FAIM

Farm Animal IMaging

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COPENHAGEN 2014

C. Maltin, C. Craigie and L. Bünger

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Contents

Introduction from the editors

The COST Action FAIM is focused on *Optimising and standardising non-destructive imaging and spectroscopic methods to improve the determination of body composition and meat quality in farm animals.*

This book is mainly a report of the third annual conference of the COST Action FA1102: FAIM which was held in Copenhagen in Denmark in September 2014. The major elements in the book are papers and posters, which were presented at the FAIM III meeting.

The book also contains reports on the meeting itself and the very interesting visit that the MC 40 management committee had to the Danish Crown factory at Horsens. Other activities during the year such as the training school held in Edinburgh, Scotland are also included.

The book also contains reports on some of the key tasks for the work groups. For work group 1 there is a report on a discussion on the use of Computed Tomography scanning for pig classification that was held at the FAIM III conference. For work group 2 there is a preliminary report of a survey to determine the range of reference methods used to determine meat quality parameters.

We hope that you enjoy this book, and feel inspired to come and join us in FAIM.

To join please contact the action Chairman, Professor Lutz Bünger using the contact details below or via the website **www.cost-faim.eu.**

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Finally, on behalf of all FAIM participants, we wish to thank COST for funding the COST Action FA1102 (FAIM) and related activities undertaken in 2013-2014.

Charlotte Maltin – Biomics Ltd, Scotland;
Cameron Craigie – AgResearch, New Zealand;
and Lutz Bünger – Scotland's Rural College, Scotland.
Editors.

Figure 1. Members of the FAIM management committee prepare to visit an abattoir in Greece

Figure 2. The FAIM III conference - Denmark 2014

FAIM III
Copenhagen meeting report

Charlotte Maltin

The COST FAIM III conference for 2014 was held in Denmark and was organised by Marchen Hviid and Bjarne Ersbøll and hosted by the Danish Meat Research Institute, the Technical University of Denmark and sponsored by Carometec.

Figure 3. The Danish Meat Research Institute

Prior to the start of the conference the MC 40 management group visited the Danish Crown abattoir at Horsens. Danish Crown is a very large International business, it is the largest meat processing company in Europe, and the worlds largest exporter of pork.

The slaughter house at Horsens was built in 2004 and is the largest pig abattoir in Europe, killing around 100,000 pigs per week and employs around 1,300 people. The abattoir is very open and welcomes visitors to view the whole process from the arrival of the pigs in the lairage, to the final packaging of the product from a cleverly constructed viewing gallery that runs throughout the plant.

The MC 40 management group were treated to an excellent lunch courtesy of Danish Crown, and then after an introduction to the whole business, were taken on a very interesting tour of the factory via the well laid out visitors' gallery viewing windows.

The speed and efficiency of the processing was impressive. The factory uses modern technologies and robotics to ensure fast and hygienic processing and works round the clock with two 9 hour slaughter shifts and a 6 hour cleaning shift. Everyone on the tour was very impressed with the high standard, slick operation of the factory, and how the company makes sure that every part of the pig is used.

Figure 4. Viewing window at Danish Crown, Horsens, Denmark

The scientific part of the conference was kindly hosted by the Danish Meat Research Institute (DMRI) in their new facilities. The DMRI employs around 110 people and works closely with industry and provides the essential link between technological innovation and industry uptake; introducing both technical knowhow and new ways of innovating to solve the challenges faced in food production.

The Institute has a special strength in slaughter technologies, automation and measurement systems, food safety and quality in relation to meat, and has an excellent range of laboratories and equipment which help DMRI to maintain its position of excellence.

The new building in which the conference was held was opened this year and provided an excellent venue for the meeting which was held over two days. The 94 participants came from 21 countries (table 1).

Figure 5. The Autoform from Carometec

The conference reception was kindly sponsored by Carometec who also brought their ultrasound image analysis based equipment Autofom. This equipment is used on line in Danish Crown and other pig abattoirs worldwide to automatically give an accurate prediction of the commercial value of pig carcasses.

Throughout the conference, the hosts DMRI and sponsors Carometec made sure that the delegates maintained body composition by providing an excellent spread of the best of Danish food and drink for the FAIM participants. We are most grateful to our hosts for keeping us so well fed and watered.

The main scientific areas covered at the conference were:

1. The valuation of body composition and meat quality in living animals; especially the challenges in large animals.
2. The benefits of using Computed Tomography as part of a breeding programme.
3. Reference methods for carcass classification and meat quality measurements.
4. Vision based classification of chicken carcasses
5. The use of spectral technologies to assess carcass quality and meat quality in raw and cooked products.
6. Image based robotic cutting of carcasses.
7. New methods for image segmentation and tissue quantification.
8. Data flow, and animal movement in relation to traceability.

Figure 6. Lunch!

Figure 7. On the meat quality tour

Country	Persons
Denmark	30
Norway	5
United Kingdom	12
Spain	6
France	6
Germany	6
Slovakia	3
Portugal	3
Sweden	4
Belgium	3
Ireland	1
Italy	1
Switzerland	1
Poland	1
Croatia	2
Australia	1
New Zealand	1
Greece	1
Lithuania	1
Slovenia	1
21	**94**

Table 1: Countries of participants

Figure 10. Gerard and Maria during the talks

Figure 8. FAIM's MC8 minus one

Figure 9. Flying into Copenhagen

Figure 11. Marchen and Lutz ready for the next presentation

Figure 12. Talking all day makes us hungry and thirsty

Figure 13. Jorgen defines meat quality

Figure 14. During the conference

Figure 15. Bjarne “WG3 knows it all ;-)”

Figure 16. Viewing window at Danish Crown, Horsens, Denmark

COST Action FAIM training school in Edinburgh is a success!

Lutz Bünger

The latest COST FAIM training school at SRUC in Edinburgh attracted more than 20 trainers and trainees from across Europe

The COST Action FAIM chair Lutz Bünger was one of the hosts, and the trainers were Chris Glasbey, from BioSS; Kirsty McLean, from SRUC; Michael Judas, from Kulmbach - Germany; Mathieu Monziols, from IFIP - France; Anton Bardera, from the University of Girona – Spain and Tobias Schwarz, from the University of Edinburgh all of whom are active FAIM participants.

The training school concentrated on Computed Tomography (CT) which can measure accurately muscle/meat, fat and bone in both living animals and in carcasses. Through being able to do this CT can be useful in the measurement of traits which are relevant to meat and animal production. Some examples of this are the measurement of muscle density as a proxy for meat quality, the number of chops in an animal based on the spine characteristics, lambing difficulties from pelvic dimensions and possibly methane output from gut characteristics.

People with a variety of backgrounds and interests ranging from students, researchers to industry representatives attended the training school. They were trained in the used of a number of software packages for image analysis and extraction of information from images.

The COST Action FAIM has allowed the exchange of ideas and the development of expertise in the identification and use of the most practical software packages to meet the needs of the users.

As Lutz pointed out, although there is not a CT scanner in every EU member state, there are ways to get round this so that it is possible for everybody to get their farm animals CT scanned. For example there are a number of mobile CT scanners which are available within mainland EU and in the UK. So there are many individuals and organisations that are interested to learn the capabilities and challenges of the various software packages now available for analysing and interpreting images.

Figure 17. At SRUC's CT Unit

The training school has developed a lot of information and software that is useful for working with images. If anyone would like access to the material, via agreement, please contact Lutz Bünger by email to **Lutz.Bunger@sruc.ac.uk** to sign up.

As part of the visit to Edinburgh, the particpants of the training school had the opportunity to tour the city in the evening. Lutz and his SRUC colleagues guided the visitors through the Royal Mile and they all had a Scottish dinner!.

Lutz and the team would like to thank everyone for their input to another successful FAIM training school.

FAIM III participants discussion on use of CT in carcass classification

Danish Meat Research Institute 26-9-2014

The background and key issues for discussion were presented by two FAIM experts from WG1 - Gerard Daumas and Eli Olsen followed by a discussion among all FAIM III participants; the session was chaired by Kim Matthews.

The background of the meeting was the possible use of Computed Tomography for the measurement of pig carcass composition, the ongoing discussions within the EU regarding changes in the current EU regulations on pig carcass classification and the FAIM WG1 milestone 7.

The focus of the **first presentation** was the question:

Would it be possible to develop a short term approach for the optional use of CT as a reference method to measure lean meat percentage in pig carcasses?

It was suggested by the first speaker that four key steps would be needed.

1. A recommended CT reference measurement method would need to be chosen and fully described.

2. A comparison between the recommended CT reference method and manual dissection reference would need to be made.,.

3. A description of various candidate CT reference measurement methods used in different laboratories and countries would be needed

4. A comparison between these candidate CT references (identified in 3 above) and the recommended CT reference would need to be made.

These 4 steps were presented with a detailed background and some worked examples. A further in depth discussion is also contained in the paper by Daumas *et al*. on page 52 of this report.

The **second set** of points offered several principles which should be considered by the FAIM participants.

1. If CT is to be used as an optional reference (i.e can be chosen as the reference in a trial without any manual dissection) then there is a need for a metrological framework i.e. an estimate of the reproducibility (especially an estimate of the variation between scanners) and traceability to the SI system (hence volume might be considered a better reference measure).

2. The use of CT should provide an advance over current approaches.

3. There is a need to calibrate existing CT scanners in Europe (about 10) using a set of existing phantoms.

4. There would be a need for standard protocol to scan this phantom set in all different labs and to use also the “in house protocol”.

5. The relationship between the reference CT method and manual dissection needs to be fully described.

6. There seems to be value in considering tissue volumes as the reference rather than tissue weights.

The Chairman of the session suggested the FAIM group could first consider two questions.

Q1. Is there general agreement that CT should be used as an optional EU reference method?

Q2. If so is there an agreement that comparison should be made between the reference CT methods, manual dissection and the candidate CT methods used in the various laboratories and different countries?

In response to these questions the FAIM participants made the following points.

- The tolerances for the reference CT methods must be tighter than those offered as standard on general equipment, otherwise it is not a reference method.
- CT is already permitted as a reference method in current regulations, so is the proposal to make CT the only reference method, not an optional method.
- The CT method to be considered as the recommended CT reference methods must have been calibrated against dissection.
- What are the issues if manual dissection is abandoned as a reference?
- CT should be related to full, not partial dissection. If dissection is abandoned there will be increased bias.
- CT cannot be used as a stand-alone reference method in current regulations and must be calibrated against manual dissection.
- Manual dissection would only be abandoned by those preferring the CT reference but manual dissection would stay a possible reference
- Manual dissection already has considerable bias between butchers and between countries. It would be expected that CT would reduce bias.
- It was suggested that the information on variability of manual dissection available in the output of the EUPIGCLASS project (www.eupigclass.net) should be reviewed as background information.
- There may be a need to understand the effects of considering partial versus full dissection for both CT and manual approaches.
- There is a need to standardize CT protocols across the EU as there are already several national protocols, if CT is to be a "stand-alone" EU reference method.
- Each scanner would need to be calibrated against a fixed standard (such as a phantom).
- The current standard phantoms are too small. Any error in measurement will lead to a large error in estimate, so larger, carcass size standards should be used.
- Once the CT scanners are calibrated against appropriate fixed standards (eg set of phantoms), then any machine based error can be corrected for in the analysis software or by changing machine settings.
- The set of phantoms should be volume calibrated with an independent SI-approved method. Eventually before assembly of the individual phantoms. The phantoms must challenge the CT determination of volume with respect to artefact generation and mixed voxels.
- Calibration should focus on volume, not density. Density is the main focus of medical uses of CT.

Conclusions

There was general agreement that CT was a reliable method, well tested and proven in terms of science.

It was proposed that CT should be allowed as an optional reference, i.e. independent of manual dissection, as the accuracy and precision of CT has been shown many times.

However, some participants pointed out that CT and manual dissection should provide comparable levels, as required by the present regulation.

It was generally agreed that further discussions were needed on the issues raised in the meeting by the FAIM participants, in particular on appropriate fixed standards (phantoms) and that a specific workshop should be held as soon as practically possible.

Workgroup 1

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Evaluating body composition in living horses: where are we up to?

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Value for Industry

- Production targets within the European Equine industry are remarkably diverse. Although of great economic and social importance, this sector has been scientifically marginalised.
- Equine body composition is a key determinant of health and morbidity, reproductive and growth efficiency, work performance and carcass quality.
- Whether horses are considered working, leisure or food animals, productivity and welfare would be improved by an ability to quantify and monitor body composition in living animals and to evaluate equine carcass compositions.
- Work is urgently needed to develop robust, non-invasive methods to predict body composition in living *Equidae* and to standardise methods for carcass evaluation.

Background

The equine industry is unique among farmed animal enterprises in its diversity of form and purpose. In terms of impact, the Industry occupies pivotal socio-economic roles and is an important part of the economy of all Member States (Liljenstolpe, 2009, Figure 1). With >5 million horses in Europe and an estimated 200,000 slaughtered annually for human consumption (Eurostat Database, 2012), the importance of the horse as an agricultural and food animal is recognized by the EU. These diverse roles overlap and occasionally conflict in areas where equine productivity, in its wider sense, also encompasses working, performance and leisure animals (European Parliament, 2008; Liljenstolpe, 2009). Diversity within the sector is further promoted by differences in animal breeds. Mature animals range across an order of magnitude in body mass (BM, 100 – 1000kg) and breed profiles differ markedly between Member States. Horses are long-lived (up to 40 years) and intensely seasonal; factors which influence management practices and production targets (Argo, 2009). Geographic differences in socio-ethical expectations for horses also serve to fragment the Industry and have constrained cohesion in promoting generic beneficial scientific advances.

Figure 1. The European Equine Industry is remarkably diverse.

Irrespective of animal types and performance targets, productivity and welfare are inextricably linked to body composition in equines. As for all mammals, adipose tissue and skeletal muscle comprise the most labile energy reserves within the body (Harris *et al.* 1986). The conflation of inter-breed differences in fat partitioning, diverse environmental, nutritional and management practices with seasonal and work-related drives on energy storage and mobilisation make adipose

tissue the most variable component of the equine body in terms of both mass and percentage (Webb and Weaver, 1979; Znamirowska, 2005; Dugdale *et al*. 2010). In a study of 107 Polish slaughter horses (half carcass mass range; 165–418 kg), carcass dissection indicated that equine half carcasses contained between 1.2–18.5% white adipose tissue (Znamirowska, 2005). A detailed study of 7 Welsh Mountain pony mares ranging in body condition score from 1.25/9 to 7.0/9 (Kohnke, 1997, where 1 = emaciated and 9 = obese) suggested that the contribution of white adipose tissue to empty BM (live BM less digesta), ranged between 2 and 30% (Dugdale *et al*. 2010).

Why work is needed

For equines, the extremes of the body fat spectrum (obesity and emaciation) represent serious risk factors for decreased production efficiency and carcass grading and increased morbidity / mortality (Henneke *et al.* 1983; Geor, 2008; Argo, 2013). Impacts are both direct, via physical and physiological constraints on visceral function, breeding efficiency, athletic performance and carcass value and indirect, through the obesity-associated development of insulin dysregulation and laminitis, a debilitating and painful condition of the feet which commonly warrants euthanasia (Geor, 2008). The causes of emaciation are multi-factorial, ranging from disease and neglect to old age and seasonal constraints on feed availability, while generalised obesity is a consequence of nutrient overprovision (Argo, 2013). Industrialised States are numerically dominated by the leisure sector and the uncoupling of animal maintenance from base economics has resulted in an epidemic of obesity to parallel that of the human populations (Wyse *et al.* 2008). This comprises a serious welfare issue and requires that bodyweight management is incorporated into standard husbandry practice (Argo *et al.* 2012). Mechanistic links between adiposity and disease have not been clearly elucidated. In man, specific regional fat depots have been associated with increased risk for triggering insulin dysregulation and the metabolic syndrome (Vega *et al.* 2006). In horses, fat partitioning between anatomical depots is highly variable between individuals (Webb and Weaver, 1979; Martin-Rosset, 2008; Dugdale *et al*. 2010).

An ability to quantify body fat content and to evaluate adipose tissue distribution within the body of living horses would define normal ranges, improve our understanding of associations with disease risk, direct management advice, allow targeted monitoring of high-risk individuals and selection of appropriate slaughter animals. Equine welfare organisations and clinicians urgently seek methods to evaluate associations between body composition and health in order to generate evidence-based guidelines. Finally, the equine slaughter industry lacks a cohesive carcass grading scheme. Although the meat to bone ratio in horse carcasses is relatively high (68.5% vs 52-55% for pork and beef, Ferguson, 1993), it is marketed as a lean meat. Fat trimming impacts on profitability while trimmed carcass fatness alters target markets. Emaciation is incompatible with human consumption. These are important considerations for an industry which recruits slaughter-animals at the end of their working lives.

In life, the sheer size of these animals prohibits the application of many imaging technologies (e.g. DEXA, CT, MRI), bar transdermal ultrasound. The longevity and value of individual horses and requirements for serial study, limit the usefulness of *post-mortem* approaches. The equine industry would greatly benefit from the development of novel imaging modalities capable of quantifying total body fat and the comparative contributions of regional white adipose tissue depots in living animals.

Methods used and results obtained

By necessity, progress has focused on *post-mortem* carcass dissection and proximate analysis of carcass homogenates to validate indirect methods of estimating total body fat in living horses. Initial attempts to identify proxy measures for the estimation of equine body fat content adapted work in the pig and bovine to evaluate associations with ultrasound-generated measures of subcutaneous fat depots (Price *et al.* 1960; Watkins *et al.* 1967; Westervelt *et al.* 1976). Data from 8 ponies confirmed the precision of ultrasound-generated vs. *post-mortem* measures of subcutaneous rump fat (supragluteal) depths at an anatomically defined site (r = 0.85, Westervelt *et al.* 1976). Two studies explored associations between ether extractable lipid contents of the entire empty BM (eBM, less digesta) of 8 horses (336-559 kg) and 11 Shetland ponies (~150 kg). Good linear associations were reported (horses, r^2 = 0.86; ponies, r^2 = 0.64) but intercepts differed appreciably with comparable eBM extracted lipid content being associated with lower rump fat depths in horses (Westervelt *et al.* 1976). Concerns for the wider application of this technique include; notable differences in fat depths between breeds, adherence to specific anatomical landmarks, transferability of landmarks between breeds and measurement errors associated with thin depots (range 0.1 - 3.0 cm, image interpretation, operator pressure, machine/calliper accuracy).

For practical purposes, subjective body condition scoring (BCS) systems, first introduced for the prediction of meat content (muscle and fat) in food animals, have been adapted as estimators of body fatness in horses. Several systems exist but the method developed by Henneke *et al* (1983) and later modified by Kohnke (1992) is preferred. This system offers clear descriptors for 6 key body areas which are independently scored (1 = emaciated to 9 = obese) to accommodate individual differences in regional fat deposition and the mean of the regional scores is used to provide an overall BCS value. Nuchal crest fat is one of the regions evaluated by the Kohnke (1992) BCS system. A simplified BCS system which evaluated only crest fatness (5 point scale) demonstrated good associations with other markers of adiposity including plasma leptin concentrations (Carter *et al.* 2009). The usefulness of crest fat as an index of total adiposity is supported by Znamirowska's work (2005) in Polish slaughter horses which identified an association between crest (nape) fat depth at the level of the 4th cervical vertebrae in split carcasses and the percentage of the carcass comprised of dissected white adipose tissue (WAT). In a study of 114 UK slaughter horses of mixed breed, gender and BCS, Morrison *et al.* (2013), reported that *post-mortem* measures of crest and ventro-abdominal retroperitoneal adipose depots were strong predictors of *ante-mortem* BCS while omental, retroperitoneal, epicardial and tailhead fat were more weakly associated.

Understanding of the usefulness and constraints of BCS systems for the quantification of adiposity in living horses was only achieved following validation of the deuterium oxide dilution method for the quantification of equine body composition (Dugdale *et al.* 2011b). This complex study used seven, rigorously-phenotyped, mature Welsh Mountain pony mares to investigate associations between *ante-mortem* measures of BCS and total body fat mass (TBFM), with *post-mortem* data defining the percentage BM as both chemically-extracted lipid and dissected WAT (Dugdale *et al.* 2011a; Figure 2). Strong linear associations were evident between all objective measures of body fat content but associations between percentage total body fat and BCS were curvilinear, confirming earlier data for both horses and cattle (Gregory *et al.* 1998; Martin-Rosset *et al.* 2008).

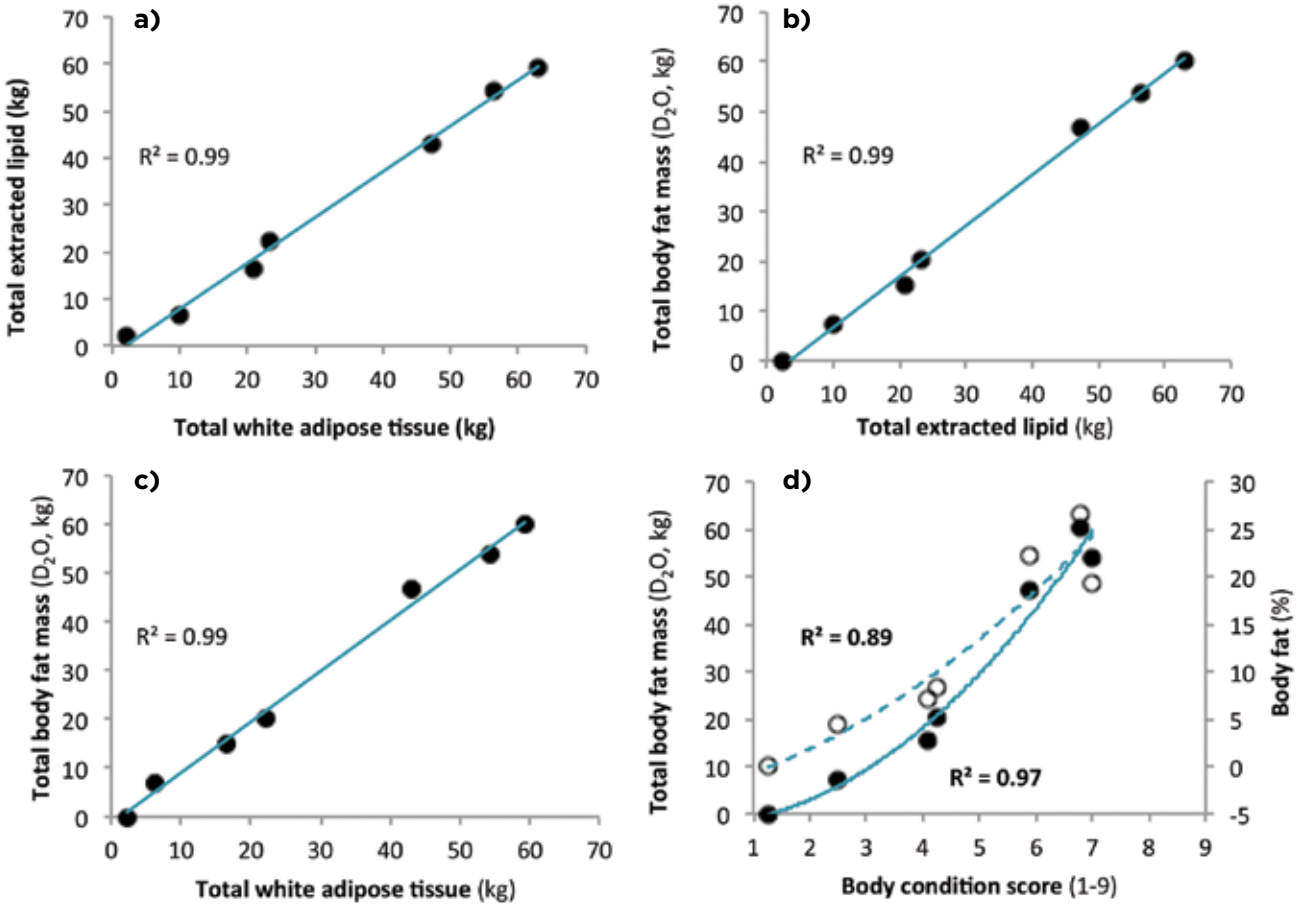

Figure 2. Linear regressions and coefficients of determination demonstrating the relationships between a) chemically-extracted lipid and dissected WAT b) TBFM determined by D_2O dilution and extracted lipid c) TBFM and WAT in 7 mature pony mares that ranged in BCS from 1.25–7/9. d) Exponential associations between body fat content and BCS.

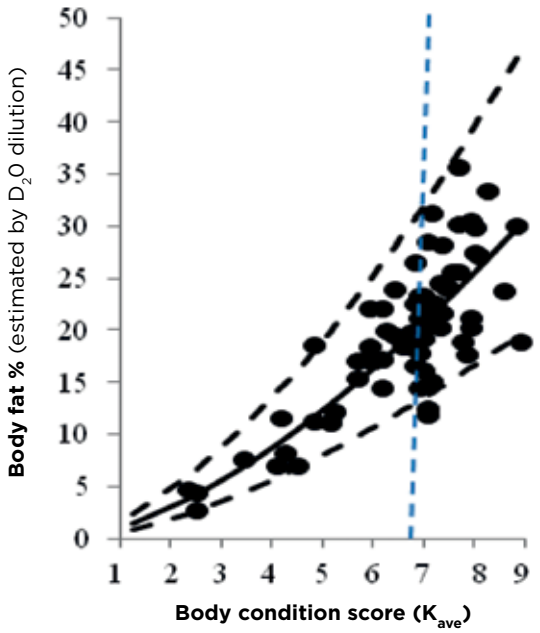

Figure 3. The relationship between BCS and body fat % in 77 horses and ponies estimated following D_2O dilution. Solid line represents fitted-values for the univariable regression model. Broken lines are 95% confidence limits for forecast predictions.

Dugdale *et al's* (2011a,b) studies highlighted two important considerations. First, body fat appeared equally-distributed between the 'externally-palpable' fat depots which are subjectively measured using BCS and 'covert' internal compartments, emphasising the need to quantify internal fat distributions. Secondly, the exponential nature of the association made it likely that the accuracy of BCS as a predictor of body fat content would decrease with increasing adiposity. The same authors used deuterium oxide dilution methods to evaluate TBFM and BCS in a mixed population of horses and ponies across the full spectrum of BCS (Dugdale *et al.* 2012; Figure 3). While BCS was a useful predictor of body fatness in thin and moderate animals, BCS and body fat content were not associated in overweight and obese individuals (Figure 3). This severely constrained the usefulness of BCS as a monitor of TBFM in animals undergoing corrective management for obesity, a finding which supported previous data from the same group (Dugdale *et al.* 2010) Receiver operator characteristics indicated the optimal cut off of BCS 6.8/9 (sensitivity 82.5%; specificity 70.8%) was appropriate for identifying animals likely to comprise ≥20% body fat and therefore in need of dietary management (Dugdale *et al.* 2012).

Validation of the deuterium oxide dilution method for the estimation of TBFM, allowed this same group of authors to re-evaluate ultrasound measures of rump fat depth in same-breed ponies mares undergoing weight-loss management (Dugdale *et al.* 2010; Figure 4).

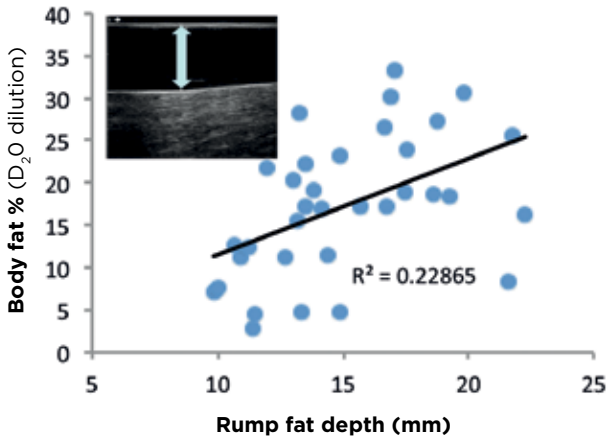

Figure 4. The relationship between ultrasound-generated measures of rump fat depth and body fat percentages calculated following D_2O dilution in 34 ponies. Inset shows transcutaneous ultrasound-generated image of the rump fat depot (arrow).

It was noteworthy that the strong relationship between chemically extracted lipid and rump fat depth demonstrated by Westerwelt (1976) in ponies which had been held on a constant or increasing plane of nutrition was greatly weakened during weight-loss. Further caution in using a single fat depot as a marker of total body fatness, where the nutritional history of an animal is unknown was offered by Argo *et al.* (2012). Ultrasound-generated measures of superficially accessible fat depots (ventro-abdominal retroperitoneal and subcutaneous rump and rib [12th intercostal space]) were recorded for animals of mixed breed and gender undergoing weight loss in winter. Data indicated that while the retroperitoneal 'storage' fat depot was depleted with time, BM and TBFM loss, the subcutaneous WAT depots paradoxically deepened (Figure 5). Increased depot depths were considered to be adaptive thermoregulatory responses, redistributing mobilised 'internal' fat to the subcutaneous compartments to limit energy losses (Argo *et al.* 2012). Although the precise location of rump fat measures differed slightly between studies, application of the pooled Westervelt (1976) equation for the estimation of body fatness to these data was biologically insensible. On the basis of rump fat, these animals would have been considered to have increased body fat by 14.9 ± 4.3%). over 16 weeks of weight-loss management. However, 'gold standard' deuterium oxide dilution data indicated that TBFM actually decreased by 18.3 ± 3.1% in the same period (Argo *et al.* 2012). Stable isotope methods remain the only accurate mechanism for total body fat assessment in living *Equidae* but are of limited value outwith research purposes and provide no information with respect to fat patterning.

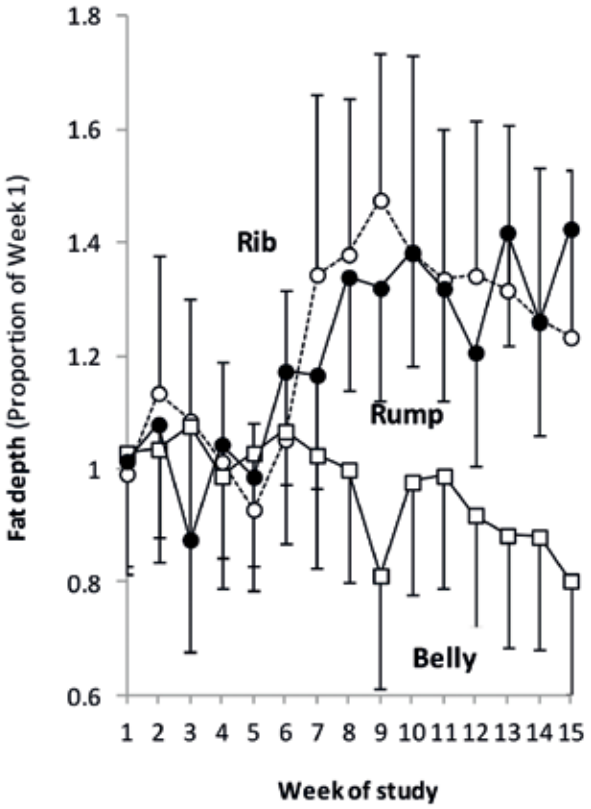

Figure 5. The relationship between ultrasound-generated measures of rump, rib and belly fat depths over time in 12 horses and ponies undergoing weight loss management in winter (Argo *et al*. 2012). The data are presented as weekly mean values (*n*=12 animal), the 95% confidence intervals are shown as error bars.

The next steps

The recent development of five point, scoring systems for key regional adipose tissue depots (nuchal crest, tailhead, retroperitoneal, epicardial, omental, mesenteric) is offering a cohesive scheme for the semi-quantitative evaluation of regional depots at surgery and *post-mortem* (EQUIFAT, Morrison *et al*. 2013). Data suggest that while crest and retroperitoneal fat scores were useful predictors of BCS irrespective of animal type, omental and tailhead scores were only weakly associated while epicardial and mesenteric depots were unrelated. While the rolling out of EQUIFAT will maximise *post-mortem* and surgical data collection, data reinforce the likelihood of functional differences between adipose tissues in horses. Exploration of depot differences and their implications to animal health and productivity would be greatly facilitated by the development of robust imaging modalities to enable non-invasive serial evaluations of horses *in vivo*.

Acknowledgements

The authors would like to acknowledge financial support for cited studies from World Horse Welfare, BBSRC and WALTHAM. Thanks also go to the UK equine slaughter industry for their willingness to assist with post-mortem data collection.

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Classification of broilers using vision technology

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Value for Industry

- Online measurement of standard carcass weight and weight and yield of breast fillet meat of broilers.
- The equipment measurements can be used in value based payment to farmers.
- Potential for measuring weight and yield of other parts as well.

Background

In the pig and cattle industries, classification of slaughtered animals has been used for many years and payment based on the classification is widely used. Danish broiler slaughterhouses pay the farmers by the live weight of the animals. The transport truck with the live chickens is weighed and the weight of the truck and the cages are subtracted. This approach gives an imprecise estimation of the live weight since varying amounts of litter, manure, water, snow etc. may be included. Even more importantly, the quality of the chickens is generally not included in the payment system, despite the fact that the value of the chickens depends not only on the weight but also on the quality. In particular, the slaughter yield and the meat content are of value and these quality parameters are highly affected by primary production factors such as the composition of the feed.

Why this work is needed

To obtain more valuable products, the broiler industry wishes to use a payment system that encourages the farmers to use production methods that will result in chickens with more meat and less fat and thus with more value. A classification system which can measure the valuable parts of the broilers is needed.

The methods used

This study has looked at the possibilities of a new classification system for broilers on which the payment can be based. To overcome the inadequacies of the current live weight based system, an estimation of the carcass weight and the total breast fillet content was chosen for the new classification system. The breast fillet is the most valuable part for the Danish slaughterhouses, and because of the high slaughter speed (approx. 12,000 per hour), vision technology was chosen.

Classification equipment

As classification equipment, the VTS2000 from E+V technology (E+V Technology GmbH, 2014) was selected. The equipment is placed on the slaughter line just after the plucker and before the evisceration. The equipment includes two cameras taking a digital image of the back and of the front side of each chicken. Computers collect the images and calculate a number of different points, lengths and areas that can be used in the estimation of the classification parameters.

Reference material

In order to calibrate the equipment, 259 Ross 308 chickens were produced. To ensure large variation in weight and breast meat content, the chickens were distributed into 10 weight groups (target live weight: 1040, 1349, 1596, 1853, 2115, 2380, 2643, 2988, 3239 and 3480 g) and 4 feeding/parent groups (low wheat / parent category 0, high wheat / parent category 0, norm wheat / parent category +1 and norm wheat / parent category -1). The chickens were fed a concept feed with low, norm or high addition of wheat. The parent category represents the age of the mother hen when the egg was laid where +1 is 24-29 weeks, 0 is 30-45 weeks and -1 is 46-65 weeks. The chickens were divided between two slaughterhouses where they were slaughtered and measured using the VTS2000 equipment.

A standard cutting procedure was developed. After slaughter, the carcasses were cut in a standard presentation, weighed and then cut into parts with all parts also being weighed. The weight of the carcass in standard presentation served as reference for the *carcass weight*. The combined weight of both outer and both inner breast fillets served as reference for the *total fillet weight*. The

combined weight of both outer and both inner breast fillets divided by the carcass weight cut in standard presentation and multiplied by 100 % served as reference for the *total fillet yield*.

Classification equations
Classification equations for carcass weight, total fillet weight and total fillet yield were made based on measurements from both slaughterhouses / VTS2000 equipment and the corresponding references using regression analysis (details are confidential). The measurement error of the classification was calculated as RMSED (Root Mean Square Error of Deviation):

$$RMSED = \sqrt{\frac{\Sigma_{i=1}^{n}(\hat{y}_i - y_i)^2}{n}}$$

and the Bias was calculated as:

$$Bias = \frac{\Sigma_{i=1}^{n}(\hat{y}_i - y_i)}{n}$$

where

$\hat{y}_i$ = the predicted value of chicken i,
y_i = the reference value of chicken i and
n = the number of chickens

The precision of the classification was calculated as:

Precision = 2 x RMSED

It was tested if Bias=0 using proc ttest in SAS (SAS Institute).

The above calculations were made on the calibration data set. Ideally, they should have been made on an independent validation data set but unfortunately, that was not possible within the scope of the study.

The results obtained

Figure 1 shows an example of the two images taken by the VTS2000 equipment. Note that the images are taken *before* evisceration. This is done because the carcasses at this point are more rigid and uniform in their presentation and any accidental damage due to the evisceration is omitted from the classification.

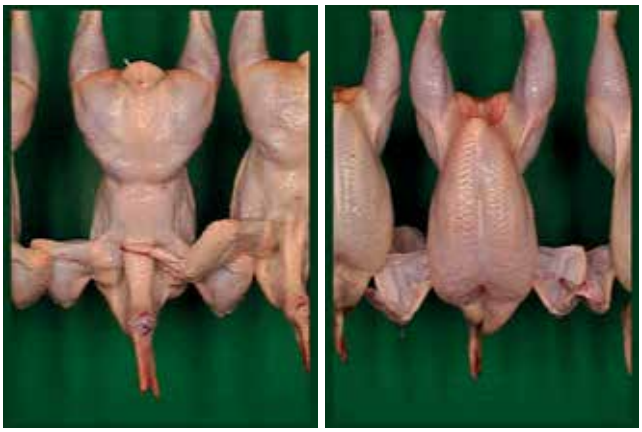

Figure 1. Images of back and front of a chicken taken by the VTS2000 equipment.

Reference material
As reference material, a standardized way of cutting the chickens was developed. Figure 2 shows a carcass from the reference material in standard presentation. The use of a standard presentation of the carcass in the reference material is essential, since this allows for a more uniform classification of the carcass weight no matter how the slaughterhouses may choose to cut the chickens as a product or as raw material for further cutting into parts. Although, the broilers are not measured in the standard presentation (see Figure 1), it is still the weight of the carcass in standard presentation, which is calculated by the equipment.

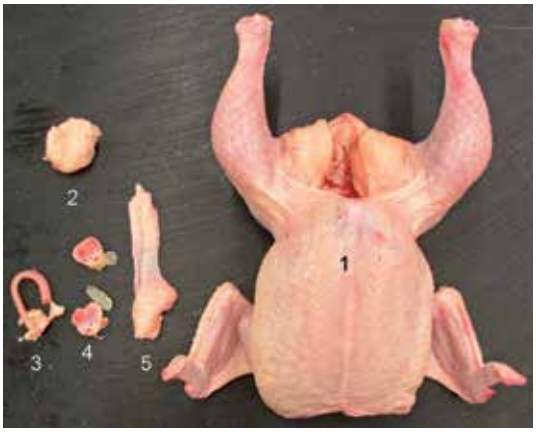

Figure 2. Carcass in standard presentation (1). Cut off are rests of leaf fat (2), neck and oesophagus (3), rest of the feet (4) and neck skin (5).

As further reference, the carcass is cut into parts in a standardized manner as shown in Figure 3. The sum of both outer and both inner fillets (1 and 2 in Figure 3) is the reference for the total fillet in the classification.

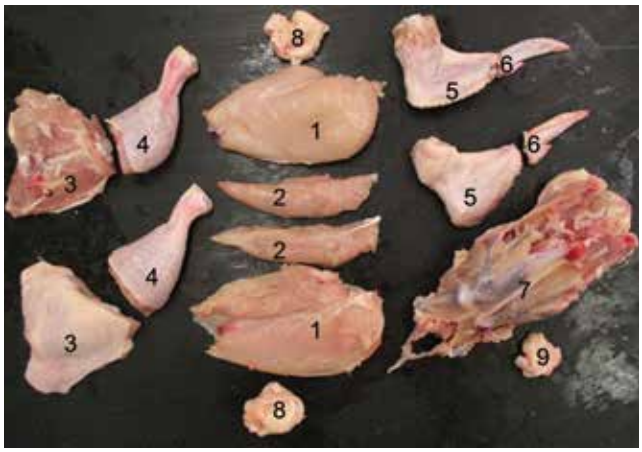

Figure 3. Reference cutting of carcass into parts. Outer and inner fillet without skin and fat (1, 2), thigh (3), drumstick (4), wing 2-joints (5), wing tip (6), carcass shell (7), scraps (skin and fat) from filet (8) and scraps (skin and fat) from thigh (9).

Table 1. shows some results from the reference cutting.

	Mean	Stand. dev.	Range
Slaughterhouse A (n=136)			
Carcass weight (g)	1728	522	824-3193
Total fillet weight (g)	530	174	240-1024
Total fillet yield (%)	30.5	1.9	26.3-35.4
Slaughterhouse B (n=123)			
Carcass weight (g)	1805	554	882-3082
Total fillet weight (g)	546	173	247-972
Total fillet yield (%)	30.2	1.8	25.6-34.0

Table 1. Reference material
Carcass weight, total fillet weight and total fillet yield for the two slaughterhouses/ equipment (N=259).

Classification equations
The classification equations for carcass weight, total fillet weight and total fillet yield were developed using measurements and reference data from both slaughterhouses (two separate installations of equipment). Table 2 shows the bias and the precision of the classification parameters for the individual slaughterhouses and for the two combined.

Slaughterhouse	A	B	Both
Carcass weight (g)	1.8 ±155	-3.6 ±121	0 ±140
Total fillet weight (g)	2.4 ±78	-4.9 ±74	0 ±76
Total fillet yield (%)	0.11 ±1.3	-0.04 ±1.5	0 ±2.76

Table 2. Classification equations
Bias and precision for the two slaughterhouses and combined.

None of the biases are statistically significant (t-test, all $p > 0.1$). For the entire data set, the carcass weight is estimated with a precision of 3140 g (95% certainty). The total fillet weight is estimated with a precision of 376 g and the total fillet yield with a precision of 32.76 %. At slaughterhouse B the precision is slightly better than at slaughterhouse A for carcass weight and fillet weight and slightly poorer for fillet yield. The differences between the two slaughterhouses are so small that they have no practical implications. They are probably due to differences in the slaughter process before the classification but could also be due to small, unintended differences in the reference material or in unknown differences between the two equipment installations, although much was done in order to make both reference material and equipment identical.

The precision of the classification may not seem too impressive for the individual chicken but the classification is to be used in payment of flocks of many thousands. The precision of the average classification of a flock depends on the flock size (N), the standard deviation of the flock (STD) and the RMSED in this way:

$$Precision_{flock} = \pm 2 \times \sqrt{\frac{STD_{flock}^{2}}{N} + \frac{RSMED_{chicken}^{2}}{N}}$$

It can be seen that the precision of the flock average will be better when the flock is larger. To have a meaningful payment system, the precision of the classification should be small compared to the variation (STD) of the flock. The variation within flocks of normal production is not known yet. However, based on the known live weight and the reference data, we may assume that the standard deviation will be approx. 220 g for slaughter weight, 75 g for fillet weight and 1.3 % for fillet yield. If, as an example, we assume that the precision should be smaller than 5 % of the standard deviation, the flock size should be at least 2,000 chickens for the carcass weight, 3,000 for the fillet weight and 4,000 for the fillet yield. Therefore, if the payment were to include fillet yield, the flock size should not

be smaller than 4,000. In that case, the precision would be 37.3 g, 32.7 g and 30.06 % for the average flock carcass weight, fillet weight and fillet yield respectively.

