.10)	(0.13)	(0.07)
C18:1n-9	-0.01	0.08	-0.22		-0.54	-0.71	0.29	-0.38
					(0.07)	(0.06)	(0.16)	(0.08)
C18:2n-6	-0.77	0.26	-0.53	-0.49		0.96	0.40	0.78
						(0.02)	(0.17)	(0.05)
C18:3n-3	-0.56	0.15	-0.37	-0.46	0.77		0.17	0.69
							(0.20)	(0.06)
n-6/n-3	-0.22	0.09	-0.24	0.06	0.21	-0.33		0.40
								(0.17)
LMP	-0.51	0.25	-0.43	-0.27	0.65	0.51	0.16	

[a] See Table 1 for trait abbreviation definitions.
The parameters came from 21 multitrait analyses.

Table 5b *Phenotypic correlations (below diagonal) and genetic correlations (above diagonal; standard errors between brackets) for main fatty acids and LMP[a] in Duroc*

	C16:0	C16:1n-7	C18:0	C18:1n-9	C18:2n-6	C18:3n-3	LMP
C16:0		-0.24	0.61	-0.39	-0.74	-0.57	-0.53
		(0.13)	(0.09)	(0.11)	(0.06)	(0.12)	(0.09)
C16:1n-7	-0.15		-0.91	0.56	0.09	-0.30	0.12
			(0.03)	(0.09)	(0.13)	(0.15)	(0.12)
C18:0	0.39	-0.84		-0.77	-0.21	0.18	-0.13
				(0.05)	(0.12)	(0.15)	(0.12)
C18:1n-9	-0.24	0.54	-0.69		-0.31	-0.55	-0.31
					(0.11)	(0.11)	(0.11)
C18:2n-6	-0.68	0.06	-0.21	-0.35		0.97	0.78
						(0.04)	(0.06)
C18:3n-3	-0.39	-0.14	0.03	-0.39	0.67		0.73
							(0.10)
LMP	-0.46	0.03	-0.14	-0.22	0.64	0.43	

[a] See Table 1 for trait abbreviation definitions.

The parameters came from 15 multitrait analyses.

Table 6a *Phenotypic correlations (below diagonal) and genetic correlations (above diagonal; standard errors between brackets) for the analysed traits[a] in Landrace*

	C16:0	C18:1n-9	C18:2n-6	Moisture	L_Fat	a_Fat	b_Fat	LMP
C16:0		-0.13	-0.71	-0.48	0.73	-0.01	0.04	-0.57
		(0.10)	(0.04)	(0.09)	(0.07)	(0.16)	(0.19)	(0.07)
C18:1n-9	-0.01		-0.57	-0.29	0.39	-0.33	-0.26	-0.36
			(0.07)	(0.10)	(0.10)	(0.14)	(0.17)	(0.08)
C18:2n-6	-0.77	-0.52		0.69	-0.88	0.20	0.15	0.78
				(0.07)	(0.05)	(0.15)	(0.19)	(0.04)
Moisture	-0.37	-0.28	0.53		-0.62	0.10	0.12	0.77
					(0.11)	(0.17)	(0.20)	(0.06)
L_Fat	0.47	0.19	-0.52	-0.21		-0.43	-0.25	-0.77
						(0.15)	(0.21)	(0.06)
a_Fat	0.02	-0.12	0.04	0.08	-0.27		0.38	0.10
							(0.20)	(0.15)
b_Fat	0.11	-0.02	-0.08	0.12	-0.05	0.59		0.28
								(0.18)
LMP	-0.52	-0.27	0.65	0.48	-0.47	0.02	-0.02	

[a] See Table 1 for trait abbreviation definitions.
All parameters came from one multitrait analysis.

Table 6b *Phenotypic correlations (below diagonal) and genetic correlations (above diagonal; standard errors between brackets) for the analysed traits[a] in Duroc*

	C16:0	C18:1n-9	C18:2n-6	Moisture	L_Fat	a_Fat	b_Fat	LMP
C16:0		-0.38	-0.65	-0.19	0.43	-0.13	0.03	-0.49
		(0.11)	(0.06)	(0.14)	(0.14)	(0.16)	(0.17)	(0.10)
C18:1n-9	-0.25		-0.35	-0.31	0.31	-0.28	-0.35	-0.31
			(0.11)	(0.12)	(0.15)	(0.14)	(0.15)	(0.11)
C18:2n-6	-0.67	-0.36		0.48	-0.67	0.27	0.30	0.78
				(0.11)	(0.11)	(0.15)	(0.17)	(0.06)
Moisture	-0.28	-0.17	0.44		-0.45	0.46	0.61	0.69
					(0.16)	(0.15)	(0.15)	(0.08)
L_Fat	0.33	0.10	-0.40	-0.09		-0.76	-0.44	-0.48
						(0.10)	(0.18)	(0.13)
a_Fat	-0.10	-0.12	0.15	0.13	-0.48		0.56	0.36
							(0.14)	(0.14)
b_Fat	-0.01	-0.09	0.06	0.23	-0.23	0.56		0.47
								(0.14)
LMP	-0.46	-0.21	0.63	0.52	-0.34	0.22	0.18	

[a] See Table 1 for trait abbreviation definitions.
All parameters came from one multitrait analysis.

Paper 4

Genetic parameters between slaughter pig efficiency and growth rate of different body tissues estimated by computed tomography in live boars of Landrace and Duroc

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Abstract

In this study, computed tomography (CT) technology was used in large-scale measurement to calculate body composition on live pigs for breeding purpose. Norwegian Landrace (L; n=*3835) and Duroc (D;* n=*3139) boars, selections candidates to be elite boars in a breeding programme, were CT-scanned between August 2008 and August 2010 as part of an ongoing testing programme at Norsvin's boar test station. Genetic parameters in the growth rate of muscle (MG), carcass fat (FG), bone (BG) and non-carcass tissue (NCG), from birth to approximately 100 kg live weight, were calculated from CT data. Genetic correlations of growth of different body tissues scanned by CT with more traditional production traits such as average daily gain from birth to 25 kg (ADG1), average daily gain from 25 kg to 100 kg (ADG2) and feed conversion ratio from 25 kg to 100 kg (FCR) were also estimated from data on the same boars. Genetic parameters were estimated based on multi-trait animal models using the AI-REML methodology. The heritability estimates (SE 0.04-0.05) for the various traits were as follows: MG (0.19 and 0.43 for Landrace and Duroc, respectively), FG (0.53 and 0.59), BG (0.37 and 0.58), NCG (0.38 and 0.50), LMP (0.50 and 0.57), ADG1 (0.25 and 0.48), ADG2 (0.41 and 0.42) and FCR (0.29 and 0.42). Genetic correlations for MG with LMP were 0.55 and 0.68, and genetic correlations between MG*

and ADG2 were -0.06 and 0.07 for Landrace and Duroc, respectively. LMP and ADG2 were clearly unfavourable genetically correlated (L: -0.75 and D: -0.54). These results demonstrated the difficulty to jointly improve LMP and ADG2. ADG2 was unfavourably correlated with FG (L: 0.84 and D: 0.72), thus indicating to a large extent that selection for increased growth implies selection for fatness. Diseases may result in very lean pigs, and selection for leanness (i.e. LMP) may therefore be suboptimal. Selection for MG is not expected to increase ADG2, but will yield faster growth of the desired tissues and a better carcass quality. Hence, we consider MG to be a better biological trait in selection for improved productivity and carcass quality. CT is a powerful instrument in conjunction with breeding, as it combines the high accuracy of CT data with measurements taken from the selection candidates and the high selection intensity. CT also allows the selection of new traits such as real body composition, and in particular, the actual MG on living animals.

Keywords: Computed tomography, body composition, lean meat growth, muscle growth, genetic parameters

Implications

The use of computed tomography (CT) makes it possible to obtain accurately carcass composition from live pigs. Since these young boars are selection candidates for elite boars, this information is expected to substantially improve the accuracy of selection for carcass traits and their growth. The CT picture analysis is automated, so the individual data on each boar will be available shortly after scanning. This new technology allows accurate prediction of breeding values for new traits such as body composition and size, weight, and density of carcass and internal organs, and makes it possible for Norsvin (the Norwegian Pig Breeders Association) to breed for actual muscle growth.

Introduction

In the 1950s, systematically breeding for leaner pig meat became an important breeding goal, and since the leanness of the pigs is very heritable (ranging from 0.12 to 0.74 for 16 studies, Clutter and Brascamp, 1998), the western modern breeds have become much leaner. Selection has been performed based on measurements of ultrasound backfat and loin measurements taken from live

selection candidates or experimental dissection for carcass composition of full-sibs, half-sibs and offspring of the selection candidates (Clutter and Brascamp, 1998). The Norwegian dissection procedure has changed from the weighing of trimmed ham and loin, in addition to manual measurement of the loin eye area, to the dissection of shoulder, loin and ham into different tissues for the prediction of lean meat percentage (Gjerlaug-Enger *et al*., 2010a).

A third option to determine body composition is CT technology, which is commonly used for human diagnostic purposes, and can also be used for detailed measurements of different tissues in sheep (Afonso, 1992; Vangen and Thompson 1992), intact iced halibut (Kolstad *et al*., 2004) and different pigs breeds (Luiting *et al*., 1995; Kolstad, 2001). The advantage with CT is that it can be used as a non-destructive method without killing the animal. Phenotypes on many animals are required for genetic analyses. A few studies on sheep have used CT data for the estimation of genetic parameters (Jones *et al*., 2004; Kvame and Vangen, 2007), but to the best of the authors' knowledge, similar work has not previously been done on pigs.

Norsvin is running a boar testing station with routine CT scanning of live pigs in a breeding programme, with an annual scanning capacity of 3500 live purebred boars. The image analysis at this boar test is completely automated and features specially designed software for the removal of non-carcass tissues to obtain a virtual carcass, as well as for the calculation of several traits, e.g. determination of body composition: kilos of lean muscle, carcass fat, bone and non-carcass tissues. The lean meat percentage calculated from CT could, with only small adaptations, replace the lean meat percentage taken from dissection in the estimated breeding values (EBV).

Calculations for leanness are not straightforward, and unfavourable genetic correlations between growth and leanness (Mcphee *et al*., 1979; Cleveland *et al*., 1982) make joint improvement of these traits a challenge. Fowler *et al*. (1976) demonstrated that lean meat growth can be selected for by using an economic selection index that combines lean meat content and growth or by using lean meat growth, which is measured as lean gain per day. Clutter and Brascamp (1998) proposed that lean meat growth is the most appropriate expression of the industry's objective for market pigs, while Chen *et al*. (2002) indicated that direct selection of EBV for lean meat growth with a multi-trait model gave a higher lean meat growth than a linear index of EBV for growth, backfat and the loin eye area.

The aims of this study will therefore be to calculate genetic parameters for the growth rate of muscle, carcass fat, bone and non-carcass tissues based on CT data. The genetic correlations between those

traits and more traditional production traits for slaughter pig efficiency, such as lean meat percentage, average daily gain in early life and in the boar test period and fed conversion ratio in the boar test, will also be examined.

Material and methods

Animals

Data for the present study were collected from August 2008 to August 2010 in Norsvin's boar testing station in southeastern Norway, and Norwegian Landrace (*n*=3835) and Duroc (*n*=3139) boars was included. Boars are kept in single-breed groups of 12 pigs per pen, and fed *ad libitum* on conventional concentrates containing 161 and 136 g digestible protein and 9.68 and 9.50 MJ net energy/kg before and after 50 kg, respectively, with 1 month of mixing the two feeds to facilitate the feed change. Major feedstuff compounds are barley, oats, peas, soy meal extract and rendering (animal) fat. The full-sib group size was 1.1 for Landrace and 1.3 for Duroc, and the average half-sib group size was 35. The average start and end weight for the test was 25 and 100 kg live weight. The boars are all selection candidates to be semen elite boars based on EBV and genetic uniqueness. More detailed information about the populations was presented in Gjerlaug-Enger *et al*. (2010b).