Flock sizes are normally 30,000 chickens in Denmark in which case the precisions are 32.7 g 31.0 g and 30.02 % respectively.

The scientific conclusions

Vision classification including carcass weight and total breast fillet meat can be precise enough to be used in a broiler payment system, if it is based on the average classification of flocks. The flock size should be so large that the precision of the average classification is small compared to the variation of the flock. With the normal flock sizes in Denmark this will not be a problem.

A payment system based on vision classification can better reflect the value of the chickens than the existing payment system. The weight of sellable products is estimated more precisely and the content of the most valuable meat can be included.

The precision of the classification of the individual animals may not be good enough to be used in an individual sorting of the chickens to different products at the slaughterhouse but – as for the payment – *flocks* may be sorted for different use based on the average classification. The variation of the flock may also be of use.

It is important to underline that the classification equations developed in this study are only valid for chickens that are comparable to the reference chickens of the study. If other types of chickens are to be classified, new equations must be developed. The same is true if the chicken population changes considerably over time, for example as a result of the new payment system. The equations should be checked from time to time.

As can be seen in Figure 3, the reference cutting included other parts than the total breast fillet. It is therefore possible to develop classification equations for these other parts as well, based on the collected reference data.

A detailed description of the study and its results can be found in the final report of the study (Claudi-Magnussen, 2010).

The next steps

The Danish broiler industry is presently implementing the vision classification and a payment system based on the vision classification is being developed.

The introduction of vision on the slaughter line may have other applications than classification – for example veterinary control, which is currently being investigated at DMRI.

Acknowledgements

This study has been done thanks to financial support by the Danish Poultry Levy Fund, Rose Poultry A/S (now HK Scan Denmark), Lantmännen Danpo A/S (now Scandi Standard) and E+V Technology GmbH.

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Integrating Computed Tomography (CT) into commercial sheep breeding in the UK: cost and value

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Value for industry

- For the selective breeding to improve lamb carcass quality, studies recommend a dual approach using both Ultrasound (US) and Computed Tomography (CT) scanning. A two stage process is recommended, based on comprehensive US scanning of all selection candidates followed by CT scanning of about 10% to 15% of the best, as identified by US.
- The availability of a CT scanning service to the UK sheep industry, allowing non-invasive, fast and accurate analysis of body composition of live sheep provides an enormous opportunity for industry. As an integrated procedure in sheep genetics evaluation, it produces substantial additional selection responses for compositional and conformation traits to improve carcass quality.
- New traits CT-based traits and their genetic basis are being investigated and it may be possible to embed them into future selection programs. The economic benefit of such new traits can be estimated after these initial investigations, but uptake into the industry will depend on the ability of the abattoirs/ supermarkets to reward farmers for these traits. This will require suitable on-line measurement technology to be in place in the abattoirs.
- As a "co-product" of CT scanning, for commercial and research purposes, there is a valuable and ever-growing database (image-bank) comprising CT images and image-derived traits. This can be combined with information on other routinely-measured traits and pedigree, maintained in the central or national databases (e.g: in the UK; http://www.basco.org/beef/index/index), which offer powerful opportunities for the development of new methods, new traits and investigation of their genetic basis, enabling their subsequent use in breeding programs.
- CT has great importance in upcoming genome wide selection (GWS) approaches. It can act as a high-throughput phenotyping tool, which will allow the establishment of a training population of several thousand genotyped and phenotyped animals; an essential requirement for the implementation of GWS.
- In addition to applications in the breeding context, *in vivo* CT measurements of body and carcass composition can also be used to determine and model optimal slaughter times and weights, and therefore to produce a final product which better meets industry and consumer demands.
- *In vivo* CT can also be used in longitudinal studies, as a non-invasive tool to model protein and fat deposition during the growing period, providing information to optimise sheep breeding, nutrition, feed efficiency and whole production systems.

Background

Sheep production in the UK: The UK is the largest producer of sheep meat in Europe. In 2010 the EU produced just over 910,000 tonnes of sheep meat, of which 277,000 tonnes were produced in the UK (AHDB, 2011). This increased in 2013 to nearly 290,000 tonnes, based on 14.5M sheep slaughterings pa. In the same year, there were nearly 33M sheep in the UK. The value of gross output at basic prices of the agricultural industry produced by the sheep sector in the UK amounted to £1,020M and has an increasing tendency (Defra, 2012).

Carcass quality: For the UK sheep industry to continue as a major producer and exporter of lamb, it is essential that its economic viability is further improved. To do so, the industry has to provide carcasses that better meet market requirements, since currently overall only around 57.6% of UK lambs meet core target specifications. This figure differs in the carcass weight ranges. In 2013, 67% of all lamb carcasses in the weight range 16 to 19.9kg were meeting the target sector (E, U or R conformation and 1, 2 or 3L fatness); 18.5% were too fat and 16% had an insufficient conformation. Lower carcass weights usually have less problems with fatness, but are low in conformation (with 47 to 82% outside the target region), whilst in higher weight ranges (>20kg) there are only 43.6% meeting this target sector, with 40.1% being too fat (AHDB, 2014).

Carcass quality (composition and shape) are moderately to highly heritable (muscularity h^2= 0.3 to 0.6, lean and fat weight h^2 ~ 0.4; (Jones *et al*. 2004), and are thus a valuable target in breeding programmes (Jones *et al*. 2004; Lambe and Simm, 2014). Therefore, improving lamb carcass quality, by increasing lean meat content without a corresponding increase in fatness, has been the focus of breeding programmes in the UK since the mid 1980s. This has been achieved utilising new technologies such as ultrasound and, more recently, CT (Bünger *et al*. 2011; Lewis and Simm, 2002; Macfarlane and Simm, 2007). In this context, it is of note that a CT reference scanning approach (cross-sectional CT scans taken at a few specific anatomical locations) can accurately predict the dissected weights of lean, fat and bone in the whole carcass. Accuracies vary slightly between breeds with the highest R^2 values in meat breeds of 0.99, 0.98 and 0.89 for fat, muscle and bone, respectively, with accuracies in Scottish Blackface sheep just slightly lower (Young *et al*. 2001).

Meat quality: The preference of both consumers and processors for leaner meat and a reduction of visible fat has influenced selection practices to reduce subcutaneous fat and increase lean meat percentage in terminal sire breeds (Simm and Dingwall, 1989). However, a similar approach in selection of pigs resulted in an associated decrease in intramuscular fat (IMF) and, which was accompanied by a negative effect on the palatability of fresh pork meat (Sonesson *et al*. 1998).

A similar picture is starting to emerge from the sheep industry, mainly in terminal sire breeds (Pannier *et al*. 2014). In some UK terminal sire sheep breeds where previous selection for reduced carcass fat has been successful, such as the Texel, levels of IMF in the high-priced loin muscle of pure bred lambs are already substantially below those recommended for consumer acceptability in grilled cuts of red meat (> 2 - 3% in grilled red meat/lamb; (Heylen *et al*. 1998; Savell and Cross, 1988).

This has been confirmed in previous projects at SRUC, where Texels were slaughtered at a fixed age of 20 weeks (Lambe *et al*. 2008; Lambe *et al*. 2011) or at commercial target live weight and condition score >35kg, CS 3; (Lambe *et al*. 2008), where levels of IMF ranged from 1.4 to 1.6%. Levels of IMF were higher in crossbred lambs (Texel x Mule) slaughtered at a fixed age of 21 weeks (Lambe *et al*. 2010), averaging 2.2%, and in Scottish Blackface lambs, slaughtered at commercial target live weight and condition score (average IMF = 2.3%).

Meat quality traits can be influenced to a large extent by animal breeding, as heritabilities are usually moderate to high (Lambe and Simm, 2014), for example for IMF (e.g. h^2 ~0.5 in Duroc pigs (Hernandez-Sanchez *et al*. 2013) and in Nor-X terminal sire sheep (Hernandez-Sanchez *et al*. 2013; Lorentzen and Vangen, 2012).

It has been shown that CT based measures taken on live sheep can predict IMF with accuracies (R^2) of > 0.65 (Clelland *et al*. 2014a).

Why work is needed?

Animal breeding is a powerful tool to boost the productivity of the sheep industry and breeding schemes offer cumulative and permanent gains associated with substantial economic benefits, fitting well within sustainable livestock production systems. Modern breeding methods also provide the most economical way to simultaneously improve a suite of traits (Amer *et al*. 2007). Accurate measurement methods (phenotyping tools) allowing high-throughput, and that are not invasive, are highly valuable tools for breeding programs and in upcoming genome wide selection (GWS) approaches. Such methods need optimisation, standardisation and automation to increase their accuracies, to make them comparable, and to make them cheaper by increasing their throughput. Subsequently, the traits measured with these methods need to be investigated, regarding their genetic basis and association with other traits, to evaluate and potentially integrate them in animal breeding schemes.

Methods used

X-ray computed tomography (CT) is a non-invasive, diagnostic tool, initially developed for use in human medicine. However, as it can non-invasively measure fat, muscle and bone *in vivo* in farm animals (up to the size of sheep and pigs), it has also been adopted for use in (farm) animal science, with numerous examples since the 1980s (e.g. Bünger *et al*. 2011).

Experimental studies carried out at SRUC (formerly SAC) from 1997 to 2000 established the best way to incorporate CT scanning for carcass traits into terminal sire breeding programs in the UK (Young *et al*. 2001; Macfarlane *et al*. 2009). Suitable software procedures to extract and quantify the areas of different tissues in crosssectional images were developed using mathematical algorithms for image analysis (Young *et al*. 2002).

These involved two main steps:

1) segmentation to remove non-carcass portions of the images;
   and
2) measurement of tissue areas in the segmented images.

The complexity of removing the internal organs and identifying tissue boundaries makes segmentation challenging and time-consuming, if done manually. Automatic procedures have recently been developed (Glasbey and Young, 2002), (Navajas *et al*. 2006). Using software (STAR: **S**heep **T**omogram **A**nalysis **R**outines) developed at SAC and BioSS (Mann *et al*. 2013), both steps can be performed automatically and therefore more quickly.

CT predictions of carcass composition have been used in commercial UK sheep breeding programmes over the last few decades (Bünger *et al*. 2011). Together with ultrasound measures of fat and muscle depth in the loin region, CT estimated carcass fat and muscle weights have contributed substantially to the success of breeding for increased lean meat yield in sheep (Moore *et al*. 2011). Details on the principles and applied CT procedures can be found in previously published work (Bünger *et al*. 2011).

In short, it needs to be mentioned that the use of CT in commercial animal breeding in the UK is based on reference scanning, which was calibrated in initial trails against dissection, and involves cross-sectional CT scans at 3 specific anatomical locations only, providing high accuracies for total muscle and fat weights (R^2 ~0.98, as mentioned above) over the whole carcass. The capability to perform spiral CT scanning using SRUC's CT scanner obtained in 2002 (Siemens, SOMATOM Esprit) has provided the opportunity to achieve 100% accuracy. However, the reference scanning approach was developed with the aim of maximising accuracy of prediction of tissue weights, whilst reducing scanning time as far as possible, to minimise cost and animal welfare implications. Therefore, the benefits from achieving 100% accuracy (around 2% more than with reference scanning) does not justify the additional cost for image analysis, even using automated procedures.

The results obtained

In the UK, CT scanning has been in use in terminal sire sheep breeding programs, such as Texel, Suffolk, Charollais, Hampshire Down, MeatLinc and Beltex sheep since 2000 (Table 1). Each year approximately 400-500 lambs in total are CT scanned, either using the fixed CT scanner near Edinburgh or more recently (since 2009) a rented mobile CT scanner (http://burgessdiagnostics.com/our-service/how-we-work) that can provide a CT scanning service to breeders located at distances from the fixed scanner that previously discouraged them from using CT scanning.

	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	total
Texel	353	0	107	148	204	137	124	83	89	108	198	378	335	250	2514
Suffolk	353	0	59	100	156	168	107	36	27	128	98	110	132	109	1583
Charollais	129	0		58	122	92	134	100	20	107	117	119	242	63	1303
MeatLinc	57	0	20	25	30	26			28	24		48	47	51	356
Hampshire Down	30	0		25	25		46	21		36	27	27	41	32	310
Beltex									50	34	20	15	26	28	173
total pa	**922**		**186**	**356**	**537**	**423**	**411**	**240**	**214**	**437**	**460**	**697**	**823**	**533**	**6239**

Table 1. The number of commercial sheep scanned at the SRUC/BioSS CT Unit in Edinburgh (including mobile CT scanning).

The outbreak of diseases in the UK and associated livestock movement restrictions led to low (zero in 2001) numbers in some years.
In addition to the above results from scanning commercial sheep, the image bank also contains images from several hundred experimental sheep.

As clearly stated above, CT scanning has substantial potential to help breed sheep with better carcasses and there is no doubt about the value of CT measures as selection criteria, but as it is more expensive than ultrasound (US) based measures a cost : benefit consideration should be undertaken.

The cost to scan a lamb on the fixed CT scanner in Scotland is about £65 and on the mobile scanner about £99. These prices are not profit orientated and include the cost of image analysis and scanner rent/scanner maintenance. The price of ultrasound scanning (US) is much lower, at about £1.75 per lamb (S. Boon, personal communication). It is, however, of note that US provides 2 simple measures at one anatomical location (third lumbar vertebrae) only: muscle and fat depth, compared to CT which provides a whole suite of traits and potentially more new traits that can be measured retrospectively from stored images.

One needs also to consider the accuracy of the methods, which is about 0.98 for CT and for US about 0.5 to 0.6. This implies that if selection for lean meat weight would be practised using solely reference scan CT information vs. selection on solely US information, the US based selection would yield a response which is about 40% lower.

However, CT scanning of all available selection candidates does not seem practical in the sheep industry and a synergistic approach between these 2 methods seems more reasonable. It has been shown that much of the benefit of CT can be obtained at a fraction of the cost by the use of two-stage selection, where most selection candidates are US scanned and only those of higher genetic merit scanned by CT. The optimisation of such two-stage selection schemes depends on balancing the cost of CT with the greater genetic gain that can be achieved.

Modelling studies in NZ (Jopson *et al*. 1997) have shown that for a large nucleus breeding scheme (1,400 ewes), economic returns were maximised when all lambs were ultrasonically scanned and the best 13% CT scanned.

Similar studies at SRUC have predicted genetic response and economic returns for lean growth rate from two-stage selection in three large breeding schemes for meat sheep in the UK (Texel, Charollais and Suffolk sire referencing schemes). The number of ewes bred ranged between 2,000 to 6,100 and the number of stock rams between 130 and 340, with an average number of lambs recorded per ewe of 1.1 to 1.3. Some genetic co-variation for the *in vivo* predictors of carcass traits was assumed. Similar to Jopson *et al*. (1997), the best strategy was to US all recorded lambs, but to CT scan only a proportion of ram lambs. With the optimal number of ram lambs CT scanned in a scheme, genetic progress for lean growth rate increased by 16 to 32% over that for US alone. The economic returns (discounted over 20 years) exceeded costs when 10 to 25% of rams were CT scanned, with largest economic returns when 10 to 15% were CT scanned (Lewis and Simm, 2002).

Comparing the selection responses in flocks using two-stage selection vs. flocks using US alone is difficult in an industry situation, as bottlenecks in finances, differences in flock sizes, selection intensities and the use of common sires etc. will confound findings. However, in 2011 a study examining the ~2200 Texel rams CT scanned since 1997, found that the average EBV accuracy of CT scanned animals increased substantially when CT information was included in the genetic evaluation (compared to just US information). This increased accuracy would lead to increase in the selection responses for muscle and fat weights and muscularity by 7, 10 and 20%, respectively (Moore *et al*. 2011).

Scientific conclusions

Studies recommend a synergistic approach in the use of US and CT to improve carcass quality, in the form of a two-stage selection, based on comprehensive US scanning of all selection candidates followed by CT scanning of about 10 to 15% of the best, as identified by US.

New CT based traits (see below), suitable for use in selection programs, will certainly affect the cost: benefit ratio, and the cost of these measurements need to be put into the perspective to its benefits.

The next steps

The use of the some of the features of the spiral scanning ability of the CT scanner is allowing us to investigate some additional new traits. These are:

- **Measured** Carcass and tissue weights and proportions for muscle, fat and bone
- **Measured** Killing out %
- **3D** Gigot muscularity,
- EM area and **3D** EM muscularity
- Spine characteristics (length and number of vertebrae) (Donaldson, 2014; Donaldson *et al*. 2013)
- CT measured muscle density (and other predictors) for IMF (Clelland, 2015; Clelland *et al*. 2014b)

Once identified and characterised, these new traits can be integrated into existing genetics databases and breeding services (e.g. in the UK: http://www.sruc.ac.uk/info/120275/egenes and http://www.signetfbc.co.uk/about/index.aspx) to supplement traits already provided by us such as:

- Predicted carcass and tissue weights and proportions for muscle, fat and bone
- Predicted Killing out %
- 2D Gigot muscularity,
- Eye muscle (EM) area and 2D EM muscularity

The advent of genomics provides new opportunities for improving carcass and meat quality through the selection of animals carrying favourable genes and/or QTLs (quantitative trait loci) for important production traits. CT should be considered as a valuable phenotypic tool to quantify important traits on a sufficiently large population using high-throughput and multi-object CT scanning (Figure 1). However, for breeders to invest in its exploitation, it is vital that there is development of objective techniques that allow producers to be rewarded for any increases in lean meat yield or meat quality accompanied with individual traceability.

Acknowledgements

The recent CT scanner was jointly funded by the Biotechnology and Biological Sciences Research Council, the Meat and Livestock Commission and received support from the Scottish Executive Environment and Rural Affairs Department. SRUC receives financial support from Scottish Government's Rural and Environment Science and Analytical Services and from various research projects funded by different national funding bodies.

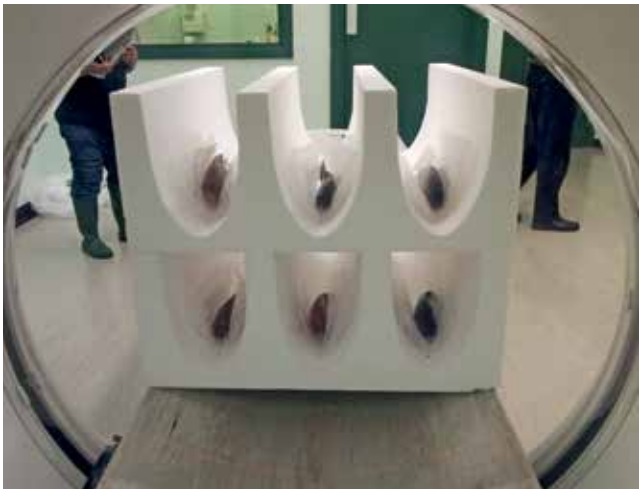

Figure 1. Multi-object CT scanning (e.g. Clelland *et al*. 2013; here Tilapia at SRUC's CT unit; collaboration with Stirling University, Khalfan Mohamed Abdullah Al-Rashdi).

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Genetic gain on body composition in pigs by Computed Tomography (CT): return on investment

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Value for Industry

- CT can provide accurate and non-invasive *in vivo* measurements of body composition of breeding pigs.
- Images from CT can be stored in a database or library, making the system backward compatible in terms of changes in phenotypes or implementation of new phenotypes.
- An estimated increase of genetic gain is around 30%, and the return of investment depending on the system size and what technology is replaced is from 3 to 6 years.

Background

The genetic gain on body composition and return on investment of new technology like CT depends on the accuracy of measurement, the level of invasiveness and direct or indirect measures. Breeding pigs are valuable animals and non-destructive techniques are highly preferred when doing measurements and these animals. Dissection provides the highest accuracy in terms of measuring body composition; however the method is invasive and destructive due to slaughtering of the animal. With respect to genetic gain, data from sibs or half-sibs are required, which reduces the gain in comparison to *in vivo* data from the animal itself. Ultrasound is the most commonly used *in vivo* method to obtain a measure of *in vivo* body composition, being non-destructive and low-cost; however, the measurements are indirect measures of body composition using fat and muscle thickness as predictors of parameters such as lean meat percentage. The accuracy of ultrasound is not very high compared to dissection. CT includes the best from two worlds, being both accurate and non-destructive. An estimated increase of 30% in genetic gain for lean meat percentage (Norsvin data; 2014) has been reported by geneticists on pigs.

Why work is needed

The genetic gain that can be made by selecting for several traits simultaneously within a group of animals is the product of (1) the selection differential, (2) the multiple correlation between aggregate breeding value and the selection index, and (3) genetic variability (Hazel, 1943). The greatest opportunity in increasing the genetic gain lies in the third, insuring that the second is as large as possible. Capturing genetic variability requires accurate data from phenotypes. Balancing accuracy with capturing of non-destructive data *in vivo* is a very challenging situation. Computed tomography (CT) and magnetic resonance imaging (MRI) are ways of measuring body composition *in vivo,* but both systems are complex, costly and require large sets of skills in people running the systems. CT has proven superior to MRI in terms of costs and speed; however MRI provides a higher degree of accuracy in soft tissues due to the physical measurements. In order to run a CT or MRI system in a breeding operation, the infrastructure, computer power and human resources need to be taken into consideration. All these costs must be accounted for when considering the return on investment.

Methods used

Since March 2008, 3,500 boars have been scanned annually. The boars are purebred Norsvin Duroc or Norsvin Landrace (Figure 1). In the Norsvin pig breeding system, all boars are CT scanned at the end of the test period at 120 kg live weight. The test period is from ~30 kg to 120 kg.

Figure 1. Norsvin Delta test station and Norsvin Landrace and Duroc boars.

Figure 2. CT scanning of Norsvin Landrace boars.

The pigs are scanned using a GE Healthcare VCT 32 scanner (Figure 2). The pigs are sedated by intramuscular injection of Stresnil Vet® 30 minutes prior to scanning. The use of sedation instead of anaesthesia is regulated by the food authorities due to the slaughtering of non-selected boars after test. After they fall asleep, the pigs are moved individually onto a fiber-glass bed used for transport of the animal. The animals are scanned using a protocol with 120 kV, an adjusted mA current based on the thickness of the pig being scanned, and a slice thickness of 1.25 mm. This will produce an image stack of approximately 1100 images per animal.

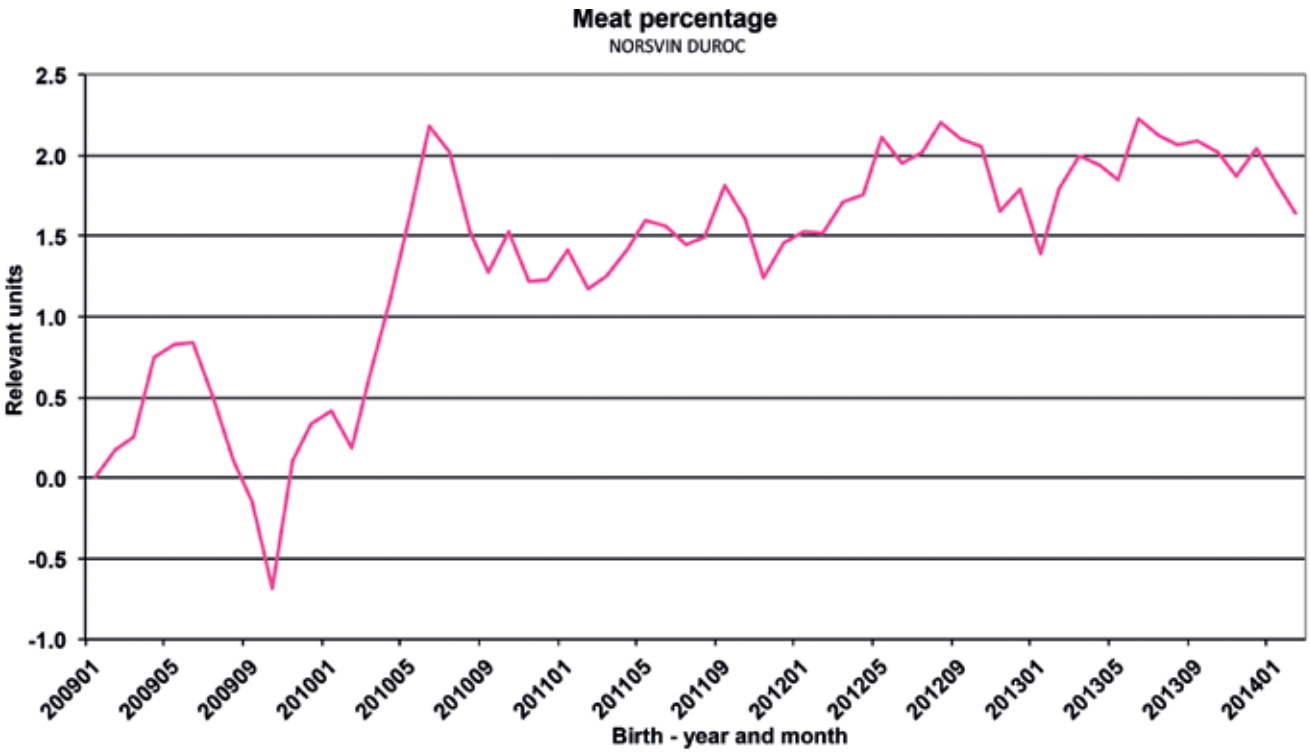

Figure 3. The genetic trend of lean meat percentage measured by CT for Norsvin Duroc.

The images are analysed using an in-house fully automatic script written in MATLAB (The Mathworks Inc, 2013). For diagnostic imaging and viewing, OsiriX DICOM viewer (Rosset *et al*. 2004) is used for this purpose. The image analysis runs every day and use approximately 3-4 minutes per pig for the current traits collected from CT; lean meat percentage and carcass yield. Leg weakness traits are recorded by human observations in the OsiriX DICOM viewer.

The results obtained

Our geneticists have observed an estimated increased genetic gain of 30% for the trait lean meat percentage. This is especially important for our terminal Duroc line (Figure 3).

The storage of images makes it possible to develop existing traits further or record new traits based on historical image data. This backward compatibility make use of historical data, and we do not have to build new datasets for each trial or study, which increase the speed of testing and implementation of new and improved traits dramatically, and reduces the cost of testing and implementation.

Increased accuracy has made it possible to reduce the weight of carcass traits in the breeding goal, making it possible to increase the weight of less heritable and difficult to increase genetic gain of, like maternal traits.

The scientific conclusions

- CT is used as part of the Norsvin breeding system where 3500 boars are tested and scanned annually.
- An estimated increased genetic gain of 30% on the trait lean meat percentage has been recorded.
- In addition to carcass traits (lean meat percentage and carcass yield), meat quality measurements like IMF and fatty acid composition are under development. Leg weakness scoring (osteochondrosis) has been implemented and an increase in heritability of the trait has increased from 0.2 to 0.3 using CT.
- The database of images provides the opportunity of backward compatibility; changes in traits or development of new traits on historical data or images is possible due to access of images back to march 2009.

The next steps

- Development of automatic script for leak weakness (osteochondrosis) scoring.
- Measurement of new traits like weight of cuts and further development of meat quality measurements like IMF and fatty acid composition.
- Integration with other technologies like ultrasound and vision systems to bring together the best data from multiple sources.
- Development of new protocols or dual-energy scans (kV) to collect better data on soft tissue composition and meat quality.

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Usage of Computed Tomography in the selection of two Hungarian rabbit breeds

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Value for industry

- Generally the carcass traits are not included among the selection criteria of rabbits.
- In the selection of rabbits the greatest advantage of using Computed Tomography is that the meat content of rabbits can be evaluated on live animals at the same age as they are slaughtered.
- The increased dressing out percentage and amount of meat means extra revenue for the slaughterhouses.

Background

The Computer Tomography (CT) lab at Kaposvár University operating since 1990 offers a unique possibility for carcass trait selection. During the last 3 decades three Pannon lines have been developed at Kaposvár University and in two of them selection has been based on CT measurements. The application of this technique in rabbit breeding is unique worldwide.

Hungary produces about 6,000 tons of rabbit meat, and 98-99 % of slaughtered rabbits are exported, so after China, Hungary is the second or third place among the main exporter countries.

Contribution of the Pannon lines is about 44% of the total rabbit population in Hungary. Therefore, the Pannon Breeding Program plays an important role in the Hungarian rabbit production and export.

The development of the Pannon White population was initiated at the Kaposvár University at the late 1980s. Selection for the carcass traits based on the CT evaluations started in 1992 (Szendrő *et al*. 1992) and that of Pannon Large in 2005.

Why work is needed

The hybrid paternal line rabbits have large adult weight and high growing performance compared to maternal lines. As a consequence, paternal rabbits have poorer dressing out percentage slaughtered at the same market weight, because they are less mature than the maternal lines with smaller adult weight. The slaughterhouses are keen to increase the dressing out percentage and meat content in the body, which could increase their revenue. Slaughterhouses pay for rabbits based on the live weight, so the dressing out percentage is not taken into consideration in Hungary.

Generally, the dressing out percentage is not measured in selection of rabbits, because it needs test slaughters and progeny test. Use of CT provides a unique and great opportunity to increase the slaughter traits of the rabbit breeds bred at Kaposvár University.

The methods used

Three rabbit breeds are selected at the Kaposvár University. Two of them are selected using CT:

Pannon White: since its establishment (1992), this line has been selected for average daily gain (replaced by 21 day litter weight from 2010) and L-value (average muscle area of *m. longissimus dorsi* was replaced by thigh muscle volume from 2004); adult body weight: 4.3-4.8 kg.

Pannon Large: since its establishment (2005), this line has been selected for average daily gain (between the ages of 5 and 10 weeks) and for thigh muscle volume; adult body weight: 4.8-5.4 kg

Genetic evaluations are performed using BLUP methodology.

To decrease the cost of CT measurement, scanning conducted on three rabbits simultaneously fixed in special holders without anaesthesia (Figure 1).

Figure 1. CT examination of rabbits for selection.

Carcass traits of rabbits are measured by CT at 10.5 weeks of age. Initially, two CT scans per rabbit (junction of the 2nd-3rd and that of the 4th-5th *lumbar vertebrae*) were reconstructed and the L-value was calculated as the average area of the *m. longissimus dorsi* and expressed in cm^2.

In 2004, L-value was replaced by thigh muscle volume (TMV). TMV is estimated with CT scans taken every 10 mm between the *crista iliaca* of the *os ilium* and the *patella*. Depending on the dimension/length of the hind legs, 11-12 scans are reconstructed. Voxel frequency of density range belonging to the muscle tissue (between +20 and +140 of the HU scale) is determined in each scan. Summing these values (of 11-12 scans), the TMV is estimated.

The results obtained

At the beginning of the selection, phenotypic correlations were estimated between 36 carcass traits and 14 CT based parameters using 12-13 week old rabbits weighing 2800 ± 50g. Strongest correlations were obtained between the L-value and the dressing out percentage (r = 0.53-0.65) and between L-value and the weight of the mid-part to carcass ratio (0.67-0.71). Compared to the total number growing rabbits ca. 16% of the female and 11 of the male rabbits were selected for CT scanning during the period with selection for L-value. The average L-value of the selected rabbits was higher by 1 and 1.8 cm^2 in the females and males, respectively, compared to that of the rabbits scanned by CT.

Estimated genetic correlations between average daily gain (ADG) and dressing out percentage, L-value or hind part ratio were negative and moderate or low (-0.32; -0.21; -0.08, respectively), the ADG showed a positive but low genetic correlation with the thigh muscle volume (0.06-0.14). When the selection targets the average daily gain and the dressing out percentage, then the negative genetic correlation is unfavourable.

A relatively high genetic correlation was reported between the L-value and dressing out percentage (0.47). This result has high importance as it proves the efficiency of the CT-aided selection.

Since 2004 the TMV has been measured and this trait is positively correlated with ADG. An unfavourable genetic correlation between the litter weight at day 21 and TMV was found (Gyovai *et al*. 2012). The estimated correlation coefficient was moderate for the 1st and 2nd parities (-0.37 and -0.37), but high for the third and fourth parities (-0.53 and -0.70).

Compared to other rabbit breeds, the Pannon White rabbits have lower amount of perirenal fat which may suggest that the CT-aided selection decreased the rabbits' fat depots (Szendrő *et al*. 2010). This finding is unfavourable and can affect the reproductive performance of Pannon White does, as it is known that energy deficit and depletion of body stores lead to a decrease in reproductive performance.

Based on this result, the selection criteria of the Pannon White breed was changed from the ADG to the litter weight at day 21. So far the genetic correlation between the thigh muscle volume and dressing out percentage was estimated and a favourable (0.59) genetic correlation was obtained between the thigh muscle volume and hind part ratio. Based on this last finding the CT-aided selection is efficient in increasing the quantity of the hind leg meat.

The estimated genetic trends for the TMV was higher in the Pannon Large (5.8 cm^3 per year) than in the Pannon White line (4.0 cm^3 per year). This finding can be the consequence of the higher TMV heritability of the Pannon Large line compared to Pannon White rabbits. When the genetic trend was converted to additive genetic standard deviation units for the Pannon Large and Pannon White lines then the magnitude of the differences decreased (0.338 vs. 0.307, respectively).

A divergent selection experiment was carried out to determine the effect of selection for L-value based on the individual performances (Szendrő *et al*. 1996). With the help of CT scanning, the best 5 (Plus sel) and the worst 5 male rabbits (Min sel) were selected on the basis of L-value at the age of 10 weeks. Thereafter randomly chosen does were inseminated with the semen of these bucks.

In the second step the best 5 males (PlusPlus sel) were chosen from the progenies of Plus bucks. The results show (Table 1) that the CT based selection improved the dressing out percentage by 1.8%, increased the weight of mid- and hind part of reference carcass (5.1% and 2.7%, respectively), and decreased the weight of skin and gastrointestinal tract (4.1% and 6.1%, respectively).

Traits	Minus selected	Plus Plus selected	P value
L-value, cm^2	19.6	20.8	<0.05
Dressing out percentage, %	62.3	64.1	<0.05
Fore part of carcass, g	397	395	NS
Mid part of carcass, g	430	452	<0.05
Hind part of carcass, g	513	527	<0.05
Skin weight, g	386	370	<0.05
Gastrointestinal tract weight, g	379	356	<0.05

Table 1. Effect of divergent selection for L-value on carcass traits (only the males were selected) (Szendrő *et al*. 1996).

Traits	Minus selected	Plus selected	P value
Thigh muscle volume, cm^3	309	336	<0.05
Feed intake (5-10w), g/d	138	128	<0.01
Body weight gain (5-10w), g/d	44.7	45.7	NS
Feed conversion ratio	3.01	2.81	<0.001
Dressing out percentage, %	60.4	61.5	NS
Fore part to reference carcass, %	30.1	29.4	NS
Mid part to reference carcass, %	30.3	30.0	NS
Hind part to reference carcass, %	36.3	38.2	<0.05
Perirenal fat to reference carcass, %	2.40	1.90	<0.01

Table 2. Effect of divergent selection for volume of thigh muscle on productive performance and carcass traits in the third generation (Szendrő *et al*. 2012).

It can be concluded that the selection on L-value is an effective method to improve the carcass traits of rabbits and as a side effect, ratios of skin and gastrointestinal tract decrease.

The effects of selection for TMV on the production performance and slaughter traits of growing rabbits were also tested by divergent selection experiment (Szendrő *et al*. 2012). Pannon White rabbits were selected to increase (Plus selected) or decrease (Min selected) their TMV during two generations. Production and slaughter traits of their offspring (the third generation) were measured.

Selection had no effect on ADG and body weight at 10 weeks of age. Plus selected rabbits had lower feed intake (FI) and better feed conversion ratio (FCR) compared to the Min selected group (Table 2). Difference between groups for TMV was 27 cm^3 to the benefit of Plus selected rabbits (336 vs. 309 cm^3). The ratio of the full gastrointestinal tract compared to body weight was lower for the Plus selected rabbits (18.1% vs. 16.7%). Compared to the reference carcass, ratios of hind part was higher, the perirenal fat was lower in the PlusPlus group.

It was thus shown that CT aided selection can efficiently increase the TMV. At the same time, the volume of fat depots decreased while FCR also improved. Results of the experiment give some evidence for effectiveness of CT based selection. If the selection objective is to increase the TMV, not only the carcass traits but also the FI and FCR will improve.

The scientific conclusions

Based on the experimental results and genetic evaluations, the CT based selection for improving the dressing out percentage of rabbits is efficient. As a result of the CT based selection the estimated genetic trend for the thigh muscle volume is about 5 cm^3 per year. Basing calculations on 2 million slaughtered Pannon rabbits per year at Hungarian slaughterhouses, 10 tons more valuable meat (thigh muscle) would be produced per year.

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Evaluating invasive and non-invasive methods to determine fat content in the laboratory mouse

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Value for industry

- *In vivo* predictions of total fat mass/percentage and non-fat mass/percentage by the use of computer tomography (CT) enables accurate phenotyping of live animals.
- Knowledge about the performance and feasibility for routine application of *in vivo* multi-object CT is of interest to industries working with small animals.

Background

Many sophisticated, non-invasive diagnostic imaging modalities are routinely used in human medicine to assess body composition and pathological conditions in a reliable and quantitative manner. These modalities include dual-energy X-ray absorptiometry (DXA), *in-vivo* CT and magnetic resonance imaging (MRI). Subsequently, in the last several decades these technologies have been adapted for use in animal models. Whilst these modalities produce high resolution images, they have a low throughput and are relatively expensive, requiring initial calibration against accepted gold standard methods such as dissection, freeze drying and chemical analysis (Table 1) (Hastings and Hill, 1989; Bünger and Hill, 1997).

Why work is needed

The use of spiral CT scanning (SCTS) in farm animal imaging is a relatively new technology used to evaluate body composition, specifically quantifying carcass tissue weight (muscle, bone and fat). SCTS decreases scan time, resulting in the production of higher resolution images in a high throughput manner (Clelland *et al.* 2013). With the use of a multi-object insert, up to 6 animals can be scanned simultaneously, thus decreasing image acquisition time further. This technique has successfully been utilised in both rabbit and fish models (Kovács *et al.* 2013; AlRashdi *et al*. 2013 Picaud *et al.* 2014), however to our knowledge the accuracy has not yet been assessed for the mouse model. The mouse is the most common model used in research as a model for other species, due to the similarities in anatomy, physiology and genetics. Moreover, mice have a short generation time, accelerated life span and their genome can be easily manipulated, thus reducing cost, time and providing a powerful tool to model other species particularly over a lifespan.

For this reason, having a standardised method for analysing body composition during growth in a high throughput, longitudinal, precise and non-invasive manner is very important.

The objectives of this study were therefore to:

1. Evaluate the robustness of multiple imaging techniques, discussing the pros and cons of each imaging method.

2. To assess the repeatability, reproducibility and accuracy of multi-object *in vivo* CT to predict total fat mass and non-fat mass.

The methods used

Mice – All animal experiments were approved by the Roslin Institute's Animal Users Committee and the animals were maintained in accordance with Home Office guidelines for the care and use of laboratory animals. 20 male inbred mice of varying body mass (20 to 40g) and age (35 to 200 days) were sacrificed by cervical dislocation and immediately weighed.

CT scanning – The body of freshly sacrificed mice were immediately CT-scanned using a Siemens Somatom Esprit CT Scanner. Multi-object (6 mice in one scan), cross-sectional CT images were taken along the length of the body (3mm apart, field of view 450mm, approximately 70 images per mouse) (Figure 1) (Luu *et al.* 2009). Sheep Tomogram Analysis Routines (STAR) software (BioSS) (Mann *et al.* 2008) was used to calculate the total area and average densities of fat, muscle and bone in each carcass image without gutting (segmenting out guts and organs), based on density thresholds (low fat: -174 HU, high fat: -12 HU, low muscle: -10 HU, high muscle: 92 HU, Bone: < 94HU).

	Dissection / Freeze Drying	PIXImus DXA	QMRI	CT	QCT
Brief Description	Individual fat pads removed from carcass and weighed. Whole carcass dried together with removed tissues.	Low energy X-rays to produce high-resolution (0.18 x 0.18 mm pixel) images.	Application of a strong magnetic field, combined with radio waves result in a detectable signal utilising the body's natural magnetic properties.	X-rays used to generate cross-sectional 2D models via rapid rotation of the X-ray tube 360° around the animal.	Synonymous to conventional CT, however produces very high resolution images allowing for "3D" microscopy.
Cost	Low	Medium	High	Medium	High
Image acquisition time (individual mice)	N/A	≈ 5 minutes	≈ 30 minutes	≈ 5 minutes (6 mice)	< 60 minutes
Manual/ Automated analysis	Manual	Automated	Manual	Automated	Manual – Some automated functions.
Expertise/ Software	Dissection skills, access to freeze dryer.	Software included.	Require MRI Radiographer, free software available for analysis.	Radiographer, Software included.	Training required. Software included.
Outcomes	Isolated fat pads highly correlated to total fat mass. Dry matter gold standard predictor of fat %.	Estimated density and mass of lean, adipose, mineralised tissue.	Precise quantification of individual fat depots. Production of 2D/3D models. Further detailed analysis possible.	Estimated mass of lean, adipose and mineralised tissue. Production of 2D/ 3D models.	Precise quantification of individual fat depots. Production of 2D/ 3D models. Further detailed analysis possible.
Destructive	Yes	No	No	No	No (*in vivo* QCT)
References	(Sharp *et al*., 1984; Hastings and Hill 1989; Bünger and Hill 1997)	(Johnston *et al*., 2005; Stevenson and van Tets 2008 http://piximus.com/)	(Berger, 2002; Hamilton *et al*., 2011; Bao *et al*., 2013; Peng et al.,2013)	(Rampersad et al., 2005; Bünger *et al*., 2011; Clelland *et al*., 2013)	(Judex et al., 2010, http://www.bruker.com/products/x-ray-diffraction-and-elemental-analysis/x-ray-micro-ct.html)

Table 1. Comparison of invasive and non-invasive methods for measuring adiposity in rodents.

These values were established from sheep calibration trials in which lambs underwent CT scanning followed by slaughter and full dissection (Young *et al.* 2001; Glasbey *et al.* 2002; Macfarlane *et al.* 2009). Mouse specific thresholds were not available and have not been reported in the literature to the best of our knowledge.

Dissection – Following CT scanning, individual fat pads were extracted and weighed. Whole mouse carcass were subsequently frozen at -20°C prior to freeze drying (FD) (Figure 1).

Freeze drying – Whole mouse carcasses and corresponding isolated adipose tissue were freeze dried to determine the dry matter weight (DM) of the carcass (Figure 1). The prediction of individual fat percentage values was calculated by regression on dry matter content (DM/BW)using an equation (FatP _DM/BW (%) x 113 - 30.2) derived by Hastings and Hill (1989). The CT based measures of tissue weights were then compared to the DM-based estimates for the fat content (fat %) and the fat free mass (FFM) in (%) using simple linear regression : $y_i = b_0 + b_1x_i$, with b_1= regression coefficient and b_0 = intercept.

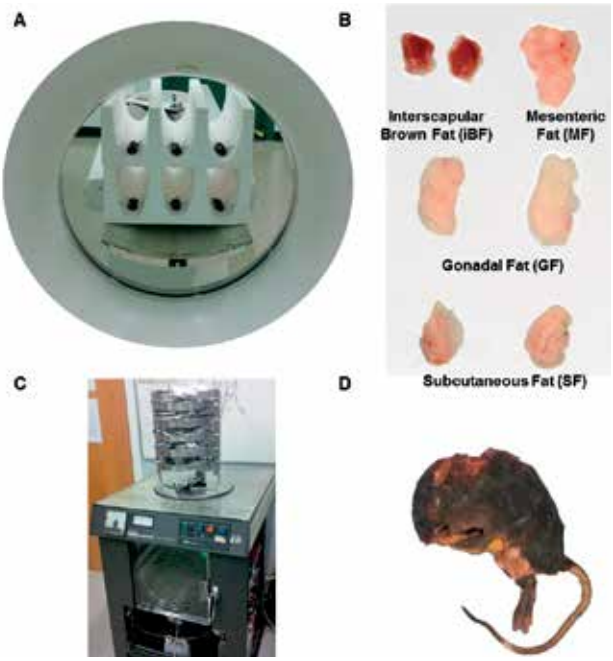

Figure 1. A. Multi-object CT scanning. B. Representative images of individual dissected fat depots. C. Freeze dryer. D. Representative Images of the freeze dried mouse.

Statistical data analysis – The data analysis has used linear regression and correlation analysis based on Excel (Microsoft Office 10) built-in functions with interval of confidence and testing of the correlation coefficients according to standard procedures described in the statistical literature (Rasch *et al.* 1978; Sharp *et al.* 1984).

Data are presented as means 3 standard error (SEM) were appropriate. Regression coefficient's are given with the intervals of confidence (P=0.05).

Abbreviations
BW: Body Weight, CT: Computer Tomography, DM: Dry matter, DS: Dissected, DXA:Dual-energy X-ray absorptiometry, FatP: Fat percentage, FatW: Fat weight, FD: Freeze dried, GF: Gonadal Fat, HU: Hounsfield unit, iBF: Interscapular Brown Fat, LTW: live tissue weight, MF: Mesenteric Fat, MRI: Magnetic resonance imaging, SF: Subcutaneous Fat, STAR: Sheep Tomogram Analysis Routines, TW: Total Weight.

The results obtained

On average the live tissue weight (LWT) of mice was 31.5g, ranging from 20.9g to 41.1g, upon separation into the various age groups (35, 120, 180 and 200 days of age) we had a large variation of fat traits with adiposity increasing with age, integral for this study. Total estimated adipose tissue by FD amounted to 3.4g (9.0%), the CT predictions were much higher (6.5g (23.6%) thus indicating that the thresholds derived from sheep dissection trials need to be refined for mice.

The simplest predictor of fatness is often live weight. Both FatW_FD and FatP_FD were highly correlated with LWT, r = 0.95 and 0.95, respectively, indicating that LWT alone allows good prediction of the fat weight and content in our cohort. LWT also correlates highly with the non-fat weight and content estimated by FD (r = 0.98 and 0.95 respectively). Moreover, unsurprisingly as the FD measures of adiposity were highly correlated with those obtained from CT (FatW_CT and FatP_CT r = 0.91,0.98) we demonstrate LWT is also a good predictor for the CT based traits, with the correlations slightly lower (r = 0.85 to 0.94).

The dissection of a single isolated fat pad from mice is a very common, invasive but highly simplistic and rapid exercise to evaluate total fat mass in mice. However the accuracy of this in C57BL/6 mice has not yet been reported. High positive correlations (r = 0.92, 0.93, 0.98 and 0.89, respectively) were found between all isolated fad pads and the FatW_FD(g) with the highest correlation between GF_DS and FatW_FD indicating that the GF seems to be the best single trait predictor for the total body fat in a mouse (r=0.98).

The scientific conclusions

We report a strong correlation between body weight alone and fat percentage in our mouse cohort (20g-40g, r = 0.95). The gonadal fat depot was identified as the most accurate single predictor of total fat mass (r = 0.931). Importantly, we observed a high positive correlation between both live tissue weight and dissected adipose tissue when correlated to CT predictions ($r \geq 0.862$), suggesting multi-object CT can accurately be used to predict total fat mass/percentage and non-fat mass/percentage in our cohort.

The next steps

- For the establishment of standardised CT methods, a comparison trial against dissection is necessary to establish mouse density thresholds, thus improving accuracy.
- With appropriate benchmarking multi-object CT will have the capability to accurately predict total muscle and bone mass (as shown in other species), thus replacing time consuming dissection in experimental design.
- Further work is required to benchmark both QMRI and QCT to dry matter based prediction or chemical analysis. These modalities, unlike CT provide high spatial resolution and contrast thus may provide additional, more detailed body composition information in a longitudinal manner.

Acknowledgements

We gratefully acknowledged the BBSRC for providing the financial support for the Ph.D. of K. J. Oldknow. We also thank COST Action FA1102 (FAIM) for support of KJO and LB. Authors thank colleagues Kirsty McLean and John Gordon from the SRUC CT unit for providing CT images, John Verth for animal assistance, Derek Ball for assistance with freeze drying and Nik Morton who provided initial guidance of dissection.

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Estimation of genetic parameters for spine characteristics, measured using computed tomography, for purebred Texel sheep

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Value for industry

- Computed tomography (CT) is a non-invasive technique that offers the opportunity to measure spine traits *in vivo*, such as vertebrae number, which are related to the number of chops.
- Estimating genetic parameters for spine traits will provide initial insight into the size and direction of the potential gain to the sheep industry if selective breeding programmes are to include spine traits.
- CT measured spine traits have low to moderate heritabilities, permitting selection responses.

Background

The formation, development, and stabilisation of the highly specialised, morphologically and genetically diverse breeds that are recognised amongst the livestock species of today have been due to both natural pressures, i.e. changes in the environment, and artificial selection, i.e. humans actively selecting the breeding animals on some desirable phenotypic trait(s) (Mignon-Grasteau *et al*. 2005; Chen *et al*. 2007; Rubin *et al*. 2012, Wilkinson *et al*. 2013). The efficiency of the latter has been greatly improved by collection of performance recordings of traits, and further still, by incorporating quantitative genetics, thus, allowing breeders more accurate predictions of, and greater influence over, the direction and magnitude of single and simultaneous trait modification through selection (Mignon-Grasteau *et al*. 2005; Chen *et al*. 2007).

The main carcass/production traits which were of interest throughout this project were body/carcass size/length and their particular association to vertebrae number and length, as these relationships may have the potential to be utilised to improve meat yield from sheep. The increasing use of computed tomography (CT) offers the opportunity to measure such spine traits *in vivo*, and the use of the technology has provided evidence of marked intra- and inter-breed vertebral variation. This has provided a good phenotypic description for spine traits.

Why the work is needed

The aim of the following study was to estimate genetic parameters for spine characteristics, along with phenotypic and genetic correlations amongst spine traits and between spine traits and other CT measured production/carcass traits for purebred Texel sheep, one of the well-established meat breeds and terminal sire lines in the industry. This should provide initial insight into both the size and direction of the potential gain to the sheep industry if selective breeding programmes were to include spine traits, and the level of emphasis that might be placed on such traits for future breed development.

The methods used

The present study used a collection of available purebred Texel lamb records (n = 461). The collection of records included information on trait performance for entire males and females born across the years 2003, 2004, and 2009, from ewes of mixed age. Lambs had been reared as singles, twins, or artificially (pet), on either a farm in Scotland (farm 1) or in Wales (farm 2) in the United Kingdom (UK). Each of the lambs used for the study group had been previously CT scanned at an average age of 117 days (range 90 – 145 days) and average weight of 34.4 kg (range 16.8 – 48.7 kg).

	Trait[2]	Description	Mean	s.d.	Min.	Max.
	CTwt	Live weight on date of CT scanning (kg)	32.42	6.037	16.80	48.70
Loin Measurements[3]	LD_W	Width of the *longissimus dorsi* (mm)	68.83	5.532	50.50	82.00
	LD_D	Depth of the *longissimus dorsi* (mm)	28.80	4.068	14.00	40.50
	LD_A	Area of the *longissimus dorsi* (mm^2)	1775	349.9	743.0	2677
Predicted tissue weights	Pfat	Predicted carcass fat weight (kg)	1.901	1.308	-0.621	6.861
	Pmusc	Predicted muscle weight (kg)	9.792	2.243	3.086	15.91
	Pbone	Predicted bone weight (kg)	2.522	0.403	1.277	3.690
	Ptotal	Total predicted tissue weight (kg)	14.21	3.704	3.820	24.00
Tissue proportions	Pfat(%)	Predicted carcass fat weight as a percentage of total predicted tissue weight	11.85	6.817	-14.21	31.07
	Pmusc(%)	Predicted muscle weight as a percentage of total predicted tissue weight	69.64	4.452	55.17	82.28
	Pbone(%)	Predicted bone as a percentage of total predicted tissue weight	18.51	3.644	12.37	38.34
	KO(%)	Killing out percentage (total predicted tissue weight as a percentage of CTwt)	43.20	4.378	22.74	53.21
	SMY(%)	Saleable meat yield percentage (predicted muscle weight as a percentage of CTwt)	30.00	2.636	18.37	35.85
Spine (region) length (SPL)	SPL_{THOR}	Length of $thoracic_{(THOR)}$ spine region (mm)	258.3	19.34	198.0	318.0
	SPL_{LUM}	Length of $lumbar_{(LUM)}$ spine region (mm)	182.1	13.75	130.0	222.0
	SPL_{T+L}	Length of $thoracolumbar_{(T+L)}$ (thoracic + lumbar) spine region (mm)	440.4	25.16	370.0	514.0
Vertebrae length (VL)	VL_{THOR}	Average length of individual $thoracic_{(THOR)}$ vertebrae (mm)	20.05	1.400	15.85	24.46
	VL_{LUM}	Average length of individual $lumbar_{(LUM)}$ vertebrae (mm)	29.10	1.558	23.86	33.00
	VL_{T+L}	Average length of individual $thoracolumbar_{(T+L)}$ vertebrae (mm)	23.00	1.337	19.47	27.05
			Median	**Q1/Q3**	**Min.**	**Max.**
Vertebrae number (VN)	VN_{THOR}	Number of $thoracic_{(THOR)}$ vertebrae	13.00	13/13	12.00	14.00
	VN_{LUM}	Number of $lumbar_{(LUM)}$ vertebrae	6.000	6.0/7.0	4.000	7.000
	VN_{T+L}	Number of $thoracolumbar_{(T+L)}$ (thoracic + lumbar) vertebrae	19.00	19/19	17.00	21.00

Table 1. Summary of CT[1] derived traits included in study. For each trait, the descriptive statistics comprising, the mean, standard deviation (s.d.), minimum (Min.), and maximum (Max.) are provided. The vertebrae count traits median and quartiles 1 and 3 (Q1/Q3) are provided in place of mean and standard deviation. The total number of records for each trait was 461.

[1] CT = x-ray computed tomography

[2] Data for all traits was derived from scans produced during CT procedure with the exception of CTwt, which was live weight physically recorded just prior to CT scanning

[3] All measured from the cross-sectional CT scan taken at the fifth lumbar vertebra

As part of the CT procedure, detailed cross-sectional reference scans (taken through the body at three positions, the ischium bone, the fifth lumbar vertebra, and the eighth thoracic vertebra) and topograms (a longitudinal, ventro-dorsal image of the body) are generated, and from these an extensive range of phenotypic data can be derived. A summary of the CT traits included in the present study, with descriptive statistics, are listed in Table 1. Analyses of CT images were carried out with the use of Sheep Tomogram Analysis Routines software (STAR; version 4.17), developed jointly by Biomathematics and Statistics Scotland (BioSS, Edinburgh, Scotland) and Scotland's Rural College (SRUC, Edinburgh, Scotland) (Mann *et al.* 2013). A mixed-linear sire model with pedigree was fitted to describe live weight (on day of CT scanning) and CT derived traits using restricted maximum likelihood procedures in ASReml (Gilmour *et al*. 2009). The final univariate model for estimating variance components (the contributions of genetic and environmental effects on traits) included the fixed effects of farm, sex, rearing rank, year born, and dam age fitted to all traits, with the addition of the fixed effect Texel muscling QTL genotype (see Donaldson *et al*. 2014) fitted to loin traits, in the analyses. Weight of the lamb at time of CT scanning (CTwt) was fitted as a covariate for loin measurement, predicted tissue weight, tissue proportion, spine length and vertebrae length traits but was excluded for vertebrae count traits. In addition, due to the nature of the data of the vertebrae count traits it was agreeable to fit them in the model as binary [0, 1] variates. The genetic effect of sire was fitted as a random factor.

Using the variance component estimates provided from the univariate analysis, the measure of heritability (h^2) for traits was calculated as the ratio of the additive genetic variance ($var\ (A)$: sire variance, $var\ (S), * \mathbf{4}$) and the total phenotypic variance ($(var\ (P): var\ (S)$ + residual variance):

$$h^2 = var\ (A) \Big/ var\ (P)$$

Further to the univariate analysis, bivariate analyses were carried out between all combinations of traits (however, this capability was not available between two binary traits i.e. between the vertebrae count traits). For the bivariate analysis between continuous traits, the model included all fixed effects, covariates, and random effects fitted as they were described for the univariate analysis above. For the bivariate analysis between a continuous and a binary trait, the fixed and random effects were fitted as for the univariate, but no adjustment was made for live weight for either trait.

Phenotypic and genetic correlations between traits were then calculated using the phenotypic and genetic variances and covariances provided by the bivariate analysis. Phenotypic correlations (r_p), between trait 1 (t_1) and trait 2 (t_2), were calculated as the ratio of the total phenotypic covariance ($cov\ (P)$) between t_1 and t_2 and the square root of the product of the total phenotypic variance of t_1 and the total phenotypic variance of : t_2

$$phenotypic\ correlation = r_p = \frac{cov(P)\, t_1, t_2}{\sqrt{var(P)\, t_1 * var(P)\, t_2}}$$

Likewise, genetic correlations (r_g) between t_1 and t_2 were calculated as the ratio of the sire covariance ($cov\ (P)$) between t_1 and t_2 and the square root of the product of the sire variance of t_1 and the sire variance of t_2 :

$$genetic\ correlation = r_g = \frac{cov(S)\, t_1, t_2}{\sqrt{var(S)\, t_1 * var(S)\, t_2}}$$

The results obtained

Heritability estimates

Heritability estimates obtained from the univariate analyses for each trait are shown in Table 2. Traits ranged widely from low (VN_{LUM}, h^2 = 0.08) to highly (VN_{THOR}, h^2 = 0.99) heritable, although with relatively large standard errors.

Trait	h^2	S.E.
CTwt	0.6520	(0.2602)
LD_W	0.3188	(0.1785)
LD_D	0.2955	(0.1797)
LD_A	0.6859	(0.2762)
Pfat	0.4230	(0.2038)
Pmusc	0.5016	(0.2180)
Pbone	0.2074	(0.1439)
Ptotal	0.2508	(0.1493)
Pfat(%)	0.5599	(0.2379)
Pmusc(%)	0.4741	(0.2132)
Pbone(%)	0.2620	(0.1576)
KO(%)	0.1794	(0.1319)
SMY(%)	0.3352	(0.1751)
SPL_{THOR}	0.3038	(0.1716)
SPL_{LUM}	0.0843	(0.0947)
SPL_{T+L}	0.1368	(0.1103)
VL_{THOR}	0.5543	(0.2412)
VL_{LUM}	0.0788	(0.0861)
VL_{T+L}	0.4431	(0.2114)
VN_{THOR}	0.9933	(0.4187)
VN_{LUM}	0.0804	(0.1246)
VN_{T+L}	0.4443	(0.2746)

Table 2 . Heritability estimate (h^2), and standard error (S.E.), for each CT derived trait.

Phenotypic and genetic correlations

A detailed presentation of the genetic and phenotypic correlations has been given elsewhere (Donaldson, 2014). To summarise, the majority of the correlations between spine region/vertebrae length traits ranged from low to very high in strength and were generally positive. Correlations between the length traits and vertebrae number traits were all low in strength and generally negative. The genetic correlations between all combinations of traits remained in the same direction as their phenotypic associations but were generally higher but also with much higher standard errors. Phenotypic correlations between spine traits and production traits were of such a small degree that they were negligible. The genetic correlations for the same spine and production trait combinations were higher but again with high standard errors. From previous analysis there has been some suggestion of positive phenotypic associations between spine traits and some production traits, however, from the results of the present study, it is difficult to distinguish any specific pattern of directional associations between these groups of traits which supports this.

The scientific conclusions

There are not yet large and powerful data sets for the dissection of the genetic basis of spine traits available. There were some restrictions (especially regarding statistical power) with the currently used data set partially due to the pedigree structure of the population studied. Nevertheless, the results support the contention that there is potential for selection on spine traits, with heritability estimates for the majority of these traits in the Texel breed fitting within the low to moderately heritable range (h^2 = 0.08 to 0.55, Table 2). Phenotypic and genetic correlations between all combinations of traits were obtained, but the genetic correlations were on the whole inconclusive and need larger data sets to be re-investigated.

The next steps

- Ideally, a much larger number of observations, from a wider genetic base, are required to obtain estimates with acceptably small standard errors (i.e. acceptable accuracy) and to fully conclude on the emphasis which should be placed on the selection of spine traits.
- This study will assist in further considerations of the use of these traits in selective breeding for genetic improvement and breed development in sheep.
- The reported findings need to be verified in larger datasets which can be extracted from the image databank mentioned in WG1T03 by Bünger *et al*.

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Prediction of carcass protein and fat chemical content using Computed Tomography in live pigs and pig carcasses

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Value for industry

- The development of a non-invasive, fast and accurate analysis based on Computed Tomography (CT) to estimate pig carcass chemical composition *in vivo* and *post mortem.*
- CT *in vivo* measurements of carcass composition can allow us to determine the optimal slaughter time and weight, and therefore to produce the ultimate product which better meet industry and consumer demands.
- CT as a non-invasive tool to model protein and fat deposition during the growing period. This information can be used to optimise pig breeding, feed efficiency and production systems.

Background

Pig carcass composition is an important parameter determining the economic value of the carcass and also for studies aiming at optimising the whole pig production system. Carcass composition is determined by its tissue composition (weight and distribution of tissues throughout the carcass) and chemical composition (protein, fat, moisture and ash content). Tissue composition has been traditionally assessed by carcass dissection while conventional chemical analyses have been used to determine chemical composition. Both types of analyses are accurate but they have several disadvantages: they are destructive, laborious, time-consuming and require the slaughter of the animal.

Computed Tomography (CT) is a non-invasive technology, based on the attenuation of X-rays in their way through the body tissues. Attenuation values depend on the different densities of the tissues and this allows the identification and quantification of lean, fat and bone tissues. CT has been successfully applied to estimate carcass tissue composition in live pigs (Szabo *et al.* 1999; Kolstad, 2001) and pig carcasses (Font i Furnols *et al.* 2009), and can be considered as a possible alternative to dissection methods. Nevertheless, there is scarce information on the use of CT to estimate carcass chemical composition of either live pigs or pig carcasses.

Therefore, the aim of this study is to evaluate the ability of CT scans of live pigs and carcasses to predict chemical protein and fat content of minced carcasses.

Why the work is needed

The chemical composition of the carcass is an important aspect in terms of quality and economic value. The numerous disadvantages of the conventional methods used to measure chemical composition make it necessary to look for an alternative technology. Research has shown the ability of CT to estimate carcass tissue composition. Nevertheless, little is known about the use of CT to determine carcass chemical composition. If successful, CT could be used to estimate carcass composition *in vivo* as well as to study and describe the growth and development of the different chemical components. This information could be used by pig breeders and meat producers in order to optimise productive parameters and improve the quality of the final product.

The methods used

Animals

This study was carried out with 92 animals with the same genetic origin, Pietrain x (Duroc x Landrace), and from four different sexes: entire males (n = 24), surgically castrated males (n = 24), immunocastrated males (n = 20) and females (n = 24). Animals were fed *ad libitum* with a commercial diet during the

experimental period (from 30 kg to 120 kg live weight). Immunocastration vaccine Improvac® was injected twice, at 12 and 18 weeks of age.

CT scanning

Pigs were scanned *in vivo* at four different target live weights (LW): 30 (n = 12), 70 (n = 16), 100 (n = 16) and 120kg (n = 48). They were fasted for at least 8 hours, weighed and anaesthetised prior to scanning. Intramuscular sedation was used at all target LW and an intravenous sedation was also applied at 100kg and 120kg to reduce breath movements. After scanning, animals were slaughtered at IRTA-Monells slaughterhouse after being stunned with 90% CO_2. Left half carcasses were scanned 24 hours *post mortem*. All the scans were performed with a General Electric HiSpeed Zx/i CT equipment located at IRTA-Monells. Images of live animals were acquired axially (1 second) and images of carcasses were acquired helically (1 pitch). Acquisition parameters for live animals and carcasses were: a voltage of 140 kV, an intensity of 145 mA and a display field of view (DFOV) from 318 to 488 mm. Image thickness was 7 mm at 30 kg LW and 10 mm at the other LW. Matrix size was 512 x 512 pixels. The procedure was approved by IRTA´s ethical committee. Figure 1 shows CT scanning of a) a live pig and b) a pig carcass.

a)

b)

Figure 1. CT scanning of: a) a live pig and b) a pig carcass.

Image Analysis

CT images were imported and analysed using Matlab software (version R2008b, The MathWorks™, Inc) to obtain the frequency of pixels associated with each Hounsfield (HU) value. Then, pixels were transformed to volume to homogenise the data.

Carcass Chemical Analysis

The right side of each carcass was used for the chemical analysis. Frozen half carcasses were first reduced into small pieces using a cutting guillotine (Model D, Spain). Teeth were removed from each carcass during this step. Then, the different pieces were gradually placed in an industrial mincer with 160 mm head (Grinder Cato-pa160, Spain). Each carcass was minced four times through plates with different pore diameter (until 3mm plate) to ensure an adequate processing of the different components. The minced side was then thoroughly homogenised for five minutes using a mixer. After that, samples were vacuum-packed and stored at -20˚C until analysis. Minced carcass samples were analysed for protein and fat content. The determination of protein content was based on total nitrogen content by Kjeldahl procedure using a nitrogen/protein analyzer (Büchi Distillation Unit B-324 and Digestor Unit B-414, Flawil, Switzerland). Fat content was determined by ether extraction (Soxtec 2050, Tecator, Höganäs, Sweden). Protein and fat content were quantified in duplicate and expressed as grams per 100 grams of carcass tissue.

Statistical Analysis

All data were analysed using SAS software (version 9.2, SAS Institute Inc, Cary, NC). Prior to the statistical analysis, the individual histograms of each scanning were plotted in order to detect outliers. No outliers were identified for live animals. For carcasses, one of them was not correctly scanned and therefore it was not used for the calculations. Chemical protein and fat data were also studied in order to detect outliers, and two carcasses were removed from the data set. Once outliers were removed, Partial Least Square (PLS) regression was applied in order to obtain prediction equations for protein and fat content. Cross-validation was used to select the optimal number of PLS factors. The variables used in the PLS models were volumes associated with attenuation HU values. The range included from -149 to +140 HU for live animals and from -100 to +120 HU for carcasses. These ranges were selected according to previous studies (Font i Furnols *et al.* 2009; 2014). The root mean square error of prediction ($RMSEP_{CV}$) was obtained by leave-one-out cross-validation using the macro presented in Causeur *et al.* (2003).

The results obtained

Descriptive chemical data

Descriptive statistics for chemical protein and fat content of minced carcasses are summarized in Table 1. Fat content showed high variability (CVx100=30.5), which is essential to obtain successful prediction equations. However, protein content had lower variability (CVx100 = 5.1). Comparison with other studies is difficult since the present work involved pigs within a large LW range (from 30 to 120 kg). Raj *et al*. (2010) showed lower values of protein content (16.5g/100g) and higher values of fat content (23.9g/100 g) in minced carcasses of pigs slaughtered at 90, 110 and 130kg LW.

CT Prediction equations

Table 2 shows statistics of CT prediction equations for chemical protein and fat content of minced carcasses. In general prediction equation statistics were similar for both studied methods: CT in live animals and CT in carcasses. Results showed that accurate equations can be obtained for predicting carcass chemical fat content. The $RMSEP_{CV}$ was 1.31 g/100g for CT scans of live animals and 1.34 g/100g for CT scans of carcasses (with an average fat content of 17.2 and SD of 5.3 g/100g, Table 1). The Residual Predictive Deviation (RPD) statistic, used to evaluate the predictive ability of the models, was higher than 3 for both methods, which is the value recommended in literature for suitable prediction models (Williams, 2001). The determination coefficients (R^2) of the regression between chemical fat content measured in the lab and predicted by CT were also high for both methods (0.94). The prediction equations for carcass chemical protein content were less accurate. The $RMSEP_{cv}$ was 0.65 g/100g for CT scans of live animals and 0.67 g/100g for CT scans of carcasses (average protein content of 18.2 and SD of 0.9 g/100g, Table 1). The RPD values were low for both methods, probably due to the narrow range of variation of this parameter (Table 2). In the same vein, the R^2 were low for both methods, although slightly higher for CT scans of carcasses (0.54 in live animals and 0.63 in carcasses).

There are few studies on the estimation of pig chemical composition using CT. Szabo *et al.* (1999) show, in their review, R^2 values of 0.83 and 0.89 for protein and fat content (data expressed as percentage of body tissue), respectively. More recently, Arthur *et al.* (2011) obtained prediction models with R^2 values of 0.92 and 0.98 for chemical protein and fat content of growing pigs, respectively, using CT tissue weights (kg). The higher accuracies found by these authors might be explained by the fact that they used CT muscle and fat weights (kg) as explanatory variables and the chemical composition was expressed in quantity (kg).

Parameter	N	Mean	SD	CVx100	Min	Max
Protein	90	18.2	0.9	5.1	15.8	20.3
Fat	90	17.2	5.3	30.5	8.7	31.9

Table 1. Descriptive statistics for chemical protein and fat content of minced carcasses (g/100g).
SD: standard deviation; CVx100: coefficient of variation; Min: minimum; Max: maximum.

Parameter	N	Variable nº (range)	$RMSEP_{cv}$	RPD	R^2
CT in live animals					
Protein	90	290 (-149 to +140 HU)	0.65	1.42	0.54
Fat	89	290 (-149 to +140 HU)	1.31	4.02	0.94
CT in carcasses					
Protein	89	221 (-100 to +120 HU)	0.67	1.37	0.63
Fat	88	221 (-100 to +120 HU)	1.34	3.93	0.94

Table 2. Statistical parameters of Computed Tomography (CT) predictions for chemical protein and fat content of minced carcasses.
$RMSEP_{CV}$: root mean square error of prediction; RPD: residual predictive deviation = SD/$RMSEP_{CV}$; R^2: determination coefficient.

The scientific conclusions

Accurate predictions were obtained for carcass fat content using CT in the live animal and in the carcass. Less accurate predictions were obtained for carcass protein content using both methods. Similarities within the prediction parameters obtained in both methods (*in vivo* and in the carcass) indicate the versatility of this technology. Moreover, these results suggest the possibility of measuring carcass chemical composition in live animals using CT. Nevertheless, further research is required in order to improve the prediction ability of the models and test its application in other sets of data.

The next steps

- To include more experimental data in order to improve the model prediction ability mainly for protein content.
- To study the use of CT to estimate other chemical components in the whole carcass such as moisture, ash and mineral (calcium and phosphorus) content.
- To validate the developed prediction equations on an independent data set.
- To apply the prediction equations on live animals to model protein and fat deposition during the growing period.

Acknowledgements

This research was funded by the Spanish National Institute of Agricultural Research (INIA) corresponding to Project No. RTA2010-00014-00-00 "Evaluación *in vivo* del crecimiento alométrico de los tejidos muscular y adiposo de los credos según la genética y el sexo mediante tomografía computarizada". INIA was also thanked for the scholarship to Anna Carabús. COST Action FAIM FA1102 "Optimising and standardising non-destructive imaging and spectroscopic methods to improve the determination of body composition and meat quality in farm animals" is greatly acknowledged. The authors thank A. Rossell, A. Quintana, C. Francàs and C. Pedernera for their valuable technical assistance.

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Predicting intramuscular fat content in the loins of divergent sheep breeds using X-ray computed tomography

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Value for industry

Computed tomography has:

- led to the development of a fast, non-destructive analysis tool to accurately estimate meat (eating) quality traits *in vivo;*
- provided a method to enable across breed evaluations of meat (eating) quality traits;
- produced the means to select for lean carcass growth without compromising meat (eating) quality in crossbred lamb breeding programmes;
- accelerated genetic improvement of carcass traits and has similar potential for meat (eating) quality traits in maternal and crossbreeding selection programmes.

Background

The fat content of meat plays a significant role in the acceptability of major meat quality attributes considered by both the processor and consumer. Generally, four major fat depots are recognised in the carcass of an animal: subcutaneous (under the skin); internal organ associated (surrounding the kidneys and other internal organs); intermuscular (between muscles and muscle groups); and intramuscular (IMF, within the muscle and between muscle fibres), the latter having the greatest association with meat (eating) quality (Smith and Carpenter, 1974; Savell and Cross, 1988).

Continued consumer preference for leaner meat, and a reduction of visible fat (subcutaneous and intermuscular) alongside the drive for a reduction in necessary fat trimming (subcutaneous), have influenced current selection practices to reduce subcutaneous fat and increase lean meat in sheep (Simm *et al.* 1985).

A similar approach in selection practices of pigs resulted in an associated decrease in IMF and in turn a negative effect on the palatability of fresh pork meat (Sonesson *et al.* 1998). A similar picture seems to be starting to emerge from the sheep industry, mainly in terminal sire breeds (Lambe *et al.* 2008; Pannier *et al.* 2014).

Why the work is needed

Given that the majority of UK commercial lamb meat production is from crossbred lambs, computed tomography (CT) has the potential to be of interest in the acceleration of genetic improvement of carcass traits in maternal and crossbreeding selection programmes (Conington *et al.* 2006), because of its higher accuracy compared to the sole use of ultrasound techniques. The purpose of this study was to investigate the accuracy of prediction equations developed using data from a terminal sire sheep breed (Texel) in two divergent breeds/crosses: a typical UK commercial crossbred slaughter lamb (TexX; Texel sires crossed with Scotch Mule ewes) and a UK hill sheep breed (SBF; Scottish Blackface).

The methods used

Data were derived from previous studies. The studies provided data comprising information from pure-bred Texel lambs (n = 377) of both sexes (female and entire males) produced over three separate years, 2003 and 2004 lambs were reared to finishing (ready for slaughter depending on condition score and live weight) and slaughtered (Lambe *et al.* 2008) and 2009 lambs were reared to weaning and slaughtered at approximately 20 weeks (Lambe *et al.* 2011). SBF lambs (n = 233) comprised of entire males and females reared to finishing and slaughtered (Lambe *et al.* 2008), and finally TexX lambs (n = 168) comprising of entire males and females reared to approximately 20 weeks of age, weaned from their mothers, and slaughtered (Lambe *et al.* 2010). Details of CT and slaughter traits in all breeds can be found in Table 1.

		Tex (n=370)			SBF (n=230)			TexX (n=165)		
	Trait Description	**Mean**	**Min-max**	**s.d.**	**Mean**	**Min-max**	**s.d.**	**Mean**	**Min-max**	**s.d.**
CT Traits										
CTLWT	Live weight at time of CT scanning (kg)	35.35	20.6-49	4.87	34.36	28.1-43.6	3.14	39.62	23.8-51.6	4.64
CT_Age	Age at CT (d)	133	93-202	21.01	145	105-202	23.86	144	132-152	4.62
LV5MD	Average muscle density in 2D scan at the 5th lumbar vertebra (HU)	48.30	41.8-55.9	2.65	44.68	38.7-50.3	2.11	46.45	41.2-53.2	2.06
TV8MD	Average muscle density in 2D scan at the 8th thoracic vertebra (HU)	44.68	36.5-54.7	2.98	39.90	32.2-51.1	2.53	41.99	37.3-51.4	2.37
LV5STD	Average soft tissue density in 2D scan at the 5th lumbar vertebra (HU)	36.22	-1.6-49.5	8.09	18.91	-14.4-44.6	12.27	22.62	-15.6-46.5	11.14
TV8STD	Average soft tissue density in 2D scan at the 8th thoracic vertebra (HU)	21.84	-21.1-46.2	11.35	2.54	-26.6-33.9	12.07	5.41	-27.7-34.4	12.34
ISCSTSD	s.d. of soft tissue density in 2D scan at the ischium (HU)	40.34	29.3-57.9	5.66	49.40	33.9-66.4	6.02	49.04	34.8-60.9	5.58
LV5STSD	s.d.of soft tissue density in 2D scan at the 5th lumbar vertebra (HU)	40.33	30.4-64.7	6.19	51.46	31.3-69.1	8.09	51.27	-15.6-46.5	8.44
TV8STSD	s.d. of soft tissue density in 2D scan at the 8th thoracic vertebra (HU)	50.56	34.1-68.1	6.70	58.01	41.6-68.8	5.49	59.34	42.5-71.9	6.40
Pr_Cfat	CT Predicted total carcass fat weight (kg)	2.34	0-6.9	1.11	3.01	1.2-5.9	1.00	3.54	0.4-7.3	1.21
Pr_IMF_A	*M. longissimus lumborum* CT predicted extracted intra-muscular fat (%)	1.48	0.04-3.3	0.56	2.19	1.1-3.5	0.44	2.07	0.4-3.5	0.48
Pr_IMF_B	*M. longissimus lumborum* CT predicted extracted intra-muscular fat (%)	1.48	0.1-4.1	0.56	2.64	1-4.9	0.77	2.29	0.7-4.8	0.68
Slaughter Traits										
Chem_IMF	*M. longissimus lumborum* chemically extracted intra-muscular fat (%)	1.48	0.3-3.9	0.68	2.28	0.2-4.6	0.82	2.14	0.7-3.9	0.61
SL_Age	Age at slaughter (d)	150	99-234	23.3	163	113-230	27.16	149	139-157	4.56

Table 1. Trait descriptions, means and standard deviations (s.d.) in Purebred Texel (Tex), Scottish Blackface (SBF) and Texel cross Mule (TexX) lambs.

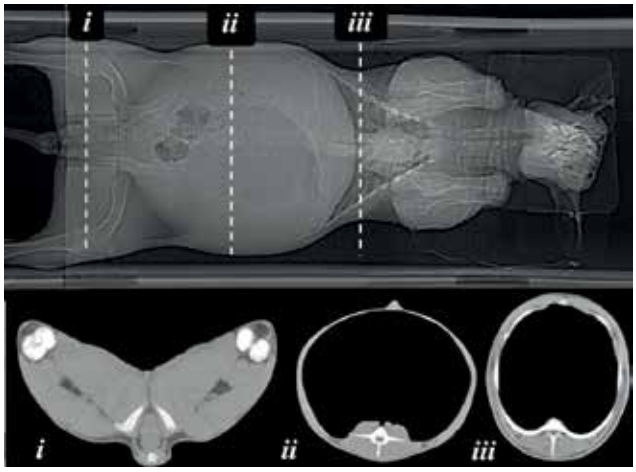

Figure 1. Topogram (Top) and single slice CT scan images (bottom) at the ischium (ISC) (i), 5th lumbar vertebra (LV5) (ii) and 8th thoracic vertebra (TV8) (iii).

All lambs were CT scanned pre-slaughter. In each of the studies, two-dimensional (2D) cross sectional scans (FOV = 450mm, resolution = 512x512 pixels) were taken at three defined anatomical positions; ISC, LV5 and TV8 (Figure 1). Image analyses were performed to separate carcass from non-carcass tissues (Glasbey and Young, 2002) and the density of each pixel (0.7mm^2) in the carcass portion was allocated to fat, muscle or bone, according to density thresholds using Sheep Tomogram Analysis Routines (STAR) software (Mann *et al.* 2013). Average densities (Hounsfield unit; HU) of each tissue in each 2D image were calculated, as well as standard deviations for the density values of all pixels allocated to each tissue. A novel average soft tissue density (and its standard deviation) was also calculated, combining the information from all pixels allocated as fat or muscle.

Carcass fat, as a measure of subcutaneous and intermuscular fat, was also predicted (Pr_Cfat) from these 3 single slice CT scans using breed-specific prediction equations developed from previous research (Lambe *et al.* 2006; Macfarlane *et al.* 2006a, Macfarlane *et al.* 2006b).

Intramuscular fat (Chem_IMF) was measured post-slaughter in a cross-sectional slice taken from the cranial end of the *M. longissimus lumborum.*

Equations used here for the prediction of IMF (Pr_IMF) were derived and validated using various CT parameters from single slice CT scans taken at the ISC, LV5 and TV8 in purebred Texels (Clelland *et al.* 2014). Results from this study identified two (A, B) optimum prediction equations based on available CT information with prediction accuracies of Adj R^2 = 0.66 and 0.68 respectively, in Texel lambs, these were;

A; CT Predicted IMF (Pr_IMF_A) (%) = 6.920 + (Pr_Cfat*0.2425) – (LV5MD*0.0654) – (TV8MD*0.0637)

B; CT Predicted IMF (Pr_IMF_B) (%) = 7.320 + (Pr_Cfat*0.0565) – (LV5STD*0.0626) – (TV8STD*0.03585) + (ISCSTSD*0.02209) – (LV5STSD*0.0565) – (TV8STSD*0.0303)

The results obtained

Summary statistics of CT traits included in the prediction of IMF, alongside meat quality (MQ) and production traits for Texel, SBF and TexX data sets can be found in Table 1.

Model A, which included information from Pr_Cfat, average muscle density in the fifth lumbar vertebra scan (LV5MD) and average muscle density in the eighth thoracic vertebra scan (TV8MD), performed well when validated in the SBF data, resulting in a prediction accuracy of R^2 = 0.64, but saw a significant reduction in prediction accuracy when validated using the TexX data (R^2 = 0.37). Prediction accuracies for Model B, which included information from Pr_Cfat, average soft tissue density in the fifth lumbar vertebra and eighth thoracic vertebra scans (LV5STD, TV8STD) and the standard deviation of soft tissue density in the ischium, fifth lumbar vertebra and eighth thoracic vertebra scans (ISCSTSD, LV5STSD, TV8STSD), were significantly reduced when validated against both the SBF data and the TexX data (R^2 = 0.57 and 0.36 respectively). Validation results can be found in Table 2

Model	Texel	SBF	TexX
	Adj R^2 (RMSEP)	R^2 (RMSEP)	R^2 (RMSEP)
A	0.66 (0.40)	0.64 (0.49)	0.37* (0.48)
B	0.68 (0.39)	0.57* (0.54)	0.36* (0.49)

Table 2. Validation of selected models across SBF and TexX data sets.
*Coefficient of determination (R^2) is significantly different from development data (Texel) (P<0.05).

The scientific conclusions

The results of this study show that prediction equations derived from a terminal sire (Texel) data set are transferable in the prediction of IMF in divergent breeds (SBF and TexX). The transferability and resulting accuracy of prediction was better in SBF than in TexX, however levels of accuracy were still at an acceptable level in the TexX. These results provide evidence that the prediction equations derived from purebred Texel lambs for successfully predicting IMF from CT parameters can be transferred across different breed types, producing correlations of 0.61 to 0.80 (R^2 = 0.37 to 0.64) in the breed types included in this study.

Acknowledgements

The authors would like to acknowledge the support of EBLEX, Quality Meat Scotland and Meat Promotion Wales.

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A pragmatic short-term approach to establish a Computed Tomography (CT) based reference method for the measurement of lean meat percentage (LMP) in pig carcasses

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Value for industry

- Computed Tomography (CT) can provide accurate and precise measurements of carcass and body composition of some farm animal species (e.g. rabbit, chicken, sheep, pig). Accuracy and precision depend on the applied CT protocol (e.g. slice thickness, sequential or spiral CT, scan locations).
- Due to its high accuracy and precision CT has the potential to be a primary reference for lean meat percentage (LMP).
- If the EU adopts CT, as primary reference for LMP it could be used in all member states to calibrate new equipment against this CT standard and this could avoid labour intensive manual dissections.
- Building an international CT-based reference would increase the accuracy of LMP evaluation and comparisons between countries, and consequently improve the market transparency and the efficiency of the whole pork chain on a national and EU level.

Background

In the EU, methods of classifying of pig carcasses must be calibrated against a reference. While this reference has always been LMP obtained by manual dissection, its definition has evolved over time. For 20 years LMP has been based on partial dissection, using a standardised cutting and dissection of the four main joints according to Walstra and Merkus (1996).

CT has been used to measure body composition in farm animals for many years by numerous researchers, in particular, in sheep and pig breeding (Bünger *et al.* 2014; Kongsro, 2014). Ten years ago, the EUPIGCLASS project recommended the introduction of CT as a reference method for pig carcass classification in the EU. This was a starting point for new research in this area. Several countries developed their own CT protocols and measurement methods for pig carcasses (Christensen and Borggaard, 2005; Daumas and Monziols, 2011; Font i Furnols *et al.* 2009; Judas *et al.* 2006; Romvári *et al.* 2006), but without any concerted EU framework. In 2008 an additional reference LMP, based on total dissection of a half-carcass without the head, was introduced (EC regulation 1249/2008). The possibility to use CT was included in this regulation, "provided that it gives satisfactory comparative dissection results". This has been interpreted by the pig grading expert group as meaning that member states that wish to use CT must calibrate it against dissection. Given the known high accuracy of CT it is the view of the authors of this paper that CT should be recognised as a reference method to determine LMP without the need for manual dissections.

One of the objectives of the COST Action FAIM, is to further the use of CT as a primary reference method for measuring body/carcass composition. All relevant reference methods for evaluation of LMP in pig carcass classification were identified at FAIM II (Daumas *et al.* 2013). The accuracies of reference methods based on CT and manual dissection were compared, with manual dissection showing the lowest accuracies (Olsen and Christensen, 2013).

Finally, the European Commission has decided to revise the EU pig carcass classification regulation and thus started a debate on these issues. The aim of this paper is to contribute to this debate by outlining how the authors feel the process should proceed. It is hoped that this paper will stimulate a debate on this issue at the FAIM III workshop which will continue and result in a final consensus among the group of experts.

Why work is needed

- The present EU pig carcass classification regulation needs improvement.
- Calibrating CT against manual dissection (as a standard) generates a greater complexity of the protocol and additional costs that are not always scientifically justified.
- CT and knife are two tools allowing the (virtual or real) dissection of whole carcasses or carcass parts (main joints).
- Virtual dissection by CT offers considerable advantages compared with manual dissection.
- To allow the maximum benefits of this new technology to be realised CT should be recognized as a standalone LMP reference equivalent to manual dissection.

The timetable of the European Commission to modify the EU pig carcass classification regulation makes this work urgent because autumn 2014 is likely to be the deadline for the final discussions.