The CT technology

A General Electric (GE) multi-slice VCT LightSpeed scanner was preformed for the CT analysis. The protocol used is a helical scan with a 1.25 mm slice thickness and soft tissue algorithm for reconstruction of the x-ray signal to image. The scanning of a pig takes 15 seconds and gives 1100 pictures (Figure 1). Different tissues have different CT values, and a Hounsfield unit (HU) defines fat as having HU values from -200 to 0, muscle as HU values from 0 to 200 and bone as HU values from 200 and higher (www.medcyclopaedia.com). The images were analysed using an in-house MATLAB (The MathWorks, Inc, 2010) programme (developed by Kongsro, Norsvin, 2008). The non-carcass tissues were removed to obtain a virtual carcass using distance measurements in the images relative to skin and internal fat. Previous carcass studies have shown that the volume and weight of different tissues (fat, muscle and bone) can be estimated directly from a stack of images with a constant slice thickness (Kongsro *et al*., 2008). The volume of different tissues was estimated using the number of pixels classified as muscle, fat and bone, multiplied by slice thickness (volume) and average density of muscle, fat and bone tissue to get the mass of the different tissues. The pixels were classified using a pixel value and column-wise neighbourhood operation classifier (The MathWorks, Inc, 2010) to classify

pixels relative to "real" tissues. The final parameters used in this study were kilos of lean muscle, carcass fat, bone and non-carcass tissues (the sum of internal organs and fat).

The traits analysed were:

1. **Muscle growth (MG) -** Average daily growth of muscle measured with CT from birth to date of scanning, approximately 100 kg live weight. The trait was defined as $\frac{\text{kg muscle from CT}}{\text{age}_{100\text{kg}}}$.
2. **Carcass fat growth (FG) -** Average daily growth of carcass fat measured with CT from birth to date of scanning. The trait was defined as $\frac{\text{kg carcass - fat from CT}}{\text{age}_{100\text{kg}}}$.
3. **Bone growth (BG) -** Average daily growth of bone measured with CT from birth to date of scanning. The trait was defined as $\frac{\text{kg bone from CT}}{\text{age}_{100\text{kg}}}$.
4. **Non-carcass tissue growth (NCG) -** Average daily growth of non-carcass tissue measured with CT from birth to date of scanning. The trait was defined as $\frac{\text{kg non - carcass tissue from CT}}{\text{age}_{100\text{kg}}}$.
5. **Lean meat percentage (LMP) -** The mass of muscle tissue was divided by the total mass of the carcass, and all the variables were measured with CT. The trait was defined as $\frac{\text{kg muscle from CT x 100}}{\text{kg muscle} + \text{carcass - fat} + \text{bone from CT}}$.
6. **Average daily gain from birth to 25 kg (ADG1) -** Young boars start the test a couple of days after entering the testing station, and the pigs' age at test start is corrected to age at 25 kg. Individual weights are automatically recorded by the Feed Intake Recording Equipment (FIRE) system (Osborne Industries INC., Kansas, USA). The trait was defined as $\frac{25\text{kg}}{\text{age}_{25\text{kg}}}$.
7. **Average daily gain from 25 kg to 100 kg (ADG2) -** The boar test ends when the pigs reach 100 kg, as measured through the FIRE system. The pig's final test weight is approximately 100 kg, and is corrected to 100 kg. The trait was defined as $\frac{75\text{kg}}{\text{age}_{100\text{kg}} - \text{age}_{25\text{kg}}}$.

8. **Feed conversion ratio from 25 to 100 kg (FCR) -** Through the FIRE system, individual daily feed intake is recorded for the boars. All visits to the feeding dispensers are logged, which is the basis for the individual feed consumption on a daily basis and for the entire testing period. The pigs are fed two types of feed in a distribution of approximately 1:2, and the feed energy consumed is estimated assuming 9.56 MJ net energy/kg feed. The trait was defined as $\frac{\text{feed intake (MJ)}}{75\text{kg}}$.

The statistical analyses

Least square means for breed - SAS Proc GLM with least square means procedure (SAS Inst., Inc., Cary, NC, USA) was used to estimate the effect of breed. An analysis was preformed with both breeds in one analysis, but without herd effect, due to confounding between herd and breed. The fixed effects were: breed, year x month, mother's parity number and number of live born in the litter the boar originated from.

Quantitative genetic analyses - Initial computations were performed on each breed separately using SAS Proc GLM to evaluate non-genetic factors to be included in the model. For the analysed traits, the tested fixed effects were herd x year, year x month, parity number and number of live born. Only significant effects were included in the final models, and various sub-models of the full model were used for further genetic analyses.

The significant effect of pen was included as a random effect in models for all traits. The significance of the random effects of pen was tested through a likelihood ratio test. A random effect of litter was not included due to small full-sib groups. In the quantitative genetic analyses, each breed was analysed separately. The traits (MG, FG, BG, NCG, LMP, ADG1, ADG2 and FCR) were analysed separately, and a general multi-trait animal model was used for the genetic analyses:

$$\mathbf{Y} = \mathbf{X\beta} + \mathbf{Zu} + \mathbf{Qp} + \mathbf{e}$$

where: **Y** is a vector of observations, **β**, **u**, **p** and **e** are vectors of fixed, random additive genetic, random pen and residual effects, respectively, and **X**, **Z** and **Q** are known incidence matrices. An estimation of (co)variance components was performed using multi-trait animal models and analysed with restricted maximum likelihood (REML) methodology. The DMU 6.7 software package (Madsen and Jensen, 2008)

and the average information algorithm were used in the estimation. Asymptotic standard errors of (co)variance components were computed from the inverse average information matrix.

Due to computational constraints because of linear dependency between the growth traits, all traits could not be analysed simultaneously in a multi-trait analysis. To obtain the correlations between MG, FG, BG and NCG, these four traits were analysed based on a multi-trait model, and presented in a first matrix. The second set of analysed traits (MG, FG, BG, NCG, LMP, ADG1, ADG2 and FCR) were analysed in four analyses. Each analysis consists of LMP, ADG1, ADG2 and FCR plus MG, FG, BG or NCG was added to each of the four analyses.

The pedigree file contained all tested animals and their ancestors traced back to 2002. The final pedigree files included 9336 Landrace and 6403 Duroc pigs.

Results

Fixed effects

Average figures for the traits studied are presented in Table 1. Initial analyses on fixed effects for the traits produced the sub-models presented in Table 2, and the significant effects were included in the final models. Among the fixed effects, year x month had a significant effect on all traits analysed. The herd of origin showed a large effect on the growth traits analysed. On average, the fixed effects account for 20.5% of the total variation (R^2) of the traits analysed here.

Breed effects

Least square means of the effect for breed are presented in Table 3. Landrace had higher MG and NCG, as well as lower BG and FG, than Duroc. Landrace was the most efficient breed, with the highest LMP, ADG1 and ADG2, and lowest FCR.

Heritability estimates

The CT and FIRE technology used to measure production efficiency gave moderate to high heritability estimates for all traits in both breeds (Tables 4a and 4b). In general, Duroc had a higher additive genetic variation for the traits analysed than Landrace, in addition to higher estimated heritabilities. The estimated heritabilities for MG, FG, BG, NCG, LMP, ADG1, ADG2 and FCR ranged from 0.19 to 0.53 in Landrace and from 0.42 to 0.59 in Duroc.

Genetic correlations

The genetic correlations between the growth of different tissues for the live pigs are shown in Table 5. MG is negatively correlated to FG and uncorrelated to NCG with both breeds. FG and NCG are highly correlated. BG is positively correlated to all other traits (MG, FG and FG).

The estimated genetic correlations between traditional production traits and the growth rate of different tissues for the live pigs at 100 kilos are shown in Tables 6a and 6b. With only a few exceptions, the sign and magnitude of the parameters were similar for both breeds. Genetic correlations between FCR and the other traits seem to differ most between the two breeds. Based on current analyses, LMP is positively correlated to MG, and negatively correlated to FG, BG and NCG. ADG1 is positively correlated to MG, FG, BG and NCG, while ADG2 is uncorrelated to MG. The ADG2 is highly unfavourable and positively correlated to FG and NCG, and moderately to positively correlated to BG. FCR demonstrated a moderate to high favourable correlation to MG in Duroc.

Discussion

Breed differences

Landrace is a highly efficient breed, and Norwegian Landrace is bred today as a dam line. Due to the breed's high efficiency and leanness (Table 3), genetic gain for increased leanness is not desirable. The Norwegian Duroc is bred as a sire line, but due to the high weighting on meat quality traits and shorter selection history for this breed, the efficiency and leanness are not at the same level as Landrace.

Heritability estimates

This study showed that the CT technology yielded medium to high estimated heritabilities for MG, FG, BG, NCG and LMP, as well as medium high heritabilities for ADG1, ADG2 and FCR estimated with data from the FIRE technology. There were no works found in the literature on FG, BG nor NCG, but in this study all these traits showed higher heritabilities than MG in both breeds. MG is less heritable than LMP, which may be due to the fact that FG is highly heritable and seems to explain the majority of the genetic variation in LMP. To the best of the authors' knowledge, MG, at least the way we have defined it, has not been previously measured. Nevertheless, the lean meat content can be predicted from backfat and the loin eye area, which can then be used for the prediction of lean meat growth (PLMG). This parameter is similar to our MG, but with data from CT, MG can be measured with a higher accuracy. In other studies of PLMG, heritability estimates ranges from 0.37 to 0.47 (Stern *et al*., 1993; Cameron,

1994; Chen *et al*., 2002). These estimates are in the range of our estimate for Duroc, while Cameron and Curran (1994) found a lower heritability (0.25) in the same magnitude as our estimate for Landrace. All those parameters for PLMG originate from ultrasound measurements of backfat and loin. So, the heritability of MG from CT does not give any higher heritability than the PLMG from ultrasound measurements. Still, the CT measures the muscle directly and might more correctly give the true variation of the trait.

This is the first study presenting heritabilities for LMP estimated from large-scale CT scanning of pigs, although a similar size in heritability is reported by Kvame and Vangen (2007), who studied genetic variation in a Norwegian sheep population with CT. Duroc exhibited a larger genetic variation for LMP than Landrace. The heritabilities for LMP in this study are slightly higher than the heritabilities estimated for these two breeds with the previous method used in Norsvin: an experimental dissection for carcass composition of half-sibs tested females and castrates (Gjerlaug-Enger *et al*., 2010a). Estimated heritabilities for LMP in our study were consistent with the corresponding estimates (ranging from 0.41 to 0.68) reported by Ducos *et al*. (1993), Sonesson *et al*. (1998) and Gilbert *et al*. (2007). All studies calculated an LMP based on a cutting procedure and the weight of lean meat for different premium cuts expressed as a percentage of the half carcass weight. Traits associated with fatness used to have a high heritability, and estimated heritabilities for ultrasonic backfat ranged from 0.35 to 0.72 in some studies by Ducos *et al*. (1993), Hoque *et al*. (2007) and Solanes *et al*. (2009).

Heritability estimates were higher for ADG1 in Duroc than in Landrace, and these heritabilities were higher or comparable to those obtained for average daily gain from birth to weaning and average daily gain from weaning to 12 weeks (0.15 and 0.27, respectively) as reported by Chimonyo and Dzama (2007). Hermesch *et al*. (2000) tested pigs from 3 to 18 weeks, thereby overlapping our ADG1 and ADG2 periods, and the heritability estimates were 0.10 in Large White and 0.48 in Landrace. This study also demonstrated the importance of the litter effect. In our study, the heritabilities for growth in the testing station period (ADG2) were of the same magnitude (ranging from 0.30 to 0.52) as reported by Ducos *et al*. (1993), de Vries *et al*. (1994) and Suzuki *et al*. (2005).

The estimated heritabilities for FCR, particularly for Duroc, were larger than the estimated heritabilities (ranging from 0.15 to 0.27) reported by and Hoque *et al*. (2007), Hermesch *et al*. (2000) and Ducos *et al*. (1993). The FCR in our study was adjusted to a fixed growth period from 25 to 100 kg live weight. Pre-correction of data by valid methods was important, as correcting for animal size as a fixed effect in

the variance component analysis could reduce some additive variance. The data are regionally used in breeding so the quality of the data was good.