Furthermore, one important aim of FAIM is to help to develop a CT reference method independent of manual dissection. One of the key elements underpinning this aim is Milestone 7: "Metrological documentation and handbook of reference methods for possible and relevant post mortem measurements of carcass composition in pig, sheep, beef and poultry". This paper, representing the views of some members of WG1, is an important contribution to this milestone.

Selection for breeding and the classification of carcasses are two fields with a very active use of CT, but the need for a common reference is more essential for classification. Moreover, since carcass classification is more advanced in pigs classification than in the other species, it seems logical to start to remove existing barriers in pigs first.

The methods used

The philosophy of our approach consists of giving CT, in the short term, the same status as manual dissection, i.e. EU reference method for pig carcass classification. The main problem is that most of the countries with CT facilities wish to continue the use of their own CT method/protocol. This makes it difficult, in the short term, to agree on a common CT reference method. To circumvent this difficulty, we propose a validation of "in-house" CT methods with a common CT reference method. Obviously, some criteria, which still need to be defined, should be fulfilled for these "in house" procedure to be authorised .

These criteria have to be discussed and agreed considering the whole calibration process of the classification methods. The most important objective of a reference method for LMP is to calibrate various on-line classification methods, which can have a prediction error up to 2.5% in LMP. The importance of the 'reference LMP' has thus to be put into perspective. Moreover, the criteria have to be set with respect to the accuracy of manual dissection, which suffers from a level of uncertainty, which is not negligible (Nissen *et al.* 2006). To be consistent, it seems reasonable to accept a comparable level of uncertainty for LMP measured by different CT protocols.

The approach suggested by the authors of this paper includes four steps listed below, and subsequently described in detail:

1. Choice and description of a recommended CT reference measurement method,
2. Comparison between the recommended CT reference and manual dissection reference,
3. Description of various candidate CT reference measurement methods,
4. Comparison between the candidate CT references and the recommended CT reference.

Step 1. The recommended, agreed CT reference should be chosen according to the following criteria

- A stand-alone reference, designed independently of manual dissection.
- Simplicity and therefore easily usable by trained personnel.
- Accuracy/Precision (A/P) and a reasonable ratio between cost and A/P. A/P should be determined by the use of an agreed phantom (ideally a set of phantoms) and using spiral scanning with varying slice thickness (from high density to lower density).
- Robustness.
- To "be equivalent to dissection" (see step 2 below).

Step 2. The recommended, agreed CT reference should be compared with the dissection reference

Comparison should be based on the criteria related to international standards, and thresholds have to be agreed on. To be consistent, it seems reasonable to choose thresholds corresponding to the criteria estimates for manual dissection.

Concerning accuracy and precision, acceptable levels should be comparable/ consistent with the present situation. The average bias between the two present references, i.e. LMP from partial dissection and LMP from full dissection, was assessed to be about 1% LMP. This same bias could be tolerated between the recommended CT reference and manual dissection. As total and partial manual dissection are authorised, the same should be allowed in the case of CT reference. The same bias could be tolerated between the recommended CT of the whole carcass and CT of primal cuts.

Step 3. The candidate CT references should be chosen according to the following criteria

- A/P should be determined like in step 1.
- To "be equivalent with the recommended CT reference" (see step 4 below).

Step 4. All candidate CT references should be compared with the recommended CT reference

Comparison should be based on the criteria related to international standards, and thresholds have still to be agreed. To be consistent, it seems reasonable to choose thresholds corresponding to the estimates for manual dissection.

The proposal is open for further discussion and adaptations, except for the concept of CT as a stand-alone reference method (Figure 1a and b).

Figure 1a. CT scanning a pig half-carcass.

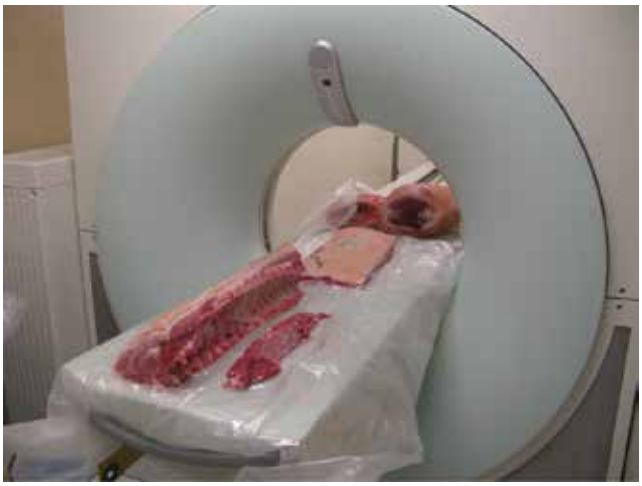

Figure 1b. CT scanning the four main joints.

The results obtained

To illustrate the proposed approach an example is described below, which includes the four steps mentioned above.

Firstly, let us consider the following recommended CT reference measurement method. Until now, only one CT method has been developed as a stand-alone reference, independent of dissection, to measure the LMP of pig carcasses (Daumas and Monziols, 2011). Image analysis can be easily performed by using a very common technique (thresholding) available in some freeware software packages (like ImageJ). This is used as the basis of our example. Then, in order to make the recommended CT reference accessible to a maximum number of institutions, we propose ranges for the acquisition parameters that are less sensitive than image analysis. The ranges are a reasonable compromise between the CT parameters used by the various institutions.

Figure 2 shows histograms obtained from scanning very different pigs - from pure Piétrain entire males (very lean) to heavy Italian pigs (very fat), described in Daumas *et al.* (2013). Despite the huge carcass composition variability the proposed HU interval seems to be adequate in all cases.

LMP calculation

LMP = d VMUS / WENT

Where:

- WENT is the weight of the scanned entity (either carcass or the four main EU joints),
- VMUS is the muscle volume of the scanned entity,
- "d" is a multiplicative factor, corresponding to the muscle density: d = 1.04 (ICRU, 1989).

Acquisition parameters

- Voltage: Range 120 kV – 140kV,
- Current intensity: minimum of 70 mA,
- Slice thickness: maximum of 10 mm,
- Spiral scanning,
- Reconstruction filter for soft tissues.

Image analysis

- Thresholding and classification of pixels,
- VMUS = Number of pixels in the interval [0 – 120] Hounsfield units (HU) multiplied by the volume of pixels.

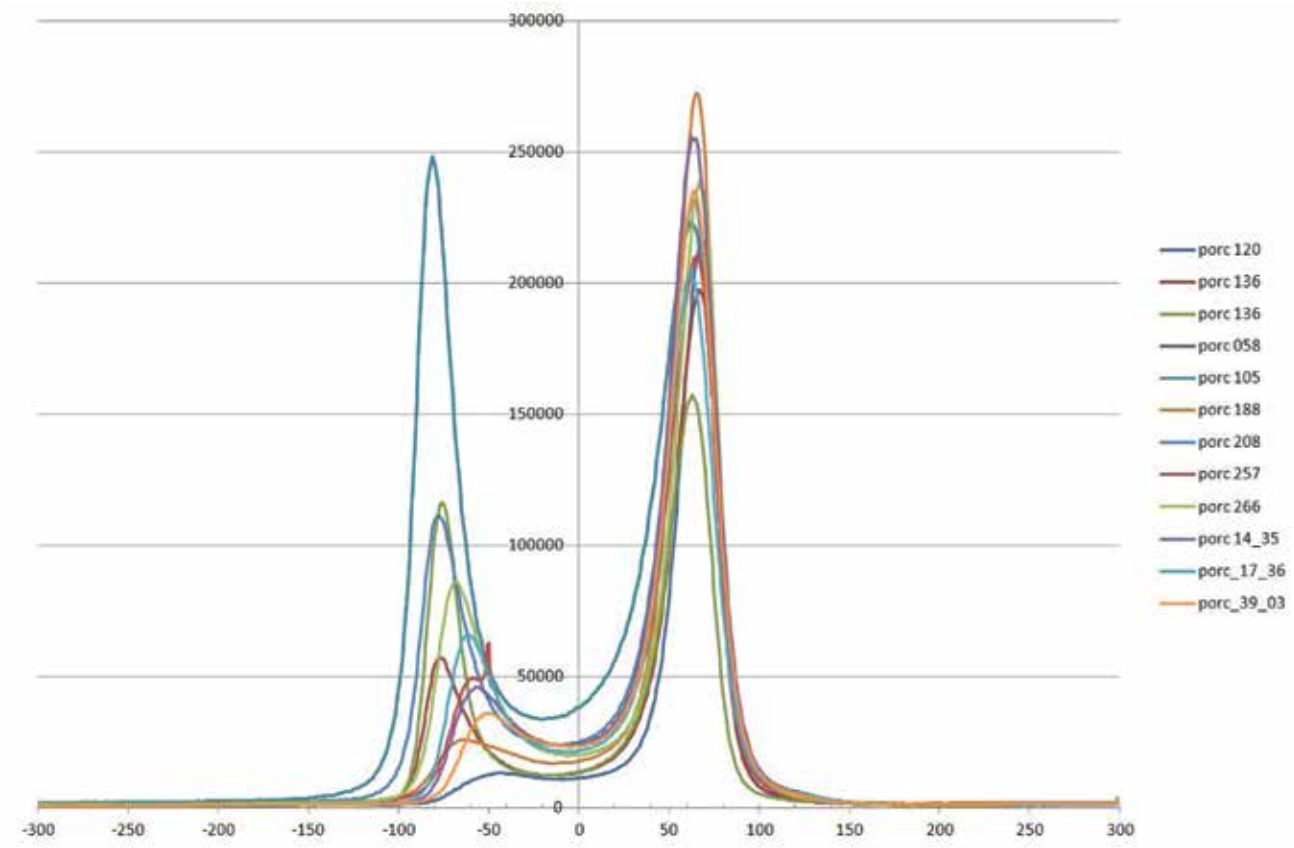

Figure 2. Histograms of HU values for 12 very different pigs.

Secondly, let us assume that bias would be the single criterion for evaluating the reference method. A study performed by Daumas and Monziols (2011) on 63 pigs demonstrated a non-significant bias of 0.6% LMP between the above recommended CT reference and the manual dissection reference for the four main joints. If we assume that the agreed threshold for a bias was ≤ 1% LMP then the above CT reference method would be recommended.

Thirdly, a candidate CT method may be proposed. It can differ from the above recommended method in acquisition parameters (axial scanning, different tube voltage or current...) and/or in image analysis (contextual analysis, specific image pre-processing, PLS calibration with dissection ...). Let us consider the following candidate CT procedure, which differs only in the upper limit of the muscle:

- LMP = 1.04 VMUS / WENT
- Acquisition parameters in agreement with the recommended CT reference (slice thickness = 3 mm),
- Image analysis: VMUS = [0 – 200] HU

Fourthly, let us compare the candidate and the recommended CT references, assuming that bias would be the single criterion for evaluating the reference method. The impact of an upper limit of 200 HU, instead of 120 HU, resulted in a median relative LMP difference of 1.8% (Daumas et al. 2013); which corresponds approximately to an absolute bias of 1.1% LMP. If we assume that the threshold on bias was agreed as a maximum of 1% LMP, then the candidate CT method would not be accepted. But if we assume that the threshold on the bias would be agreed as a maximum of 2% LMP, then the candidate CT method would be acceptable.

The scientific conclusions

- Previous CT studies support the contention that CT can be at least as accurate as manual tissue dissection (by knife) as a reference method for pig carcass classification, and can replace it when a CT scanner is available.
- The proposed approach shows one practical solution to facilitate the use CT instead of manual dissection as a reference method in the short-term.
- A possible relevant CT reference method that could be recommended for pig carcass classification has also been proposed.
- Several candidate CT procedures are being used in different countries and their accuracy for LMP determination needs to be established in order for them to be accepted as a CT reference.
- Some criteria and thresholds have been presented to illustrate the feasibility of the approach.

The next steps

- A roadmap based on this paper could contain the following steps:
  - A group of experts from pig classification and CT fields should propose and discuss two recommended CT references: one for the LMP of a pig carcass and one for the LMP of its four main joints.
  - The acceptance procedure of the candidate CT methods should be discussed in detail.
  - This discussion within the FAIM action may provide additional insights for the EU that could inform the debate about the EU regulations for pig classification and the role of CT as a primary reference method.
  - Amongst the points for discussion will be the different types of possible references; four could be specified;
    1. The LMP from manual dissection of the four main joints,
    2. The LMP from manual dissection of the carcass,
    3. The LMP from virtual CT dissection of the four main joints,
    4. The LMP from virtual CT dissection of the carcass.
  - It is obvious that each reference will have to be documented and that reference 3 will be based on the agreed cutting points of reference 1.
- The proposed approach for the LMP by CT could be expanded to other tissues, other species, and other non-invasive imaging techniques, such as MRI.
- Further investigations aimed at delivering an improvement in this approach and involving a focus on phantom use should be carried out.
- A set of phantoms should be selected and circulated among all CT units in Europe. These phantoms should be scanned according to the suggested standard protocol and with the lab specific protocols in use in the different countries.

Acknowledgements

Funding by the COST Action FAIM has assisted progress on this issue of a common CT reference for pig carcass classification.

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Determination of meat quality with non invasive technologies in live animals

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Value for Industry

- Measuring *in vivo* meat quality is a big challenge for animal and meat production.
- Correlated or confounding between traits i.e. carcass, production and maternal traits, requires high quality and accurate phenotyping of meat quality.
- Moving meat quality measures from carcass and meat samples, towards live animals, will reduce costs and make selection of animals based on their meat quality possible.

Background

Consumer preference for meat has moved towards leaner meat maintaining juiciness, flavour and tenderness. In addition, nutritional value of meat with respect to health is of great interest, i.e. fatty acid composition (FA). Leanness of meat, juiciness and FA are often negatively correlated to each other, in addition to negative correlation with animal production, carcass and maternal traits. This presents one of the biggest challenges in animal breeding and production. There are a large number of publications showing measurements of meat quality at slaughter and meat samples, however this paper will not focus on these measurements. Indirect means of assessing meat quality are considered in several publications. These show the relationship between production traits like growth and meat quality, where high growth rates was found to induce large fiber diameters, lower proteolytic potential and reduced water holding capacity of poultry meat (Dransfeld *et al*. 1999). Handling of animals may induce stress related responses in animals like postmortem acidification, leading to dark cutting beef in cattle (Warriss, 1990). The feeding of animals also affects meat quality, i.e. fatty acid levels in feed diets have a significant effect on meat from both ruminants and mono-gastric animals (Wood *et al*. 2003). Alternative strategies like nutrigenomic approaches have also been presented to cope with meat quality challenges related to feeding (Andersen *et al*. 2005). In terms of non invasive and non-destructive measurements, several papers have shown the potential of fast sensors which may be deployed, based on the development of biophysical methods for assessing meat structure and quality (Dames and Clerjon, 2008). Many of these methods may be transferred to an *in vivo* measurement situation. Ultrasound seems to be the preferred technology used with respect to *in vivo* meat quality; predicting the level of intramuscular fat (IMF) in both cattle (Aass *et al*. 2006) and swine (Newcom *et al*. 2002). Computed tomography (CT) and magnetic resonance imaging (MRI) has proved to be a very accurate and valuable tool in estimating body composition in farm animals (Szabo *et al*. 1999), and CT was found to predict both intramuscular fat and fatty acid composition in beef cuts (Prieto *et al*. 2010), intramuscular fat in Texel lambs (Clelland *et al*. 2014). In a paper by Kongsro and Gjerlaug-Enger (2013), CT was found not to be a feasible method for *in vivo* prediction of IMF in swine. However, the prediction equations used in this paper did not include information from body fat. Nuclear magnetic resonance (NMR) spectroscopy was used to measure IMF *in vivo* using biopsy samples, however, the biopsy method was considered to be invasive from the animal welfare point of view.

The biggest challenge seems to be the ability to make a prediction of meat quality without the confounding effect of carcass or body composition, like fatness, muscle depth etc. The main focus must be to develop «stand alone» measurements which enable us to make robust and accurate prediction without confounding effects from other correlated measurements or traits.

Why work is needed

In vivo measurements of meat quality are of great importance in order to meet consumer demands for higher leanness and nutritional value, and increased juiciness, flavour and taste. In order to cope with negative correlations within different meat quality traits, and between meat quality traits and

production, carcass and maternal traits, or to avoid confounding between traits, more accurate «stand alone» measurements are needed. This means that prediction equations containing body or carcass fat cannot be sustainable in predicting IMF or FA, due to the confounding or correlation between IMF, FA and body fat. One cannot obtain genetic progress in these traits without solving the problem of confounding. These measurements must be non-invasive, accurate, fast and cost-effective in order to meet both the breeders, farmers, meat industry and consumers demands for an effective meat supply chain.

Evaluation of non-invasive technologies Method used and results obtained

The accuracy of non-invasive technologies depends on, i.e. the degree of variation in the trait being measured, time between measurement and slaughter, and operator errors. Selecting for leanness will result in a lower level and lower variation in for example IMF, which will result in poorer prediction results. The difference in the animal populations between Europe or the US will strongly affect the results of the different technology with respect to meat quality. One needs to take into consideration, both the animal population and the samples from it, and the ability of the technology to predict meat quality traits.

Ultrasound

The first papers on ultrasound date back to the 1950s and enabled researchers to visualize the cross-section view of the near-skin tissues (Szabo *et al*. 1999). Ultrasound utilizes high-frequency sound signals and the reflection is measured on the interface between different tissues (i.e. fat and muscle). Ultrasound has been used extensively to measure fat and muscle depth *in vivo* on farmed animals as an estimate of body composition. With respect to meat quality, Newcom *et al*. (2005) reported a correlation of 0.34 to 0.60 between ultrasound and intramuscular fat percent from chemical analysis. Other studies have shown low accuracies, and the main reason may be the low degree of variation in IMF. The traits being measured must be reflected by some kind of reflection by the sound signals. The advantage of ultrasound is the low cost and ease of use, distribution worldwide and widely accepted as *in vivo* measures of body composition.

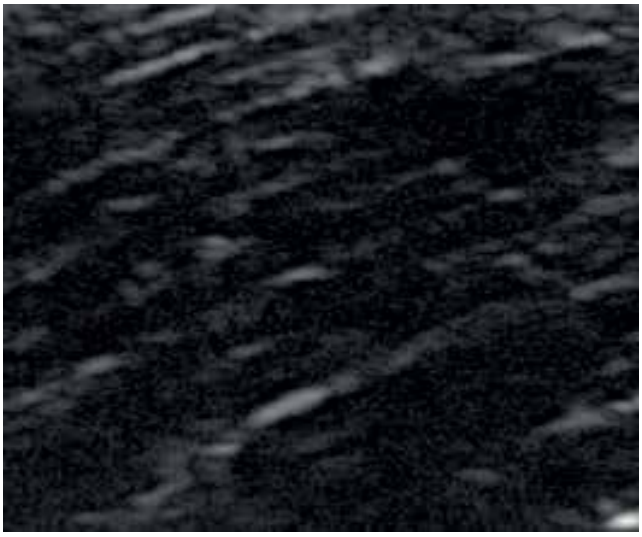

Figure 1. Ultrasound image loin sample marbling. (Courtesy of Norsvin)

Computed Tomography (CT)

CT was first applied for farmed animal studies in Norway in the early 1980s (Skjervold *et al*. 1981). Since then, CT has established itself as a very accurate technology for predicting or estimating body and carcass composition. CT utilizes the rates at which the tissues in the body attenuate X-rays. The images generated from CT from reconstructing these attenuation profiles by rotation, consist of a matrix of pixels, each pixel having a CT-value or gray scale. These CT values represent the level of attenuation within this pixel, and are highly correlated with the density. With respect to meat quality, the trait being measured must be reflected by some kind of difference in CT values, either on a pixel scale, or by patterns in pixels i.e. texture. Both Prieto *et al*. (2010) and Clelland *et al*. (2014) reported accuracies of 0.76 and 0.65 for beef cuts (*post mortem*) and Texel lambs (*in vivo*) when predicting IMF using CT. Kongsro and Gerlaug-Enger (2013) reported a low accuracy for *in vivo* prediction of IMF in pigs using CT, and CT was not considered to be a feasible tool for this purpose. The results from all studies depend on the variation in IMF, which was lowest in the pig situation, the confounding between body fat and IMF, and the validation method used in the papers. There are still a lot of possibilities for further development of CT techniques with respect to meat quality. Changing energy levels or applying different filters might improve the accuracy, but at the end of the day it all depends on the variation of meat quality traits in the samples and if they are related to some kind of difference in densities, either on the pixel level or matrix/texture level.

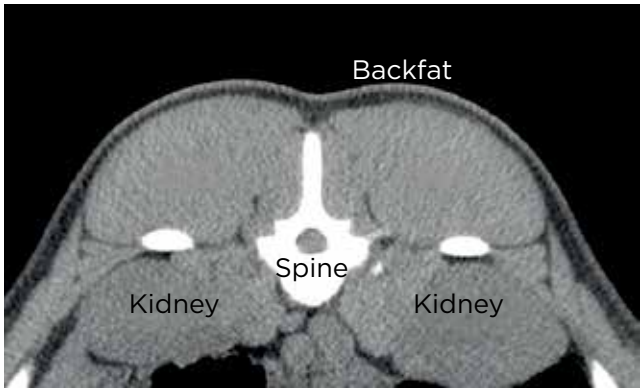

Figure 2. CT image loin sample.
(Courtesy of Norsvin).

Magnetic Resonance Imaging (MRI)
Like CT, MRI is based on images consisting of a matrix of pixels, but the principle and physics behind these images are entirely different. In nuclear magnetic resonance (NMR) the nuclei of atoms, positioned in a strong magnetic field, absorb and re-emits energy as a function of time (Szabo *et al*. 1999). Based on the magnetic properties of the hydrogen atoms, MRI can provide cross-sectional images of bodies (Monziols *et al*. 2006). The contrast in pixels within soft tissues is considered to be higher for MRI compared to CT (Ballerini *et al*. 2000). This gives MRI an advantage in terms of measuring meat quality. MRI has proved to be an accurate tool in predicting and estimating *in vivo* body composition (Baulain, 1997), but the cost, speed and complexity of measurement are higher compared to CT.

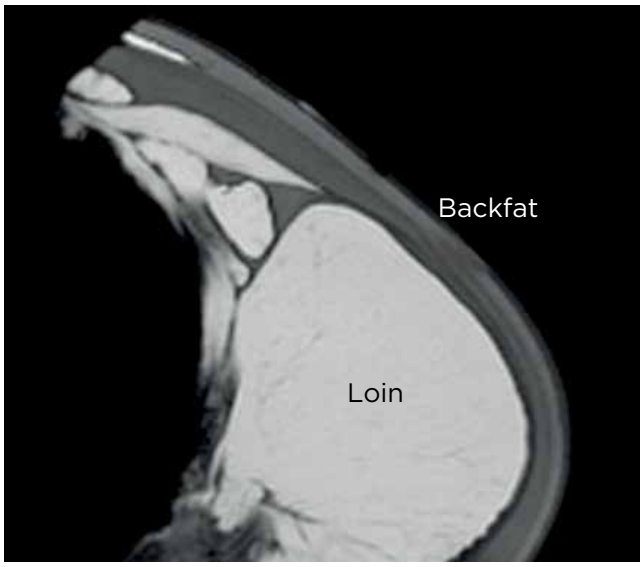

Figure 3. MRI image loin sample.
(Courtesy of Stephane Quellec, IRSTEA).

Biopsy
A biopsy is an *in vivo* procedure to remove a piece of tissue or sample of cells from a body to be analyzed in a lab. Valin *et al*. (1982) presented a method of predicting lamb meat quality traits based on muscle biopsy fibre typing. The authors found that biopsy can be used for predicting meat quality assumed that the muscle fibre types do not change between biopsy and slaughter. This is also valid for other types of technologies. Ville *et al*. (1997) showed that biopsies can be used as samples from an animal on which to perform meat quality measurements in a more controlled environment using i.e. NMR. The main obstacle may be that biopsy is considered somewhat invasive, which may affect the welfare of animals. Further studies are needed to study the feasibility, accuracy and to ensure animal welfare.

Emerging technologies
Near infrared reflectance (NIR), immuno-based assays and biosensors are often discussed as emerging technologies to determine meat quality (Mullen and Troy, 2005), and may be feasible for *in vivo* measurements. Lundstrom *et al*. (2009) studied the potential of chemical sensor arrays (electronic nose) to classify samples of boar taint. There are still too high a percentage of false negatives, but future studies could improve the use of electronic nose, particularly for *in vivo* application, to predict boar taint, which is regarded as a big challenge for the pork industry in the years to come. The development of new and emerging technologies depends on a multi-disciplinary approach, where knowledge from areas like medicine, engineering, computer science and biology is joined together to meet the challenges animal and meat production are faced with in the near future.

Figure 4. Electronic nose. (Courtesy of Amazon.com).

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Quality assessment of pig adipose tissue at the abattoir using NIRS

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Value for Industry

- Swine fat tissue is an important ingredient for processed meat (e.g. cured sausages, salami) and its fat content and fatty acid composition are crucial for processability and shelf life of the derived products.
- It is well established that with increasing levels of unsaturated fatty acids in the fat tissue the firmness decreases and the susceptibility for oxidation and therefore rancidity increases. Thus, fat tissue destined for meat processing has to contain low amounts of unsaturated fatty acids.
- Suggested thresholds for the percentage of polyunsaturated (PUFA) and saturated fatty (SFA) acids range from 15-17% and 34-36%, respectively.
- In order to obtain this information, meat processors need a fast and reliable method to monitor the quality of the fat tissue of the pigs delivered to the abattoir.

Background

For almost 30 years now, quality assessment of fat tissue from pigs has been determined in the larger Swiss abattoirs at the slaughter line using the fat score (originates from the German expression "Fettzahl"), a method derived from the iodine value (Margosches *et al.* 1924). This fat score originates from a simplification of the original iodine value method (Scheeder *et al.* 1999), intended to enable an automated titration; hence, a reduction in the complexity and costs of the routine analysis. The threshold for an acceptable quality was set at a fat score < 62 (Prabucki, 1991, Wenk and Prabucki, 1990). Greater values are penalized by a reduction of the market price ranging from €0.08 up 0.83/kg hot carcass weight. Thus, Swiss pig producers need to pay attention not only to the carcass weight and its lean tissue proportion but also to the fat quality. In monogastric animals, such as the pig, PUFA tissue level closely depends on the amount of ingested dietary PUFA (Bee, 2004, Bee, 2005). Thus, feeding recommendations for grower-finisher pigs take also into account the fat score (Stoll and Bee, 2002, Stoll *et al.* 2004). Consequently, diets fulfilling the recommendations are more costly because of the restricted use of components with greater amounts of PUFA such as corn, vegetable oils and even barley. Therefore, the fat score threshold is always subject of intense discussion between producers and meat processors.

Why work is needed

For many years alternatives to the fat score such as determination of the actual fatty acid composition of the fat tissue were discussed. This opportunity emerged with the fast developing and affordable NIRS (near-infrared spectroscopy) technology. Therefore, the Swiss branch organization of the meat processors Proviande, together with all major players of the Swiss Swine production chain and research institutions, launched a 2 year project aiming to establish new threshold values based on fatty acid determination in fat tissue using NIRS. The goal was to determine new thresholds for the fatty acid composition of the fat tissue based on its technological properties during processing and sensory appreciation of derived meat products and to elaborate reliable NIRS calibrations.

The methods used

Origin of the fat samples

Together with the largest feed mills and 15 pig farms an on-farm feeding trial was organized. For the experiment, grower finisher diets were formulated which covered a wide spectrum of PUFA and monounsaturated fatty acid (MUFA) levels. From a total of 154 slaughter batches (on average 40 pigs per batch), adipose tissue samples were collected from the outer layer of the backfat close to the hips according to the procedure of Proviande (2003) and stored at -20°C for further analysis of the fatty acid

profile via the gas chromatography (GC, reference method) and the acquisition of NIRS spectra.

In addition, from selected batches, meat as well as fat samples were collected for the production of various meat products (salami, salametti, bacon). These were subsequently used to monitor traits like melting behaviour and oxidative stability as well as sensory analysis. For detailed information on the performed measurements the final report of Scheeder and Muller (2014) is available on line.

Reference method for PUFA fraction and iodine value (IV)

The reference method used for the determination of the fatty acids profile in the backfat samples is described by Ampuero Kragten *et al.* (2014). Briefly, the samples are transesterified *in situ* in acidic media with 5% HCl in methanol and toluene for 3 h at 70°C. During a cleaning step with a LiChrolut Si (40-63 mm) SPE cartridge (Merck, Germany) the newly obtained FAME molecules (fatty acid methyl esters) are eluted with dichloromethane. Finally, a solution of purified FAME in pentane is injected into a GC-FID equipped with a Supelcowax polar column (Supelco, Sigma-Aldrich Co.). Individual FAME molecules are quantified against an internal standard (C19). PUFA is the sum of all polyunsaturated FAME expressed in percent of total FAME. The IV is calculated taking into account the content (in %) and the molar mass of each MUFA and PUFA.

NIRS system

Four NIRFlex N-500 (Büchi Labortechnik AG, Flawil, Switzerland), a FT-NIR spectrometer, were used. The spectra in diffuse reflectance mode were taken between 1'000 and 2'500 nm (10'000 et 4'000 cm^{-1}), with a resolution of 8 cm^{-1}. Each data point is in fact the average of 32 spectra. Mixed backfat samples, free of skin, were thawed and then scanned at ambient temperature in a glass measuring cell of 3.4 cm diameter x 2.5 cm, using a metallic accessory on the surface for homogeneous pressure on the sample (Figure 1). Almost all samples were measured simultaneously in three different laboratories of which one was located at a slaughterhouse.

Around 1500 spectra were used for the calibration models and around 600 spectra for the validation. Calibration and validation samples were different. Calibration models were developed with NIRCal® software (Büchi Labortechnik AG, Flawil, Switerland) using PLS algorithms with various mathematical pre-treatments, e.g. nle (normalisation to unit length), db1 (1st derivative BCAP 5 points), dg2 (second derivative Savistky Golay 9 points), snv (standard normal variate).

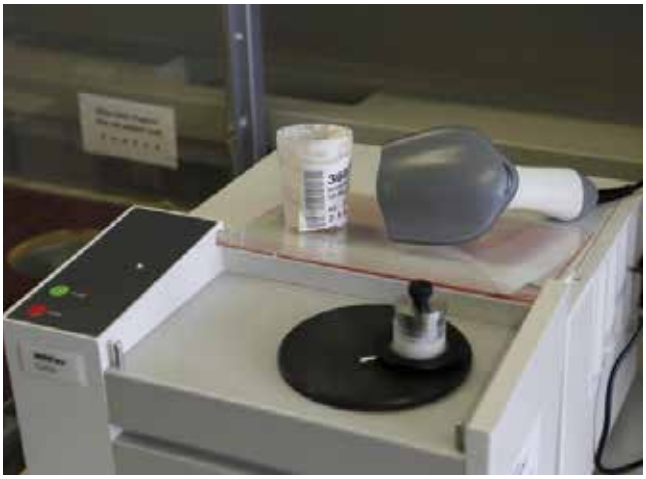

Figure 1. NIRFlex N-500 (Büchi Labortechnik AG, Flawil, Suisse) equipped with a glass measuring cell of 3.4 cm diameter x 2.5 cm with a metallic accessory placed on the surface of the fat sample for homogeneous pressure on the sample.

The results obtained

NIRS and IV calibrations

In Figures 2 and 3 NIRS predictions for PUFA and IV versus the respective reference values in calibration and validation samples are represented. The calibration set of samples for PUFA ranged from 8.0 to 22.3%. This model presented an RER (Range Error Ratio (RER = range of calibration samples/ SEP where SEP = standard error of prediction; measures the predictability of the prediction model.)) of 36.6, well over 20 which is considered as essentially quantitative (Williams, 1987); hence, showing a good prediction ability of the NIRS model. The determination coefficient for the calibration model was 0.9816; whereas SEC (standard error of calibration set) and SEP (standard error of prediction) were 0.36 and 0.39%, respectively. The validation set of samples showed a bias of 0.05%.

The calibration set of samples for IV ranged from 56.3 to 78.2 and presented an RER value of 43.5 showing again a good predictability of the NIRS model. The determination coefficient for the calibration model was 0.9920; whereas SEC and SEP were both 0.50. The validation set of samples showed a bias of 0.03.

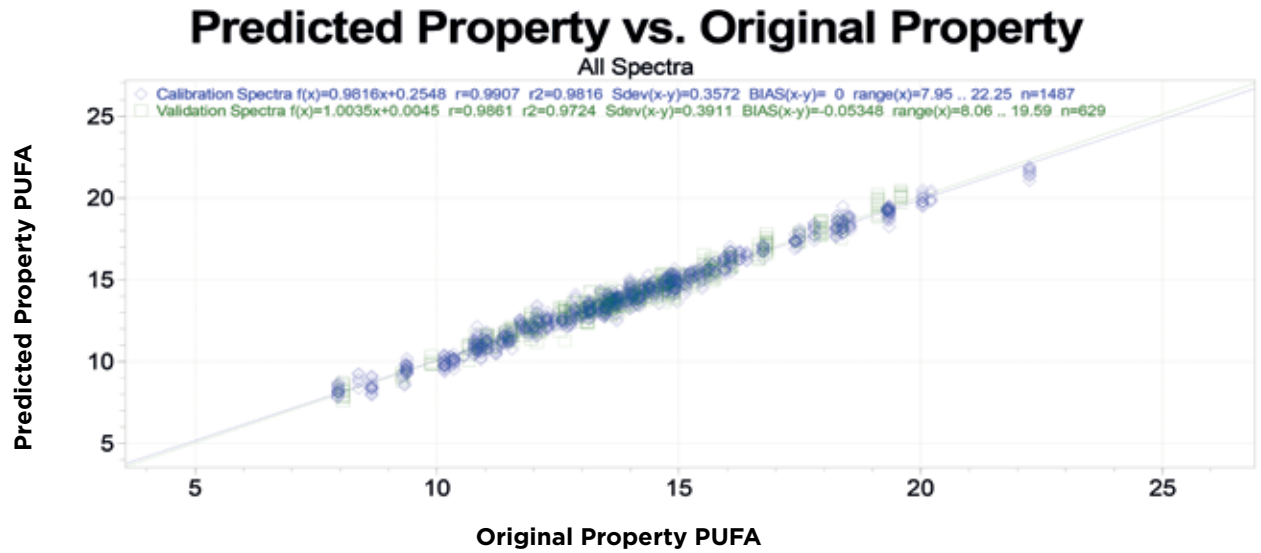

Figure 2. NIRS predictions for PUFA in calibration and validation samples, *versus* reference values.

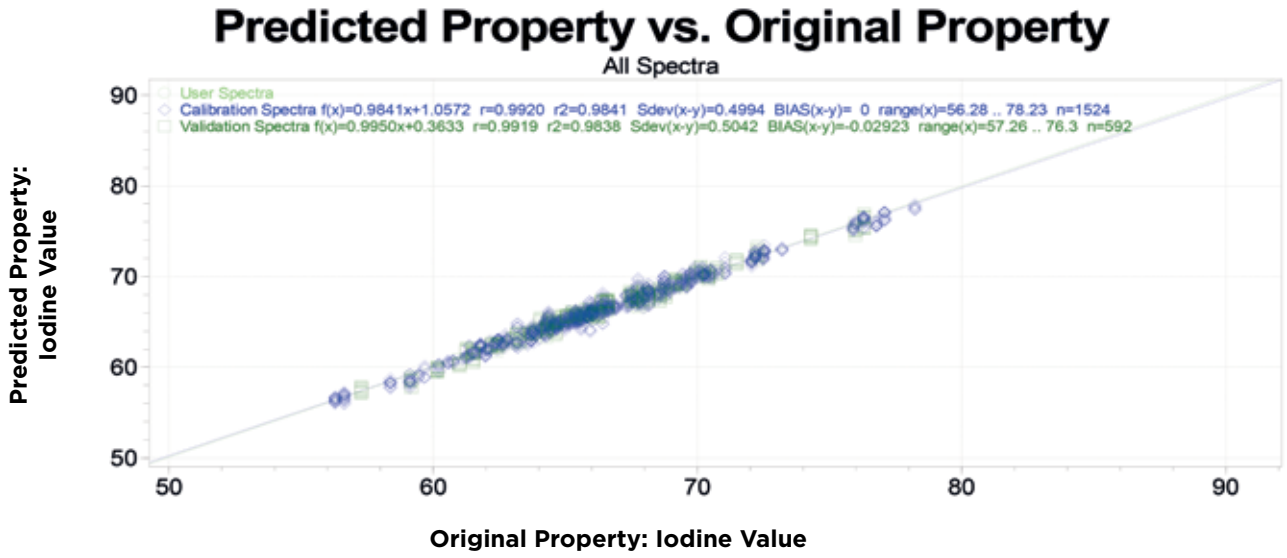

Figure 3. NIRS predictions for iodine value in calibration and validation samples, *versus* reference values. Iodine value was calculated based on the monounsaturated and polyunsaturated fatty acid methylester level.

The scientific conclusions

Derived new thresholds

A clear linear relationship between melting behaviour of the fat tissue and its PUFA content and IV value were observed. However, the results didn't allow to establish a clear cut off point. Based on subjective perception, processability of the fat tissue during meat fabrication was not impaired with increasing PUFA levels and IV of the fat tissue. Similarly, oxidative stability of the meat products determined using the thiobarbituric acid reactive substances assay didn't markedly decrease with decreasing degree of saturation. However, in bacon a curvilinear decrease in the oxidative stability (Rancimat method) with increasing PUFA level was observed. This was even more evident when samples were stored at -20°C for up to 6 months. The breaking point was at the level of 17% PUFA. Sensory evaluation of meat products, performed by a trained panel of the School of Agricultural Forest and Food Sciences (HAFL) and a consumer panel recruited by HAFL revealed negative appreciations of the products when PUFA levels of the fat tissues were > 20%.

Based on these findings, the interested parties in the pig production industry agreed on new thresholds values for PUFA (< 15.5%) and IV values (< 70; indirect measurement of SFA). These new threshold values are now valid since July 1 2014 (Christen, 2014).

The next steps

One can see that the NIRS calibration sets nicely surround the PUFA fraction and IV of 15.5% and 70, respectively. One limitation of the current available data set is the low number of samples with greater PUFA > 17% and IV > 73. An ongoing study, aims at generating this kind of samples, which will help to improve the calibration for more unsaturated fat samples.

NIRS technology shows a good aptitude for predicting fat quality in swine carcasses. The measurements are now performed routinely at the large abattoirs in Switzerland as a fast and reliable technique.

Acknowledgements

The data presented in this text are part of the project entitled "*NACHHALTIGE SICHERUNG DER FETTQUALITÄT BEI MASTSCHWEINEN*" which was managed by Dr. Martin Scheeder and Martina Müller of the Bern University of Applied Sciences, School of Agricultural, Forest and Food Sciences HAFL and SUISAG, Sempach.

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NitFom – rapid on-line assessment of pork fat quality

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Value for Industry

- NitFom is an invasive handheld measuring device which predicts the iodine value and fatty acid profile of pork fat with a measurement cycle of 3 seconds.
- Prediction models for iodine value and several fatty acids are developed both in warm and cold fat, making the equipment useful on-line at the slaughterhouse but also at retailers.
- The NitFom is suitable for quality control of fat raw material and enables the industry to respond rapidly and systematic to the different markets having different demands and quality criteria for fat quality.

Background

The chemical composition of pork fat influences several quality traits important for the slaughter industry and hence is of great economic importance to the abattoirs. Adipose tissue is composed mainly of triglycerides – esters derived from glycerol and three fatty acids. Fatty acids can be categorized in three groups: saturated, mono- or poly-unsaturated. The number of C=C double bonds in the aliphatic chain indicate the degree of saturation. Saturated fatty acids do not contain C=C double bonds, whereas one or more C=C double bonds are present in mono- and poly-unsaturated fatty acids, respectively. The content of unsaturated and saturated fatty acids affects fat texture, color and product shelf life. Pork fat with a high content of poly-unsaturated fatty acids is soft, yellow and is more prone to oxidation during storage. Soft fat texture results in poor technological quality as the lack of firmness causes layer separation between fat and muscles which greatly reduces cutting yields and slice-ability of bacon and fermented sausages. In addition, soft fat will lead to an unpleasant wet, oily and transparent fat appearance and oxidation occurs faster, which reduces product shelf life and market value. The chemical composition of fat is affected among other things by the feeding regime. Feeding pigs with maize, rapeseed or distillers dried grain will increase the level of un-saturation in pork fat.

Traditionally, the iodine value (IV) of fat is determined by measuring how much iodine (in grams) that 100 grams of a given substance would consume by its double bonds. Thus, saturated fat would have a low IV and unsaturated fat a high IV. A widely used method is to extract fatty acid methyl esters followed by separation and quantification using gas chromatography (GC). The iodine value is calculated according to the potential number of iodine atoms added to each fatty acid following the AOCS recommended guideline (AOCS, 2009). The two methods described above are laborious, time-consuming and expensive.

During the last decades, the use of on-line near-infrared (NIR) spectroscopy has been researched intensively as a rapid method for obtaining meat and fat quality parameters (Perez-Juan *et al.* 2010). However, no real industrial application for on-line assessment of fat quality using NIR spectroscopy has previously been described. Sørensen *et al*. (2012) demonstrated that fatty acid composition and iodine value could be predicted by using near infrared transmission (NIT) spectroscopy.

The aim of the present study was to evaluate the relationship between the iodine value in pork fat from hot and cold fat samples obtained by chemical analysis and on-line rapid near infrared transmission spectroscopy. In addition, the ability to predict fatty acid profile of hot samples on-line was investigated.

Why work is needed

Early prediction of iodine value and fatty acid profile enables the slaughter industry to quantify fat quality and thereby use the information for product sorting resulting in increased production efficiency and economic gain.

The methods used

The experimental test described below was performed in collaboration with Danish Crown (Herning, Denmark). The test was divided in two phases; first a calibration test was performed and later the resulting calibration model was validated. A total of 100 pig carcasses were selected for the calibration study. Carcasses were selected by Danish Crown aiming at obtaining a large span of iodine values. Approximately 30 minutes after slaughter, each carcass was measured with the NitFom device. The measurement was made in the neck region of the back fat next to the shoulder blade – approximately 7 cm from the split line. Samples measured on the day of slaughter (hot samples) were used for developing hot calibration models. On day 1 after slaughter, all samples were measured once more with NitFom (cold samples) and included in the development of cold calibration models. References were obtained by chemical analysis (GC-FAME) at Danish Technological Institute - Danish Meat Research Institute (Taastrup, Denmark). The iodine value was calculated from the fatty acid profile according to the AOCS recommended practice (AOCS, 2009). The final calibration data set consisted of 100 iodine and fatty acid references each linked to a NIT spectrum allowing partial least squares (PLS) models to be developed for both hot and cold fat samples using Matlab R2013b. Six weeks after the calibration trial, 50 new carcasses were chosen for validation of the calibration models.