Relationships among growth of different tissues and slaughter pig efficiency

Genetic correlation between MG, FG, BG and NCG - In general, we expected positive correlations between growth of different body tissues. Animals with a high growth rate will necessarily need a high growth for all their body components. However, MG was lowly correlated to the other traits, especially NCG, while FG and NCG were highly positively correlated in both breeds. The only negative correlation was estimated for the genetic correlation between MG and FG, thus an antagonistic relationship exists between the growth of carcass fat and muscles.

Genetic correlation between LMP and MG, FG, BG and NCG - As expected, only MG was positively correlated to LMP. The highest correlation was nevertheless between FG and LMP, not significant different from -1. This means that the selection for LMP mainly affects FG, but it also affects NCG and MG to a higher and smaller degree, respectively, though the smallest effect was LMP on BG.

Genetic correlation between ADG1 and MG - Selection for growth has different effects on the different tissues of the pig. ADG1 is positively correlated with all different types of tissue in the small pig, which indicate a general response in body size when selecting ADG1. Kolstad (2001) found no differences in leanness early in life (10-25 kg) between three lines that were very different in fatness as finished slaughter pigs. The three lines, the Norwegian Landrace, Duroc and a “backfat-line cross” were CT-scanned several times from 10 to 105 kg. The “backfat-line cross” was a crossbreed between Norwegian Landrace and a line from a selection experiment in which a high backfat and low growth rate were selected for (Vangen, 1977). This “backfat-line cross” ended up with twice the amount of total fat compared with ordinary Norwegian Landrace (Kolstad, 2001). Consequently, the high degrees of uniform distribution of different tissues in small pigs of different lines and breeds are obvious, and support our results with medium high genetic correlations between ADG1 and growth of all the different tissues.

Genetic correlation between ADG2 and FG - When looking back at the parameters for Norwegian Landrace in earlier periods, the genetic correlations between backfat and ADG2 were less unfavourable with ultrasonic backfat measurements than with CT backfat measurements. This is shown in Figure 2, and all the analyses from earlier periods were done simultaneously in 2010, so the software and statistical models were the same for all periods. One year extra for pedigree was used for all periods.

The backfat from CT was measured in the CT picture, and the backfat measurement from ultrasound was measured at the last rib on the live boars. The genetic correlations between backfat and ADG2 have increased, with an increased heritability for backfat measured with CT in the last analysed period. An increased accuracy with CT data clearly demonstrates the true high connection of backfat and ADG2 in modern pigs, although the largest part of fat reduction in Norwegian Landrace was done before 1992, and only a few millimetres (ranging from 8.1 to 5.9 mm backfat on Landrace boars at 100 kg) of fat reduction was achieved during this period. Even so, we see a reduction in phenotypic variation of backfat thickness for this selection period, as well as a reduction in heritability for backfat for the period of ultrasound measurements until 2008. Estimated heritabilities for ADG2 increased of this period (1992-2010) despite reduced phenotypic variance. A change from manual to electronic (FIRE) weighing was done in the data period, which positively affected the quality of the data.

The theory of growth is that selection for growth is expected to produce leaner animals because a selection for growth is a selection for animals with steeper growth curves (Krieter and Kalm, 1989; Kohn *et al*., 2007). For that reason, selection for growth is expected to result in a higher adult weight and in less mature, leaner animals at a fixed weight, as fat growth is expected to increase around puberty. Back in the 1960s and 1970s, estimates on the fat lines of pigs in relation to the genetic correlation between backfat and growth were favourable for Landrace (Vangen, 1979). In a study by Hetzer and Miller (1972) on relatively fat pigs, the results indicated that a selection for lower fatness should improve the growth rate in Duroc, whereas in Yorkshire, a higher leanness would result in slower growing pigs. A favourable correlation between growth and leanness is, however, far from what we can interpret from our results. Modern, fast growing, lean pigs such as the Norwegian Landrace and Duroc have a highly unfavourable genetic correlation between ADG2 and FG or LMP, which is in agreement with results reviewed in a paper by Cleveland *et al*. (1982). A mean correlation between the average daily gain and backfat was 0.12, ranging from 0.26 to 0.55 for 11 studies from 1962 to 1994 (Clutter and Brascamp, 1998).

Wild pigs and other mammals living in temperate climates are often born in the spring. To escape from predators is an important trait, so MG is an early priority in the summer months. But before the winter comes, FG is important for energy reserves and isolation against the cold since its helps to maintain body temperature. Subcutaneous fat insulates animals because of the low rate of heat transfer in fat, a trait which is especially important for animals living in cold climates. This applies for all mammals in temperate regions, and can explain the high genetic correlation between FG and ADG2 for pigs in our study.

Early dissection studies on farm animals at different ages has made it possible to produce growth curves of different tissues, and the maximum growth rate for bone is reached before that of muscle, while fat is the latest to develop (Hammond, 1950). Modern pigs are fast growing animals, and in our study, FG increased in the ADG2 period from 25 to 100 kg live weight. In addition, a pig is different from other farm animals with its high volume of subcutaneous fat in the carcass, while e.g. ruminants have more internal fat. Further, Hammond (1950) compared pigs with high/low versus low/high growth, both early (before 16 weeks) and late (from 16 to 34 weeks) in a growth period to 200 lb live weight (90.7 kg). He found very different frames, despite having an equal final weight for the two tested groups. The pigs that had grown high/low had more bone and muscles, while the pigs that had grown low/high had a higher fat content. Although this was a feeding experiment, it supports our findings that pigs that grow fast in the beginning (ADG1) have higher MG, whereas pigs that grow fast in the later period (ADG2) have a higher FG. Despite the very high age in this study (100 days longer than for our pigs), the final weight was almost similar at only 10 kg lower.

Genetic correlation between MG and ADG2 - Bennett (1992) stresses the difference for predicted and actual measurements. If lean meat growth could be measured directly, then selecting for lean meat growth would be straightforward. However, composition is often predicted by indirect means. The relationship between PLMG and other traits can be different from the relationship of actual lean meat growth (ALMG) for the same traits. Hence, a selection for ALMG may be desirable, whereas a selection for PLMG may be undesirable. Therefore, ALMG is distinguished from PLMG, partly because fat affects PLMG more than ALMG.

Chen *et al*. (2002) estimated very high favourable genetic correlations (ranging from -0.80 to -0.86) between growth (days to 113.5 kg, not average daily gain, and therefore the opposite sign) and PLMG in Yorkshire, Duroc, Hampshire and Landrace. Mrode and Kennedy (1993) also found a high genetic correlation (0.96) between growth (average daily gain from 30 to 90 kg) and PLMG in Yorkshire, Duroc and Landrace. This is far from the parameters estimated in our study, with genetic correlations close to zero between ADG2 and MG for both breeds. So what is the effect of trial (breed and environment) and what is the effect of the method and technology when correlations between ADG2 and MG in our study are so different from similar parameters, i.e. PLMG, from other studies? We have attempted to illuminate this in some preliminary analyses, in which the equation for PLMG (National Pork Producers Council (NPPC), 2000) from a study by Chen *et al*. (2002) was used to make NPPC's PLMG for our Norwegian Landrace and Duroc pigs. Measurements for backfat and loin area were then taken from analyses of CT images. The NPPC's PLMG equation gave similar results for the Norwegian breeds in

terms of similar means and standard deviations as MG, but with different genetic correlations to other traits. Estimated genetic correlations between NPPC's PLMG and ADG2 were 0.32 (Landrace) and 0.21 (Duroc) points higher and more positive than corresponding correlations between MG and ADG2, while genetic correlations between NPPC's PLMG and LMP were 0.35 (Landrace) and 0.27 (Duroc) points lower and less positive than corresponding correlations between MG and LMP. Higher genetic correlations between MG and LMP than between NPPC's PLMG and LMP are expected since LMP is measured with volume of different tissues from CT, but the high genetic correlations between NPPC's PLMG and ADG2 are more problematic. The high correlation between ADG2 and PLMG from ultrasound in other studies might be overestimated as a result of the technology used. The growth period used in all studies was not identical, and growth early in life was more positively correlated to MG; nonetheless, this does not explain the differences between the correlations discussed here. As seen in Figure 2, traits measured with CT can produce very different genetic correlations than ultrasonic traits because of the high accuracy of CT technology and image analyses.

Genetic correlation between FCR and MG vs. FG - Webster (1977) compared the energy required for protein deposition and fat deposition, and concluded that the same amount of metabolic energy is deposited in 1 kg of fat as in approximately 8 kg of fat-free muscle. This supports the favourable genetic correlations between FCR and MG, especially in Duroc, as also reported by Hoque *et al*. (2007). In contrast, more energy is required to maintain muscle tissue due to the higher metabolism in that tissue, while fat has a low metabolic activity. Webster (1981) found the energy demand for maintenance to be less per kg metabolic body weight in obese than lean rats, but the differences disappeared when the maintenance was expressed as a function of lean mass. Kolstad and Vangen (1996) found a higher maintenance requirement in Landrace, which was a faster growing breed, than in Duroc. This indicates that a selection for improved MG may increase the maintenance requirement. In our study, genetic correlations between FCR and growth of different tissues were small, except for FCR and MG in Duroc, which had a medium growth. The reason for the small differences in genetic correlations in Landrace could be that the low energy demand required for MG is outweighed by the greater need for maintenance, while FG costs more to grow but requires less energy to maintain. The high genetic correlation between FCR and MG in Duroc was favourable, but only to a certain level. In the long term, a reduced FCR can reduce the ability of MG due to reduced appetite and feed intake. The minimum feed intake capacity should not be lower than the feed intake that maximises the potential for protein deposition and therefore MG, which was thoroughly discussed in papers by Hermesch *et al*. (2003) and Hermesch (2004).

Genetic correlation between ADG2 and NCG - A large volume of non-carcass tissues is undesirable, as there is no value for this tissue at slaughtering. The high genetic correlations between ADG2 and NCG, particularly in Landrace, may indicate that the pigs need larger internal organs to achieve fast growth. The estimated parameters in this study clearly demonstrate the unfavourable connection between ADG2 and NCG. NCG is not discussed in earlier studies, but our results are in accordance with Bidanel and Ducos (1996) and Norsvin's selection parameters, in which unfavourable genetic correlations between growth and dressing percentage were estimated. Dressing percentage is almost the inverse trait to the percentage of non-carcass tissues. However, a high ADG2 is highly influenced by FG, and the high genetic correlation between NCG and FG may thus point towards a connection between subcutaneous fat and internal fat. Therefore, large non-carcass tissues may consist of a high amount of internal fat and a lesser degree of internal organs such as lungs, heart, stomach, intestines and liver, as these vary less between animals (pers. comm. Kongsro, 2010). A selection against NCG or for a higher dressing percentage is controversial since the internal organs are needed to achieve a high production. Still, an increased amount of internal fat is bad energy utilisation for the pig industry. To keep the dressing percentage unchanged, a balanced selection may be one strategy for this biological challenge.

What improvements can the new CT technology make in a practical breeding programme?

Norsvin has long experience in the selection for LMP and ADG2 among other traits and EBV clearly demonstrate the antagonistic relationship between growth and leanness in practical breeding. Hence, a selection based on EBV and total merit index may not be straightforward. One of the main problems with LMP is that the trait does not distinguish between pigs of different size, e.g. larger pigs with large muscle mass vs. small and skinny animals that are low in fat due to diseases. ADG2 may be used to balance this, but pigs with a high ADG2 are often fat. An optimal selection based on these two unfavourably correlated traits is not uncomplicated and depends heavily on the chosen economic weights. Additionally, even in cases in which these two traits are optimally combined in a total merit index, breeders often have individual preferences for the specific traits in the breeding goal, thus potentially yielding variable and unpredictable selection responses for the different traits. The overall goal, however, is to develop a pig with an efficient meat production low in fat, which can be achieved by a selection for improved MG. Moreover, when selecting for MG, diseased pigs (being small and lean) will not be chosen. For this reason, MG seems to be a biologically robust trait for genetic selection. Selection for increased MG may be accompanied by selection for reduced FG, which will be rather straightforward due to the favourable genetic correlation between these two traits.

An efficient selection for MG is not optimal without the new technology presented in this study. The CT and software developed for image analysis have created huge changes for operative breeding programmes. The largest advantages of the current CT method in comparison with previous methods of dissection are that the phenotypes are acquired on the selection candidates. In addition, we observe increased heritabilities and a larger genetic variance on the carcass traits. The combination of these factors makes the selection better adapted for increased slaughter pig efficiency, i.e. large muscle growth on healthy slaughter pigs.

Conclusions

It is well known that an antagonistic genetic relationship exists between growth and leanness in the modern pig. This may be explained by ADG2 being highly correlated with FG, though hardly correlated with MG. Hence, selection may be more efficient and accurate by selecting for an increased MG in combination with a reduced FG for fat breeds, which can be measured on selection candidates using CT. Furthermore, the latter traits exhibit a favourable genetic correlation, thereby resulting in selection on both traits being easy to implement. Since MG and low FG are what the pig industry struggle for, the best way to acquire genetic gains is to select directly for these traits. On the other hand, selection for ADG2 is highly problematic because of its unfavourable genetic correlation to FG and NCG. High MG is a result of large muscle mass, and skinny, slow growing animals will not be rewarded using this trait. With CT technology, we are able to select for the actual MG, which is the best biological trait among the traits studied in this paper. Additionally, with regard to the phenotypes on the selection candidates, the genetic progress can become very high.

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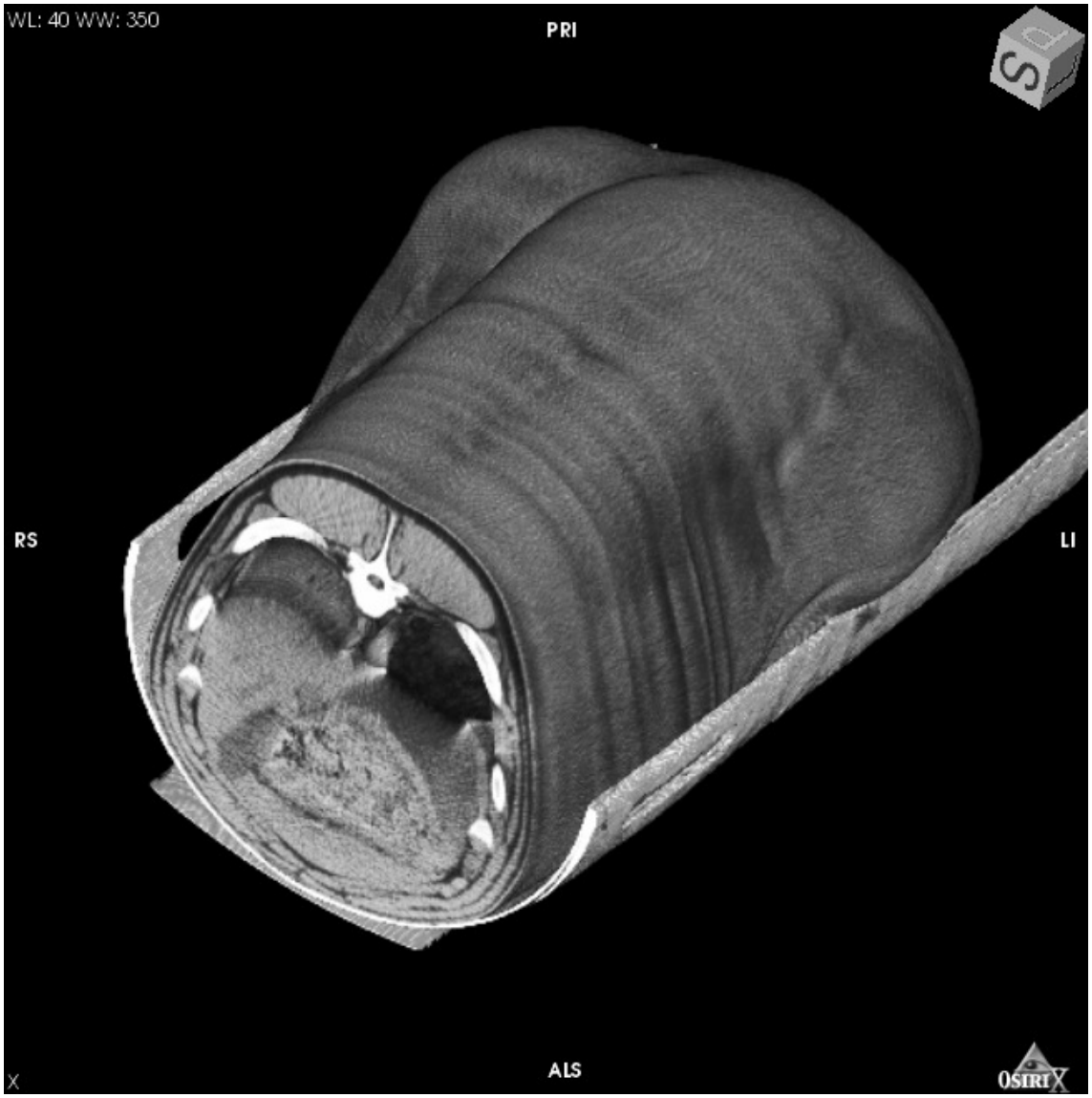

Figure 1 *Spiral scan of a boar, 1100 slices. The images start at the middle of the pig, lying on the belly. The black field in the middle is the air in the lungs, above the stomach*

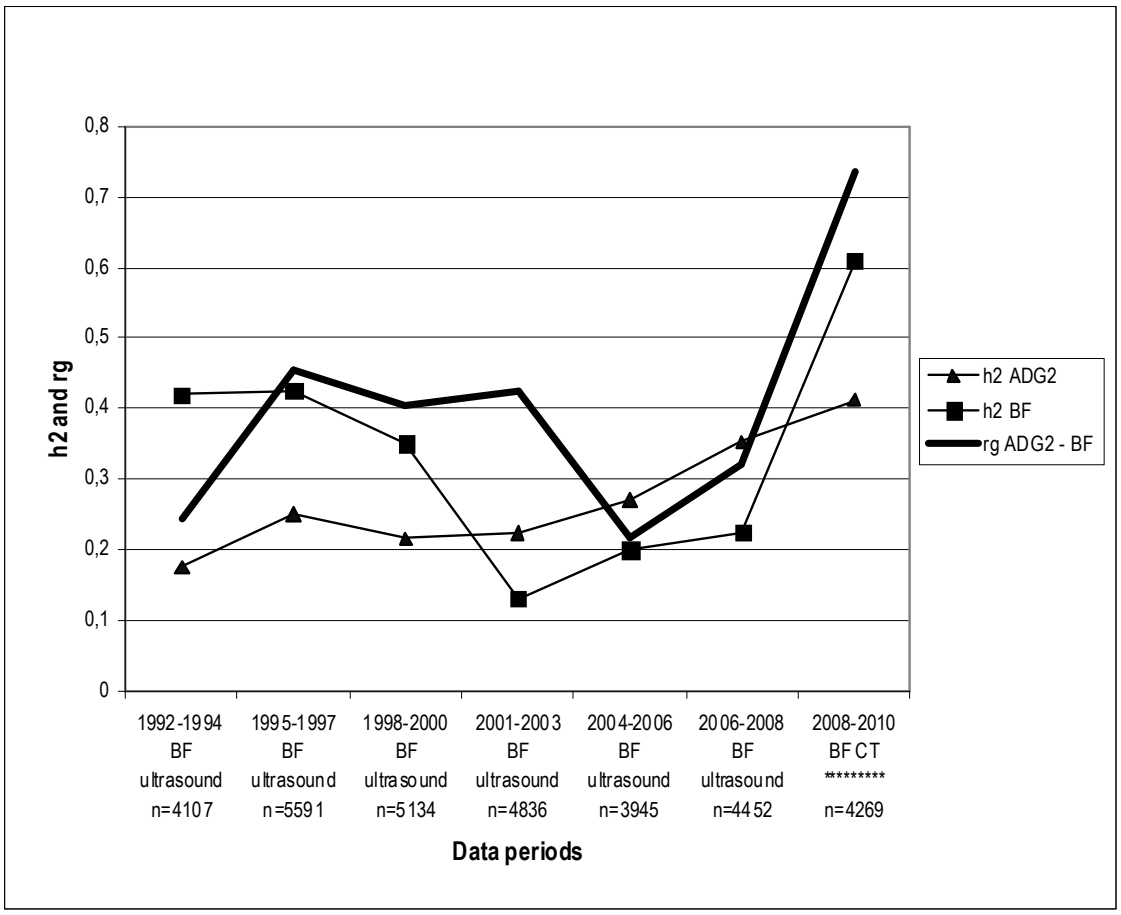

Figure 2 *Different levels of heritabilities for ADG2 and BF (backfat thickness), and genetic correlations between ADG2 and BF for different data periods for Norwegian Landrace*

Table 1 *Numbers of animals per trait, mean, standard deviations of means, minimum and maximum for Landrace and Duroc*

TRAIT			N	Mean	SD	Min.	Max.
Muscle growth from birth to 100 kg (g/d)	MG	Landrace	3835	278	21	168	360
		Duroc	3139	233	21	160	304
Carcass fat growth from birth to 100 kg (g/d)	FG	Landrace	3835	98	17	47	172
		Duroc	3139	112	20	47	190
Bone growth from birth to 100 kg (g/d)	BG	Landrace	3835	50	5	36	70
		Duroc	3139	53	4	39	73
Non-carcass tissue growth from birth to 100 kg (g/d)	NCG	Landrace	3830	219	22	142	319
		Duroc	3136	214	22	142	311
Lean meat percentage at 100 kg (%)	LMP	Landrace	3835	65.3	3.4	51.6	75.7
		Duroc	3139	58.6	4.2	44.4	73.9
Average daily gain from birth to 25 kg (g/d)	ADG1	Landrace	3832	390	44	223	635
		Duroc	3125	365	38	241	543
Average daily gain from 25 kg to 100 kg (g/d)	ADG2	Landrace	3835	905	74	631	1174
		Duroc	3139	874	73	613	1361
Feed conversion ratio from 25 kg to 100 kg (MJ/d)	FCR	Landrace	3604	20.1	1.2	14.2	27.9
		Duroc	2956	20.6	1.4	13.0	27.8

Table 2 *Fixed effects of the models for the analysed traits [a] in Landrace and Duroc*

	Year x month	Herd x year	Parity number [b]	Live born [c]	R^2 Landrace	R^2 Duroc
MG	x z	x z	x z	x z	0.21	0.19
FG	x z	x z	z	x z	0.20	0.16
BG	x z	x z	x z	x z	0.29	0.18
NCG	x z	x z	x z	x z	0.30	0.21
LMP	x z	x z			0.20	0.16
ADG1	x z	x z	x z	x z	0.31	0.18
ADG2	x z	x z	z	x	0.17	0.12
FCR	x z	x z	z	x	0.22	0.19

[a] See Table 1 for trait abbreviation definitions.

x = significant effect (P <0.05) for Landrace.

z = significant effect (P <0.05) for Duroc.

Empty cells = non-significant (P >0.05).

[b] Parity number of the litter the pig originated from, ranged from one to three.

[c] Live born of the litter the pig originated from, ranged from 1 to 23

Table 3 *Least square means[a] for effect of breed for the analysed traits[b] in Landrace and Duroc*

	Landrace	Duroc	Significance
MG	281.3	234.1	***
FG	99.5	113.1	***
BG	50.9	53.0	***
NCG	221.4	214.7	***
LMP	65.2	58.5	***
ADG1	401.5	369.1	***
ADG2	907.4	874.0	***
FCR	20.1	20.7	***

[a] The fixed effects in GLM models were: breed, year x month, mother's parity number and litter's live born.