The NitFom consist of stainless steel twin-probes mounted in a probe house (Marnø and Sørensen, 2013). Each probe is knife-tipped and designed to penetrate 4 cm into the carcass through the skin. Optical fibers connect the emitter probe to a light source while the receiver probe is connected to a NIR spectrometer. The two probes have windows facing each other allowing light to be transmitted through the fat tissue. As the probe head is ejecting itself from the carcass, NIR transmission spectra are recorded at several depths. A built-in algorithm facilitates differentiating tissue types (meat vs. fat). Only spectra recorded in fat tissue will be part of the predicted fatty acid profile and iodine value of the carcass. The speed with which the NitFom probe is retracted from the carcass determines how many spectra are obtained.

The results obtained

Figure 1 shows the relationship between the NitFom predicted iodine value of hot (Figure 1A) and cold (Figure 1B) carcasses and the reference samples. A high accuracy of NitFom predictions in both hot (R^2=0.94) and cold (R^2=0.93) samples was found. Both models have a RMSECV of 1.8 IV meaning that 95% of the samples would be predicted correctly within ±3.6 IV using the NitFom fast-track method.

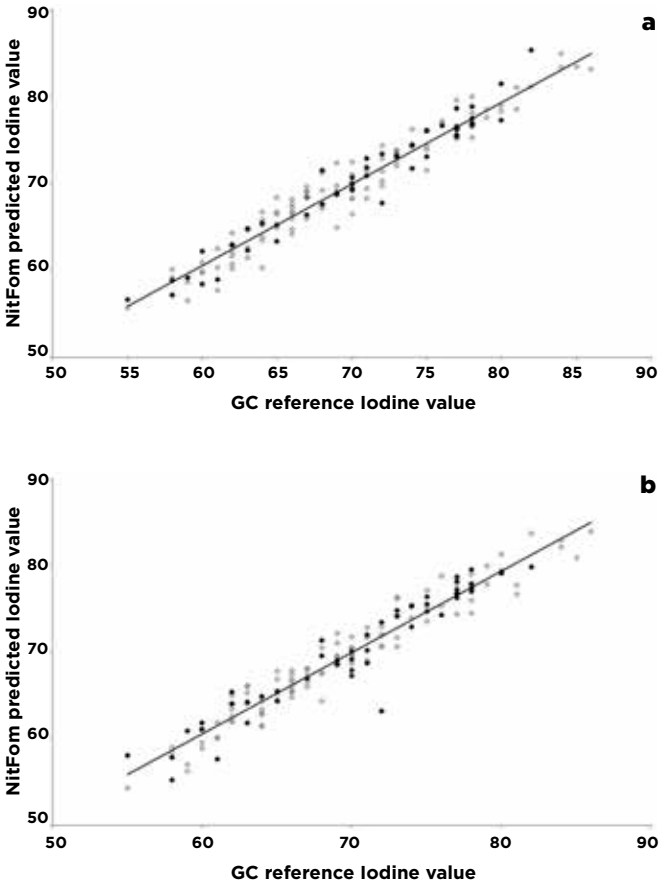

Figure 1: Relationship between GC reference and NitFom predicted iodine value in hot (a) and cold (b) fat samples. Open symbols represent the calibration data set (n=100) and filled symbols represent the validation data set (n=50).

Full validation of the calibration models was performed using 50 additional fat samples from hot and cold carcasses (Figure 1). This resulted in a RMSEP value of 1.5 IV for hot samples and 2.0 IV for cold samples. Hence, the calibration models for prediction of iodine value in hot and cold unknown samples would be highly acceptable for practical implementation.The ability of the NitFom device to predict individual fatty acids was explored in hot fat samples (table 1).

	Prediction model				Reference GC statistics			
Trait	**#PC**	**R^2cv**	**RMSECV (%)**	**RMSEP (%)**	**Avg (%)**	**Std (%)**	**Min (%)**	**Max (%)**
Omega 6	2	0.914	1.172	1.052	15.6	4.00	7.8	25.2
Omega 3	2	0.733	0.391	0.474	1.70	0.76	0.6	4.0
Polyunsat	2	0.939	1.145	1.288	17.3	4.61	8.4	28.1
Monounsat	3	0.558	1.452	2.106	42.8	2.19	36.0	46.8
Saturated	2	0.821	1.445	1.415	38.9	3.43	31.3	46.4
C18:3	2	0.733	0.299	0.281	1.50	0.58	0.6	3.4
C18:2	2	0.915	1.110	1.056	14.8	3.82	7.4	23.8
C18:1 (9)	3	0.456	1.387	1.655	37.7	1.88	32.1	41.6
C18:1 (11)	1	0.366	0.204	0.198	2.50	0.25	1.9	3.3
C18:0	2	0.655	0.978	1.091	13.6	1.67	10.3	17.4
C16:0	2	0.812	0.780	0.710	23.5	1.80	19.3	27.1

Table 1. NitFom prediction statistics of different fatty acids obtained from hot fat samples.

Table 1 reveals that especially poly-unsaturated and saturated fatty acids are nicely predicted with the NitFom device in hot fat samples. However, the correlation between mono-unsaturated fatty acids in reference samples and the NitFom predicted values are rather poor. It remains to be elucidated why the relationship between reference and NitFom prediction is poor for mono-unsaturated fatty acids.

The scientific conclusions

In conclusion, rapid prediction of iodine value in pork fat can be performed using an on-line fast-track measuring device based on near infrared transmission spectroscopy (NitFom). Prediction models have been developed both in hot and cold samples enabling prediction of iodine value early after slaughter and in cold samples after the carcass is split into primals. In addition, the NitFom device is suitable for rapid on-line assessment of individual fatty acids.

The next steps

The NitFom is now commercially available by Carometec A/S and enhanced prediction of entire carcass composition from linking NitFom derived information on iodine value in separate fat layers to volumetric information derived from the AutoFom will be further explored. In addition, the potential use of NitFom for prediction of meat quality traits will be evaluated.

Acknowledgements

Danish Crown (Herning, Denmark) is acknowledged for their collaboration in the study and Danish Technological Institute - Danish Meat Research Institute (Mrs. Kirsten Jensen) for performing GC analysis.

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Quantification of intramuscular fat in fish by MRI

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Value for Industry

Magnetic resonance imaging can provide:

- A non-invasive, rapid and accurate method for measurement of the spatial distribution of fat in fish;
- High-throughput measurements that are achievable for selective breeding programs;
- Methods which are potentially extendable to other kinds of meat or food.

Background

The measurement of lipid distribution in fish is relevant for researchers in fish physiology and also for the breeders and the fish industry. Indeed, the stocks of lipids vary in the tissues according the age or the season and research on fish biology would benefit from a better knowledge of the lipid quantity and repartition. Moreover, lipids, and particularly intra-muscular lipids, are also linked to the nutritional quality and highly influence the sensory perception and the taste/flavour aspects of the flesh. Furthermore, in order to optimize the quality of fish products, improvement by genetic selection has become a promising strategy (Gjedrem, 1997). Thus, in the case of selective breeding programs, typically involving several thousand individuals, a rapid method is needed.

X-rays computer tomography (CT) and magnetic resonance imaging (MRI) are likely to meet these requirements. Indeed, both quantity and distribution of fat tissues can be measured within a large field-of-view allowing the analysis of several samples together. They both have been used for estimation of fat content in fish (Mathiassen *et al.* 2011). CT technique is based on the difference of density between lipids and muscle and is limited to one contrast. On the other hand, MRI is a more flexible technique which can provide, in the case of high magnetic field system, the characterization of the triglycerides. Moreover, since it does not involve the use of ionizing radiation, MRI would be harmless in case of repeated examinations for *in vivo* studies. Thus MRI seems a promising technique for the measurement of fish lipids.

Why work is needed

MRI spin echo T1-weighted images have been used to quantify the lipid distribution in the flesh of trout at 0.2T (Toussaint *et al*. 2005) and on trout cutlets at 1.5T (Collewet *et al*. 2013). The method was based on the contrast between muscle and fat. Contrary to X-rays for which the Hounsfield units can be used, the signal in MRI is system dependent. Thus a calibration step was needed in order to convert the image intensity into fat proportion. Moreover, since this approach is very sensitive to radio-frequencies (RF) inhomogeneities, a correction scheme was used in order to get rid of position effects. The calibration led to a low root mean square error (RMSE) of 0.8%. The major drawback of this protocol is that it depends of both the MR system and the RF antenna and thus requires a calibration step.

Another approach is called “water/fat separation” the result of which is a set of two images, one corresponding to the fat image, the other to the water image. This method is based on the difference of the signal frequency between hydrogen protons of water and fat. The phase of the signal measured for one voxel will depend on the quantity of water and fat inside. This approach has become the standard for clinical imaging in liver or muscles (Hu *et al*. 2012). Beyond the advantages of using a standard method, this one seems much more appropriate for high throughput analysis since it naturally compensates the RF inhomogeneities. Indeed, the proportion of fat is computed as the ratio between the “fat image” and the “water image”, both corrupted by the same multiplicative bias. This would allow the use of a larger field of view and thus a highest throughput. Moreover, contrary to the

"contrast" approach, the measurement should not depend on the MR system since it corresponds to the amount of fat protons to water protons ratio.

Regarding fish applications, this technique has been used for the quantification and localization of fat in Atlantic mackerel at 1.5T (Brix *et al.* 2009). The authors succeeded in globally visualizing the fat. However, they found no agreement between fat quantified by MRI and gas chromatography analysis and could not clearly explain why. The aim of the current study was to explore the possibility of using the water-fat separation approach for fat quantification in fish. For this, we used nuclear magnetic resonance (NMR) as a reference to validate the results obtained by MRI.

The methods used

Fish

Cutlets of 2 cm thickness were taken from three different Scottish farmed salmons. The skin and the bones were then removed so that the subsequent NMR analysis, (which requires samples to be easily ground), would be done on exactly the same material. Twenty-seven cutlets were used as is. Three others were "artificial" cutlets made of an assembly of the leanest parts of the fish. This was done to get a larger variability. This led finally to a total of 30 samples.

MRI measurements

Samples were arranged horizontally in trays positioned one above the other so that four samples were put together in the MRI system (Siemens Avanto 1.5T). The samples were maintained at a temperature of 4°C in order to avoid water loss. Images were acquired with a head coil, using a gradient echo sequence at 6 different acquisition times: 2.35, 4.85, 7.35, 9.85, 12.35 and 14.85 ms. The repetition time was 3000 ms and we made 2 accumulations of the signal in order to increase the signal-to-noise ratio. The field-of-view was 192x96 mm^2 and the matrix size 128x64. The slice thickness was 5 mm. This led to a spatial resolution of 1.5x1.5x5 mm^3. The total acquisition time for 4 samples was 6 minutes 24 sec. For qualitative evaluation purpose (see Figure 1) images of a cutlet before the removing of the skin and bones and with a slice thickness of 3 mm were acquired.

Water and fat images reconstruction

In each voxel the acquired complex signal $s(t)$ at time t can be modeled by the following equation:

$$s(t) = \left(W + F \sum_{k=1}^{K} a_k e^{i 2\pi f_k t} \right) e^{\frac{-t}{T_2^*}} e^{i 2\pi \varphi t}$$

Where W and F are the signals of the water and of the fat and are the unknowns of interest. Fat is a complex molecule which implies that all hydrogen protons do not have the same frequency. This is taken into account by the sum over k of all the frequencies. Since the relative amplitudes a_k and the frequencies f_k for a salmon depend on the breeding conditions we computed an average of the data found in the literature (Aursand *et al.* 2000). In particular, we used $K = 9$ to describe the fat spectrum. T_2^* represents the relaxation time of the signal. φ corresponds to an additional phase due to the perturbation of the magnetic field lines induced by the presence of the samples inside the magnet. Thus, in each voxel 4 unknowns have to be estimated: W, F, T_2^* and φ. This estimation problem is not straightforward since it requires the minimization of non-convex criteria. Many methods have been proposed in the literature and we used the one proposed by Hernando *et al.* (2010).

Fat fraction computation

Since NMR provides a mean fat fraction, we computed the mean MRI fat fraction by averaging over all the voxels belonging to the samples. These were easily segmented thanks to the high contrast between the samples and the background.

Fat fraction is classically logically expressed in g/g. However, W and F are not proportional to weight but to the number of hydrogen protons. Moreover, many macromolecules such as proteins do not give any signal in MRI. However they have to be taken into account if we want to measure a fat fraction in g/g.

So, two fat fractions were computed. We computed the mean proton density fat fraction

$$PDFF_{MRI} = \overline{F} / \left(\overline{F} + \overline{W} \right)$$

as well as a fat fraction in g/g, FF_{MRI}, obtained by correcting $PDFF_{MRI}$ with the proton density of fat and water and with the hypothesis of an average water content (70%) for each cutlet. This information of average water content allows compensating the absence of signal from the proteins. The proton density of fat was computed considering the model of the fat used in the model.

NMR measurements

After MRI acquisition, the samples were grounded and homogenized and placed in cupels. These were weighed and put in a ventilated electric oven overnight at 103°C in order to obtain the dry residues. The moisture of each sample was measured at the same time. The dry samples were powdered and placed in test tubes. These tubes were analysed by NMR using the method described in (Toussaint *et al*. 2002). This measurement gave the mass of lipids in the dry samples. Thanks to the moisture measurement, the relative mass of lipids of each sample, noted FF_{NMR}, was determined.

The results obtained

Qualitative evaluation

Figure 1 shows a cartography of fat fraction (in g/g) obtained on a cutlet before the removing of skin and bones. This result is coherent with the expected repartition of lipids in the cutlet with high content of fat in the subcutaneous area and a decreasing gradient towards the area around the central fin. It shows that MRI is a suitable tool to observe the spatial repartition of lipids.

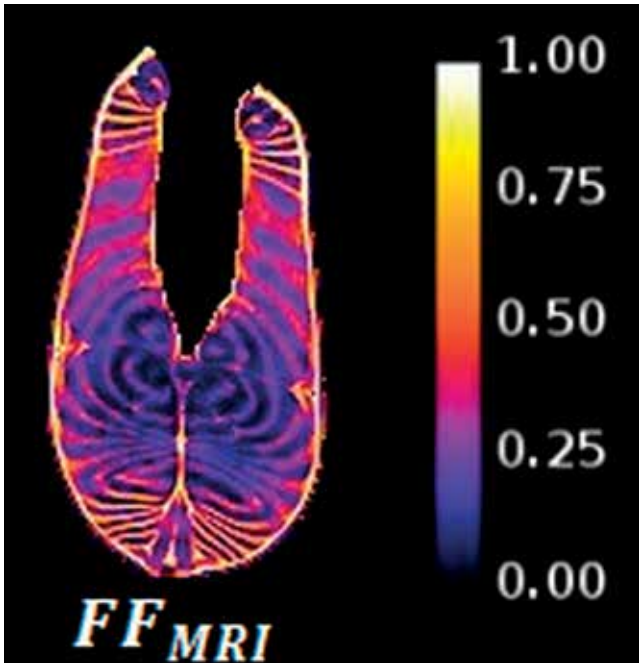

Figure 1. Fat fractions (in g/g) on a cutlet. The spatial resolution is 1.5x1.5x3 mm^3

Quantitative comparison of MRI and NMR

The values of FF_{NMR} were in the range of 4% to 18%. Figure 2 shows $PDFF_{MRI}$ and FF_{MRI} in function of FF_{NMR}.

The R^2 and the slope of the linear relation between $PDFF_{MRI}$ and FF_{NMR} were respectively 0.97 and 1.49. Between FF_{MRI} and FF_{NMR} they were equal to 0.96 and 1.03.

Very high correlations were found between MRI and NMR. The slope of the linear correlation between FF_{MRI} and FF_{NMR} was very close to 1. This showed that MRI could be used even without any calibration step since only average water content information and an average fat model was used to convert $PDFF_{MRI}$ in FF_{MRI}. Using this linear relation, a RMSE of 0.7% was found which is quite satisfactory.

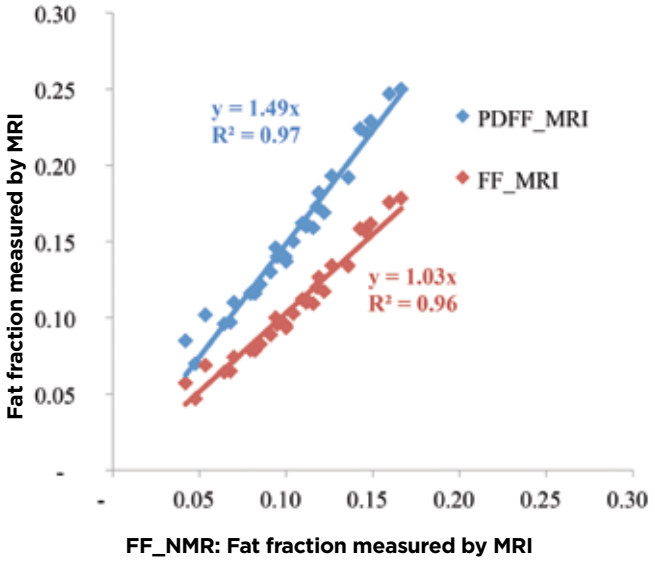

Figure 2. $PDFF_{MRI}$ and FF_{MRI} in function of FF_{NMR} and respective linear trend lines.

The scientific conclusions

In this study we showed that "water-fat separation" approach in MRI was able to measure fat content in salmon cutlets with a very good accuracy. Moreover, with some additional information such as average water content and an average fat model, the method could give quite accurate measure in g/g. These results were validated by comparing the mean MRI value with NMR. A qualitative evaluation on a whole cutlet showed very coherent spatial distribution, demonstrating that MRI measurements were very probably accurate at the voxel level too.

The method is non sensitive to RF inhomogeneities which means that several samples could be analysed simultaneously without any bias due to the position. For example, it seems feasible to analyse simultaneously 18 cutlets using a large coil and 3 sets of 6 superposed trays. This would lead to an acquisition time of less than 13 minutes for 18 samples, i.e. less than one minute per sample.

These results were obtained in a range between 4% and 18% of fat content which is quite standard for salmon. However, a study on lower fat content should be carried out in order to address other fish species such as trout which has lower fat content.

Fish present some advantages regarding MRI fat quantification compared to other species. Indeed, even at low temperature such as 4°C, fat in fish is not crystallised since it is not saturated. In the case of pig for example, fat would be partly crystallised at 4°C. Since crystallised protons do not give any signal in MRI, quantification would be probably less accurate. Thus, quantification of fat in other species should be led at higher temperature with the risk of water loss or meat degradation.

The next steps

First of all some refinements of the method will be tested. In particular, some bias may exist for low fat fraction due to the statistical properties of the noise in MRI (Liu *et al.* 2007).

Evaluation of the sensitivity of the method to the knowledge of the fat spectrum will be led through simulations. It is important to know if this knowledge is of major importance or not. In this case, we will work on a method to estimate this spectrum along with the other unknowns.

We will also lead experiments on the robustness of the method regarding the position in the MR system in order to check that the method is effectively suitable for high-throughput analysis.

Finally, another challenge is to test this method on other kinds of meat (beef, pork, lamb) or other kind of food containing lipids.

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Effect of high pressure and raw meat pH_{24sm} on water distribution of dry-cured ham as studied by a multiple spectroscopic approach

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Value for industry

- High Pressure Processing (HPP) is known to be an efficient treatment to ensure microbiological safety of dry-cured ham.
- However, HPP may affect the microstructure of the ham and produce changes on the sensory properties of the product, such as increase of saltiness perception and hardness.
- A better understanding of the bio-physical effect related to HPP of dry-cured ham could aid the industry to produce safe, salt reduced products with fewer incidences of defects.

Background

HPP is currently being used to extend shelf-life and to improve the safety of commercially processed meat products. Pressure levels applied for the pressurization of meats and meat products in industry range from 400 to 600 MPa (Cheftel and Culioli, 1997). HPP treatment can influence meat protein conformation and induce protein denaturation, aggregation or gelation, due to the rupture of non-covalent interactions within protein molecules, and to the subsequent re-formation of intra- and inter- molecular bonds within or among protein molecules (Sun and Holley, 2010) Pressurization also causes a loosening of the meat protein matrix and alterations in the water distribution in meat (Bertram *et al*. 2006) and proton nuclear magnetic resonance (NMR).
The effects of HPP on colour (Cava *et al*. 2009), on volatile compounds (Rivas-Cañedo *et al*. 2009), on protein and lipids oxidation (Fuentes *et al*. 2010). on texture and microstructure (Garcia-Gil *et al*. 2014) in dry-cured ham have also been studied before.

Why work is needed

Despite the previously mentioned studies, the effects of different pressure levels and different raw meat pH_{24SM} (pH taken in *Semimbranosus* muscle at 24 hours *post mortem*) on the intrinsic water populations and the accompanying impact on the structure of dry-cured ham at the end of the process have scarcely been studied. For this reason in this work, a multiple spectroscopic approach including Nuclear Magnetic Resonance relaxometry (NMR) (technology that by means of the alignment of the water molecules in a constant magnetic field can provide information of the water compartmentalization thus differentiating if it is more or less bound), Time Domain Reflectometry (TDR) (technology based on the use of a microwave pulse for the determination of dielectric properties) and Multispectral Imaging (MI) (technology based on the acquisition of images for recording spectral reflection properties) were used on dry-cured ham with 2 different pH_{24SM} (low and high) and subjected to 3 high pressure levels (200, 400 and 600 MPa) in order to achieve detailed physico-chemical knowledge of the impact of HPP.

The methods used

To conduct the approach mentioned above, the experimental work was divided into two experiments.

In Experiment 1, 10 dry-cured hams with pH pH_{24SM} ranging between 5.63 and 5.76 were selected to study the effect of different pressure levels.

In Experiment 2, in order to study the effect of pH, 20 additional dry-cured hams were selected and grouped into two classes according to their pH_{24SM}: 10 hams with low pH_{24SM} (5.42-5.54) and 10 hams with high pH_{24SM} (6.09-6.40). *Biceps Femoris* muscles were sampled and portions pressurized at 200, 400 or 600 MPa for 5 min with water at 10 °C as pressure-transmitting medium (Hyperbaric Wave 6500/120, N.C. Hyperbaric, S.A., Burgos, Spain).

Proton NMR T2 relaxation measurements were performed on a Maran Benchtop Pulsed NMR Analyzer (Resonance Instruments, Witney, UK) operating at 23.2 MHz. T2 relaxation data were analysed using distributed exponential fitting analysis, which revealed the presence of three water populations: water closely associated with macromolecules (T_{2B}), myofibrillar water (T_{21}) and extra-myofibrillar water (T_{22}). The TDR device RFQ Scan 3.0 (Sequid GmbH, Bremen, Germany) was used to obtain time domain curves. VideometerLab vision system (Videometer A/S, Denmark) was used to acquire multi-spectral images on 18 different wavelengths ranging from UV (405 nm) to short wave NIR (970 nm).

Salt and water contents were chemically determined (ISO 1841-2,1996 and AOAC, 1990). For water loss determinations, samples were centrifuged and supernatant volume was expressed as the sample weight loss percentage (Picouet *et al*. 2012).

A one-way analysis of variance (ANOVA) was performed with XLSTAT-Pro (Win) 7.5.3 (Addinsoft SARL, Paris, France). Statistical analysis was run with a confidence level of 95%. Comparisons between treatments and pH were evaluated with the Tukey test.

The results obtained

Effect of high pressure levels

Salt (4.27 ± 0.33%) and water content (52.72 ± 1.16%) of the samples were similar, forming a homogeneous group of study. Different high pressure levels produced a significantly different effect on water loss, showing a steady linear increase when the pressure level was increased (Table 1). This can be related to crucial changes in the meat protein structure, such as modification of myofibril ultrastructure and protein denaturation, which become more severe with an increase in pressure level (Garcia-Gil *et al*. 2014; Picouet *et al*. 2012; Bertram & Andersen, 2007). A decrease in the water-binding properties (increase of water loss) was also evident in the NMR results obtained.

Significant differences in the T_{2B} and T_{21} areas (representing water closely associated with macromolecules and myofibrillar water, respectively (Bertram *et al*. 2001)), between the untreated samples and the HPP treated samples were found (Table 1). Changes in the T_{2B} population are the direct result of the protein conformation changes whereas changes in the T_{21} population reflect a pressure-induced change in the functionality of the myofibrillar proteins, resulting in changes in the amount of water entrapped within the myofibrillar matrix.

	Control	200 MPa	400 MPa	600 MPa
Water loss (%)	4.74 ± 0.68^{c}	5.45 ± 0.94^{b,c}	6.33 ± 1.07^{a,b}	7.30 ± 0.59^{a}
T_{2B} area	4.59 ± 0.35^{a}	4.02 ± 0.94^{b}	3.79 ± 0.50^{b}	3.65 ± 0.55^{b}
T_{21} area	92.06 ± 0.29^{b}	92.74 ± 0.50^{a}	93.02 ± 0.68^{a}	93.15 ± 0.63^{a}

Table 1. Mean ± standard deviation of water loss (%) and the area of T_{2B} and T_{21} water populations of samples subjected to different pressure levels.

^{a,b,c} Different letters indicate significant differences ($P \leq 0.05$) between pressure treatments.

In contrast, although TDR response is dependent on the water linking, no differences between the non-pressurized samples and the HPP-treated samples were found in the present study.

Areas under the reflectance spectra between 405 and 970 nm and L* value showed a significant increase when the pressure level was increased (Table 2). While no significant difference was found between non-pressurized and 200 MPa samples, a significant increase between samples pressured at 400 MPa or higher was evidenced. This was attributed to changes in the surface protein conformation that may have affected the light scattering. These results are consistent with previous studies elucidating the detailed changes in protein structure as function of pressurization. Bajovic *et al*. (2012) reported that meat muscular proteins including myofibrillar proteins were unfolded up to a pressure of 300 MPa. Picouet *et al*. (2012) estimated a point of inflection at 340 MPa in dry-cured ham determined by the sigmoid equation for τ_{opt}. Besides, differences in L* value were significant from 200 MPa, showing a point of inflection at pressures between 200 and 400 MPa (Table 2). Mor-Mur and Yuste (2003) related the increase in L* value of pressurized meat products to a new arrangement of surface proteins promoted by HHP due to the coagulation or denaturation of proteins, which would have increased the reflected/absorbed light ratio. In addition, higher L* values in raw pork meat have also been ascribed to higher water losses (Traore *et al*. 2012), which are be consistent with the present results.

Effect of pH_{24SM} and high pressure levels

Salt and water content in low pH group (3.88 ± 0.34% and 57.24 ± 1.04% respectively) and in high pH group (4.25 ± 0.36% and 58.60 ± 1.82% respectively) were comparable but differed from the batch of hams used in Experiment 1.

Table 3 shows the water losses of the samples with different pH_{24SM} subjected to different pressure treatments. In both pH groups, samples treated with 600 MPa were significantly different from the rest of the pressure treatments. However, no significant differences between the water losses of the two pH groups were found (Table 3).

In contrast, areas of the two water populations (T_{2B} and T_{21}) between low and high pH groups were significantly different. Raw meat pH_{24SM} affects the dynamics of the curing process resulting in different properties of the dry-cured ham at the end of process.

Statistical analysis of the areas under the reflectance spectrums and L* value showed significant differences between different HPP levels and different raw meat pH_{24SM} (Table 4). For both pH groups, significant differences in reflectance area and L* value were evidenced between 200 and 400 MPa. Significant differences between the two groups of pH were also found (Table 4). The reason for these differences needs to be further investigated.

	Control	200 MPa	400 MPa	600 MPa
Reflectance area	512.36 ± 19.19^{b}	514.42 ± 12.19^{b}	555.99 ± 21.79^{a}	577.84 ± 28.82^{a}
L*	41.71 ± 1.85^{b}	42.95 ± 0.94^{b}	46.71 ± 1.65^{a}	47.80 ± 2.38^{a}

Table 2. Mean ± standard deviation of the reflectance area between 405 and 970 nm and L* value of samples subjected to different pressure levels.

^{a,b} Different letters indicate significant differences (P ≤ 0.05) between pressure treatments.

		Control	200 MPa	400 MPa	600 MPa
Water losses (%)	Low pH	3.12 ± 0.65^{c}	4.45 ± 1.1^{b}	3.62 ± 0.85^{b,c}	7.42 ± 0.92^{a}
	High pH	3.53 ± 1.00^{b}	4.01 ± 0.80^{b}	3.49 ± 0.62^{b}	8.86 ± 2.37^{a}
T_{2B} area	Low pH	3.42 ± 0.78^{y}	3.18 ± 0.75^{y}	3.31 ± 0.58^{y}	2.81 ± 0.75^{y}
	High pH	4.35 ± 0.78^{a,x}	4.54 ± 0.43^{a,x}	4.20 ± 0.46^{a,b,x}	3.79 ± 0.56^{a,b,x}
T_{21} area	Low pH	94.97 ± 0.71	95.13 ± 0.89^{x}	95.11 ± 0.63^{x}	95.47 ± 0.68^{x}
	High pH	94.25 ± 0.97	93.71 ± 0.61^{y}	94.30 ± 0.65^{y}	94.17 ± 1.00^{y}

Table 3. Mean standard deviation for water loss (%) and the areas of T_{2B} and T_{21} water populations of samples from the low and high pH groups subjected to different pressure levels.

^{a,b,c} Different letters indicate significant differences (P ≤ 0.05) between pressure treatments.

^{x,y} Different letters indicate significant differences (P ≤ 0.05) between low and high pH groups within each parameter analysed.

		Control	200 MPa	400 MPa	600 MPa
Reflectance area	Low pH	576.32 ± 28.88^{b,y}	592.50 ± 22.66^{b,y}	711.68 ± 65.14^{a}	725.00 ± 58.88^{a,y}
	High pH	642.32 ± 28.88^{b,x}	664.26 ± 42.68^{b,x}	753.34 ± 48.22^{a}	790.58 ± 58.58^{a,x}
L*	Low pH	48.87 ± 3.24^{b,y}	49.83 ± 2.12^{b,y}	56.61 ± 3.64^{a}	57.67 ± 3.52^{a,y}
	High pH	51.57 ± 2.01^{c,x}	52.85 ± 2.64^{c,x}	57.45 ± 2.96^{b}	61.03 ± 3.02^{a,x}

Table 4. Mean standard deviation of the reflectance area between 405 and 970 nm and L* value of samples from the low and high pH groups subjected to different pressure levels.

^{a,b,c} Different letters indicate significant differences (P ≤ 0.05) between pressure treatments.

^{x,y} Different letters indicate significant differences (P ≤ 0.05) between low and high pH groups within each parameter analysed.

The scientific conclusions

Water loss, T_{2B} and T_{21} water populations, reflectance curves between 405 and 970 nm and L* results showed significant differences between high pressure levels, with an inflection point between 200 and 400 MPa, as well as between the two pH groups. These differences were probably due to changes in the meat protein structure, which in turn produces modifications on the mobility of the intrinsic water.

The next steps

Further studies are needed to better understand the mechanisms of how high pressure and pH influence the binding properties of water in dry-cured ham.

Acknowledgements

This work has been supported by projects RTA2010-00029-CO4-01 and RTA 2013-00030-CO3-01 from INIA (Spain). HCB wishes to thank the Danish research council FTP for its financial support to the NMR activities throughout project #274-09-107.

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An approach to predict chemical composition of goat *longissimus dorsi* muscle by near-infrared reflectance spectroscopy

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Value for industry

- NIRS have value as a tool to assess rapidly the composition of goat fresh meat.
- NIRS could improve the efficiency of processing of goat fresh sausages.
- NIRS could help the selection of raw meat to reach the best quality processed products.

Background

The near-infrared spectroscopy (NIRS) using the Fourier transform (FT) is a technology known by the end of 1960s when a computerized spectrophotometer NIR was developed, and its applicability to the analysis of meat was shown (Ben-Gera and Norris, 1968).

NIRS technology is currently a highly versatile tool used in diverse fields including the food industry and particularly in animal science to predict the chemical and physical composition of meat of different species (Weeranantanaphan *et al.* 2011). Due to this high versatility, the technology is being used for large-scale meat quality evaluation to predict chemical composition (Prieto *et al.* 2009) and to identify and authenticate different homogenized meat muscle from beef, pork, lamb and chicken species (Damez and Clerjon. 2013). In goats, as far as we know, there are no studies about the reliability and accuracy of this technology to characterize the meat composition.

Why work is needed

In Portugal, as in other Mediterranean countries, ewes and goats are produced under an extensive system and females are milked to produce milk for cheese. As the kids and lambs cannot compete with milk production for cheese manufacturing they are slaughtered at 1 to 3 months of age, producing light carcasses that are very appreciated by consumers. These carcasses are traditionally commercialized as quality brands as protected designation of origin (PDO) and protected geographical indications (PGI). However there are animals that are outside the specifications of these quality brands, particularly culled animals or those with weight or age that cannot be classified within a PDO or PGI label. These animals have very low consumer acceptability and consequently a low commercial value. A strategy to give value to those animals would be welcome by producers as well as butchers, meat industry or supermarkets.

Two new products, a raw fresh meat sausage and a processed meat product “manta” from Serrana goats and Churra Galega Transmontana ewes were developed, opening new opportunities of new markets. The project involved a partnership agreement arranged between a research centre (Carcass and Meat Quality and Technology Laboratory of Agrarian Scholl of Bragança), two breeder associations (ANCRAS - Serrana National Association of Breed Producers and ACOB - Bragançana National Association of Breed Producers) and a meat manufacturing industry

(Bísaro Salsicharia Tradicional) to add value to these animals, creating two new products, a raw fresh meat sausage and a processed meat product "manta" from Serrana goats and Churra Galega Transmontana ewes.

Good background knowledge about the chemical characteristics of raw meat is important for industry to be able to achieve adequate quality of new products such as sausages.

This work was a first approach to study the ability of NIRS to estimate the protein, moisture, connective tissue, ash and fat content in the *Longissimus dorsi* (LD) muscle of goat meat.

The methods used

Samples (n = 240) of LD muscle were taken from the 8th to 13th rib of the goat carcasses. Samples were minced in a power mill (BÜCHI B-400) specific for meat and meat products obtaining homogeneous samples. Then the samples were scanned in a FT-NIR Master™ N500 (BÜCHI) equipped with a 360 degrees rotation system using a petri dish and over a NIR spectral range of 4000 to 10000 cm^{-1} with a resolution of 4 cm^{-1}. Three 3 spectra per sample were collected (Figure 1).

Subsequently chemical analyses were performed at the Carcass and Meat Quality Laboratory of ESA-IPB. The protein content (NP 1612:1979), moisture (NP 1614:2002), ash (NP 1615:2002), fat content (AOAC, 1997) and collagen (hydroxyproline) (NP 1987: 2002) of the samples were determined.

Using version 5.5 of NIRCal BÜCHI software, chemometric calculations were developed to obtain a robust calibration. For calibrations, sets of calibration (C-set) and validation (V-set) spectra were used and a partial least square regression (PLS) model was developed. A normalization by closure, normalization between 0 to 1 and MSC full were performed as mathematical pretreatments to reduce baseline variations caused by scattering and first derivative Savitzky-Golay 9 Points was also used to reduce baseline effects and to increase smaller absorption peaks particularly eliminating the linear ordinate offset in spectra with very sharp absorption bands with high noise spectra.

Models using principal components analysis (PCA) and PLS have also been used previously in several muscles of different species (Cozzolino and Murray, 2004).

Figure 1. Process sample preparation and subsequent NIR Master analysis.

The results obtained

The range of chemical parameters measured are presented in Table 1. All are within the expected values for fresh goat meat.

	N	Mean	SD	Range (Min/Max)
Protein	165/66	21.17/21.19	1.11/1.11	18.45-23.38 / 18.65-23.04
Moisture	152/62	76.25/76.27	1.94/1.90	70.25-80.12 / 71.52-80.02
Ash	130/57	2.44/2.45	0.36/0.4	1.32-3.07 / 1.38-3.05
Connective Tissue	84/42	0.87/0.82	0.36/0.32	0.19-1.77 / 0.24-1.31
Fat	50/16	2.37/2.56	1.01/0.92	0.5-3.93 / 1.05-3.73

Table 1. Statistical parameters (C-set/V-set).

The best calibration models are presented in Table 2. The consistency parameter describes the relationship between the standard errors of calibration (SEC) and validation (SEV) sets respectively. Values between 70 and 110% are acceptable. The Q-Value is a measure of the quality of the calibrations and it ranges from 0 to 1. This measure is useful as a tool for the judgment when different calibrations were compared. For values higher than 0.6 the calibrations became acceptable.

	Protein	Moisture	Ash	Connective Tissue	Fat
Pretreatment	Normalization Between 0 to 1	MSC Full	1. Normalization by Closure 2. First Derivative Savitzky-Golay 9 Points	1. Normalization Between 0 to 1 2. First Derivative Savitzky-Golay 9 Points	1. First Derivative Savitzky-Golay 9 Points 2. MSC Full
Wavelength cm^{-1}	4200-10000	4200-10000	4200-10000	4200-10000	4200-10000
Method	PLS	PLS	PLS	PLS	PLS
SEC	0.33	0.54	0.08	0.19	0.41
SEP	0.43	0.48	0.28	0.24	0.49
C-set R	0.91	0.92	0.94	0.72	0.83
V-set R	0.87	0.94	0.52	0.46	0.60
Consistency	77.45	112.67	30.25	78.71	82.84
Q-value	0.63	0.74	0.44	0.50	0.56

Table 2. Calibration and validation parameters to goat fresh meat.

With the exception of ash content, all calibrations' models show relatively good predictability. The calibrations for moisture and protein show the best values.

The calibration models obtained show similar accuracy as the results reported in lambs by Sun *et al.* (2012).

This study combines the NIRS with chemometrics as a method to discriminate the geographical origin of lamb meat samples. Also good results were accounted by Guy *et al.* (2011) that use the NIRS to predict lamb meat fatty acid composition.

The scientific conclusions

The calibration models obtained are important as a first attempt to predict the chemical composition of goat meat by NIRS.

The NIRS technology combined with chemometrics will be a useful tool to raw goat meat selection and to improve the quality of sausages processing.

More experimental data will be needed to improve the accuracy of these calibrations.

The next steps

This paper considers the reliability and accuracy of NIRS technology to characterize the goat meat quality. The calibration models for chemical characteristics of goat raw meat would be useful for industry before processing the sausages, to enusre adequate quality of new products. Nevertheless more experimental data are needed to improve the accuracy of these calibrations and more calibrations of physical, chemical and sensory meat attributes will be necessary.

Acknowledgements

COST Action FAIM FA1102 "Optimising and standardising non-destructive imaging and spectroscopic methods to improve the determination of body composition and meat quality in farm animals" and BISOVICAP (Project QREN SI I&DT Co-Promotion nº 21511/201) are greatly acknowledged.

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Capability of near-infrared spectroscopy to predict quality of fresh pork for cooked ham production

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Value for industry

- The use of Near Infrared Spectroscopy (NIRS) as a selection tool for pale, soft and exudative (PSE) pork could ensure a better utilization of fresh hams when processing high quality cooked ham.
- Implementation of NIRS in the production line of cooked hams as a classification tool for fresh hams could result in batches with a more uniform quality.
- NIRS as prediction tool for the quality of fresh pork can allow a more efficient processing of cooked hams with lower slicing losses.

Background

Presence of pale, soft and exudative (PSE) pork is still a concern for the fresh pork and processing industry. Not only the use of extreme PSE pork when processing meat products, e.g. cooked ham, causes problems. Also the use of meat with intermediate PSE characteristics can lead to lower processing yields and texture defects (Torley *et al*. 2000). Although efforts are made to separate pork with PSE characteristics and process it to obtain final products with improved properties and consumer acceptability (Lesiów & Xiong, 2013), currently used methods are still not selective enough to distinguish intermediate PSE categories from good quality meat.

Destructured zones in cooked ham can be associated with PSE pork meat in terms of biochemical properties as both defects are characterized by a higher protein denaturation in the fresh meat (Laville *et al*. 2005). Since muscles which are more sensitive to protein denaturation are present in the core of the ham, destructured zones are not visible during visual control of the fresh ham, and are only detected after the production of the cooked ham.

Hence, a more reliable technique is needed to give information on the technological quality of the fresh ham and thus to predict its suitability for producing high quality cooked ham. Previous studies showed the potential of NIRS to measure pork meat quality and to categorize meat and meat products based on quality (Prieto *et al*. 2009).

The aim of this study was to evaluate the use of NIRS to 1) detect PSE pork under normal operating conditions and 2) classify fresh hams into different classes of end-quality.

Why work is needed

As it is well known that the use of fresh hams with PSE characteristics can lead to cooked hams with lower and non-acceptable end-quality or can result in high slicing losses, it is recommended that meat companies perform objective quality measurements (e.g. pH, conductivity) in addition to the visual inspection so that meat quality defects can be early detected.

Belgian cooked ham factories sometimes measure pH at the reception. As it is generally accepted that ultimate pH of PSE meat, measured 24 hours after slaughter, is comparable to the pH value of pork with normal quality, the pH measurements done at the reception of the companies are not adequate to detect meat with intermediate PSE characteristics, which can lead to the production of cooked hams

with destructured zones. These structural defects are frequently observed when high quality cooked hams, i.e. with a minimum of added technological aids, are produced (Franck *et al*. 1999; Vautier et al. 2004) and are often correlated with slicing problems. Therefore, a more accurate technique is needed for the early detection of (intermediate) PSE fresh ham so that slicing losses can be reduced and less non-consumable products will be produced.

Materials and methods

In a first experiment, a total of 15 pigs were randomly selected at the slaughterhouse of which the *Longissimus Dorsi* (LD) muscles from both carcass halves were hot deboned. PSE condition was induced by incubating the deboned LD muscle, sampled at 30 minutes post mortem, from one half of the carcass at 40°C for 4 hours followed by chilling to 4°C. The deboned LD muscle from the other half of the carcass was immediately cooled at 4°C. During the following 8 hours, pH and temperature were monitored in the slaughterhouse after which the LD samples were transported to the laboratory and further stored at 4°C. After 24 hours, ultimate pH, PQM and CIE $L^*a^*b^*$ color values were measured. From all LD muscles, infrared spectra were collected using NIRS technology equipped with a fiber optics probe over the range 4000 to 12000 cm^{-1}. An aliquot of the LD samples was kept at -80°C to determine the sarcoplasmic and myofibrillar protein solubility according to Claeys *et al.* (2002).
In a second experiment, 26 *Semimembranosus* (SM) muscles, showing visual PSE characteristics, and 24 normal SM muscles were selected at the cutting room of a Belgian cooked ham factory. All the selected SM muscles were provided with the *Adductor* (AD) muscle. After collecting the meat quality parameters (pH, PQM, $L^*a^*b^*$) and NIR spectra at the cutting room of the production plant, salt injection was executed. Afterwards, the SM samples were transported to the laboratory where they were prepared to cooked meat products in a pilot plant for cooked ham production.

In a third experiment, a total of 48 fresh hams were selected based on pH measured 20 minutes post mortem in the SM muscle of which 24 hams were subjected to early post mortem electrical stimulation (ES) to induce PSE pork meat. At 48 hours post mortem, traditional meat quality parameters as well as NIRS measurements were performed on both intact and deboned fresh hams. Protein solubility was determined at the transition zone between SM and AD muscle. Afterwards, raw hams were prepared to high quality cooked hams and industrially sliced. The sliced hams were visually classified according to whether destructured zones were present or not.