[b] See Table 1 for trait abbreviation definitions.

*** = P < 0.001.

Table 4a *Heritabilities (h^2) for the analysed traits[a] with standard errors, genetic and phenotypic standard deviations (σ_a, σ_p) in Landrace*

	h^2	SE	σ_a	σ_p
MG	0.19	0.04	8.49	19.51
FG	0.53	0.05	11.78	16.24
BG	0.37	0.05	2.43	3.99
NCG	0.38	0.05	12.19	19.67
LMP	0.50	0.05	2.26	3.20
ADG1	0.25	0.04	19.08	37.79
ADG2	0.41	0.05	45.67	71.07
FCR	0.29	0.04	0.63	1.17

[a] See Table 1 for trait abbreviation definitions.

Table 4b *Heritabilities (h^2) for the analysed traits[a] with standard errors, genetic and phenotypic standard deviations (σ_a, σ_p) in Duroc*

	h^2	SE	σ_a	σ_p
MG	0.43	0.05	12.88	19.65
FG	0.59	0.05	14.74	19.23
BG	0.58	0.05	3.10	4.08
NCG	0.50	0.05	14.65	20.72
LMP	0.57	0.05	3.02	4.00
ADG1	0.48	0.05	25.08	36.13
ADG2	0.42	0.05	45.91	70.51
FCR	0.42	0.05	0.88	1.36

[a] See Table 1 for trait abbreviation definitions.

Table 5 *Genetic correlations for Landrace (above diagonal) and Duroc (below diagonal) for the analysed traits*[a]

	MG	FG	BG	NCG
MG	~	-0.37	0.21	-0.02
FG	-0.35	~	0.34	0.87
BG	0.33	0.37	~	0.44
NCG	0.17	0.77	0.60	~

[a] See Table 1 for trait abbreviation definitions. SE ranged from 0.03 to 0.12.

Table 6a *Genetic correlations for Landrace for the analysed traits*[a]

	LMP	ADG1	ADG2	FCR
LMP	~			
ADG1	*-0.25*	~		
ADG2	*-0.75*	*0.15*	~	
FCR	*0.14*	*0.27*	*-0.53*	~
MG	0.55	0.40	-0.06	-0.11
FG	-0.97	0.35	0.84	-0.21
BG	-0.38	0.52	0.38	-0.06
NCG	-0.78	0.54	0.87	-0.29

[a] See Table 1 for trait abbreviation definitions. SE ranged from 0.03 to 0.14.

Letters in cursive are average values.

Table 6b *Genetic correlations for Duroc for the analysed traits*[a]

	LMP	ADG1	ADG2	FCR
LMP	~			
ADG1	*-0.27*	~		
ADG2	*-0.54*	*0.40*	~	
FCR	*-0.31*	*-0.08*	*-0.34*	~
MG	0.68	0.45	0.07	-0.53
FG	-0.92	0.52	0.72	0.09
BG	-0.25	0.71	0.39	-0.03
NCG	-0.56	0.75	0.87	-0.22

[a] See Table 1 for trait abbreviation definitions. SE ranged from 0.03 to 0.14.

Letters in cursive are average values.

General Discussion

The aims and practical implementation of this thesis

In this study, the possibilities of breeding for meat, fat and carcass quality was investigated and genetic parameters were estimated for 30 different traits through Papers 1, 3 and 4. Paper 2 describes the development of the calibration work for the NIRS-predicted traits used for the genetic analysis in Paper 3. Paper 4 focuses on the possibilities for CT technology and the new CT traits of growth of different tissues, and includes genetic correlations to traditional traits for slaughter pig efficiency.

The combination of new technology, the large number of tested animals and the genetic parameters available for selection on meat, fat and carcass quality traits will hopefully make this thesis useful for future pig breeding programmes.

Storing and flow of data

Most of the meat and fat quality traits recorded were established as part of this research project. The quality of data and computer technology were given a high priority when new measurement methods were established. This is important for the implementation of this study and for the further use of these methods. Also, in order to utilize the data in estimation of EBVs and selection, automatic storage of data is important. For all traits recorded, a barcode was used for identification of the meat and fat samples, and the animal ID was scanned into a computer together with the measurements at the abattoirs working station, shown in Figure 5. This barcode was used as standard identification from the abattoir, and could therefore withstand handling a wet environment. Since all the different traits were connected to suitable software, the hour and time of recording was automatically stored and operator ID was connected to all the records. The data was first stored in a computer at the pilot scale abattoir, and was later submitted to another computer for automatic storage in an Oracle database at Norsvin (Figure 5). New tables in this database as well as programmes handling the various types of files with data, e.g. Excel and Html, were developed to handle the large amount of data produced for the new meat and fat quality traits in this thesis. Data stored in the Oracle database has a high degree of security. Developed SAS SQL's were used to access the Oracle database, which is efficient for the use of the data for, e.g. genetic analysis.

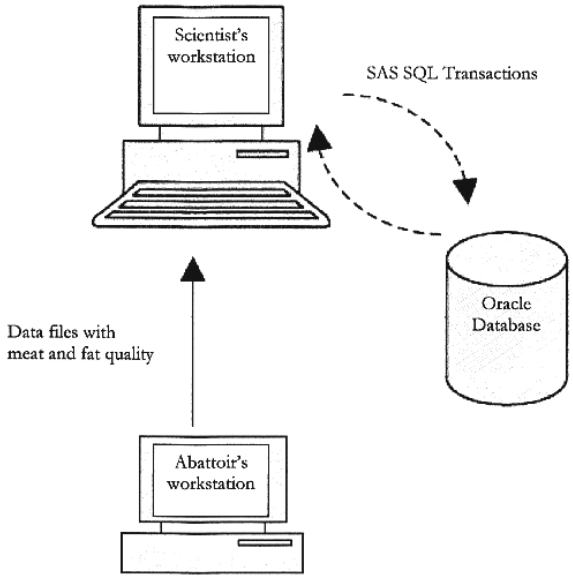

Figure 5 - The graphic shows the data flow.

Rapid methods and large-scale analyses

The measurements should be implemented at low costs, so rapid methods and large-scale analyses are needed. This is also important for the future use of fat and meat quality in commercial breeding programmes. The NIRS technology has a great potential for analysis of organic samples. Investment costs in high-throughput equipment are high, but their future use will generate small additional costs. The sample preparation used (e.g. homogenization of LD and melting of SF) and scanning through the samples (transmission/transflectance) requires more time than measurements with NIRS reflectance probes, which are available for both NIRS instruments used in this thesis: a FoodScan near infrared spectrophotometer and an XDS near infrared rapid content analyser. It was not a major issue in this study that the samples were homogenised and the higher accuracy has a large impact the genetic parameters, particularly the estimated heritabilities. The use of the microwave method to obtain the total lipids of the SF was rapid in comparison to fat extraction with solvents, and makes the method used for the NIRS-predicted traits environmentally friendly, safe and user-friendly. The methods used are also rapid and low cost compared with chemical methods, generally reducing costs by 80% to 90% when compared to traditional reference methods used for analyses at laboratories. The colour measurement of meat and fat is also fast and the version of Minolta used (CR400) had transfer data to Excel spreadsheet which was combined with barcode identification of the samples. The drip loss measurement with the EZ-DripLoss method uses simple but high quality equipment such as the circular knife and special containers to ensure equal treatment for all samples. This method is rapid compared

to the traditional bag method, and in this study the connection of a weight and a barcode scanner to a computer and a developed Excel spreadsheet helped make this recording even more efficient.

Ethical issues

Animal welfare has a high priority in Norsvin's test station and at the slaughterhouse. The approximate zero incidents of PSE and DFD meat confirm good routines for transport and treatment of pigs at slaughterhouses. The absence of chemical solvents for meat and fat quality analysis makes the methods environmentally friendly, and made it possible to conduct the analyses of meat and fat quality in a cutting plant with strict hygiene. The handling of pigs at the CT scanning follows strict procedures and each boar has single pens when they are anaesthesised. The radiation used in this study only has a slightly higher energy than that of the recommended upper levels for humans, so one scanning per lifetime for the pigs is completely unproblematic. The safety of the workers is taken into account as a radiation safe room was built for the CT scanning. The amount of radiation emitted from regular medical x-rays, such as the one used in this study, is lower than from older CT equipment.

Selection for meat, fat and carcass quality

The meat quality is of increasing importance for modern consumers. The antagonistic relationship between carcass quality and meat and fat quality has been focused on for many years (Frøystein, 1985), and a close cooperation between the breeding and meat industries have had a large impact on traits included in the breeding programmes (Hvamstad *et al*., 1978; Norsvin, 2009). Still, the relative weighting of carcass versus meat and fat quality has often gone in the direction of a higher lean meat content for Norwegian Landrace. To a high degree, this has been desired, and the change in carcass quality for this breed was successful from early on (see Figure 1 in the general introduction and Paper 4).

In Norway, Duroc has a much shorter selection history and different breeding goal compared with Landrace. A balanced selection for meat quality traits vs. LMP and ADG has preserved a characteristically high level of IMF and a good WHC for the breed as presented in Paper 1. Genetic changes from 2000 to 2008 was increased IMF by 0.3%, LMP by 4% and ADG2 by 45 g/day, while decreasing the FCR by 1.8 MJ/kg for pigs in test stations (Norsvin, 2009). The change in these traits is large, but an underlying change in muscle fibre types has never been documented. Still, the high weighting for IMF and ultimate pH in Duroc has retained some characteristics which make this breed different from the Norwegian Landrace in regard to meat and fat quality traits as presented in Papers 1 and 3. Larzul *et al*. (1997) found high heritabilities in the percentage for fibre types (I, IIA and IIB) and cross-sectional areas for the fibres, and ultimate pH were highly negative when correlated to type IIB fibre percentages, and the IMF was not related to any fibre type composition, but could be more related to the maturity of the pigs.

In this thesis, genetic parameters for meat, fat and carcass quality traits are analysed, and many traits seem to be suitable for breeding purposes. To be able to include these traits in breeding programmes, genetic parameters related to production and reproduction traits are needed. All traits with high genetic correlations to each other should be analysed in multi-trait analysis, and further optimisation work could determine the economic weight of the traits on the basis of the maximised total profit per slaughter pig per year. Since no production traits were analysed with the meat quality traits in Paper 1 and only the LMP was included in the genetic analysis of fat quality traits in Paper 3, further analyses are needed before any recommendations can be given for the new traits in this thesis. New multi-trait analyses will provide the genetic correlations between production, carcass, meat and fat quality traits.

Estimated genetic correlations for ADG2, FCR, CT-LMP, IMF, EZ-DripLoss, C18:1n-9 and C18:2n-6 are presented in Table 1 (Gjerlaug-Enger, 2011, unpublished). The heritabilities for these traits are presented in Papers 1, 3 and 4, and other traits are not included due to computational constraints with large multi-trait analyses. For example, MG is left out due to linear dependence to other traits as LMP and ADG.

Table 1 - Genetic correlations between production, carcass, meat and fat quality traits.

Landrace		**ADG2**	**FCR**	**CT-LMP**	**NIRS-IMF**	**EZ-DripLoss**	**C18:1n-9**	**C18:2n-6**
N	*Desired direction*	*increasing*	*decreasing*	*increasing*	*increasing*	*decreasing*	*increasing*	*decreasing*
24840	**ADG2**	~						
12788	**FCR**	*-0.61*	~					
4525	**CT-LMP**	**-0.57**	0.05	~				
6565	**IMF**	*0.21*	**0.18**	**-0.72**	~			
6565	**EZ-DripLoss**	0.10	**-0.29**	**0.33**	*-0.40*	~		
3220	**C18:1n-9**	-0.11	**0.21**	-0.09	*0.14*	*-0.23*	~	
3220	**C18:2n-6**	0.04	**-0.45**	**0.57**	*-0.47*	*0.28*	*-0.52*	~
Duroc								
16294	**ADG2**	~						
9626	**FCR**	*-0.39*	~					
3624	**CT-LMP**	**-0.39**	*-0.38*	~				
7213	**IMF**	*0.18*	**0.34**	**-0.82**	~			
4345	**EZ-DripLoss**	-0.01	**-0.27**	**0.32**	*-0.39*	~		
1764	**C18:1n-9**	**-0.28**	**0.33**	0.03	0.06	0.05	~	
1764	**C18:2n-6**	*-0.27*	**-0.26**	**0.37**	*-0.37*	-0.02	*-0.27*	~

Bold number = unfavourable genetic correlation; Cursive number = favourable genetic correlation; No formatting = no significant correlation

Among the production and carcass traits studied here, the ADG2, FCR and CT-LMP were unfavourably correlated to most of the meat and fat quality traits in Table 1. The highest unfavourable genetic correlation was estimated between CT-LMP and NIRS-IMF. The average literature values of the genetic correlations between LMP and IMF (Sellier, 1998) were lower than our results. The LMP was also unfavourably genetically correlated to EZ-DripLoss and C18:2n-6. The genetic correlations between LMP and EZ-DripLoss are in agreement with previous studies by Knap *et al*. (1997), Sonesson *et al*. (1998) and Hermesch *et al*. (2000). Gjerlaug-Enger *et al*. (2010) found high positive genetic correlations between the moisture content in meat and CT-LMP, and the opposite correlations to NIRS-IMF as

reported in Table 1. This is supported by Lo *et al*. (1992) and Cameron *et al*. (1990), and indicates some changes in muscle fibre characteristics from the selection on LMP.

In general, the selection for increased leanness yields more PUFAs and less SFAs in the SF. In relation to soft lard and oxidative stability, the modern pig shows a change in fatty acid composition (Rosenvold and Andersen, 2003). This is in agreement with literature studies comparing the leanness and fatty acid composition of slaughter pigs (Fernandez *et al*., 2003; Sellier *et al*., 2010). The MUFAs have intermediate values, less correlated to the LMP in both Landrace and Duroc (Table 1 and Paper 3).

The genetic correlation between production traits (ADG2 and FCR) and meat and fat quality traits was generally low-to-medium high. Low favourable genetic correlations between ADG2 and NIRS-IMF are found for both breeds, which is supported by Lo *et al*. (1992) and Kadarmideen *et al*. (2004), although opposite low unfavourable genetic correlations are also reported (Sonesson *et al*., 1998; Hermesch *et al*., 2000), which are an indication of no clear genetic relationship between the growth rate and meat quality traits. The other correlations between ADG2 and meat and fat quality traits are small, and are not consistent for the two breeds. Sellier *et al*. (2010) also found the ADG to be low in terms of its genetic correlation to fatty acid composition supporting our results. Generally speaking, a decreased FCR is unfavourable for all of the meat and fat quality traits presented in Table 1, which is in agreement with the genetic meat quality parameters reported by Hermesch *et al*. (2000), Kadarmideen *et al*. (2004) and Gilbert *et al*. (2007). To the best of the author's knowledge, genetic correlations between fat quality and FCR are not available in the literature.

The results from Table 1 have twice the amount of data for IMF and EZ-DripLoss as presented in Paper 1, and size of the correlations between these traits are the same as in Paper 1. Negative genetic correlations between IMF and DRIP were also reported by de Vries *et al*. (1994) and van Wijk *et al*. (2005).

In Table 1, the genetic correlations between meat and fat quality traits are presented, though such parameters have not been previously presented in this thesis. Except for one correlation which was not significant different from zero, all the genetic correlations between meat and fat quality trait were favourable. This is supported by Sellier *et al*. (2010), with high positive genetic correlations between the percentage lipids in SF and LD, and low-to-medium positive genetic correlations between the percentage moisture in SF and LD.

In order to have a sustainable breeding for carcass and production traits, the meat and fat quality must be taken into consideration. Many meat and fat quality traits measured with rapid large-scale methods demonstrate a high genetic variation that makes it possible to select for such traits in pig breeding programmes.

Potential candidate traits for pig breeding programmes

The EZ-DripLoss in meat

Estimated heritabilities for EZ-DripLoss (Figure 6) were higher than ultimate pH in LD muscle, and are a direct trait of the WHC in the meat. The pH in meat is a challenging trait, as meat temperature has a large impact on the predicted pH (Bendall and Swatland, 1988; Karlsson and Rosenvold, 2002). In view of the results in Paper 1, the EZ-DripLoss trait is therefore expected to be more efficient in pig breeding programmes. EZ-DripLoss is favourable correlated with IMF, and is an important trait for the consumers' perception of meat appearance, texture and juiciness (Bryhni *et al*., 2003).

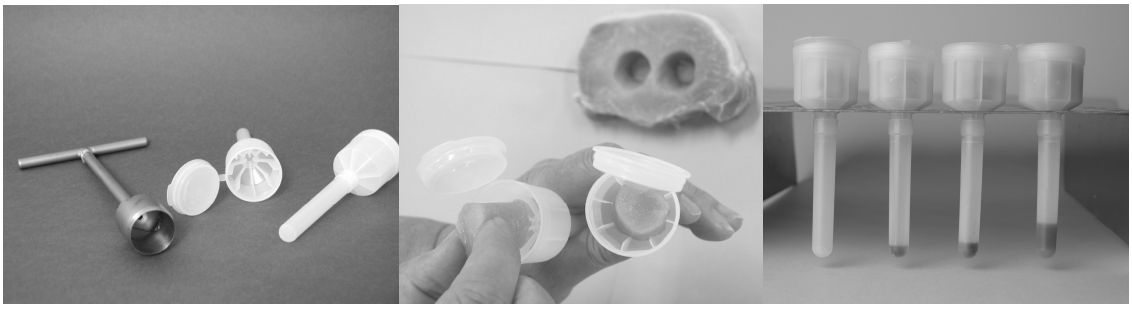

Figure 6 - The EZ-DripLoss method, which was developed at the Danish Meat Research Institute, is used for the determination of drip loss in LD muscle. Photos: Danish Meat Research Institute.

Drip loss occurs at abattoirs, cutting plants, food industry units and in consumers' kitchens, and the economic value of 1% reduction in drip loss is substantial. The kilos of pig carcasses produced in Norway is 124 million and with an LMP of 59.5% (Animalia, 2009). A 1% increase in WHC yields 738 tons of extra meat. The values for the meat are highly variable within the value chain, hence an estimate has not been calculated.

Previously, WHC was only an important trait for fresh meat. For processed meat, additives could improve the technological quality of the meat. It is expected that that the consumer will not accept additives as phosphate and salt, and the modern meat industry has a high requirement for meat quality.

The EZ-DripLoss trait was implemented in the breeding goal for Norwegian Landrace and Duroc in 2010, and the optimisation work has given the trait a relatively high value.

The EZ-DripLoss is a direct, rapid and low labour-intensive method in comparison with other traits that measure WHC or drip loss from meat and is therefore associated with low costs; in real terms, this amounts to NOK 40 per animal. Depreciation costs are low for this trait.

The a* value in meat

We already know that consumers do not prefer to pale meat (Risvik, 1994; Bryhni *et al*., 2003). As a correlated trait to the content of myoglobin and some hemoglobin in the muscle, the a* value is more important (Figure 7). Oksbjerg *et al*. (2004) fond a high genetic correlation (0.59) between pigment and the a* value in pig meat. These organic molecules contain iron, which is a mineral important to human nutrition (Egelandsdal and Haug, 2010).

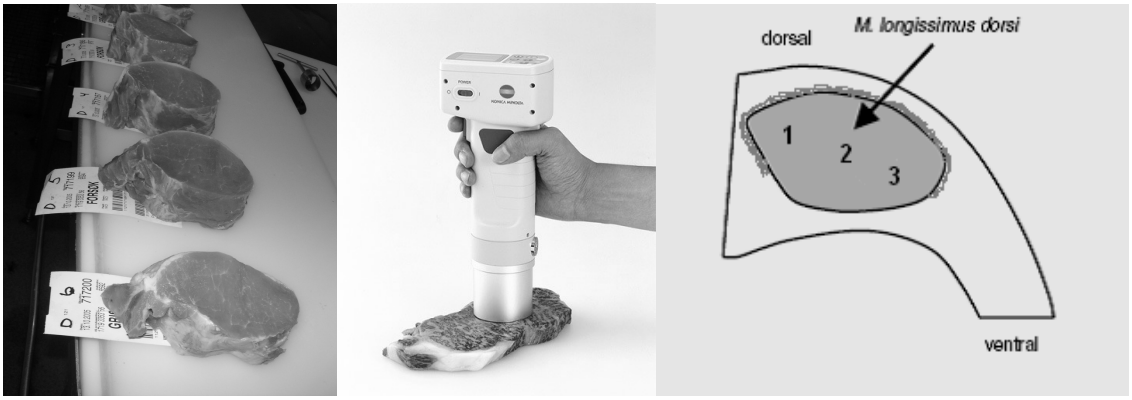

Figure 7 - The meat colour was measured using (2) a Minolta Chroma Meter CR-400 on (1) bloomed chops of LD muscles. (3) Measuring positions, with duplicate measurement in three positions.
Photos: (2) www.konicaminolta.com, (1, 3) Gjerlaug-Enger.

Worldwide, iron deficiency, which in a morbid condition is called anaemia, is considered to be one of the main nutritional deficiency disorders (Lopez *et al*., 2002; www.weightloss.com.au). At the opposite end of the scale, iron excess also creates health problems, e.g. an increased risk of colon cancer (as reviewed by Lopez *et al*., 2002), with a varied diet being the ultimate solution to iron deficiency (Hunt, 2005; Egelandsdal and Haug, 2010). The heme iron from meat, particularly red meat, is of higher value than the non-heme iron found in plant foods (30% down to 1-10%, respectively) (Lopez and Martos, 2004; Walczyk and Blanckenburg, 2005).

As a result, the redness in meat could therefore have some potential in pig breeding programmes, but to the best of the author's knowledge, it is not commonly included in breeding goals. In Paper 1, this trait

had medium heritability and did not exhibit any large unwanted correlations to other meat quality traits in Norwegian Landrace and Duroc pigs.

The colour measurement was done using just an ordinary colour measurement with a Minolta instrument. The automatic transmission of data to a computer reduces the time for analysis and NOK 25 per animal is the variable cost. With large-scale measurements, the depreciation costs are low.

The intramuscular fat

IMF content is an important factor in relation to eating quality, including tenderness, juiciness and flavour, and 2-3% IMF is recommended for optimal eating quality (Bejerholm and Barton-Gade, 1986; Fortin *et al*., 2005). Estimated heritabilities of 0.40 to 0.61 for chemical IMF and NIRS-IMF (Figure 8), respectively, presented in Paper 1 made it logical to replace chemically analysed IMF (lipid extraction) with NIRS-IMF in the breeding programme for Norwegian Duroc in 2006. The size of these heritabilities has been shown to be at these levels when analysing these traits for extended data series over longer time periods. In 2007, the NIRS-IMF was implemented as a new trait in the breeding programme for Norwegian Landrace. Despite a low SD for IMF for Landrace, the relatively high heritability made this possible.

On the basis of the results in Paper 1 and the experience of the use of NIRS-IMF in practical breeding programmes for Norwegian breeds, NIRS-IMF seems to work better than the reference method for chemically analysed IMF. The author's recommendations are that NIRS-IMF determination should be further validated, and perhaps replace lipid extraction as reference method for IMF. Consequently, the predicted level of IMF from real-time ultrasound, CT and marbling scores, etc. should all be evaluated to the NIRS-IMF, and not to the lipid extractions.

Figure 8 - (1) The FOSS FoodScan near-infrared spectrophotometer (FOSS, Denmark) used for the determination of fat, moisture and protein in (2) the LD muscle. (3) Approximately 180 g of ground samples were placed in a 140mm round sample dish, and the dish was placed in the NIRS instrument. A barcode was used for identification purposes. Photos: Gjerlaug-Enger and Kringberg.