Discriminant analysis (SPSS Statistics, IBM, version 21) was carried out in order to investigate the possible use of traditional meat quality parameters or NIR spectral data, to classify 1) the LD samples in normal and PSE-induced samples, 2) the SM samples according to their external PSE characteristics, 3) the fresh hams according to the visual score given to the cooked hams after slicing.

Results and discussion

Results of meat quality parameters on both normal and PSE-induced pork LD muscles are shown in Table 1.

Meat quality parameter	Normal LD		PSE LD		
	Mean	SD	Mean	SD	P
pH_{30min}	6.34	0.30	6.22	0.28	0.270
$pH_{4h30min}$	5.79	0.26	5.29	0.07	< 0.001
pH_{24h}	5.37	0.07	5.41	0.07	0.080
PQM_{24h}	6.63	2.88	16.07	1.30	< 0.001
L^*_{24h}	51.21	2.95	59.47	3.52	< 0.001
a^*_{24h}	6.53	1.17	6.99	1.03	0.270
b^*_{24h}	13.40	0.75	15.54	0.86	< 0.001
Sarcopl. prot. sol. (mg/g)	74.11	2.43	62.29	4.07	< 0.001
Myofibr. prot. sol. (mg/g)	14.93	0.53	11.91	0.89	< 0.001

Table 1. Mean and standard deviation (SD) of the meat quality parameters of normal (n = 15) and PSE-induced (n = 15) pork LD muscle

Although the pH_{30min} was not different between both groups, incubating the LD muscles at 40°C for 4 hours resulted in a lower pH value 4.5 hours post mortem ($P < 0.001$). As the pH fell below 5.8 while muscle temperature was above 35°C, the muscle was sensitive to heat denaturation and thus PSE. The ultimate pH for both chilling regimes was similar. PSE-induced pork also had significantly higher PQM, L^* and b^* values ($P < 0.001$) measured 24 hours after slaughter.

A reduced protein solubility in PSE-induced LD samples than in normal LD muscle was observed ($P < 0.001$). Both traditional meat quality parameters and NIR data were used to classify the LD muscles according to the chilling regime to which they had been subjected. Classification results after cross-validation are shown in Table 2. Using the raw NIR spectral data resulted in a 93.3% correct classification after cross-validation. The classification results obtained with NIR were very good, however not as conclusive as those achieved with the traditional meat quality parameters PQM_{24h} and b^*_{24h}.

Parameter	Normal LD	PSE LD	Total
PQM_{24h}	86.7	100	93.3
L^*_{24h}	93.3	86.7	90.0
b^*_{24h}	93.3	100	96.7
$PQM_{24h} + b^*_{24h}$	100	100	100
Protein solubility	100	93.3	96.7
NIR spectra	100	86.7	93.3

Table 2. Percentage correct classification after cross-validation for normal (n=15) and PSE-induced (n=15) LD samples.

Results of meat quality measurements on both normal and PSE SM muscles are shown in Table 3.

PSE SM samples had significantly lower pH and a^* values ($P < 0.001$) and significantly higher L^* and b^* values ($P < 0.001$) compared to normal SM samples. A reduced sarcoplasmic protein solubility in PSE SM samples than in normal SM muscle was observed ($P < 0.001$). Both meat quality parameters and NIR spectra were used to classify the SM muscles according to the presence of visual PSE characteristics (Table 4). Using the raw NIR spectra resulted in 90.0% correct classification after cross-validation which is a promising result, however still not as good as that observed with a combination of the L^* value and PQM.

Concerning the PSE induction experiment by ES on fresh hams, 25 hams of the original 48 were visually classified into normal quality hams; 23 hams showed presence of destructured zones and were therefore classified into inferior quality hams. Results of meat quality parameters, measured on the intact and deboned fresh hams, of both normal and inferior classified cooked hams are shown in Table 5.

Cooked hams exhibiting destructured zones had significantly lower pH_{48h} ($P < 0.01$) measured on the SM muscle of the intact fresh ham than normal classified hams. After deboning, inferior classified hams showed significantly higher L^*_{48h} ($P < 0.05$) and b^*_{48h} ($P < 0.01$) values, measured on the *Biceps femoris* (BF) fresh muscle, compared to normal quality hams. Classification was performed based on the conventional and NIRS measurements carried out 1) on the raw, intact hams and 2) after deboning. Measuring NIR spectra on the BF muscle after deboning resulted in a 60.0% and 56.5% correct classification after cross-validation for normal and inferior classified hams respectively. Using the traditional meat quality parameters, the best classification was obtained using the b^*_{48h} measured on the BF muscle after deboning, i.e. 76.0% and 60.9% for normal and inferior hams respectively.

Meat quality parameter	Normal SM		PSE SM		
	Mean	SD	Mean	SD	P
pH	5.70	0.13	5.50	0.07	< 0.001
PQM	12.53	3.82	13.55	3.83	0.347
L^*	49.08	2.98	62.49	3.04	< 0.001
a^*	11.43	2.43	10.05	1.51	< 0.050
b^*	16.90	1.84	19.17	0.98	< 0.001
Sarcopl. prot. sol. (mg/g)	65.55	16.78	43.75	7.28	< 0.001
Myofibr. prot. sol. (mg/g)	13.39	5.19	14.04	8.44	0.748

Table 3. Mean and standard deviation (SD) of the meat quality parameters of normal (n = 24) and PSE (n = 26) pork SM samples.

Parameter	Normal SM	PSE SM	Total
L^* + PQM	100	100	100
Protein solubility	83.3	88.5	86.0
NIR spectra	79.2	100	90.0

Table 4. Percentage correct classification after cross-validation for normal (n = 24) and PSE (n = 26) SM samples

Intact		Normal		Inferior		
	Muscle	Mean	SD	Mean	SD	P
pH_{20min}	SM[1]	6.07	0.14	6.10	0.11	0.440
pH_{60min}	SM	5.71	0.24	5.74	0.23	0.671
pH_{48h}	SM	5.74	0.11	5.66	0.07	< 0.010
pH_{48h}	BF[2]	5.78	0.21	5.69	0.10	0.057
PQM_{48h}	SM	8.25	1.50	7.73	1.46	0.225
PQM_{48h}	BF	8.32	1.14	8.40	0.74	0.788
Deboned						
pH_{48h}	SM/AD[3]	5.80	0.16	5.72	0.09	0.053
pH_{48h}	BF	5.82	0.18	5.76	0.11	0.142
PQM_{48h}	SM/AD	8.93	0.82	8.84	0.57	0.667
PQM_{48h}	BF	8.72	0.68	8.59	0.70	0.506
L^*_{48h}	SM/AD	58.73	4.47	61.37	4.61	0.050
L^*_{48h}	BF	49.42	6.04	53.62	6.18	< 0.050
a^*_{48h}	SM/AD	8.09	1.89	8.27	2.30	0.768
a^*_{48h}	BF	10.82	2.07	10.91	1.64	0.870
b_{48h}	SM/AD	16.99	1.28	17.55	1.62	0.192
b^*_{48h}	BF	16.26	1.42	17.69	2.00	< 0.010
Sarcoplasmic protein solubility (mg/g)	SM/AD	50.37	7.78	50.03	6.71	0.872
Myofibrillar protein solubility (mg/g)	SM/AD	8.71	0.62	8.44	0.57	0.115

Table 5. Mean and standard deviation (SD) of the meat quality parameters, measured on the intact and deboned fresh hams, of both normal (n = 25) and inferior (n = 23) classified cooked hams after PSE induction by ES

[1] SM: *M. Semimembranosus*
[2] BF: *M. Biceps femoris*
[3] AD: *M. Adductor*

Scientific conclusions

The classification results obtained, showed that NIRS can be used to discriminate PSE from normal quality at 24 hours post mortem. However, further improvement is needed to develop a more robust classification tool that can be implemented at industrial level. The use of NIRS as classification tool for the quality of fresh pork for cooked ham production in terms of destructured zones should be further investigated. The poor classification of fresh hams according to the presence of destructured zones in the cooked ham can be attributed to the non-uniform PSE induction.

Future research

Future research could involve an expansion of the rather limited dataset and a more uniform methodology to induce PSE pork meat.

Acknowledgements

This research was financed by IWT Flanders (Brussels, Belgium), Belpork npo, several meat companies and suppliers of NIRS equipment. The authors would like to thank the staff of the participating slaughterhouse and cooked ham companies for their assistance. The NIRS equipment provided by Bruker (Evere, Belgium) is gratefully acknowledged.

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Lesiów T, Xiong YL (2013). A simple, reliable and reproductive method to obtain experimental pale, soft and exudative (PSE) pork. Meat Science, 93, 489-494.

Prieto, N, Roehe, R, Lavín, P, Batten, G, Andrés, S (2009). Application of near infrared reflectance spectroscopy to predict meat and meat products quality: A review. Meat Science, 83, 175-186.

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Vautier A, Minvielle B, Boulard J, Bouyssiere M, Houix Y (2004). Viandes déstructurés: Effet du système d'abattage et des conditions météorologiques. TechniPorc, 27, 19-23.

Reference methods used to determine meat quality parameters: preliminary overview

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Value for industry

- Knowledge of meat quality parameters is of interest to the meat industry because it helps the production chain optimize its processes and, consequently improve product quality which benefits consumers.
- Several non-destructive imaging and spectroscopic technologies may be suitable for on line use to determine meat quality parameters, which would offer high value to the industry. However, these technologies have to be calibrated against a reference for each quality parameter by means of standardized method.
- Due to differences in the methods currently in use there is a need for a handbook providing a collection of most of the methodologies used as a reference, as well as variations carried out in different laboratories.

Background

Within the COST Action FAIM, relevant meat quality parameters for pork, beef, lamb and poultry have been identified (milestone 2 and 4) through the use of a questionnaire and a discussion session amongst all the participants in FAIM II Conference in Kaposvár (FAIM, 2013) . In addition, a preliminary literature review of imaging and spectroscopic technologies currently used for the evaluation of raw and processed pig, beef, sheep and poultry meat quality was performed (Font-i-Furnols *et al*. 2013). Some of these technologies are suitable to be used on line, so would produce important benefits for the meat industry. In general, the technologies and methodologies that are applied have different accuracies when used for the determination of meat quality parameters. Knowledge of accuracy is necessary for calibration and validation trials against a reference. There are several methodologies used as a reference but they are not always comparable and/or standardized among laboratories. Some of the methods are based on standards such as ISO (International Organization for Standardization), AOAC (Association of Official Analytical Chemists) and National Standards, while others are based on methodologies published in scientific journals or based on the experience of the laboratory.

Some reviews have been published considering one or more reference methods for meat quality parameters. For instance, Honikel (1997, 1998) published a review of reference methods for the determination of water holding capacity, colour and tenderness. These methods are still used in several laboratories. Nollet and Toldrà (2009) were the editors of the 'Handbook of Muscle Foods Analysis' in which several meat quality attributes were considered. However some changes and modifications are applied in some laboratories to these standardized and published methodologies and one of the FAIM deliverables is to collect all such methodologies into a handbook of standardised references.

Why work is needed

Imaging and spectroscopic technologies need to be calibrated against a reference. Due to the high variability in methods used as a reference to determine meat quality traits, sometimes comparison between them can be difficult. It is important to identify and collect these references, and, if possible, work to make them (and the units used to measure them) comparable and standardized. Moreover, it would be of interest for users of new technologies to have a handbook collection of methodological

references for meat quality analysis, because this would help them in the determination of these parameters and, consequently, in the calibration and validation of their devices based on imaging and spectroscopic technologies.

The methods used

A questionnaire was sent to all the FAIM participants, who belong to different Research Centres, Universities or Industry, in order to obtain information about the reference method used in their laboratories to determine the selected meat quality parameters in pork, beef, lamb and poultry. A template and some examples were provided in order to obtain information in a standardized format.

The results obtained

Up to the end of July 2014, we have received feedback from 15 research centres or universities in Austria, Belgium, Croatia, France, Germany, Italy, New Zealand, Norway, Portugal (2 centres), Slovenia, Slovak Republic, Spain, Switzerland and United Kingdom. Each of them has provided information about reference analysis of up to 11 meat quality parameters.

In considering the results, the following are relevant:

(1) For some meat quality attributes more than one methodology is used, even in the same laboratory, depending on the species and the characteristics of the project in which measurement has to be obtained.

(2) Some references are used in several laboratories with or without modifications.

(3) Other references are specific for one laboratory.

(4) Furthermore, for several attributes, some centres have reported their own methodology, without a published reference.

(5) Most of the references are used in several species with or without changes among species.

A summary of the references used for each meat quality parameter is detailed below. It should be noted that these references can have modifications and that modifications are not presented here but they will be included in the methodological handbook. Moreover, some methodologies have been reported to be used only in some species while other methodologies in all the species of interest. Finally this is a first draft of the information received and further work is planned.

pH (Figure 1)

All the different laboratories measure pH with a pH-meter. Between the centres, the muscle to be evaluated varies, as do the times of the evaluation and the type of devices used. Furthermore, within muscle the pH also can vary considerably.

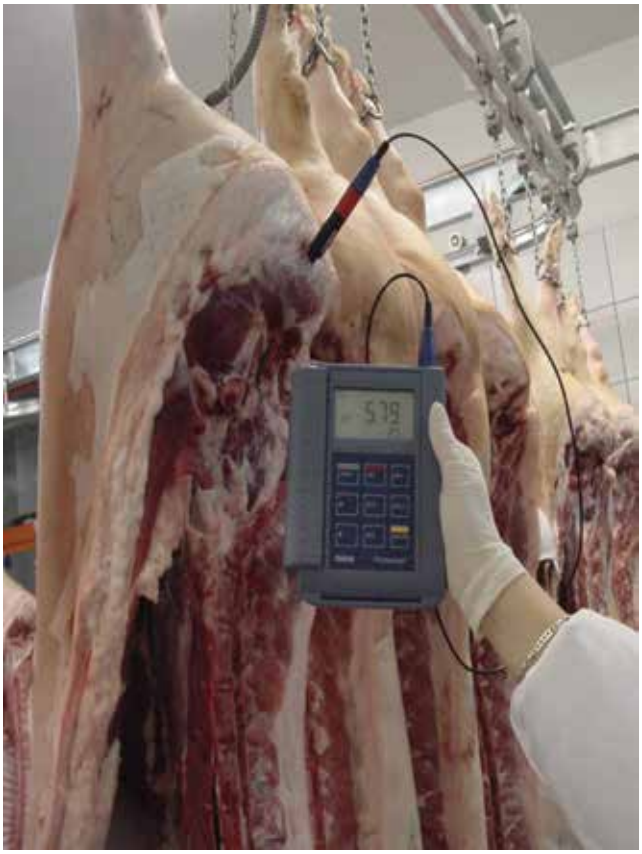

Figure 1. Measure of pH on muscle *Semimembranosus* at 24 h *post mortem*.

WATER HOLDING CAPACITY

Information has been received on methods used to evaluate water holding capacity. Different methods look for differently bound water, so methods are not always well correlated.

Drip loss

- Allison C P, Ritter MJ, Doumit ME (2002). Techniques for quantification of loin muscle water-holding capacity. Proceedings of the Third Pork Quality Improvement Symposium.
- Christensen LB (2003). Drip loss sampling in porcine m. longissimus dorsi. Meat Science, 63, 469-477
- Grau R, Hamm R (1957). Über das Wasserbindungsvermögen des Saugetiermuskels II. Lebensmittel-Intersuchung Und-Forschung 105, 446-460.
- Honikel KO (1997). Reference methods supported by OECD and their use in Mediterranean meat products. Food Chemistry, 59, 573-582.

- Honikel, K.O. (1998). Reference methods for the assessment of physical characteristics of meat. Meat Science, 49, 447-457.
- Kauffman RG, Cassens RG, Sherer A, Meeker DL (1992). Variations in pork quality. NPPC Publication, Des Moines, U.S.A. pp 1-8.
- Rasmussen AJ, Anderson M (1996). New method for determination of drip loss in pork muscles. In Proceedings of the 42nd ICoMST, pp 286-287. Lillehammer, Norway. (Figure 2)

Figure 2. Determination of drip loss in pork loin according to EZ method.

Cooking loss

- Honikel KO (1997). Reference methods supported by OECD and their use in Mediterranean meat products. Food Chemistry, 59, 573-582.
- Honikel, K.O. (1998). Reference methods for the assessment of physical characteristics of meat. Meat Science, 49, 447-457.
- Wheeler TL, Shackelford SD, Koohmaraie M (2005). Shear Force Procedures for Meat Tenderness Measurement. http://www.ars.usda.gov/SP2UserFiles/Place/54380530/protocols/ShearForceProcedures.pdf
- Unpublished "in-house" methods or adaptations

Grill loss-Thaw loss

- Honikel, K.O. (1998). Reference methods for the assessment of physical characteristics of meat. Meat Science, 49, 447-457.

INTRAMUSCULAR FAT

- AOAC International PVM 4:1997
- ASTN (1988). Total fat extraction in certain food products according to AOAC. Application Short Note. Tecator, Hoganas, Sweden.
- Folch J, Lees M, Sloane-Stanley C (1957). Simple method for the isolation and purification of total lipids from animal tissues. The Journal of Biological Chemistry, 226, 497–509.
- ISO 1443:1973, Meat and meat products - Determination of total fat content.
- ISO 6492:1999, Animal feeding stuffs – Determination of fat content.
- NP 1613 (1979)
- Schormüller J (1968). Handbuch der Lebensmittelchemie, Band III/2. Teil, Tierische Lebensmittel Eier, Fleisch, Fisch, Buttermilch. Springer-Verlag, Berlin, Heidelberg, New York, S. 1201-1202.
- § 64 German code of Law for Food and Animal Feed, LFGB 2011, Beuth-Verlag, Berlin.

COLOUR

Instrumental colour: (Figure 3)

- CIE. 1986. Colorimetry. 2nd ed. CIE Publ. No. 15.2. Commission International de l'Eclairage, Vienna, Austria.
- Honikel KO (1997). Reference methods supported by OECD and their use in Mediterranean meat products. Food Chemistry, 59, 573-582.
- Boccard R, Buchter L, Casteels E, Cosentino E, Dransfield E, Hood DE, Joseph RL, MacDougall DB, Rhodes DN, Schön I, Tinbergen BJ, Touraille C (1981) Procedures for measuring meat quality characteristics in beef production experiments. Report of a working group in the commission of the European communities' (CEC) beef production research programme. Livestock Production Science 8 (5) 385-397.

Figure 3. Determination of L*, a* and b* colour parameters with a Minolta colorimeter.

Visual colour:

- ISO 8586-1:2009, Sensory analysis. General guidance for the selection, training and monitoring of assessors. Part 1: Selected assessors.
- ISO 8586-2:2009, Sensory analysis. General guidance for the selection, training and monitoring of assessors. Part 2: Experts.

- Nakai H, Saito F, Ikeda T, Ando S, Komatsu A (1975). Standard models of pork colour. Bullettin of National Institute of Animal Industry, 29, 69-74.

Metmyogobin reducing activity/Oxygen consumption

- Kim Y H, Hunt MC, Mancini RA, Seyfert M, Loughin TM, Kropf DH, Smith JS (2006). Mechanism for lactate-color stabilization in injection-enhanced beef. Journal of Agricultural and Food Chemistry, 54(20), 7856-7862.
- King DA, Shackelford SD, Rodriguez AB, Wheeler TL (2011). Effect of time of measurement on the relationship between metmyoglobin reducing activity and oxygen consumption to instrumental measures of beef longissimus color stability. Meat Science, 87(1), 26-32.

Chemical fat colour:

- Boccard R, Buchter L, Casteels E, Cosentino E, Dransfield E, Hood DE, Joseph RL, MacDougall DB, Rhodes DN, Schön I, Tinbergen BJ, Touraille C (1981) Procedures for measuring meat quality characteristics in beef production experiments. Report of a working group in the commission of the European communities' (CEC) beef production research programme. Livestock Production Science 8 (5) 385-397.

TENDERNESS (Shear Force)

We will focus on the information received on the determination of instrumental tenderness, mainly by means of shear force parameter. Other information has also been received for the determination of indirect measure of tenderness by means of indicators such as sarcomere length and collagen content and solubility.

- Bérard J, Kreuzer M and Bee G 2008. Effect of litter size and birth weight on growth, carcass and pork quality, and their relationship to postmortem proteolysis. Journal of Animal Science 86, 2357-2368.
- Chrystall NB, Devine CE (1991). Quality assurance for tenderness. Meat Industry Research Institute of New Zealand Publication No. 872.
- Honikel KO (1998). Reference methods for the assessment of physical characteristics of meat. Meat Science, 49, 447-457. (Figure 4)
- Liu Y, Lyon BG, Windham WR, Lyon CE, Savage EM (2004). Principal component analysis of physical, color, and sensory characteristics of chicken breasts deboned at two, four, six, and twenty-four hours post-mortem. Poultry Science, 83, 101–108.
- Ruiz de Huidobro F, Miguel E, Blázquez B, Onega E (2005). A comparison between two methods (Warner-Bratzler and texture profile analysis) for testing either raw meat or cooked. Meat Science, 69, 527-536.
- Shackelford SD, Wheeler TL, Koohmaraie M (1999). Tenderness classification of beef. II: Design and analysis of a system to measure beef longissimus shear force under commercial processing conditions. Journal of Animal Science 77, 1474-1481
- Wheeler T L, Shackelford SD, Koohmaraie M (2005): Shear Force Procedures for Meat Tenderness Measurement. http://www.ars.usda.gov/SP2UserFiles/Place/54380530/protocols/ShearForceProcedures.pdf
- Unpublished "in-house methods or adaptations"

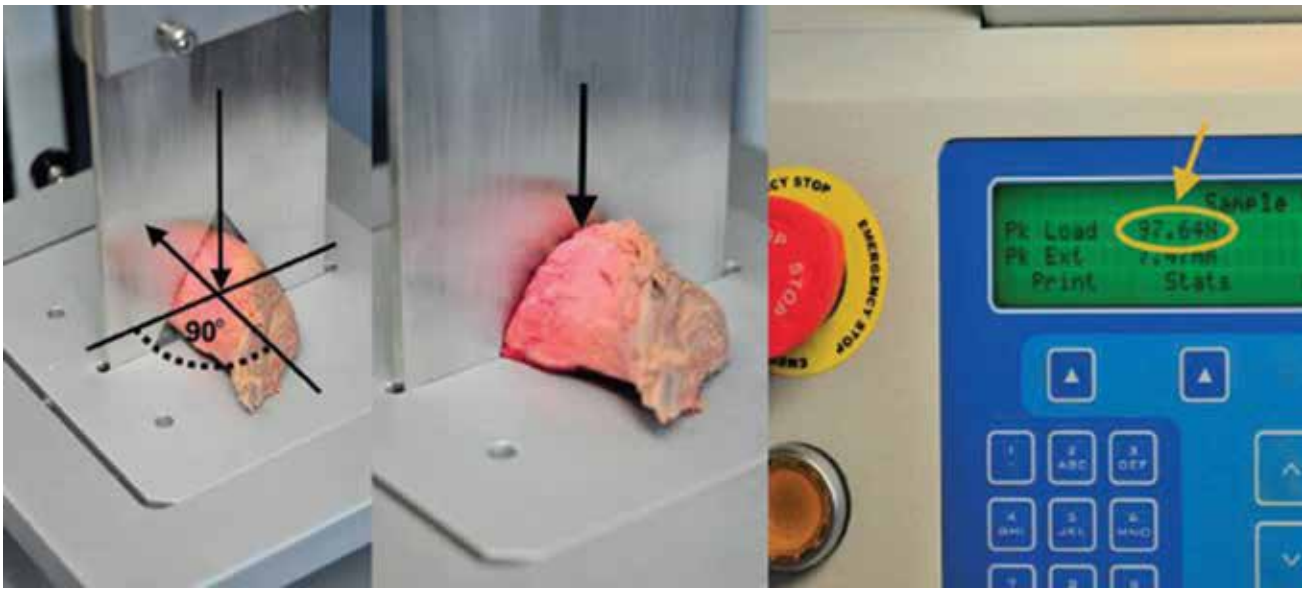

Figure 4. Steps in the determination of Warner-Bratzler shear force with a texturometer.

TENDERNESS AND JUICINESS SENSORY AND FLAVOUR (includes Taste and Taint)

For sensory assessment there are several methodologies of meat preparation which will depend on the objective of the evaluation. These methodologies are published in several scientific papers which are not included here because there are a large number of them.

Assessors training:

- ISO 8586-1:2009, Sensory analysis. General guidance for the selection, training and monitoring of assessors. Part 1: Selected assessors.
- ISO 8586-2:2009, Sensory analysis. General guidance for the selection, training and monitoring of assessors. Part 2: Experts.
- ISO 87025:1996, Sensory analysis. Methodology. Texture profile.
- ISO 4120:2008, Sensory analysis. Methodology. Triangular test.
- ISO 5495: 2009: Sensory analysis. Methodology. Paired test.
- ISO 8587:2007: Sensory analysis. Methodology. Ranking.

Unpublished "in-house methods or adaptations

Reference scale and evaluation procedure:

- ISO 87025:1996, Sensory analysis. Methodology. Texture profile.
- ISO 4221:2006, Sensory analysis. Guidelines for the use of quantitative response scales.
- Meilgaard M, Civille GV, Carr BT (1987). Sensory evaluation techniques. CRC Press, Inc. Boca Raton, Florida, USA (pp. 281).

Unpublished "in-house methods or adaptations

FATTY ACID COMPOSITION

Extraction methodology:

- Ampuero Kragten S, Collomb M, Dubois S, Stoll P (2014). Zusammensetzung von Fettsäuren in der Tierfütterung-Analysenmethoden. Agrarforschung Schweiz, September 2014.
- AOAC International PVM 4:1997
- Folch J, Lees M, Sloane-Stanley C (1957). Simple method for the isolation and purification of total lipids from animal tissues. The Journal of Biological Chemistry, 226, 497–509.
- Schulte E, Weber K (1989). Rapid preparation of fatty acids methyl esters of fats with trimethylsulfonium hydroxide or sodium methylate. Fat Science Technology, 91, 181-183

Fatty acids methylation:

- Ampuero Kragten S, Collomb M, Dubois S, Stoll P (2014). Zusammensetzung von Fettsäuren in der Tierfütterung-Analysenmethoden. Agrarforschung Schweiz, September 2014.
- AOAC International PVM 4:1997
- Machery-Nagel (2014). Methylation with TMSH. Consulted on 24.06.2014 in: http://www.mn-net.com/tabid/10234/default.aspx
- Morrison WR, Smith LM (1964). Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. Journal of Lipid Research, 5, 600-608.
- Raes K, De Smet S, Demeyer D (2001). Effect of double-muscling in Belgian Blue young bulls on the intramuscular fatty acid composition with emphasis on conjugated linoleic acid and polyunsaturated fatty acids. Animal Science, 73, 253-260.
- Schulte E, Weber K (1989). Rapid preparation of fatty acids methyl esters of fats with trimethylsulfonium hydroxide or sodium methylate. Fat Science Technology, 91, 181-183

Fatty acids analysis:

- Ampuero Kragten S, Collomb M, Dubois S, Stoll P (2014). Zusammensetzung von Fettsäuren in der Tierfütterung-Analysenmethoden. Agrarforschung Schweiz, September 2014.
- AOAC International PVM 4:1997
  Unpublished "in-house methods or adaptations

WATER CONTENT

- AOAC (2005). Official Methods of Analysis. Official method 950.46B(a) 18th Edition.
- ISO 6496:1999, Animal feeding stuffs - Determination of moisture and other volatile matter content
- NP 1614 (2002) (Portuguese standard)
- Schormüller J (1968). Handbuch der Lebensmittelchemie, Band III/2. Teil, Tierische Lebensmittel Eier, Fleisch, Fisch, Buttermilch. Springer-Verlag, Berlin, Heidelberg, New York, S. 1200-1201.
- § 64 German code of Law for Food and Animal Feed, LFGB 2011, Beuth-Verlag, Berlin.

ASH CONTENT

- AOAC (2000). Official Methods of Analysis. Official method 920.153.
- ISO 5984:2002 Animal feeding stuffs -Determination of crude ash.
- NP 1615 (2002) (Portuguese standard)
- Schormüller, J. (1968). Handbuch der Lebensmittelchemie, Band III/2. Teil, Tierische Lebensmittel Eier, Fleisch, Fisch, Buttermilch. Springer-Verlag, Berlin, Heidelberg, New York, S. 1201.
- § 64 German code of Law for Food and Animal Feed, LFGB 2011, Beuth-Verlag, Berlin

PROTEIN CONTENT

- AOAC (2000). Official Methods of Analysis. Official method 976.05. 17th ed.
- ISO 5983-2:2005: Animal feeding stuffs - Determination of nitrogen content and calculation of crude protein content – Part 1: Kjeldahl (N x 6.25), and Part 2: Block digestion/steam distillation method
- NP 1612 (1979) (Portuguese standard)
- Schormüller J (1968). Handbuch der Lebensmittelchemie, Band III/2. Teil, Tierische Lebensmittel Eier, Fleisch, Fisch, Buttermilch. Springer-Verlag, Berlin, Heidelberg, New York, S. 1203.
- § 64 German code of Law for Food and Animal Feed, LFGB 2011, Beuth-Verlag, Berlin

The scientific conclusions

- Standardized and well described references are needed to determine meat quality attributes to make them comparable between laboratories and use in industry.
- References are needed to calibrate and validate imaging and spectroscopic devices for the determination of meat quality parameters and to determine its accuracy in the prediction.
- Meat quality parameters can have different reference methods for its determination depending on (a) facilities and equipment necessary for the determination, (b) relation between time and personal required for the analysis and recourses of the laboratory, and (c) objectives and characteristics of the work in which is going to be used.
- Nowadays, some standards and modifications of them are used in the different European laboratories and it is of interest of the researchers and the industry to join them in a handbook to make them accessible to all the interested parties.

The next steps

This paper is just a short overview of methodologies used as references in different laboratories for the determination of meat quality parameters in beef, pork, lamb and poultry. In the follow up, this information will be described, completed and presented as a handbook to the scientific and industrial community.

Acknowledgements

This work has been possible due to the collaboration of FAIM participants who have shared information about the references used in their laboratories. Thanks to all of them. Financial support of COST Action FAIM FA1102 “Optimising and standardising non-destructive imaging and spectroscopic methods to improve the determination of body composition and meat quality in farm animals” is greatly acknowledged.

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Font-i-Furnols M, Prevolnik M, Fulladosa E, Čandek-Potokar M (2013). Spectroscopic and imaging technologies to evaluate meat quality: a preliminary review. In: Farm Animal Imaging, Kaposvár 2013. Ed.: C. Maltin, C. Craigie & L. Bünger. pp. 45-48.

Honikel KO (1997). Reference methods supported by OECD and their use in Mediterranean meat products. Food Chemistry, 59(4), 575-582.

Honikel KO (1998). Reference methods for the assessment of physical characteristics of meat. Meat Science, 49(4), 447-457.

Nollet LML, Toldrà F (editors) (2009). Handbook of Muscle Food Analysis. CRC Press, Taylor & Francis Group. United States. pp. 968.

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Vitamin A, marbling and connected sets

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Value for industry

- Marbling is an important trait for beef consumers.
- Quantitative characterisation of intramuscular fat allows standardization of marbling appearance.
- Understanding the structure of marbling will facilitate biological understanding and the role of beef production systems in marbling development.

Background

The quantity and distribution of intramuscular fat in beef, known as marbling, is an important selection criterion for consumers, with Asian markets generally preferring many, fine, evenly distributed marbling flecks and US markets preferring little marbling (Hart, 2001). Marbling scores are routinely assigned by professional graders (Harper and Pethick, 2001) but these scores are subjective and simplistic in that they do not capture complex marbling appearance such as the size and shape of marbling flecks or their spatial distribution (Shiranita *et al*. 2000). Automatic systems based on image analysis are a natural way forward to standardise detailed descriptors of marbling. Such systems have been considered (Albrecht *et al*. 1996; Yoshikawa et al. 2000; Shiranita *et al*. 2000; Toraichi *et al*. 2002; Maeda *et al*.2014) but are subject to variation in lighting, glare, and image quality (Yoshikawa *et al*. 2000). Here, image analysis methods are reported for characterising marbling in beef. Analysis was conducted both in 2-dimensions (2D) on individual images of steaks and on sequences of steaks forming a 3-dimensional (3D) representation of marbling.

Why the work is needed

Consistent, quantitative characterization of marbling is useful for scientific studies on the genetic and biochemical aspects of fat metabolism and beef quality. Automated image analysis also has the potential to improve grading of meat by providing greater consistency, more detailed information and a lower cost than standard methods for grading meat. While the use of image analysis for meat quality is growing, the methods used have not yet matured and have not taken full advantage of advances in areas such as computer vision and medical image analysis.

The methods used

The work described herein is part of a larger study on the relationship between vitamin A and marbling quality. Full experimental details of this study, including animal management are reported elsewhere (Siebert *et al*. 2006; Kruk *et al*. 2008). Briefly, 20 Angus steers were randomly allocated to two experimental groups: a vitamin A supplemented group (A+) and a vitamin A non-supplemented group (A-). Over a 10-month period, the A+ group received a weekly vitamin A supplement. Various measurements were made during this time to assess vitamin A deficiency.

After slaughter, the striploins, quartered at the 12-13th rib were collected, trimmed, vacuum packed and frozen at -20˚C. Twenty five consecutive 4mm thick slices were cut from striploin, cleaned, coded and stored. Images of these steaks were taken under controlled conditions in a purpose built photography chamber to ensure consistency in lighting and to reduce glare. Image size was 2014 x 1536 pixels with spatial resolution 75 pixels/cm. The rib eye region was extracted manually from each image using Adobe Photoshop® 7.0 and superimposed on a uniform green background (RGB = 171, 160, 10). All further image processing and image analysis steps were performed using in-house algorithms written in the scientific computing platform Matlab 7.0. The colour images were converted to gray-scale based on luminance. A linear filter was used to flatten the image and reduce any residual glare. This filter consists of a single pixel of value one at the center of a ring of pixels of radius R with the same fixed intensity value chosen so that the filter is zero-sum. Since this filter approximates the identity near the center, local features of the image are well preserved

but global variation is subtracted due to the zero-sum property. An empirically determined threshold was used to obtain a binary image showing the marbling only. Each 8-connected set of pixels in the image was taken to be a separate marbling fleck. The area of the fleck was taken as the number of pixels constituting the fleck.

Next, principal component analysis on the coordinates of the pixels comprising the fleck was used to determine the major and minor orientation axes of the fleck as well as the ratio of their lengths (determined by the eigenvalues). For every fleck, an ellipse was constructed to have the same area and the same major and minor orientation axes as the fleck. A number of shape parameters were defined using the ellipse associated with the fleck. The major and minor axes of the ellipse were used to define the length, width and eccentricity of the fleck. Superimposing the ellipse on the fleck and measuring the area of the region outside the ellipse, but inside the fleck, led to an irregularity of shape measure (irregularity, for short). This value divided by the area of the fleck was called the normalized irregularity. In addition, the location of the center of each fleck was recorded.

Significant differences between the two experimental groups were found for the mean fleck area, eccentricity, normalized irregularity, number of flecks and the length of the minor axis.
A natural extension of the methods presented thus far is to consider the marbling flecks as 3D objects instead of the 2D representations found by observing single slices. In order to carry out a 3D analysis, the 25 steaks from each striploin were scaled and aligned using two holes drilled through the frozen striploin parallel to the long axis of the striploin prior to slicing. Together, the 25 slices formed a 3D representation of the marbling with asymmetric voxels of size 0.13 mm x 0.13 mm x 4.00 mm.

Identifying individual 3D marbling flecks required finding the connected components in the 3D array. In discrete 3D space, there three types of connectedness: two voxels are called 6-connected if they share a face, they are called 18-connected if they share a face or an edge and 26-connected if they share a face, an edge or a vertex. All three versions of connectedness were tested in determining 3D marbling flecks. The size of the 3D fleck was taken to be number of voxels comprising the fleck.

The results obtained

One of the drill holes exited the striploin prematurely with the result that 2D slices for that striploin could not be aligned with confidence to form a 3D representation. Hence, results are presented for 19 striploins instead of 20.

Figure 1. The 3D representation of marbling in a single striploin. The majority of the marbling forms a single connected set. Some isolated flecks are apparent but whether these are truly separate or have been separated due to sampling is not known.

In each striploin, a single 3D fleck was found to account for nearly all of the marbling (Figure 1). The mean per cent of the total marbling volume taken up by the single largest fleck was 82.6 (SD = 14.6, n = 19) for 6-connected, 83.8 (SD = 14.9, n = 19) for 18-connected and 84.0 (SD = 15.0, n = 19) for 26-connected. With so much of the total marbling comprising a single fleck, statistical analysis of size, shape and spatial distribution parameters analogous to that performed for the 2D representation was not meaningful.

However, questions did arise regarding the correctness of this observation. Since each slice represents a distance of 4 mm, two disjoint 2D flecks in adjoining slices may erroneously be counted as a single fleck while a true single 3D fleck may be separated and counted as two distinct flecks (Figure 2).

Whether these two phenomena roughly cancel each other so that the number and sizes of 3D flecks measured by finding connected sets is fairly accurate or whether one of these phenomena dominates to skew these observations is not immediately apparent.

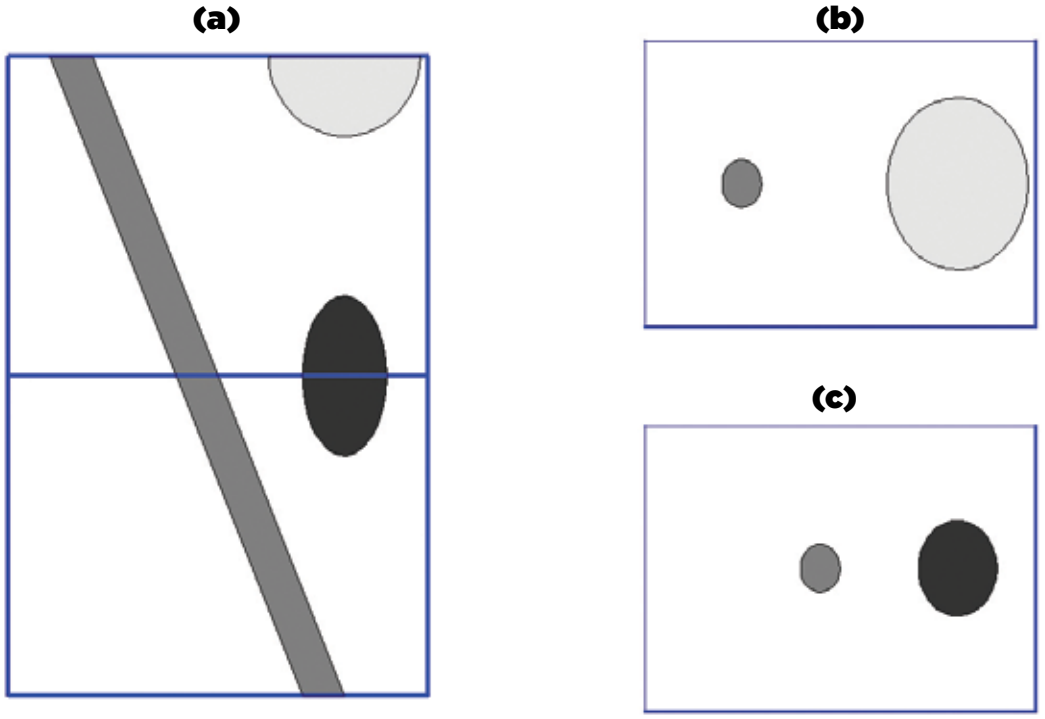

Figure 2. Connecting and disconnecting flecks due to slicing. (a) shows a schematic of two consecutive steaks on top of each other. The view is an x-ray view from the side. The diagonal bar represents a tubular marbling fleck. The dark and light ovals are other examples of 3D flecks. (b) shows the image of the top steak taken from above and (c) is the image of the bottom steak taken from above (after separating the steaks). In images (b) and (c), the connected tubular fleck is seen as two separate flecks because the two 2D representations of this fleck in the consecutive steaks do not overlap. The other two isolated flecks overlap in consecutive images and so are counted as a single fleck.

Accordingly, simulations were conducted to ascertain if the observed very large single 3D marbling flecks were likely to be due to chance alone or if they were likely to represent the true structure of marbling in 3D. For each binary striploin, the total number of marbling voxels was used to determine the density of marbling, or in neutral language, the proportion of 'on-voxels'.

A random striploin was constructed by randomly assigning voxels as 'on' or 'off' to match the proportion of on-voxels in the true striploin. The number and size distribution of the connected components in the random striploin were recorded as well as the proportion of the total number of on-voxels occupied by the largest component. This was repeated 10 times (10 different random striploins) for every one of the 19 true striploins.

For 26-connectedness, if a 3D binary array has a density of on-voxels of only 0.1, then since an on-voxel is connected to 26 neighbours, the probability of having at least one on-voxel as a neighbour is $1 - (.9)^{26} \approx 0.94$. Thus, by chance alone, a voxel is quite likely to have at least one 26-connected neighbour and so large connected components are quite likely. Indeed, the simulations showed that finding a single 3D fleck the size of the ones observed in the true striploins by chance alone was quite high.

However, for 6-connectedness and the same density of on-voxels, the chance of finding a single large 3D fleck of the size observed in the true striploins was very small. Using the measured densities of the true striploins to construct random striploins showed that single large 6-connected flecks found in the true striploins could not be explained by chance alone ($p < .001$).

A mathematical note: The problem of finding a formula for the most likely size of the largest single fleck given a certain density of on-voxels is a variation of an unsolved problem in mathematics. The numerical estimates of the largest connected components found by the simulation above are easy and reliable.

Scientific conclusions

Marbling flecks are a fraud. The simulations suggest that the very large single 3D flecks are not the result of chance due to the overall density of marbling. This suggests that most of the marbling in a striploin is concentrated in a single large structure. Since slicing at 4 mm spacing easily results in artificially disconnecting some marbling and since many of the isolated flecks appear to form chains extending from the main large fleck (Figure 1), a reasonable conjecture is that the marbling in a striploin consists of a single 3D marbling structure.

The next steps

The conjecture that marbling forms a single structure should be investigated experimentally. Future work to explain the formation of this structure may well illuminate important aspects of fat metabolism.

Acknowledgements

The authors would like to thank the Cooperative Research Centre for Cattle and Beef Quality and the Department of Natural Resources and Environment of Victoria for their support.

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Image and model based virtual cutting of lamb carcasses

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Value for industry

- To maximise the value of meat from every carcass and reward farmer suppliers appropriately requires tools to calculate the yield and value of a basket of retail cuts required by the marketplace.
- These tools will help decision making when it comes to evaluating the optimal cut mix for each processed carcass, based on the criteria of the composition (weight of meat, fat and bone) of the cut.
- As cut specifications vary between countries and over time, flexibility is required so that the virtual cutting of carcasses is as close to reality as possible.
- In order to refine the accuracy of such tools, predictive equations linking the yield of each cut with the characteristics of the carcass (grade, age, and dimension) were developed.