NIRS-IMF has a low cost in large-scale measurements. The price for NIRS-IMF is NOK 45 per animal, while the price for chemical analysis is NOK 439 per animal (SBR analysis at eurofins.no 2010). In addition, the cost of depreciation for the instrument also needs to be taken into consideration. The depreciation cost for the instrument for Norsvin, who are testing 2 - 3000 animals per year and have 10 years of depreciation time, this yields a cost per sample analysed at NOK 15 - 23 per animal.

The moisture and protein content in meat

The same NIRS analysis, which was used for NIRS-IMF, is also used for the prediction of muscle moisture and protein content. These two parameters are discussed in Paper 1. Moisture is the largest component in meat, and drip loss in not favourable for meat quality, but the moisture level itself, to the author's knowledge, has not been tested in any sensory analysis. It is indisputable that water is inexpensive to produce. Meat protein is considered as a high quality protein for human nutrition since it contains all the essential amino acids in the appropriate amounts. Even so, the small amount of knowledge that exists about the optimal level of muscle moisture and protein content in pig meat quality makes these traits less suitable as traits in breeding programmes.

The moisture in subcutaneous fat

The NIRS-predicted moisture content in SF (Figure 9) could be a potential candidate trait for pig breeding programmes. The results in Paper 3 document that this trait will improve the technological quality of fat, which is supported by the genetic parameters presented by Cameron (1990). The method described in Papers 2 and 3 is low labour-intensive and the traits show a medium heritability. The largest problem here is the unfavourable genetic correlation to LMP, so the indirect cost will be a reduction in the progress of LMP.

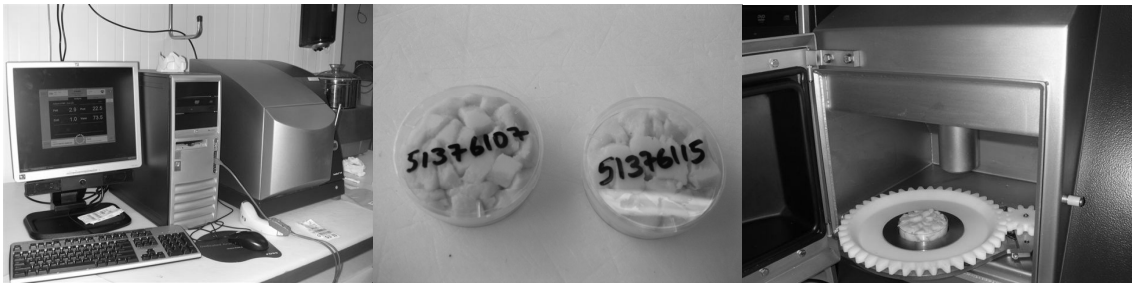

Figure 9 - (1 and 3) The FoodScan near-infrared spectrophotometer (FOSS, Denmark) was used to determine the fat moisture content in (2) subcutaneous fat samples. Photos: Gjerlaug-Enger.

The variable costs with the NIRS-predicted moisture content are about 10% of the cost of the reference method for moisture content. The NIRS-predicted moisture content in fat costs NOK 35, while the moisture analysis for the laboratory costs NOK 304 (Moisture in food analysis (reference method in Paper 2) at eurofins.no 2010). Depreciation cost per sample is equal to NIRS-IMF.

The C18:1n-9 and C18:2-6 fatty acids

Studies from Spain and Switzerland (de Pedro *et al*., 1992; Schwörer *et al*., 1999; Garcia-Olmo *et al*., 2001) used NIRS for the prediction of fatty acids (Figure 10), and this thesis has shown that the fatty acid composition could be estimated using NIRS technology. The XDS near-infrared rapid content analyser had never been used for fatty acid analysis (Jøns pers. comm.), although the spectral range of this instrument should be suitable for fatty acid analysis. The FT-IR analyses and Raman technology are also possible methods for the determination of a fatty acid profile, and were also discussed as alternative methods.

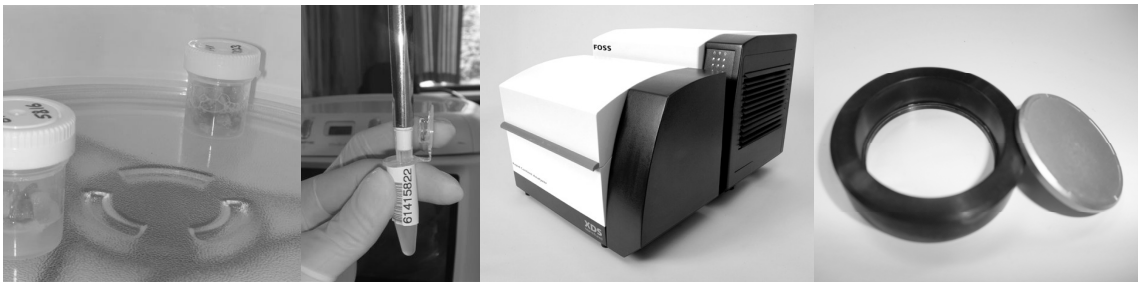

Figure 10 - (1) A microwave fat melting technique was used for the samples preparation. (2) Total lipid was added to Eppendorf tubes with a pipette. (3) The XDS near-infrared rapid content analyser. (4) Scanned once using a circular quartz cuvette and a gold plated reflector provides optical path lengths of 0.2 mm. Photos: (1, 2, 4) Gjerlaug-Enger, (3) www.foss.com.

In view of the genetic correlations estimated in Paper 3, a selection for an increased level of C18:1n-9 is possible since this fatty acid has only a slightly negative correlation to LMP, while C18:2:n-6 is highly correlated with LMP, and can be more easily manipulated by feeding (Enser *et al*., 2000; Tikk *et al*., 2007). All the fatty acids traits showed significant genetic variation, except the ratio of n-6 to n-3 fatty acids, which is not possible to change with traditional breeding. The fat level can influence the ratio of n-6 to n-3 fatty acids, due to the difference of this ratio in polar and neutral lipids (de Smet *et al*., 2004), which might be the explanation of the heritability (0.06) for the ratio of n-6 to n-3 fatty acids at Landrace. In Duroc, this ratio was approximately zero (0.01). This might also explain the correlation between this ration and LMP in Paper 3 for Landrace, but these effects are small compared to dietary effects (de Smet *et al*., 2004; Hallenstvedt, 2011).

This NIRS analysis was terminated in 2008 and the data has only been used in this thesis, but a reintroduction of this analysis is possible. The variable costs with NIRS-predicted acid composition are less than 10% of the cost for a GC analysis. The NIRS-predicted fatty acid composition costs NOK 110, while a GC analysis costs NOK 1392 in (GC at eurofins.no 2010). In addition, the cost of depreciation of the instrument must be taken into account. Norsvin, who has been testing 2 - 3000 animals per year, with 10 years of depreciation time, gives a depreciation cost per sample of NOK 16 - 24 per animal.

The fat colour

Measurements of fat colour (L*, a*, b* values) is a rapid and technically simple method for improving technological fat quality, and does not require any additional calibration work with complicated statistics or reference analysis for SF moisture or SF fatty acid contents. It is therefore also easy to start to measure fat colour, although due to the genetic parameters from Paper 3, selection against fat colour will probably be less efficient than the selection for moisture content or fatty acids. The cost per analysis is low at ca. NOK 10 per animal.

The MG, FG, BG and NCG from CT data

CT technology has the unique ability to measure body composition in live animals. The CT data used in Paper 4 originates from the ordinary testing of the boar at station with the CT scanning of all tested boars. The modern x-ray CT (GE multi-slice VCT LightSpeed scanner) takes 1100 images of a pig. The scanning of 3500 animals per year is done in three half days per week. On average, six pigs are tested per hour. The CT images require a large storage capacity (3.8 million new images per year), which holds a great importance for further research. The modern testing station is also efficient with respect to daily management, in addition to the activities related to boar testing, e.g. feed recordings.

LMP was the first trait from the CT data used in the breeding programme for Norwegian breeds. As discussed in Paper 4, there are some problems in relation to this trait. The LMP trait has been shown to be highly unfavourable correlated to ADG2 for both Landrace and Duroc. These two breeds have large differences in their selection history and physiology, so this problem may be common for other breeds as well. In addition, LMP is not a good trait for carcass quality, since a small carcass with high LMP has lower value than the same LMP on a large carcass.

Some experiments have been carried out to find a model for muscle mass, but since all animals are CT scanned at the same weight at approximately 100 kg, all weight traits are equal to percentage traits. An alternative would have been to CT scan all animals at the same age, but this is less relevant from an

industrial standpoint. We have used a simple trait definition: kg meat/age (MG) as described in Paper 4, which is the same trait as LMG in the literature. What makes the CT trait special is the actual measurement of the muscle on live pigs, while other LMGs are based on ultrasound measurements or the dissection of carcasses. MG seems to be a suitable trait for carcass quality and slaughter pig efficiency, as well as being a biologically robust trait. MG is favourable correlated to FG, which simplifies the breeding. In cases which LMP and ADG2 are optimally combined in a total merit index, the results are almost the same as selection for MG. Since MG is a compound trait of ADG and LMP, MG is expected to be less unfavourable correlated to meat and fat quality traits than LMP. This is supported in a study by Sellier *et al*. (2010) there both LMP and LMG were evaluated towards a number of meat and fat quality traits.

The author recommends using MG in the breeding goal with an economic weight for both Landrace and Duroc. The size of the economic weight should reflect the weight of the LMP and ADG2 anno 2010, see Figure 4 in the general introduction. For Duroc, this can be considered as an economic weight in the reduction of FG as well. Due to the high leanness in Landrace, the FG should be weighted towards a zero genetic change for this breed. The BG and NCG should also be included due to their importance for the functionality of the pigs, and both traits should be weighted to a zero genetic change, or a small increase as an indirect response from the selection for MG.

Which traits are not recommended?

The L* value in meat

This thesis has demonstrated the possibilities for further development in the recording of new traits in pig breeding. Nevertheless, there are problematic issues for a couple of well-established traits often used in breeding programmes. In Paper 1, there are some problems with the L* value in meat. This trait is comparable to the reflectance measured with the photoelectric instrument or the HGP2 instrument, both previously used for the selection of Norwegian breeds. In 2005, the reflectance was taken out of the breeding goal for the Norwegian breeds due to the unfavourable genetic correlation between HGP2 reflectance and IMF in Duroc. This is also shown by our results in Paper 1, in which the L* value was positively correlated to IMF in Duroc. This was not expected in view of the morphological and biochemical characteristics of the different muscle fibres. A high level of IMF is expected in muscles with a high number of oxidative muscle fibres, such as type I (slow-twitch red fibres), which have many mitochondria, a high fat metabolism, a higher ultimate pH and WHC and a darker red colour in the meat. On the other side, glycolytic muscle fibres, such as type IIB (fast-twitch white fibres), are low in IMF and energy metabolism based on glycogen, thereby producing lactate which gives a lower ultimate pH, WHC and a lighter colour postmortem (Karlsson *et al*., 1993; Karlsson *et al*., 1999; Choi *et al*., 2009; Lee *et al*., 2010). The positive genetic correlation between L* value and IMF in Duroc more than likely comes from visible fat cells detected at the colour measurement, and not from the meat colour itself. In Landrace, a breed with a low IMF level, we do not see this relationship. To the best of the author's knowledge, this is not discussed in the literature. Still, we find this to be reasonable, and this causes the reflectance and L* value in meat to be of low value for pig breeding, especially for breeds with high levels of IMF.

In general, the skeletal muscles of modern pig breeds contain a higher proportion of type IIB fibres in relation to type I fibres than observed in wild pigs (which was reviewed in two papers: Choi *et al*., 2009; Karlsson *et al*., 2009). This is most likely a consequence of the selection for improved carcass quality and lean meat content. For selection against this change in fibre types to ensure a better meat quality, the author recommends a selection for ultimate pH, and particular WHC and drip loss as an alternative to L* value and reflectance.

The growth from 25 to 100 kg live weight

ADG2 is one of the most commonly used traits in pig breeding in combination with BF (Clutter and Brascamp 1998), and ADG2 and LMP/BF are well-established parameters for Pig Breeders'. These two parameters are unfavourably correlated and the selection for these parameters is not straightforward. From the study on the growth of different body tissues from CT data in Paper 4, the genetic correlations between these traits and more traditional traits used in pig breeding programmes are investigated. The correlation between MG and ADG2 was low for both Landrace and Duroc. This makes ADG2 a problematic trait for selection when the goal is to optimise the MG, as ADG2 also showed very high genetic correlations to FG and NCG. However, ADG2 is important for a fast turnover in slaughter pig production, in addition to appetite and fitness, although the favourable effect of ADG2 on appetite and fitness is the same as for MG. Pigs with high MG have the desired carcass quality, and there is a great potential for the use of CT data with direct selection for MG, FG, BG and NCG. Each breed needs specially designed economic weight in the breeding goal, depending on the desired change in the animals and the correlations to the other traits. In view of the genetic parameters in Paper 4, the author recommends selection for MG as an alternative to ADG2 and LMP in the breeding programmes for both Norwegian Landrace and Duroc.

The quality of data and the estimated variance components

Generally speaking, the measuring methods, statistical models, data size and structure, genetic connectedness and the number of generations included in these analyses affect the estimated genetic parameters. This study has demonstrated that it is possible to estimate genetic variances of a high magnitude for traits measured with rapid methods. The amount of data utilised in this thesis is relatively large compared to many similar studies, and gave small standard errors for the estimates.

The genetic parameters for new traits include an average of 2.6 generations for Papers 1 and 3 and 1.7 generations on average in Paper 4. The genetic connectedness is good, since an artificial insemination rate of 100% has been used with elite semen boars, and all boars were tested at the same test station. The use of operative testing facilities makes the estimated parameters reliable for the Norwegian Landrace and Duroc populations.

For the use of rapid methods, especially for NIRS, it is highly recommended to monitor the analytical methods. This is often done by use of a reference method for analysing a few samples at regular intervals. Our experience with this is that the FoodScan instrument is stable. Nonetheless, the variation between laboratories is large, and the level in the reference method has often shifted when the laboratories make methodical changes. Hence, we chose not to correct for the bias between the reference method (Soxhlet) and the predicted NIRS-IMF, as well as for the newest reference method (SBR) used, as the level of the tested samples was equal to our NIRS-IMF values.

Problems were experienced with the stability of the XDS instrument. This problem was not related to the prediction of fatty acid percentages, but to spectral information in a wavelength region which was not used in the analysis, though we did not find the reason for this problem. For further use, we will have to check the predicted values carefully since other studies have reported problems with the stability over time of similar methods, as discussed in Paper 2.

An appropriate model including contemporary group effects will correct the data for problems as shifts or trends related to the environment and measuring methods. The time of recording is therefore of great importance. A good model would also correct for much instrument- and operator-related error variance, thereby giving higher estimated heritabilities.

Recommendations for further research

Determination of IMF in live animals

In the period from 2008 to 2010 from the CT scanning was started, there has been a difference in the timing of the data recorded for the two traits, the NIRS-IMF and LMP from CT data. The same phenomenon related to timing as was seen between chemically analysed IMF and LMP from dissections taken before 2006. Different time for measurements is not optimal, especially not when the traits are unfavourable correlated. Therefore, the IMF measured in live animals have a high values in combination with CT-LMP.

The idea of IMF measured using CT was described long ago, but R^2 values between 0.18 and 0.30 were below the acceptable levels for practical use (Vangen and Enting, 1990). In later studies, simple linear regressions of IMF on muscle density from CT gave on average an R^2 value of 0.36 with live sheep (Lambe *et al*., 2010), which ranged from 0.30 to 0.44 in cattle (Navajas *et al*., 2009). Multiple linear regression models for the average densities of pixels allocated as muscle density and fat density in cross-sectional scans, as well as the standard deviations of pixel density values, gave an R^2 value of 0.48 (Lambe *et al*., 2010). The accuracy in these studies is not satisfactory, thus new statistical methods are needed. A multivariate calibration method (PLS regression) was used to predict IMF in beef cuts using muscle and fat CT densities. All CT values were used, and the statistics were similar to the equations made for NIRS data in Paper 2 of this thesis. A much higher R^2 was estimated, ranging from 0.71 to 0.76 for these beef cuts (Prieto *et al*., 2010). The R^2 is highly related to the SD in the data, and the pigs, particularly Landrace, had a smaller SD for the level of IMF than cattle (Paper 1). Figure 11 (1) shows the kind of CT image we would like to use for the calibration of IMF in the muscles.

In addition, IMF can be predicted from ultrasound measurements, and a study of lean cattle gave 0.80 for the R^2_{Val} (Aass *et al*., 2009), though a similar study of Duroc did not give an R^2 higher than 0.32 (Newcom *et al*., 2002). Currently, ultrasound measurements are collected in the nucleus herds (off-test of sows) and the CT measurements are collected at the boar test station (sons of the off-tested sows). Figure 11 (2) shows an ultrasound image suitable for the calibration of IMF in live pigs.

These methods will not have the same level of accuracy as the NIRS-IMF described in Paper 1, but a predicted IMF value of the selection candidates is still of high value in a selection programme. Pigs are

routinely sampled using CT and ultrasound scanning in Norsvin, so there is a great potential using image analysis and multivariate calibration. The NIRS-IMF from Paper 1 would be a good reference method. A major challenge will be associated with an in vivo measurement, since the image quality can be reduced if the animals are not completely still.

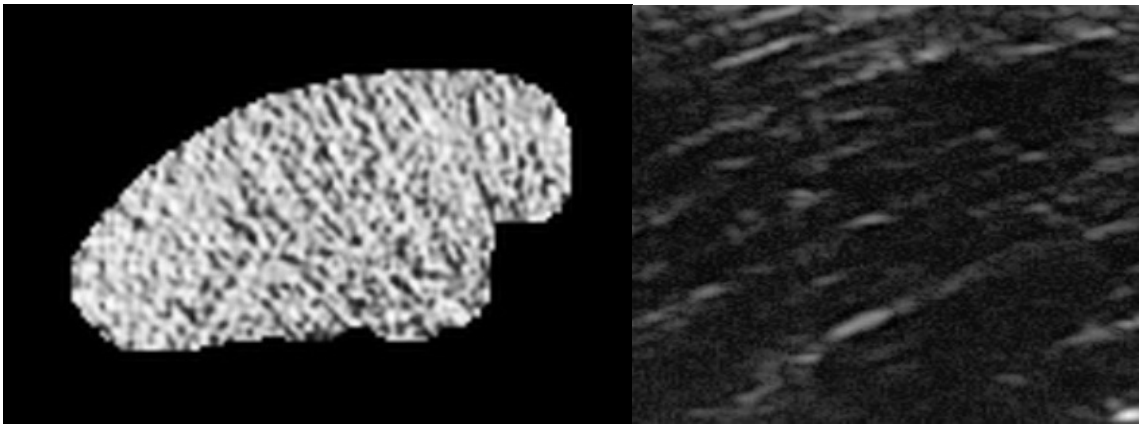

Figure 11 - (1) CT image and (2) ultrasound image, both of which show structures in the muscle and perhaps IMF. Photos: Kongsro, 2010.

Improved fatty acid composition

On average, the daily Western diet contains approximately three to four times more C18:2n-6 than the recommended level, and almost two times less than that recommended for C18:3n-3 (Simopoulos, 2000). The C18:2n-6 can be convened to C20:4n-6 (AA), and the conversion of C20:4n-6 in the body to n-6 prostaglandin and n-6 leukotriene hormones provides many targets for the biological mechanism and an excessive n-6 fatty acid intake can cause atherosclerosis, asthma, arthritis, vascular disease, thrombosis, immune-inflammatory processes, and tumour proliferation (Simopoulos, 1999; Simopoulos 2003; Haug *et al*., 2010). The n-6 to n-3 ratio in the Western diet is 10:1, which is almost five times the recommended ratio (Simopoulos, 2000). In pig fat, this ratio is also unfortunately high, often 8:1 to 12:1. This n-6 to n-3 ratio for the fat tissues of pigs is directly influenced by the fat composition of their diet (Øverland *et al*., 1996; Enser *et al*., 2000; Bryhni *et al*., 2002).

The nutritional research has demonstrated that it is possible to increase the use of fish silage in the feed for pigs (Hallenstvedt, 2011), although the use as to be restricted to maintain a high sensoric quality (Øverland *et al*., 1996; Bryhni *et al*., 2002; Hallenstvedt, 2011). The C18:3n-3 for plant oils is of particular importance since these oils in small volumes can change the ratio of n-6 to n-3 without harming the product's quality (Tikk *et al*., 2007; Beaulieu *et al*., 2009). For examples linseed and canola oil has a high content of C18:3n-3 and C18:1n-9 fatty acid.

Soya meal is an important source of protein in conventional concentrate feed given to pigs all over the world. In Nordic countries, soya imports increased when bone meal was forbidden, and soya meal displaced the volume of locally produced grain since more soya than bone meal is needed to achieve the same percentage of protein. Additionally, some soya oil or other oils high in n-6 fatty acid are often added to the feed to increase the concentration of energy. In Sweden, both the slaughter and animal feed industries have stressed these problems in relation to soybean for more than 10 years, but high pressure on the price of pig meat in the EU has made it impossible to implement any action in regard to soya. However, Linseed and canola oil can be grown in high volumes in Nordic countries, which is important to help increase self-sufficiency and the local production of food. In Norway, the pig industry has not dealt with this problem.

So why is this of interest for further research on fat quality? It is possible to measure fatty acid composition with the NIRS technology presented in this thesis (Paper 2). The fatty acids were moderate-to-highly heritable, but it is not possible to change the ratio of n-6 to n-3 fatty acids with traditional breeding (Paper 3). The SF in modern pigs eating commercial concentrates has too high content of C18:2n-6 and the ratio of n-6 to n-3 are unfavourable, which has primarily been caused by soy. If soya can be replaced with other protein feed stuff, e.g. linseed and canola seed, the ratio of n-6 to n-3 can be favourable for human health, but might be unfavourable for the technological fat quality. Breeding for more C18:1n-9, this will be positive for both human health and technological fat quality. A n-6 to n-3 ratio of ca. 2:1 is desired in order to make pig meat neutral in relation to the recommended n-6 to n-3 ratio (Simopoulos, 2000), and this is possible to achieve if the pigs are fed a concentrate containing 3-4% canola oil and 1-2% linseed oil (Egelandsdal and Haug, 2010), or whole canola seeds as a alternative lower in price. Based on results from paper 3, the NIRS technology allows us to breed for more C18:1n-9 and also perhaps less C18:2n-6. If the n-6 fatty acids are reduced and the n-9 fatty acids are increased more then the n-3 fatty acids, the iodine number decreases and a high-quality pig meat is possible to produce.

General Conclusions

The main conclusions from this study are:

- Development of rapid methods for the determination of meat and fat quality traits that demonstrate a high accuracy and reasonable genetic variance in many of the parameters studied.

- Meat, fat and carcass quality traits measured with rapid methods that show biologically sensible genetic correlations to each other and some new discoveries.

- The proposed rapid large-scale methods for meat and fat quality traits have ~80-90% reduction in costs compared to traditional laboratory methods.

- CT technology used to measure the growth of different tissues in live boars at a testing station that exhibits a high accuracy of EBVs for the selection candidates for the CT traits: growth rate of muscle, carcass fat, bone, and non-carcass.

The main recommendations for the development of pig breeding goals:

- The recommended meat and fat quality characteristics measured with rapid methods are NIRS-IMF, EZ-DripLoss, a* value in meat and NIRS-predicted moisture and fatty acids in subcutaneous fat.

- The selection for L* value and reflectance in meat seems to be discouraging, due to cofounding with marbling /IMF.

- The data from CT scanning makes it possible to control the growth of muscle, carcass fat, bone, and non-carcass components in a better way than traditional traits such as lean meat and dressing percentages.

- The growth rate in the slaughter pig period from 25 to 100 kg are highly unfavourable correlated to fat growth, and less correlated to muscle growth as calculated from CT data.

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