Background

With an annual turnover of €1.2b, Silver Fern Farms (SFF) processes 30% of all cattle, sheep, lambs & deer in New Zealand (Buchanan 2012). Silver Fern Farms uses a number of objective measurement technologies in its production lines. In 2010, Silver Fern Farms deployed the world's first X-ray analysis system for lamb, now operational across all of the company's sheep and lamb plants. The X-ray data is being used for primal weight predictions on all sites and to make cutting decisions (Buchanan 2012). The X-Ray system is being further developed with DEXA hardware for on-line meat/fat/bone analysis.

In 2013, SFF and Auckland Bioengineering Institute (ABI) started a collaboration project in which the major lamb carcass muscles and bones were described mathematically into a 3D computer model (Ho *et al.* 2013).

The model made use of a finite element cubic Hermite mesh which was superimposed onto a 3D CT image. The mesh can then be morphed to adapt to different morphologies of carcasses, thus allowing flexible use of the parametric mesh. Since then progress has been made in various aspects of carcass modelling and database construction. Recently, the project reached the completion of its third milestone, whose main objectives were to develop virtual cut software and to investigate the yield of primal/retail cuts through statistical analysis. The aim of this paper is to briefly introduce the methods used in the virtual cut software, and to highlight some unique features of this software.

The methods used

A. Musculoskeletal Model

We have been using a 3D musculo-skeleton model for the lamb carcass from an internal project (Bodley 2000). The model contains cubic Hermite surface mesh for the full skeleton and major muscles of a lamb carcass (Figure 1).

B. CT Imaging

CT scanning was performed for fifteen lamb carcasses at AgResearch, Invermay, New Zealand. The spatial resolution of the CT scans was 0.765mm x 0.765mm x 5mm (width x height x depth). Amongst these carcasses, ten carcasses were scanned with the neck string-on, and the other five were scanned with the neck string-off.

C. Model – CT Image Connection

In order to obtain the muscle/fat/bone ratio of a specific meat cut, a connection between the 3D model and CT images needs to be established. This is realised by integrating the primal cut locations (available from the online X-ray system) and the relative volume portion of each primal/retail cut from the 3D model. The virtual cut is then mapped onto the CT images of lamb carcasses of different lengths, weights and ages.

D. Graphics Engine

The graphics engine underpinning the 3D meshing and rendering techniques is OpenCMISS-Zinc/CMGUI, an advanced graphics package based on two decades of research by ABI. The graphics techniques were previously used primarily for medical and physiological applications (e.g., Bradley et al. 1997). Lamb carcass modelling is the first application of this graphics engine in the meat industry.

E. Compositional analysis

The value of a retail cut depends on multiple factors including the muscle/fat ratio and the intramuscular fat. These criteria need to be evaluated carefully and the data used for analysis such as the muscle/bone/fat ratio need to be applicable to the farmers for management decisions, as well as relevant to the market and consumer preferences for lamb cuts. Implementation of the compositional analysis tool consists of several components. Firstly, the densities of muscle, bone and fat are collected based on literature studies as well as the abattoir measurements. Secondly, after identifying the intensity range of the muscle/bone/fat in CT images, we were able to visualise the muscle/fat/bone proportions (Figure 2). Note that a 3D parametric mesh was also constructed for the carcass, as shown in Figure 2. This technique is indeed the major difference between our approach and that of other research groups. Thirdly, a 3D masking algorithm was used where all voxels inside a bounding box are marked as 1, the voxels outside as 0. This allows accurate compositional analysis for arbitrary retail cuts.

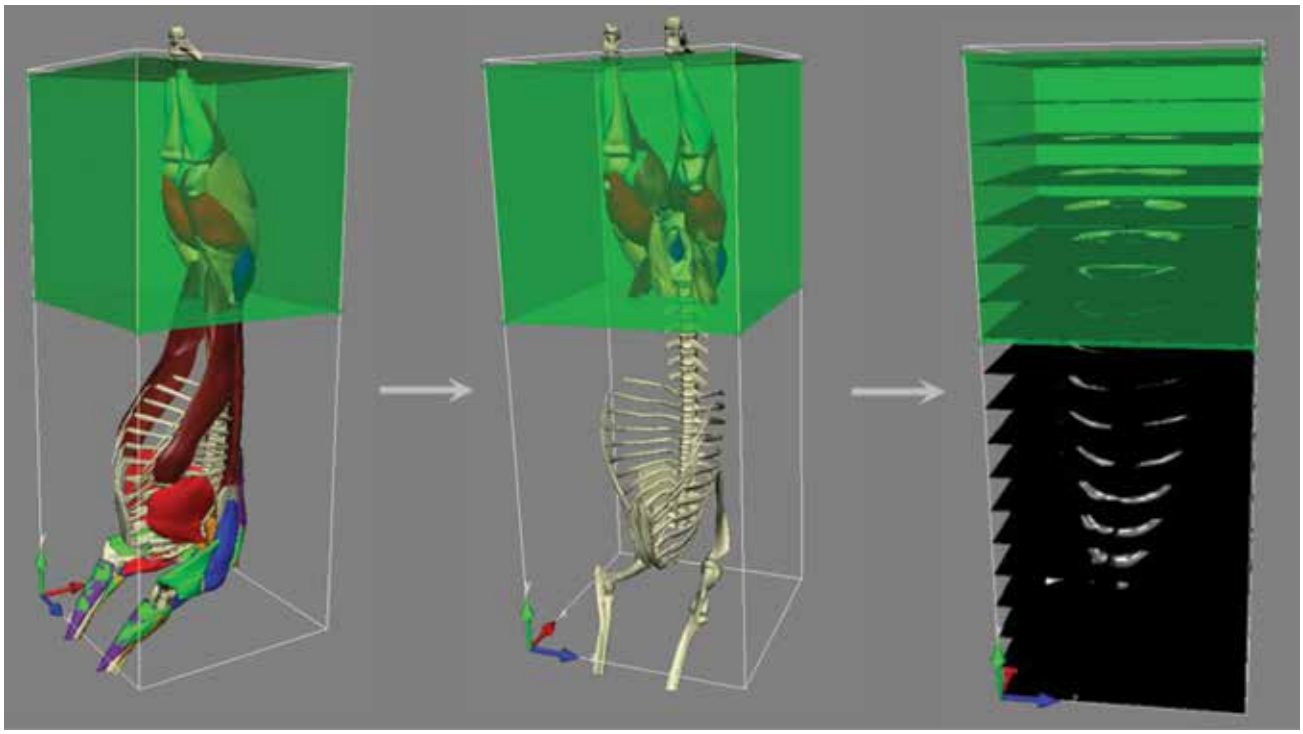

Figure 1. Virtual resection planning on the musculo-skeleton model. The virtual cutting scheme is mapped onto a 3D CT image.

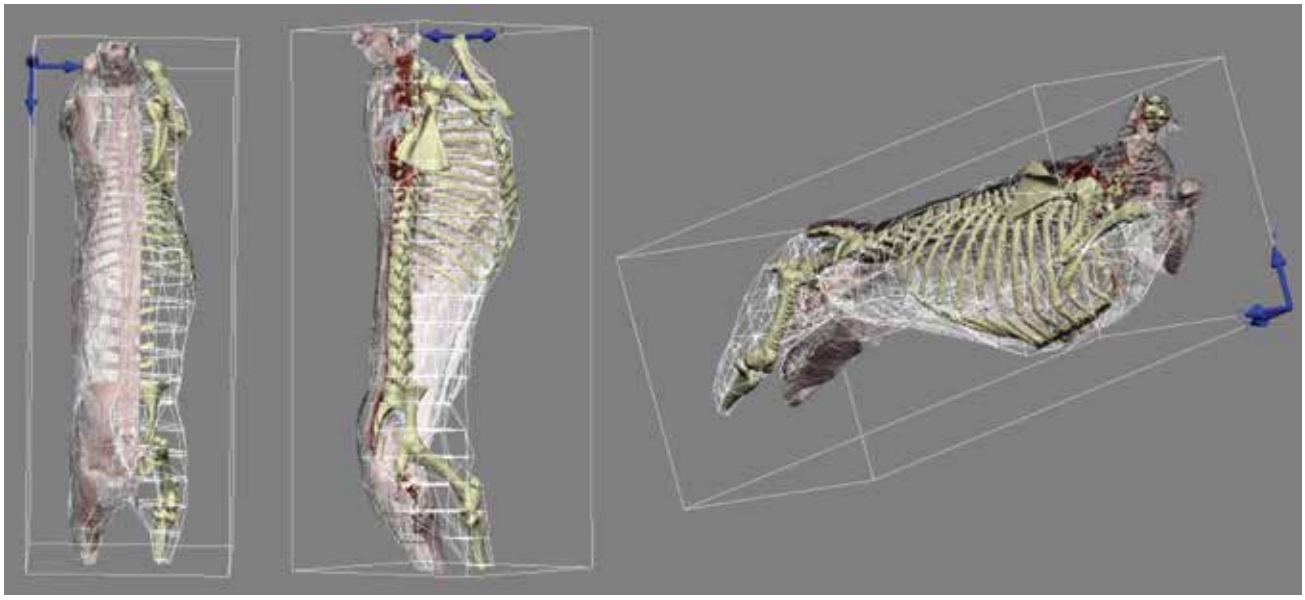

Figure 2. Compositional analysis: the muscle/bone/fat are visualised for the carcass. A parametric mesh (white lines) for the carcass was constructed which can be used for morphing.

The results obtained

We applied the above methods to the compositional analysis of 15 carcasses whose CT images were available. The yield predictions of primal cuts were highly accurate (adjusted R^2 up to 0.95) when compared to objective measurements as well as the X-ray scanning system. The yield of retail cuts was also validated through a comparison with statistical analysis of retail cut yields of 1,000 carcasses.

Figure 3 illustrates the pipeline for the analysis of a typical retail cut, the French rack:

- The 3D model and CT images are super-imposed where the model is used for cut planning, and the CT image for compositional analysis;
- The planner drafts/designs the dimension and the boundary of a specific primal/retail cut (in this case a French rack);
- The software computes the muscle/bone/fat ratio of that cut.

Currently we are in the phase to connect individual components in this pipeline seamlessly, and to allow its easy use by planners.

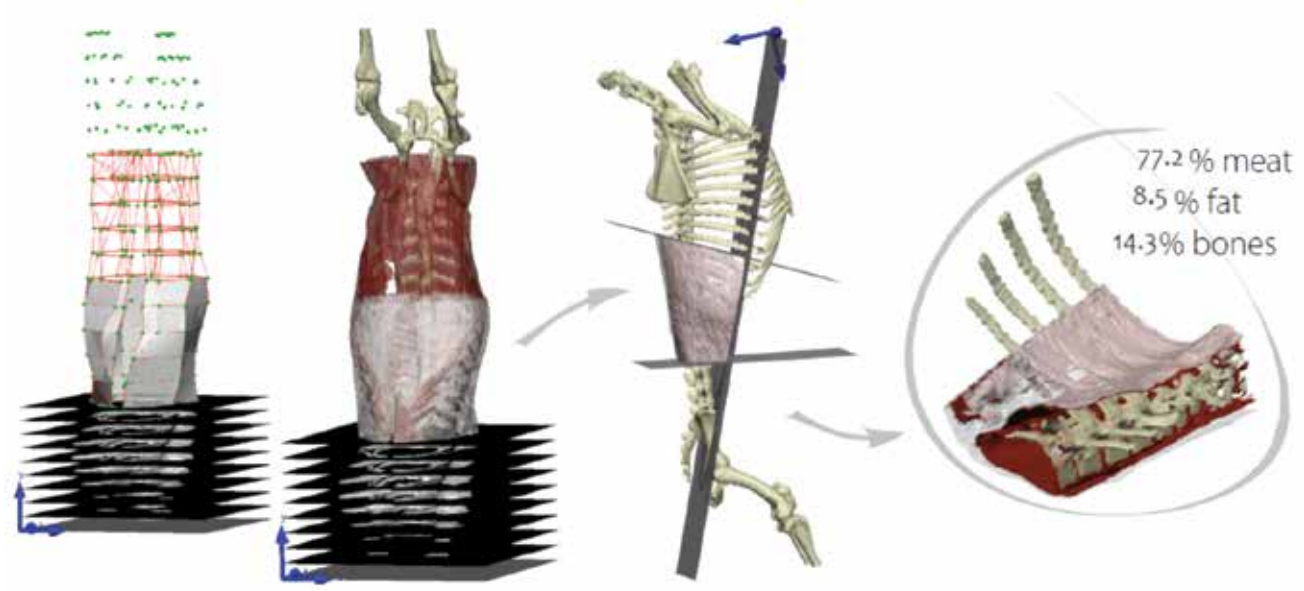

Figure 3. Illustration of the compositional analysis of a retail cut: the 3D model/CT image.

Conclusion & Future Work

Mathematical models of lamb carcasses and their major tissues have many potential applications. An example of compositional analysis for retail cuts has been shown in above sections. The next step in this project will be to develop real-time predictive cut yield equations that draw on objective measurement data (eg. weight, X-Ray, DEXA), live animal data (eg. breed, age, sex) and knowledge of lamb growth.

Acknowledgements

We thank Dr Richard Christie, Mr. Alan Wu and Mr. Hugh Sorby of ABI for their support in the OpenCMISS-Zinc/CMGUI software.

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New segmentation method for CT based selection program in rabbits

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Value for industry

- Computed tomography based *in vivo* examinations provide useful information for rabbit selection programs to increase the premium cuts in carcasses.
- Automatic segmentation process leads to fast and accurate estimations of the valuable meat cuts.

Background

The non-invasive methods that are suitable to evaluate the carcass traits and meat yields of living animals have opened new possibilities in animal breeding. One of these methods is the application of computed tomography (CT). The Institute of Diagnostic Imaging and Radiation Oncology started operation at Kaposvár University in 1990. Between 1992 and 2003 CT aided selection was practiced, improving the cross-sectional muscle area of the *musculus longissimus dorsi* (L-value) in rabbits (Szendrő *et al.* 1992). Since 2004, the volume of the thigh muscle (VTM) has replaced the L-value as the selection criterion in rabbits (Szendrő *et al*. 2012).

Why work is needed

The VTM was estimated in rabbits using CT scanning. Depending on the length of the hind legs, 11-12 CT scans were taken every 10 mm between the *crista iliaca* of the *os ilium* and the *patella*. A simple, thresholding based segmentation was used to select the voxels having densities in the range of the muscle tissue (between 20 and 140 of the HU scale) in each scan. VTM was estimated as the overall volume of the selected voxels (in 11–12 scans). However, this method has some major segmentation faults caused by the muscle like densities in the intestine area, genital area and the significant partial volume effect (Nguyen *et al*. 2012). Thus there is a need for better methods to estimate muscle volumes in rabbits more accurately.

The methods used

The aim of the method which has been developed is to estimate the muscle volume of the hind leg parts automatically and accurately.

The advances in CT technology enable the reconstruction of thin (2mm) and overlapping slices. From the detailed CT image, the muscle region is extracted by thresholding, morphological operations and utilizing the anatomical features of the skeleton in that region.

Automated segmentation methods are applied to the image. The CT scanning technology allows the simultaneous examination of 3 animals which are placed in separate troughs within the scanner. The initial preprocessing of the images involves the separation of the 3 individuals examined in one process and the removal of the trough in which the animals are positioned (Kovács *et al.* 2013). During the automated segmentation process, the body of the rabbit is assumed to lie along the axial direction of the CT scan.

The first step of the segmentation is the extraction of the skeleton by applying hysteresis thresholding with thresholds 150 and 100 on the Hounsfield unit (HU) scale (see Figure 1(a) for illustration).

The next step is the identification of the region of interest (ROI) when we determine the axial range where the valuable meat can be found. From the skeleton, the ribs and spine processes are removed by applying morphological erosion in the axial direction. The junction point of the backbone and the pelvis is identified by tracing the axial slices of the backbone towards the pelvis until the slice of the backbone becomes 1.5 times larger than that of the previous slice.

When the junction point is found, the backbone and the other bones of the upper body reion can be easily removed, thus, the pelvis and the bones of the legs are extracted. One border of the axial range of the ROI is defined as the first slice where the *crista iliaca* of the iliac-bones appears. The other border of the ROI is identified in a later step of processing.

In the axial range of the pelvis bone several industrial constraints define the region where the valuable meat resides. These constraints are implemented by building a mask and ignoring the voxels covered by it when the total volume of the meat voxels is determined. First, the axial slice of the pelvis with the least bone voxels is determined. Let the axial index of this slice denoted by p. Then, the iliac-bones are elongated downwards and towards slice p. The region enclosed by the elongated iliac-bones and the convex hull of the pelvis, backbone and tail bones are added to the mask (see Figure 1(b) for the mask covering the region that is excluded from further processing).

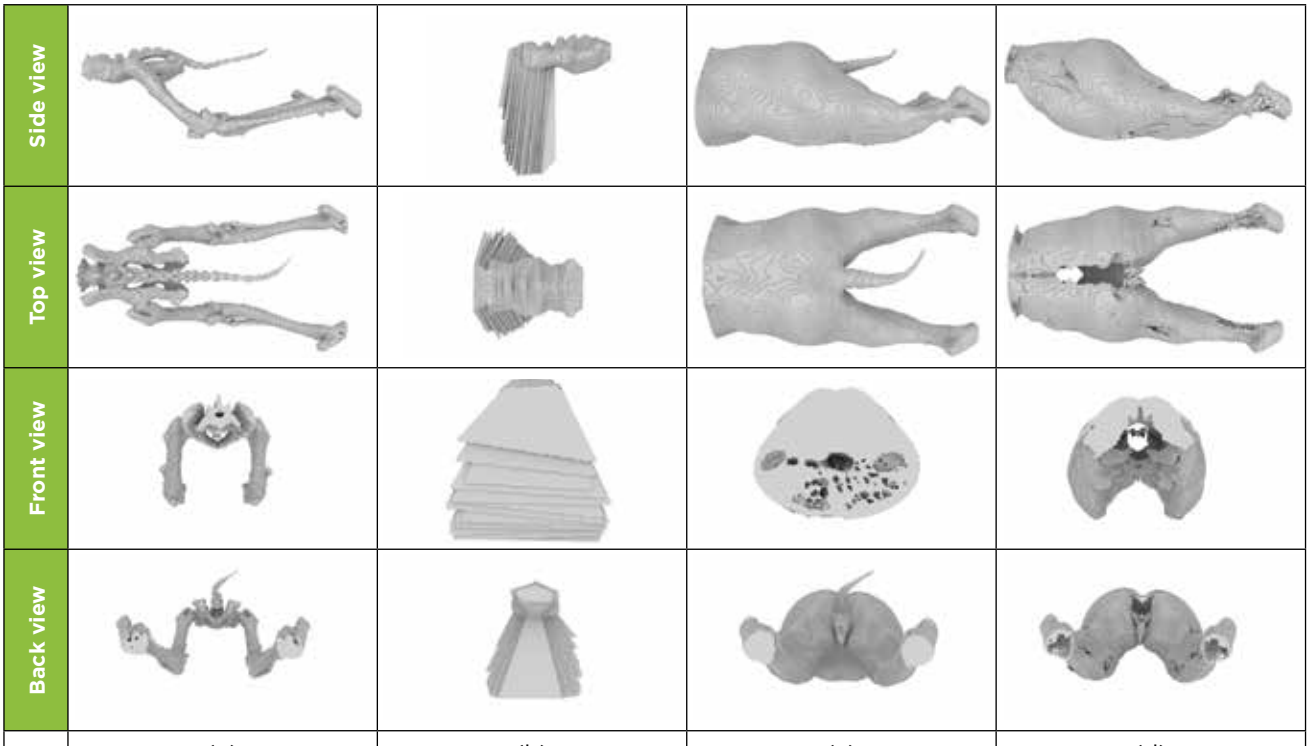
The next step is a rough segmentation of soft tissues by applying simple thresholding with a threshold level of -200 in the HU scale. The result of this rough segmentation has several deficiencies, e.g. it includes many tissues in the abdominal region (see the front view in Figure 1(c)).

In order to remove these artefacts, slice-by-slice processing is coupled with morphological operations. An arbitrary axial slice of the rough segmentation intersecting only the legs of the animal is selected as an etalon. On the next slice in the caudo-cranial direction of the animal the following operation is carried out. A voxel is accepted as muscle voxel, if and only if there is a muscle voxel in its neighbourhood on the etalon slice. This operation is repeated for each slice in the caudo-cranial direction, removing the waste majority of the muscle like tissues that are not connected smoothly to the muscles of the leg. The very same operations are performed in the opposite direction, starting from slice p.

Now, having an estimation of muscle voxels, the other border of the ROI is defined as the axial slice below the knee of the animal with the least muscle voxels.

The final segmentation is obtained by removing the skeleton from the segmentation and excluding the voxels covered by the mask. The segmentation provides a fairly good indicator of valuable meat voxels in the region of interest (see Figure 1(d) for illustration).

Figure 1: Various views from the steps of the segmentation process. The skeleton of the animal (a); the region excluded by industrial constraints (b); the muscle voxels resulting from simple thresholding (c); the final segmentation (d).



The proposed method was tested and compared to simple thresholding based technique on 152 Pannon white hybrid rabbits. The living rabbits were scanned by a Siemens Somatom Sensation Cardiac CT located at Kaposvár University (Kaposvár, Hungary). After the CT scanning, the rabbits were slaughtered, dissected and the valuable parts of the carcasses were weighed. The total hind leg muscle weight (hind leg fillets) and the results of the volumetric CT segmentations were compared directly. A linear regression model was used and the error of prediction with leave-one-out cross-validation ($RMSEP_{CV}$) was calculated by SAS software.

The results obtained

The average weight of the hind leg fillet was 405 ± 41.3g. The average volume of the thigh part of the muscles determined by the previously used simple thresholding technique became 361 ± 44 cm^3. We have also determined the mean volume of the hind leg muscles with the thresholding based technique (415 ± 50 cm^3) and using the proposed method (403 ± 37 cm^3). The proposed segmentation method obtained better prediction parameters than the simple thresholding based technique using either the thigh muscle volume or the whole hind muscle volume (Table 1).

Segmentation method	N	Segmented muscles	Slice thickness (mm)	Correlation (r)	Coefficient of determination (R^2)	RMSEPcv (g)
Thresholding	152	thighs	10	0.83	0.70	23.03
Thresholding	152	hind legs	10	0.84	0.71	22.40
Proposed	152	hind legs	2	0.93	0.86	15.83

Table 1. Evaluation of segmentation methods in the prediction of hind leg fillet's weight from segmented muscle volumes.

The scientific conclusions

Based on the experimental results, the proposed segmentation method can increase the precision of selection and speed up the breeding process.

The next steps

The current method determines the volume of the muscles with acceptable accuracy and clearly outperforms the previous, thresholding based techniques. However, the weight of the valuable meat is determined as the product of the total muscle volume and the mean muscle density. In fact, different muscle voxels have different HU values and the variance of densities can clearly affect the estimation of weight. In order to overcome this issue and increase the accuracy of estimation, we would like to apply some fuzzy approaches to the estimation of total muscle weight by fitting a proper probability distribution to the densities covered by the muscle volume and weighting the densities with the value of the probability density function in order to consider voxels with very high or low densities with lower weights.

Acknowledgements

This study was financially supported by the TECH_08_A3/2-2008-0384 (OM-00198/2008), GOP-1.3.1. (Innovative technological development at Olivia Ltd), GOP-1.1.1-11-2012-0132 and AGR_PIAC_13-1-2013-0031 projects.

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Tissue quantification using expectation-maximization algorithms

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Value for industry

- Knowledge of carcass composition is important for the different stakeholders of the meat industry because it allows an optimization of the production process, and is related to price.
- The intramuscular fat (IMF) content in pork impacts the sensory quality and, consequently, the sensory acceptability of the pork by consumers. Determination of IMF can help the meat industry direct its production to satisfy market requirements.
- Computer tomography (CT) scanning can be used to classify carcasses and measure IMF.
- Expectation-maximization algorithms can be used to determine image features from the tissue images without requiring any predefined threshold values making this a robust approach.

Background

The classification of computer tomography (CT) image voxels into tissues according to Hounsfield Unit (HU) value is a complex process. The main difficulties are due to variability associated with the scanner parameters and protocols, animal variability and the partial volume effect, that appears when a voxel contains more than one tissue. Most of the classification methods in the literature are based on thresholding techniques that consider only histogram data (Dobrowolski *et al.* 2004; Johansen *et al.* 2007; Romvári *et al*. 2006) or also voxel neighbourhood information (Lyckegaard *et al*. 2006; Vester-Christensen *et al.* 2009). These methods assume fixed thresholds and therefore do not tackle the variability problem. Despite the advances in this area, there is still no reference model that determines the HU-tissue correspondence and each country has defined its own model (Daumas and Monziols 2011; Font-i-Furnols *et al.* 2009; Romvári *et al.* 2006). Therefore, the definition of an adaptive and accurate method capable of dealing with scanner variability is of great interest.

Expectation-maximization (EM) algorithms are a computational tool which can be used to tackle the classification task. These types of algorithms are based on iterative methods for finding maximum likelihood estimates of parameters in statistical models, where the model depends on unobserved latent variables. The EM iteration is composed of two steps: the expectation (E) step, which creates a function for the expectation of the log-likelihood, evaluated using the current estimate for the parameters; and the maximization (M) step, which computes parameters maximizing the expected log-likelihood found on the E step. The obtained parameters are then used as input for the next EM iteration. A typical problem that can be solved using an EM algorithm is the estimation of the parameters of a Gaussian mixture, i.e. samples that have been generated from two or more Gaussian distributions. The present work has three objectives: (1) provide robust algorithms to quantify pork tissues, (2) analyze the robustness of these algorithms on image variability, and (3) determine errors of estimation of carcass lean meat content and loin IMF content.

Why work is needed

Determination of carcass lean meat content is crucial in the pig meat industry because the ability to measure it accurately would allow a fair payment to producers and an optimization of the production process. Moreover, IMF is a quality parameter of meat that influences palatability. Consequently, the pork industry needs a tool to quantify and classify carcasses and meat with high accuracy so it can effectively measure and market the value proposition of each pig.

The methods used

In this work, we have focused on two different problems: carcass classification and IMF quantification.

Carcass classification

For the tests, 10 half carcasses were scanned with a helical General Electric scanner located in the IRTA-CENTA centre (Monells, Spain). Acquisition parameters were a voltage of 140 kV, and intensity of 145 mA, displayed field of view (DFOV) from 460 mm to 500 mm depending on the width of the carcasses. Image thickness was 10 mm and images were taken helically every 10 mm (pitch 1). Matrix size was 512 x 512 pixels. After the scanning, carcasses were cut following the Walstra and Merkus (1995) procedure and dissected.

The processing pipeline of the method has been presented in detail in Bardera *et al. (*2014). Here, we focus on the tissue classification part. In our case, we assume that each tissue (fat, lean, and bone) follows a Gaussian distribution and, thus, the image histogram can be seen as a mixture of three Gaussian distributions. One of the main acquisition limitations is the voxel size that does not guarantee that each voxel contains a single tissue but a mixture of two or more. This image artifact has been extensively studied in the image processing field, especially for medical applications, and it is known as the partial volume effect. Van Leemput *et al.* (2003) proposed an expectation-maximization approach to simultaneously estimate the parameters of the resulting model and perform a partial volume classification applied to brain tissue classification on Magnetic Resonance (MR) imaging (Leemput *et al.* 2003). In this model, a non-observed image Y with a high resolution is composed of voxels that only contain pure tissues. Then, the real observed image Y is obtained by downscaling with a factor M this high-resolution image, which leads to the creation of voxels with the partial volume effect, but only on a limited set of possible mixtures ($M^2 - 1$) such that each of them will correspond with a Gaussian distribution. This simplification leads to an analytical formulation for the computation of the model statistics (i.e., mean and variance of each tissue and quantity of each pure and mixture classes) (Leemput *et al.* 2003). This method will lead to a final histogram model that consists of a mixture of three Gaussian distributions corresponding to the pure tissues and $(M^2 - 1)(\mathrm{k} - 1)$ Gaussian distributions corresponding to the tissue mixtures.

In our approach, we cannot directly use the original method, due to the low relative frequency of the bone tissue voxels and the large spread of their corresponding intensity values. For that reason, we assume that the bone structure corresponds to a tissue that has a Gaussian distribution with mean value 180 HUs and a standard deviation of 10 HUs. These values are not modified during the maximization step of the algorithm, but are used to model the mixture tissue of lean and bone.

Intramuscular fat quantification

A total of 363 images from commercial pigs were used for this work. A tomogram at the level of the 3rd–4th last rib was obtained from each loin using General Electric HiSpeed Zx/i computed tomography equipment at IRTA-CENTA in Monells, Spain. This tomogram was taken in a 512×512 matrix and with a using displayed field of view of 250 mm using the following different scanning procedure: Helical, 120 kV, 200 mA, rotation time 1.5 s, 1 mm thickness, and a reconstruction algorithm “Edge”.

After scanning, the *m. longissimus thoracis* was removed, and a piece from the 3rd–4th last rib region was frozen at -20°C until being used for the IMF determination. For IMF determination, loins were thawed (4°C for 24h), grounded and analyzed using the Infratec 1265 Meat Analyzer (Foss Tecator AB, Hoganas, Sweden), based on near infrared technology.

In order to quantify the fat content from the image histogram, we assume that it is composed of two tissues (fat and lean) and the corresponding mixture tissues. As in the carcass quantification algorithm, the mixture tissues model is based on the Van Leemput proposal. Here, the difficulty is that the pure fat tissue is not present in the image histogram. Thus, the EM algorithm is not able to estimate the corresponding Gaussian distribution parameters (mean and standard deviation) from the data. For that reason, we assume a “virtual” fat tissue, of which the mean and standard deviation are input parameters of the algorithm and are kept fixed in the EM algorithm. These Gaussian parameters could be estimated from other images that contain pure fat tissue or could be defined by default values. For the test presented in this paper, we have used a mean value of -65 HUs and a standard deviation of 10 HUs. From these parameters, the features of the mixture tissues can be computed following the original Van Leemput proposal.

The results obtained

We have applied different algorithms for carcass segmentation and lean-meat percentage estimation, and for IMF estimation. In the first case, we assume that the histogram is composed of three tissues (fat, lean, and bone). The second method focuses on the fat and lean HU ranges (between -120 HU and 120 HU) and only considers these tissues, with a constant mean and standard deviation for the fat tissue.

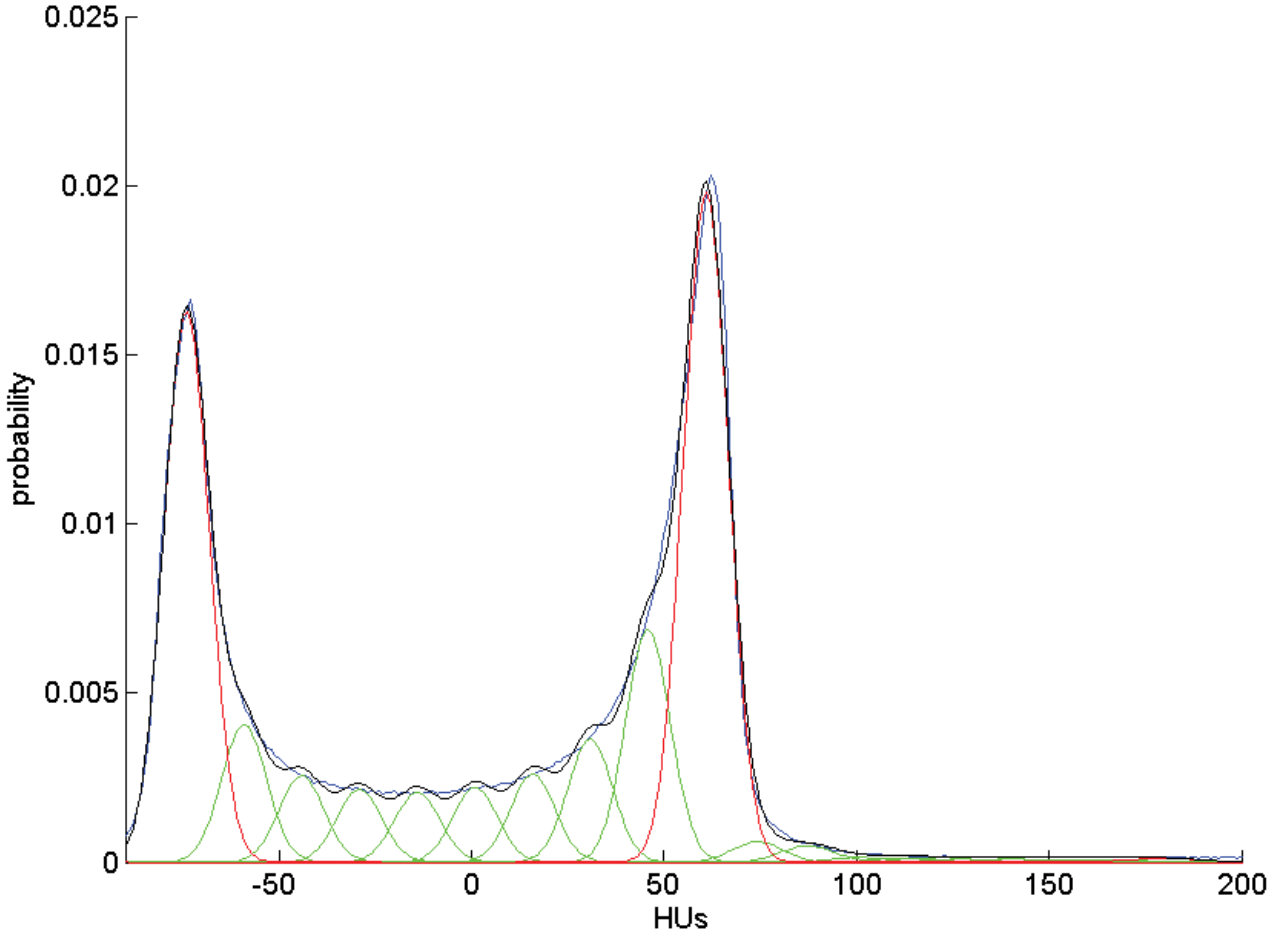

Figure 1. Normalized histogram of a pork carcass (blue), and the estimated distribution (black) with the pure tissue distributions (red), and partial volume effect distributions (green).

Figure 1 shows the normalized histogram of a pork carcass (in blue) and the corresponding pure tissues classes (in red) and partial volume tissue models (in green). Note that the histogram has two Gaussian-like peaks corresponding to the pure tissue voxels (centered in -70 and 60 HUs for the fat and lean classes, respectively) and a great plateau between them corresponding to mixture voxels. Values greater than 100 HUs correspond to the mineralized bone, but there is not a clear Gaussian-like peak.

Figure 2 (over page) shows the results of the fixed thresholding method (top row) and the expectation-maximization algorithm (bottom row) compared with the manual dissection which is considered the reference model. Columns represent the different tissues (fat, lean, and bone). Each scatter plot has the manual dissection in abscissa and the automated method as ordinate. The R^2 values of the linear regression are also given.

As it can be seen, the proposed method obtains better results than the thresholding-based techniques for the fat and lean tissues, while the results show a worse performance with the bone classification. In addition to these results, Bardera *et al*. 2014) show that this method is more robust with respect to global changes in the values of the image. The next experiments deal with the IMF estimation. Figure 3 shows the real normalized histogram of the image (in blue) and the corresponding estimated Gaussian function of pure lean tissue (in red) and partial volume tissue models (in green). The sum of the Gaussian functions corresponds to the normalized histogram of the model, which is plot in black. In addition to the estimated Gaussian functions, a dotted line is plot to show the "virtual" fat tissue mean and standard deviation, which have been used in the estimation. As it can be seen the fitting between the real histogram and the model is very close.

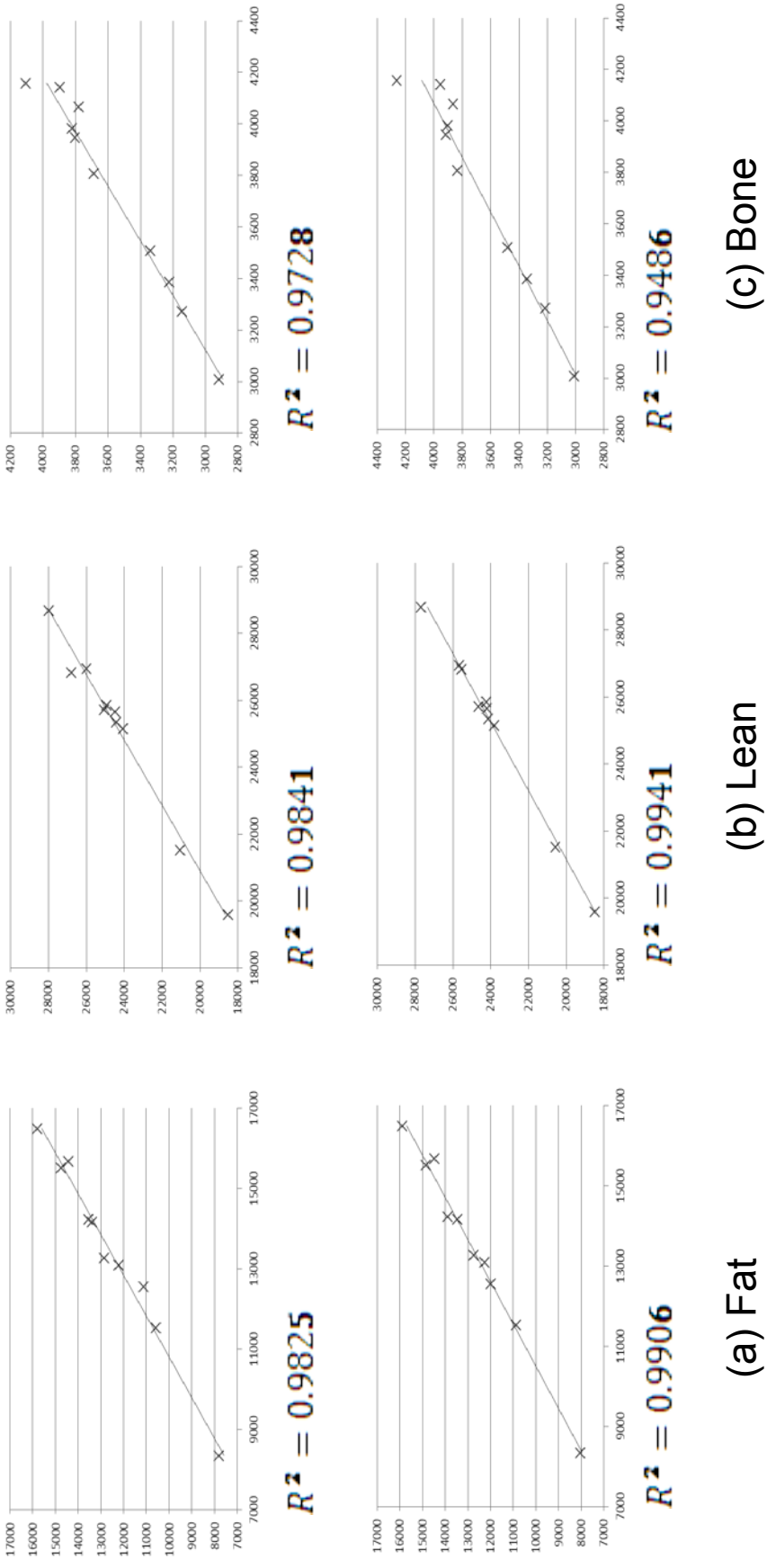

Figure 2. Estimated carcass fat (a), lean (b) and bone content (c) (Y-axis) after apply fixed thresholding method (top row) and the expectation-maximization algorithm (bottom row) versus dissected contents (X-axis).

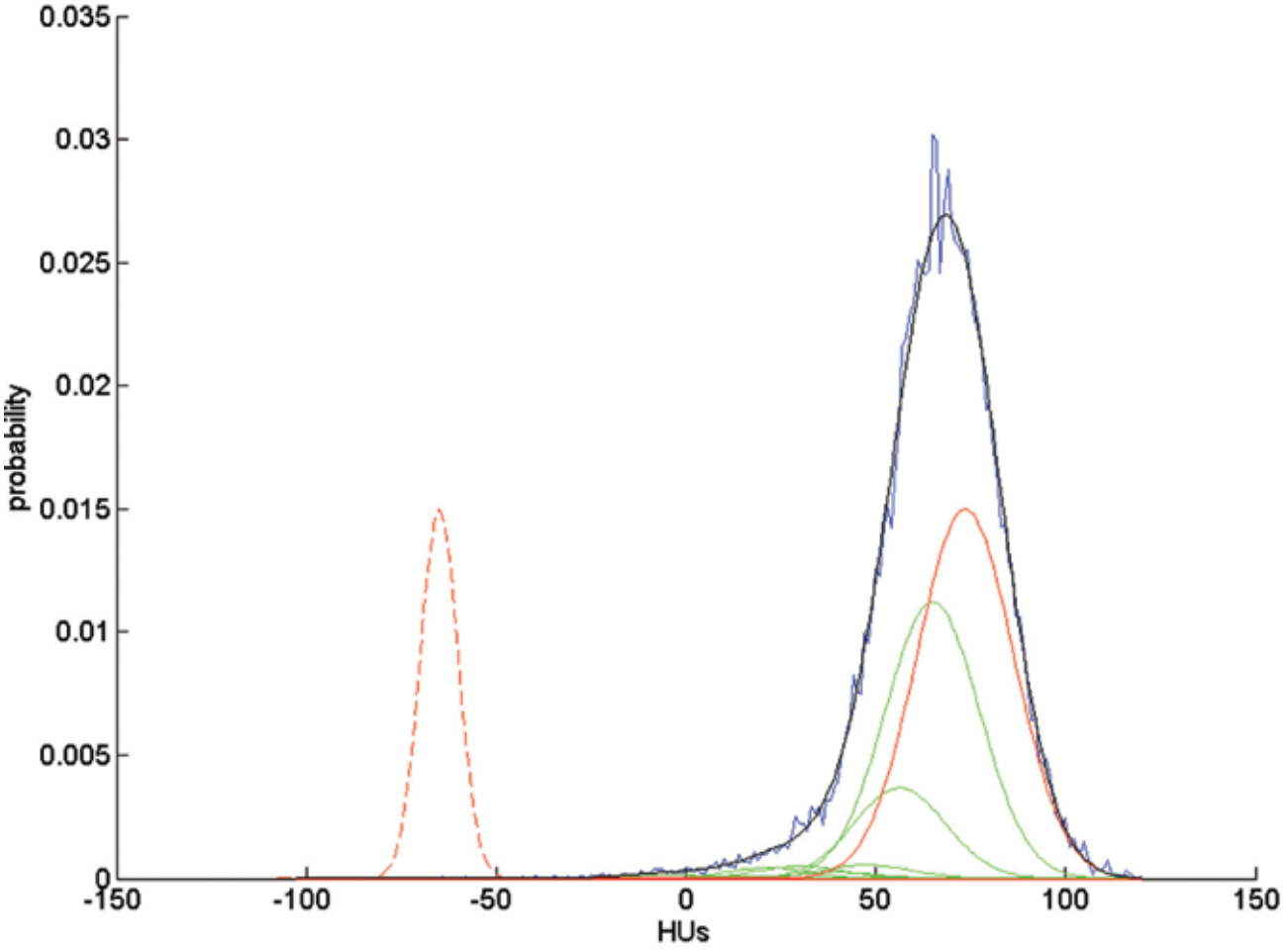

Figure 3. Normalized histogram of a loin (blue), and the estimated distribution (black) with the pure lean distribution (red), and partial volume effect distributions (green). The discontinuous red line represents a Gaussian distribution with the mean and standard deviation assumed for the fat tissue.

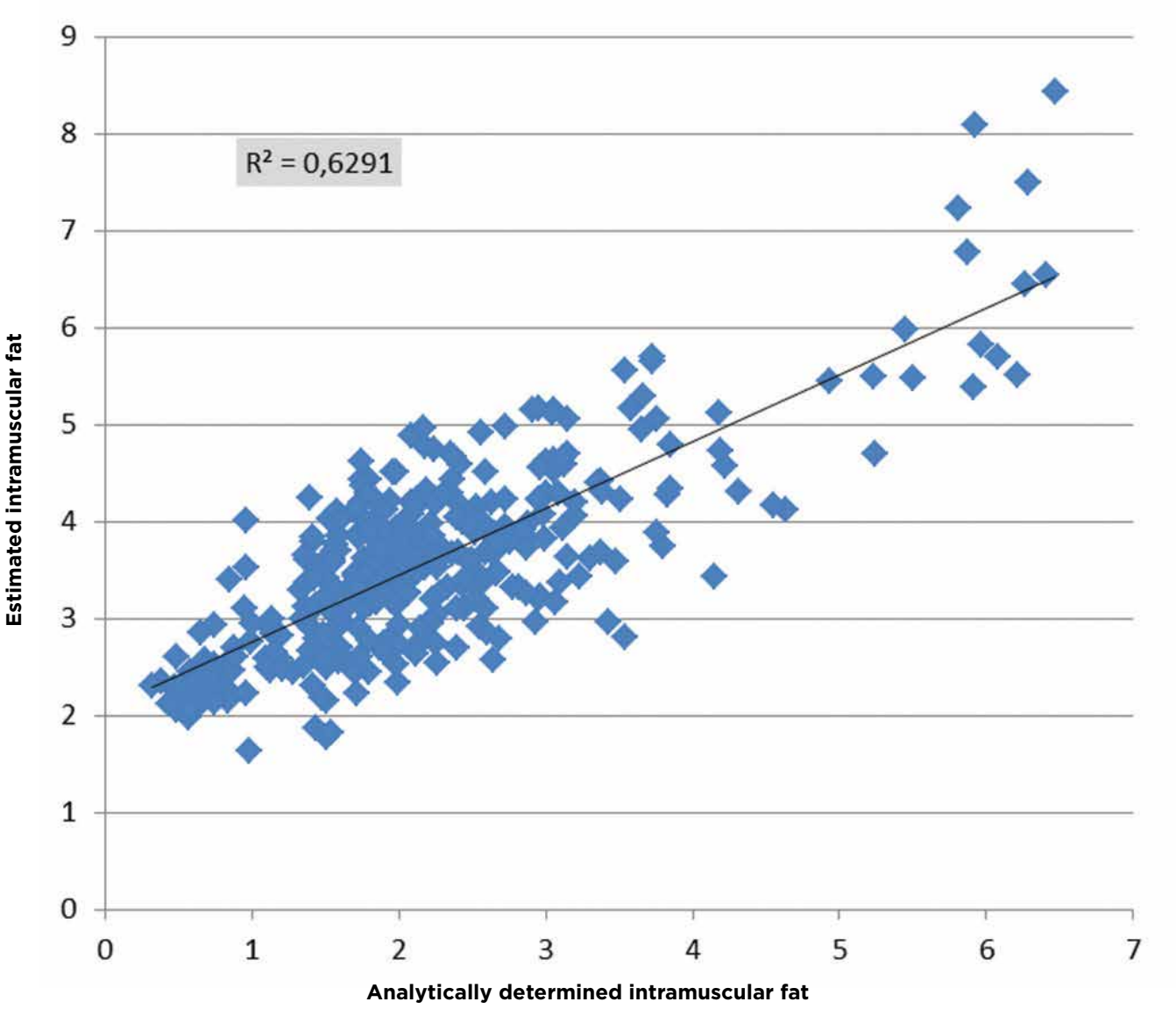

Figure 4. Intramuscular fat estimated (Y-axis) *versus* analytically determined (X-axis).

Figure 4 shows the correlation with the IMF estimated with the proposed method and the IMF measurements obtained analytically. Note that the R^2 value is 0.63, which indicates a moderate correlation. Probably increasing the amount of loins with high levels of IMF would allow a better adjustment of the algorithm and a higher accuracy in the estimation of the IMF content.

The scientific conclusions

CT is a suitable technique for determining carcass lean meat percentage and IMF of the loin. Expectation-maximization algorithms have a great potential in the quantification of total fat, lean and bone from carcasses. Expectation-maximization algorithms can be used in the quantification of intramuscular fat in loins since they deal with the partial volume effect.

The next steps

In carcass quantification, we plan to improve the bone tissue model in order to better describe the real histogram features. In IMF, we will analyze the dependence of the results on the "virtual" fat tissue parameters in more detail. In addition, a more comprehensive evaluation of the proposed methods with a large number of datasets will be undertaken.

Acknowledgements

This work has been funded in part by grants from the Spanish Government (Nr. TIN2013-47276-C6-1-R) and from the Catalan Government (Nr. 2014-SGR-1232). The research was partly supported by INIA through the project INIA-RTA2010-00014-00-00. The authors wish to thank Anna Carabús, Albert Brun, Albert Rossell, and Agustí Quintana for their invaluable assistance.

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Workgroup 4

Ruta Gronskyte

Monitoring motion of pigs

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Value for industry

- Monitoring pigs will improve animal well-being and health.
- Multiple pigs are quickly separated from the background in videos.
- Identification, optical flow and modified angular histogram is an efficient method to track the motion of pigs and their behaviour.
- Any size herds can be monitored collectively using this method.

Background

Analysis of farm animal movement can provide valuable information about animals' health and stress levels. The indicators, such as slower walking speed or irregular walking patterns, can reveal sick or injured animals while the opposite may be an indicator for excessive stress. In this project pigs' movements are of major interest, though the same methodology can be applied to any group of animals that are similar in size, shape and colour, and have a specific walking pattern with a large body.

Why the work is needed

To perform online animal surveillance the identification, motion estimation and analysis of a single video frame have to be computationally fast as the standard number of frames per second is 24, 25 or 30. Identifying individual pigs in a herd is a challenging task due to their body shape and colour similarities. When animals are in a very close proximity to each other it is difficult to identify the borders between them. To complicate matters further, animals usually have a large amount of dirt and markings on their bodies. However, pigs can be separated from the background using an image without animals as a reference.

We also show that analysing a herd of pigs will give a good understanding about animal behaviour. This can be done by estimating the motion occurring in the entire frame and then filtering away the background leaving only the motion of the pigs. For that we propose using optical flow (OF) (Wedel *et al.* 2011), which is widely used for individual human subjects and crowds ((Perko *et al.* 2013), (Kratz *et al.* 2009)), and for the estimation of the movement of a vehicle. This method does not require the identification of individual animals. For each frame in a video, a vector field is estimated by OF. The vectors represent direction and relative speed of movement. Background subtraction identifies vectors that belong to the animals, which was used for the further analysis. Due to the trot exhibited by moving pigs, vectors of the single pigs are pointing in multiple directions and symmetry between the two sides of the body is present. Thus, modified angular histograms (MAH) can be used to analyse the movement of the herd. If the individual animals can be identified, the OF together with MAH can be a useful tool in the evaluation of the individual animal's walking pattern. Such analysis can give a specific indication of injuries.

The method used

In this section a detailed description of the pig identification in a frame and pig movement analysis is described.

Identification of pigs in a frame

Identification of individual pigs is a challenging task as pigs are very similar in colour, shape and may be covered in a lot of dirt. The task becomes even more challenging as pigs can have painted markings of various colours. For this project, the videos were recorded from a stationary location and thus the background remains largely unchanged over time. A background image without pigs was used to isolate the pigs.

Figure 1. Mean background figure.

To eliminate the influence of lighting fixtures at the facility, a mean background frame was used. We used the mean image of 100 empty frames, which is presented in Figure 1. Figure 2 shows that the colour of the floor is similar to that of the dirt found on the pigs.

The RGB colour channels were adjusted to enhance the contrast allowing for a better separation of the floor and dirt. The enhanced frames were converted to HSV colour space, to separate the brightness of the image. The frames were compared per pixel yielding a mask representing the pigs. The mask was noisy at this stage, e.g. small islands of similar pixels. To reduce that noise, median filtering together with small region removal was used. The median filtering replaces the value of each pixel with the median of its neighbouring pixel values. The identified pigs are presented in the Figure 2.

Figure 2. Identified pigs in the image. Red lines represent pigs' edges, green lines holes.

Pigs' Movement Analysis

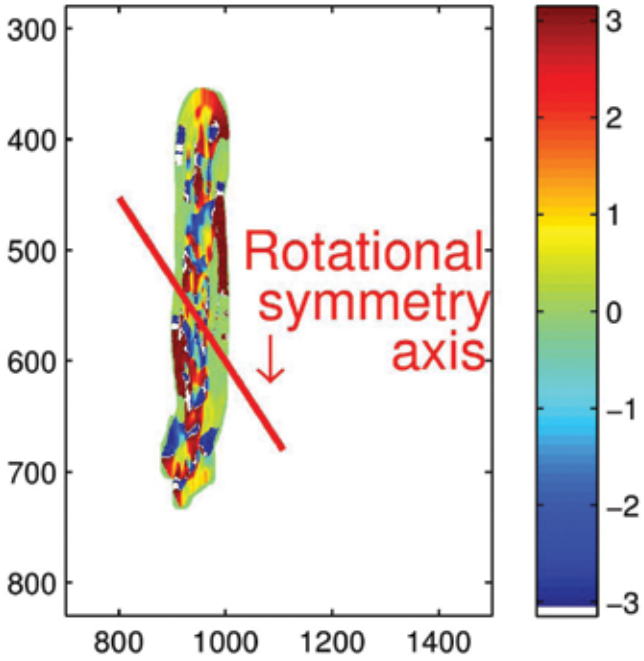

Figure 3. How a pig moves in a symmetry axis.

The movement of pigs was estimated using OF. Due to the trot the OF vectors are pointing to multiple directions as shown in Figure 3. The colour bar indicates the angle range $[-\pi;\pi]$ $[-\pi;\pi]$ in radians – i.e. the whole 360 degrees around the pig. To summarise the length and direction of the vectors we propose to use a MAH. A MAH for each frame was estimated by dividing the angle range into smaller sub ranges/bins.

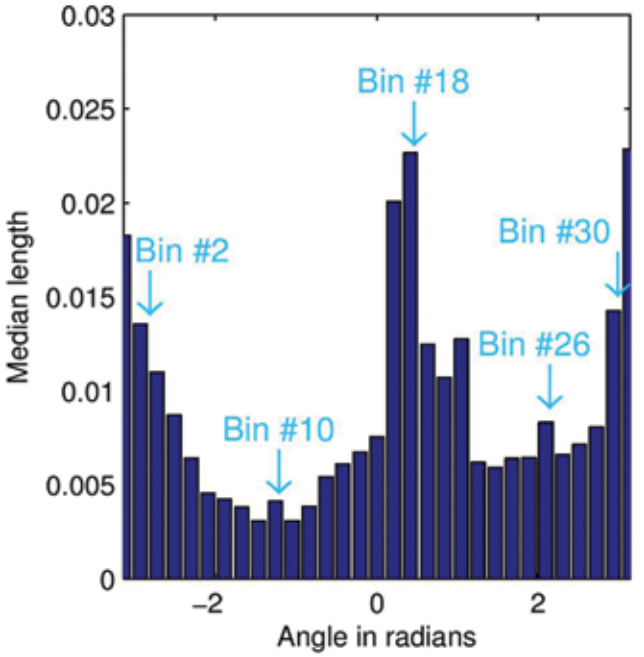

Figure 4. MAH of the pig presented in Figure 3.

In each bin the median vector length was computed. Figure 4 is the MAH of the pig in Figure 3. The MAH has three peaks and two valleys. The peaks represent body movement to the sides due to the trot. The valley at 1.5 radians represents forward movement and the right valley at -1.5 is the opposite direction. The collection of MAH can be used to establish the norms for stressed or/and injury-free herds or individual pigs. Using statistical process control techniques, these norms can be used for on-line animal monitoring.

The scientific conclusions

In this paper, identification of pigs in a single video frame for the purpose of behaviour monitoring is discussed. For monitoring to be on-line the pig identification together with other analysis steps have to be computationally fast, as there are around 30 frames per second to analyse. Due to the similarity in colour and shape, identification of individual pigs in a herd is complicated. Dirt and different markings makes the identification even more complicated. We suggest, identifying all pigs in a frame rather than individually. This was done by background subtraction. The OF was used to estimate motion in entire frame. OF vectors were filtered based on pixels that are identified as pigs. The MAH was used to summarize the OF. The norms of MAH for stress and injury free animals can be established and later used for on-line monitoring. The on-line monitoring can be performed on a single pig if it is the only pig in the frame or collectively on an entire group when multiple pigs are visible in the frame.

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From FAIM II

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Meat Quality – standard methods and new approaches

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Value for industry

- Meat quality can be assessed with a wide range of established analytical methods.
- Most of these chemical or physical methods are used in research and depend on manual sample preparation and time-consuming processes.
- Modern meat production increasingly calls for fast methods allowing the determination of meat quality parameters early in the production chain.
- Traditional analytical methods may still have a central role in basic research, but new rapid methods are applied increasingly in routine applications in the meat industry.

Background

In food production, all the steps in the production process influence product quality. Thus, animal production (e.g. housing conditions, genetics, feeding regime), conditions of transport and slaughtering, and the subsequent handling of carcasses and packaged product all affect meat quality. Quality in general, as defined by the German Society for Quality, is the "sum of all characteristic traits and attributes of a product ... which refer to their ability to fulfill given needs" (Deutsche Gesellschaft für Qualität, DGQ, 1980, translated). Meat quality, in particular, comprises all characteristic traits, i.e. quality factors, relevant for the use of meat as food. These quality factors are properties which are objective and measurable.

According to their relevance for consumption value and nutrition, as well as for hygiene and processing, quality factors of meat can be differentiated into four groups (Table 1).

In summary, meat quality is the sum of all relevant sensory, nutritional, hygienic and processing characteristic traits of the meat (Hofmann, 1973).

However when considering the intended use of the meat, it is clear that not all traits have the same importance. For processing, the water holding capacity and the consistency of the fat (and the corresponding fatty acid pattern) are of greatest interest. In contrast, the most important factors for the consumer are colour, tenderness and the typical taste of the meat.In summary, meat quality is the sum of all relevant sensory, nutritional, hygienic and processing characteristic traits of the meat (Hofmann, 1973).

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Criteria	Quality traits
Sensorics	Colour, tenderness, flavour, taste
Nutrition	Protein, fat, or water content
Hygiene and toxicology	Residues, microorganisms, shelf life
Technology	Water holding capacity, pH, texture

Table 1. Examples of quality traits grouped according to major criteria (Hofmann, 1973).

Why work is needed

For the measurement of quality factors, appropriate chemical, physical, histological, microbial or sensory methods all have to be used. To rate meat according to a special quality factor, target or threshold values have to be defined.

A well-known example is PSE meat (pale colour, soft consistency and exudative tissue), an aberration of quality especially in pork meat. PSE meat can be identified by a pH-value 45 min post mortem that is less than 5.8 in the *Musculus longissimus dorsi* between the 12th and 13th rib. The pH-value characterizes the post mortem glycolysis and thus differentiates between "normal" RFN (reddish-pink, firm, non-exudative), PSE and DFD meat (dark, firm, dry).

However, some meat samples cannot be clearly characterized by pH-value alone. Therefore, drip loss is a necessary additional criterion to assign meat to quality classes. PSE meat has a drip loss >5%, whereas it is <5% in RFN and <2% in DFD meat.

Drip loss is measured for a defined slice of a muscle that is stored at 4°C for 72 h. Drip loss is then expressed as the percent difference between initial and final weight. Thus, drip loss exemplifies that most laboratory methods are very time consuming. Consequently, there are an increasing number of studies that try to estimate quality factors using rapid methods (e.g. spectroscopy).

The methods used

Standard methods measure either physical or chemical traits of meat. Among the physical traits, meat **colour** is of importance for consumer acceptance, but it is also related to its haem content. A standard method to determine meat colour is the L*a*b*system, e.g. using a Minolta CR400 (Konica Minolta Optics Inc., Tokio, Japan). Images are taken on the fresh cut surface at a defined time after slaughter, namely 45 min or 24 h.

Shear force is an inverse measure of tenderness and correlates to meat palatability. It is measured on cooked or grilled meat samples of a defined size. We use a Warner-Bratzler shear blade for 1x1 cm^2 sample slices of 2.5 cm, with the blade running perpendicular in relation to the fibre axis.

The overall **fat content** of pig carcasses as well as the **fatty acid composition** of the adipose tissue are important chemical quality factors, especially for the processing industry. Pork fat is a major component of processed meat products and its composition determines the quality of the final product. Meat processors demand firm pork fat because of its high oxidative stability. Consistency and oxidative stability of fat result from the relative composition of fatty acids. For example, a high proportion of saturated fatty acids (SFA) results in firm fat. In contrast, increased levels of monounsaturated fatty acids (MUFA) or polyunsaturated fatty acids (PUFA) are susceptible to oxidation and rancidity, and the fat has a soft, greasy and oily texture (Wenk *et al.* 1990). On the other hand, MUFA and PUFA are connected with superior health properties (Hugo and Roodt, 2007).

The routine measurement of fatty acids in our laboratory is performed with laborious gas chromatography (GC). Samples are prepared as described in detail by Schulte and Weber (1989). Briefly, backfat samples are homogenized and melted with butylated hydroxytoluol. For transesterification from fatty acids to methyl esters, an aliquot of the lipid fat is mixed with toluene and trimethylsulfonium hydroxide (TMSH). Intramuscular fat (IMF) is extracted with a mixture of methanol and dichlormethane and then transesterified with TMSH. Then, the samples are injected into the GC system.

Total intramuscular fat content is determined by a modified method following §64 in the German code of law for food and animal feed (LFGB data collection, 2013). Fat is extracted with petroleum benzene in a Soxhlet-system without prior HCl-digestion.

The results obtained

Several studies have demonstrated that near infrared (NIR) technology is possibly a suitable tool for rapid estimation of the fatty acid profile in various tissues (Gonzalez-Martin *et al.* 2005; Galian *et al*. 2005; Müller and Scheeder, 2008; Perez-Marin *et al*. 2009).

The aim of our studies was to evaluate if fatty acids can be determined in backfat and muscles with a NIR system suited for rapid online application. We used a system from NIR-Online GmbH (Walldorf/ Bade, Germany) which has a photo diode array. The advantage of this detector is its very fast measurement, within milliseconds, compared to scanning spectrometers with measurement times of several seconds or even minutes.

Samples of backfat and of the *m. longissimus dorsi* for intramuscular fat content (IMF) were obtained from different German crossbreed as well as pure bred pigs (n = 135). The measurements were made by direct application of the spectrometer onto the backfat or the loin without any treatment or manipulation of the sample. Statistical calculations were performed with partial least squares (PLS) regression for complete spectra, or for spectral subsets.

Fatty acid estimation with the applied fast NIR system produced promising results (Bauer *et al.* 2010). For backfat, calibration had coefficients of determination of R^2 = 0.61–0.92, with standard errors of 0.09–1.12%-points. For validation, coefficients of determination were between R^2 = 0.56 and R^2 = 0.89, with standard errors of 0.11–1.20%-points. For IMF, regression results had lower coefficients of determination and higher standard errors, due to a small range of fat content of 0.4-2.8%.

Thus, the estimation of specific fatty acids with the NIR system evaluated is a promising alternative to conventional GC methods. It is a fast method used without destroying the sample tissue and so suited for online application. However, further studies are necessary to optimize prediction results and to develop robust estimation models for practical use.

At the moment, an early determination of meat quality in the production process with acceptable levels of accuracy and precision has eluded researchers. Taking into consideration an increasingly automated processing, this will become of great importance.

In a further research project (Bauer *et al.* 2013; Scheier *et al.* 2013; Schwägele *et al.* 2014), we evaluated the online suitability of different rapid methods to estimate quality parameters in pork. To this end, pH_{45} (45 min post mortem), pH_{24} (24 h post mortem), colour in the L*a*b*-system (24 h post mortem on a fresh cut), drip loss (storage at 4°C for 72 h) and shear force (cooked samples with a Warner-Bratzler shear blade) were determined for field samples of ham (n = 156). The quality parameters and the spectroscopic measurements were taken at the *m semimenbranosous* (SM). The best estimation results for the spectroscopic methods were achieved with Raman spectroscopy. Therefore, we evaluated a hand-held Raman probe described earlier (Schmidt *et al.* 2010) as a fast and non-invasive method to assess meat quality, suited for online application. PLS regression models were developed with the PLS toolbox 6.2 (Eigenvector Research Inc., Wenatchee, WA, USA) based on MATLAB 7.9.0 (R2009b, The Mathworks, Natick, MA, USA).

Based on the analysed reference parameters, we achieved an unambiguous and differentiated sorting of the hams into eight quality classes, ranging from PSE through "normal" RFN to DFD meat. Almost 60% of hams were sorted as "normal", but as many as 35% as "exudative". Spectra measured 24 h post mortem produced promising regression models both for pH_{24} and drip loss, with R^2 = 0.87 and R^2 = 0.82, respectively. Shear force could not be predicted by Raman spectra taken after 24 h, but by spectra taken 1 h post mortem. This PLS model gave the overall best correlation with R^2 = 0.95. In summary, the prediction of relevant quality parameters by Raman spectroscopy provides good potential as a non-invasive method to assess meat quality during the production process.

The next steps

Proteomic studies are a new approach to characterize meat quality. Its central idea is to find biomarkers that can be linked to reference meat quality parameters.

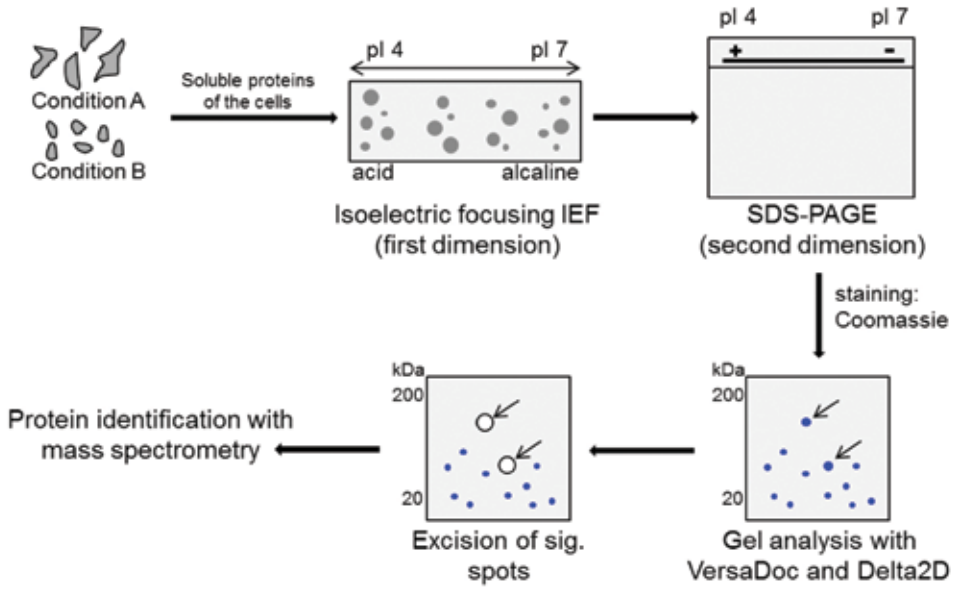
The method we use to determine the protein profiles of meat samples is the two dimensional gel-electrophoresis (Figure 1; scheme modified referring to lecture 10 at https://www.msu.edu/course/mmg/433/lecturesS2005/). This method allows the comparison of two different conditions, e.g. the comparison of RFN and PSE meat. It is crucial, that samples differ only in respect to the one variable of interest. Otherwise, it is not possible to unambiguously link the quality parameter to the protein profile.

After sample preparation, the first dimension is the so-called isoelectric focusing (IEF). In this step, the extracted proteins get separated according to their individual isoelectric point in the pH-range of the gel strip. The gel strips for the IEF have various pH-range, e.g. pH 4–7 or pH 3–11, and are available in different lengths depending on which size the gel of the second dimension should have. In the second dimension, the proteins are separated according to their molecular weight using a sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). After the two separation steps, the gels are stained (in our case with Coomassie Blue) and subsequently analysed and documented by an imaging system (VersaDoc, BioRad, Munich, Germany) and an analysis software (Delta2D, Decodon, Greifswald, Germany). The software seeks different expressions of proteins between the compared conditions. The protein spots which are found to be significantly different are excised from the gel, and finally the proteins are identified by mass spectrometry.

Summary

In summary, the well-established standard methods are still required and useful to provide reference values for the objective characterization of meat quality traits. But the introduction and development of new techniques are necessary for a deeper understanding of underlying processes of meat quality on the one hand. On the other hand, online applicable methods — like spectroscopic methods — can quickly deliver results during the production process and thus support the meat processing industry.

Figure 1. Schematic overview of the 2-dimensional gel electrophoresis in our workflow.



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Status for NEXIM New X-ray Imaging Modalities for safe and high quality food

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Value for industry

- New X-ray techniques based on grating based imaging (GBI) provide imaging modalities that allow objects that have similar absorption profiles, but different micro-structures to be distinguished from one another.
- GBI has shown great potential for investigating how heat treatment affects the final quality of meat products. The non-destructive technique allows for imaging the same sample in a raw and cooked state and the full 3D volume can be quantitatively analyzed to reveal new parameters relating to the final quality.
- Additionally GBI has proven capable of revealing foreign objects not detected by conventional X-ray absorption techniques. These materials include cartilage, paper, wood fragments, insects and plastic. Implementing GBI into an in-line scanning process could therefore greatly increase the detection of foreign objects, meeting the consumer demands of safe and high quality food.

Background

The main objectives of the NEXIM project are to develop the novel X-ray grating interferometry technique (Weitkamp *et al*. 2005; Pfeiffer *et al*. 2008) specifically towards food application and to identify the areas within the Danish food industry with the highest technological and commercial impact. The main focuses are determined to be threefold:

1) **Improving the detectability of low density foreign bodies incidentally present in food products.**
2) **Development of new modalities for assessment of quality traits in food production, for instance connective tissue and fatty acid composition.**
3) **Develop a proof-of-principle of a conveyor belt solution that can form the basis for real product development.**

In the past year the NEXIM project has focused on these three objectives, studying the applicability of GBI to meat quality assessment and foreign object detection. Some efforts have been put to developing laboratory-based setups further towards an in-line scanning system. Additionally, close co-operation with industrial partners has further emphasized the need for new techniques for quality control, product development and foreign object detection.

Why work is needed

In the food production industry, conventional X-ray scanning offers penetrating power to monitor the inside of food. X-rays can provide high contrast between fat and meat and detect foreign objects that have a sufficient difference in attenuation length from the food product. The subtle differences in soft material and foreign objects such as cartilage, wood chips, plastic fragments, insects and paper make these materials hard to distinguish using conventional X-ray techniques.

A recent survey of Japanese customer complaints on contaminants in food (Takashi *et al.* 2009) showed that these objects are the most challenging foreign materials, which still cannot be adequately detected. Further, within meat science there is a great interest in determining the effects of heat treatment and different temperature profiles on the final eating quality of meat. However, no current method has the capability of revealing 3D structural properties of soft materials such as connective tissue due to the similar attenuation properties of meat.

However, phase contrast and dark-field imaging have been shown to provide superior contrast in a study of pork rind and fat (Jensen *et al*. 2011), and potential for improved segmentation of pork back fat and beef muscle (Nielsen *et al*. 2012).

GBI provides means to both increase the detection of foreign objects and to study structural changes of food products in 3D non-destructively, such that the same sample can be measured before and after heat treatment. Until now, GBI has been performed mainly at synchrotron sources or using laboratory-setups. Neither is suitable to an industrial setting due to complexity, acquisition times and cost. Further development of the method towards an industrial standard is therefore needed, and the NEXIM project has focused its attention to bringing GBI closer to an industrial in-line scanning system.

The methods used

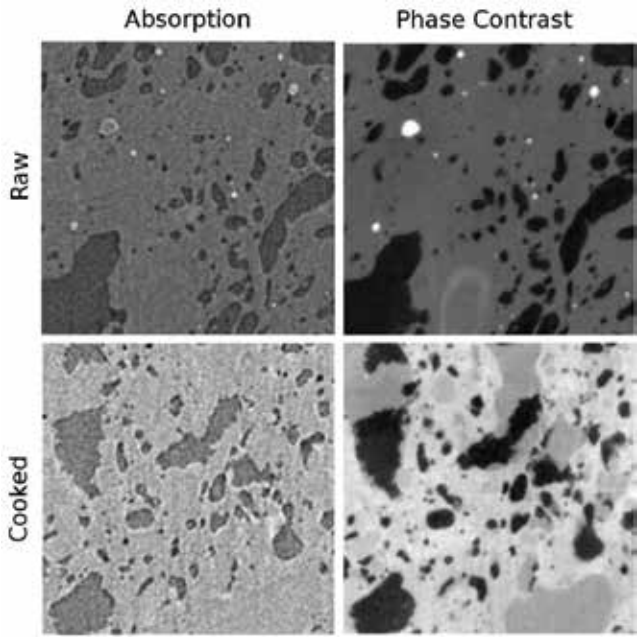
In order to determine how the industry can best benefit from GBI, data has been gathered both from synchrotrons and laboratory-based setups. This serves as an essential pre-study to whether the goal of achieving an industrial standard for GBI will be feasible. In the past year the main focus has been set on investigating the effects of heat treatment on meat products, and both whole muscle and meat emulsions were imaged at the TOMCAT beamline at the Paul Scherrer Institute, PSI, Switzerland. The full volumes obtained were 1720 x 1720 x 513 voxels with an effective voxel size of 7.4 µm x 7.4 µm x 7.4 µm.

Additionally the sensitivity of GBI to foreign objects has been investigated at the laboratory-based setup at Niels Bohr Institute using a rotating anode tube source. Several food products have been imaged including minced meat, sour cream, potatoes and spring rolls with foreign objects such as folded paper, cigarette stubs, broken glass, insects and string. The acquired data was 195 x 487 pixels with an effective pixel size at sample of 126 µm x 126 µm.

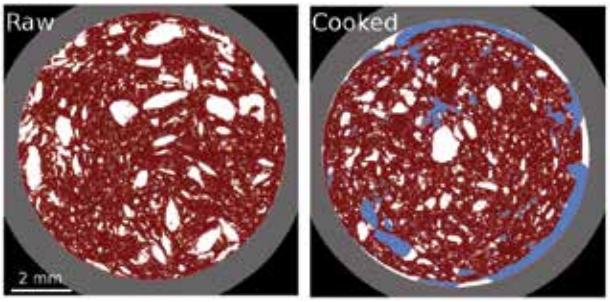
Image segmentation has played a large role in the analysis of acquired data, and multivariate algorithms have been developed to simultaneously exploit the three imaging modalities obtained from GBI. Conventional X-ray analysis typically relies on the Hounsfield scale, which is a quantitative scale describing radio density, for classification of sample materials. Since GBI also measures the electron density and scattering properties of the imaged sample, new means to classify materials is needed. Here, the multivariate nature of the data has been exploited and is modeled as a mixture of multivariate Gaussians using an expectation-maximization (EM) algorithm (Dempster *et al*. 1977). Additionally, to exploit the spatial nature of the data, Markov random fields (MRF) using graph cuts (Boykov *et al.* 2001) have been applied and further extended to obtain good segmentation results of fine structures such as connective tissue.

The results obtained

The obtained results from TOMCAT can be seen in Figure 1 where a partial slice of the meat emulsions from the absorption and phase contrast modalities are shown. The contrast obtained by measuring the refraction (phase contrast) is superior, and the expressible fluid in the emulsion can be distinguished from the protein network. As the GBI method allows for obtaining data non-destructively for the entire sample volume, the same samples are imaged before and after heat treatment. This introduces the opportunity to quantitatively determine parameters indicating the final product quality.



a) Meat emulsions.



b) Segmentation results.

Figure 1. In a) tomographic slices of meat emulsions from the absorption and phase contrast modalities are shown. The emulsions are shown in both the raw and cooked state. The phase gives clearly a higher contrast, revealing the expressible fluid in the cooked meat emulsion. In b) the segmentation results from modeling the data with the EM and MRF algorithms is shown. Reprinted from (Einarsdottir *et al*. 2013).

The stability of meat emulsions was studied where the use of two lipids types in the emulsions was analyzed and compared (Einarsdottir *et al.* 2013, Miklos *et al*. 2013). Table 1 shows the percent object volumes for the sample ingredients in the emulsion samples. The multivariate segmentation method was capable of classifying the expressible fluid making it possible to determine the cooking loss of the emulsion. These results were presented at the InsideFood Symposium 2013 in Leuven, Belgium.

Parameter	Lard raw	Lard cooked
Protein (%)	73.9	73.9
Water (%)	-	15.6
Fat (%)	25.3	25.0
Salt (%)	0.8	-

Table 1. Percent object volumes for the ingredients in the emulsion samples. The protein phase includes both protein and moisture which could not be separated due to resolution limitations.

In (Nielsen *et al.* 2013) the novel GBI approach showed how dark-field imaging revealed organic foreign materials in minced meat and sour cream. The results are shown in Figure 2. Here the absorption modality only reveals the broken glass in the minced meat. The cigarette stub is discernible in the phase contrast modality and the paper, cigarette and insects are all clearly visible in the dark-field modality.

These results proved promising, and further studies showed that wood chips in potatoes and string in spring rolls can be easily identified. Further empirical studies are needed to determine the size detection limit.

Here, the capability of the dark-field modality to reveal sub-scattering information (Pfeiffer *et al*. 2008) may prove useful in identifying foreign objects smaller than the detector resolution. Furthermore, segmentation methods based on texture analysis are in the pipeline for future work.

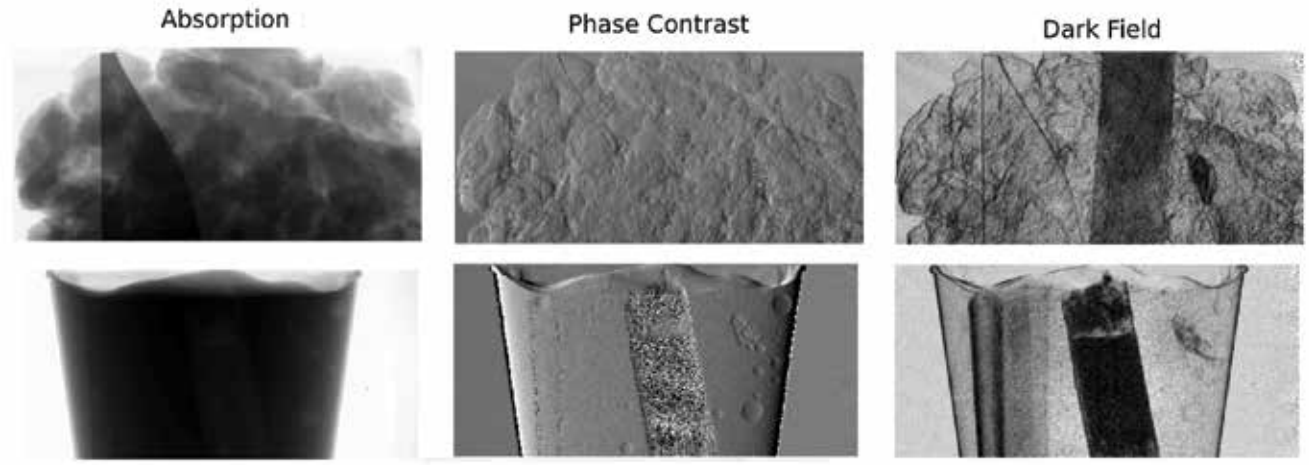


Figure 2: The results from GBI of organic foreign bodies placed in food products. Top: Minced meat with broken glass (left), 4 layers of paper (middle) and ladybug (right). Bottom: Sour cream with 8 layers of paper (left), a cigarette butt (middle) and a fly (right). Reprinted from (Nielsen *et al.* 2013)

The scientific conclusions

Food quality assessment

Studies from the preceding year have shown that GBI is a feasible method to determine cooking loss and structural changes of connective tissue in meat products after heat treatment.

Foreign object detection

Proof-of-principle experiments have demonstrated some of the potentials of GBI as a promising emerging modality for detection of organic and other 'hard-to-find' foreign bodies in food products.

In-line scanning

Some attempts have been made to further develop a laboratory-based setup towards an industrial solution. A scanning type procedure has been tested with good results.

Next steps

Several obstacles are still to be overcome before GBI becomes a feasible option for industrial use. Here acquisition times and instrument sensitivity play a big role. Future studies will focus on time resolved GBI, further development of segmentation methods and texture analysis

Acknowledgements

The authors acknowledge financial support through the NEXIM research project funded by the Danish Council for Strategic Research (contract no. 11-116226) within the Program Commission on Health, Food and Welfare.

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TAG – Technology, Agriculture and Greater efficiency

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Value for industry

- Due to the decoupling of production from subsidies sheep farming is moving from a 'headage' mentality to a market focused system, based on improving the quality of production. This evolving process requires management information as a tool to assist in this change of behaviour. Gathering flock information (both managerial and financial), and collating and acting upon relevant reports needs to be developed and adopted by industry.
- Using new IT systems to deliver on the above aspirations will assist farmers in developing their product that will fit the needs of the market. There is an urgent need to look for efficiencies of production along all links in the food chain.
- The number of lambs hitting customer specification is often quoted at 60 – 65% leading to inefficiency and wastage. Some leading dedicated lamb supply groups are conversely achieving 85 – 95% due to attention to detail, good record keeping responding proactively to carcass feedback from the abattoir.

Background

The 'TAG' project was funded as part of the Rural Development Plan for Wales (RDPW) 2007-13 Supply Chain Efficiencies Scheme (SCES). It operated from June 2009 to March 2013 with the aim of improving the efficiency of Welsh sheep production systems and the quality of lamb they produced. It operated by linking the sheep supply chain from sheep farmer, marketing agents/processor and a retailer. It was managed by Menter a Busnes, which is an independent economic development company operating in Wales.

Delivery of the project involved recruiting 80 farmers who would be willing to adopt new electronic identification (EID) recording systems for their sheep flocks and the establishment of working partnerships with key businesses within the agri-food sector. Its success required innovation by all parties both individually and in the way they collaborated. Thus it linked: farm input suppliers – producers – marketing agents/processor – retailer.

Participating farmers were organised into three groups or 'tiers' and were provided with different levels of technology (e.g. computer and EID readers) and support to record their flocks. This enabled involvement irrespective of barriers such as a confidence and ability with ICT and access to Broadband.

It also provided the data required to evaluate the practicalities of possible alternative sheep recording systems, as per the project objectives.

Project need

With the demise of the production based subsidies Welsh sheep farming has moved into an era that has to be market focused with the ability to compete with high quality products from outside Wales as regards the home market and also the main export markets on the Continent. The current buoyant lamb trade is partially due to the exchange rate and the value of the Euro in relation to the pound. Wales needs the right product to satisfy this favourable export market while still competing with lamb produced from low input sheep systems such as New Zealand. Lucrative new markets are also emerging in China and the Far East.

Welsh lamb with its PGI status continually needs to aspire to improve not just the quality of the product in terms of taste but also efficiencies of production along the whole food chain. Greater margins can be achieved on sheep farms to reduce the continual exit from sheep production due to economic pressures by reducing wastage and costs on farm and at the processor.

The lack of critical mass is of huge concern within the Welsh sheep industry and the innovation encouraged in this project should result in the greater sustainability of our core producers and processors who are often working on very tight margins as regards profitability.

In a recent IGD study, animal welfare was a prime concern for the consumer and improved recording and the acting upon issues such as lameness, ant-helmintic resistance, and lambing problems are key elements that need to be seen to be addressed on our Welsh Sheep farms.

Using new IT systems to assist in the delivery on the above aspirations will assist farmers in developing their product that will fit the needs of the market. The number of lambs hitting customer specification is often quoted at 60 – 65% while conversely some leading dedicated lamb supply groups are achieving 85 – 95% due to attention to detail, good record keeping responding proactively to carcass feedback from the abattoir.

The emergence of 'disruptive' technology such as EID makes the timing of a pilot project such as this essential to turn what could be a legislative and financial burden on a sheep industry into a positive that will lead to much greater efficiencies and sustainability.

Project objectives

1. Benchmark on key performance indicators
2. Encourage uptake of new ICT based technologies to provide flock management information
3. Assess the success of using ICT technologies on farm
4. To develop a dissemination structure on how the successful use of ICT on farm could be rolled out to further farms in future
5. Evaluate the practicalities of the three tiers
6. Provide individual flock management information to the farmer
7. Decrease the burden of record keeping by the use of electronic exchange of information
8. Enable farmers to implement a system that could assist an increase in the percentage of lambs hitting the required specifications
9. Empower farmers to investigate various information that can be collected i.e. physical data
10. Develop a good quality aggregate data on a central database

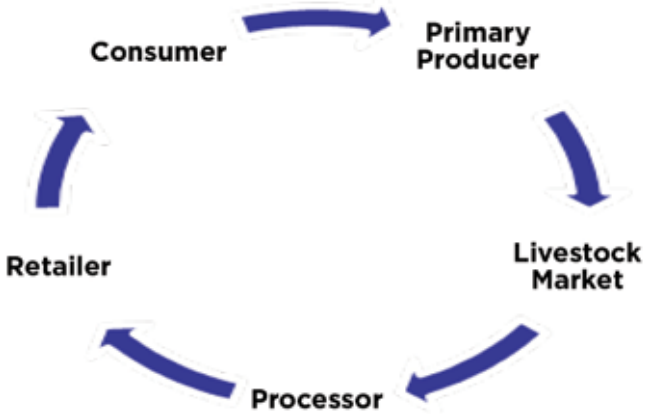


Figure 1. Project partners

The project involved two livestock markets, a major lamb processor with a throughput of one million lambs per annum and a key retailer with 16% of the UK retailer market share (Figure 1).

The three tiers

Tier 1

This group was responsible for the whole process from initial tagging to analysing data. Suitable farmers were recruited and the TAG management team encouraged them to fully embrace the concept of sheep management data. These farmers were already IT inclined and were further trained and mentored in the use of specialist equipment.

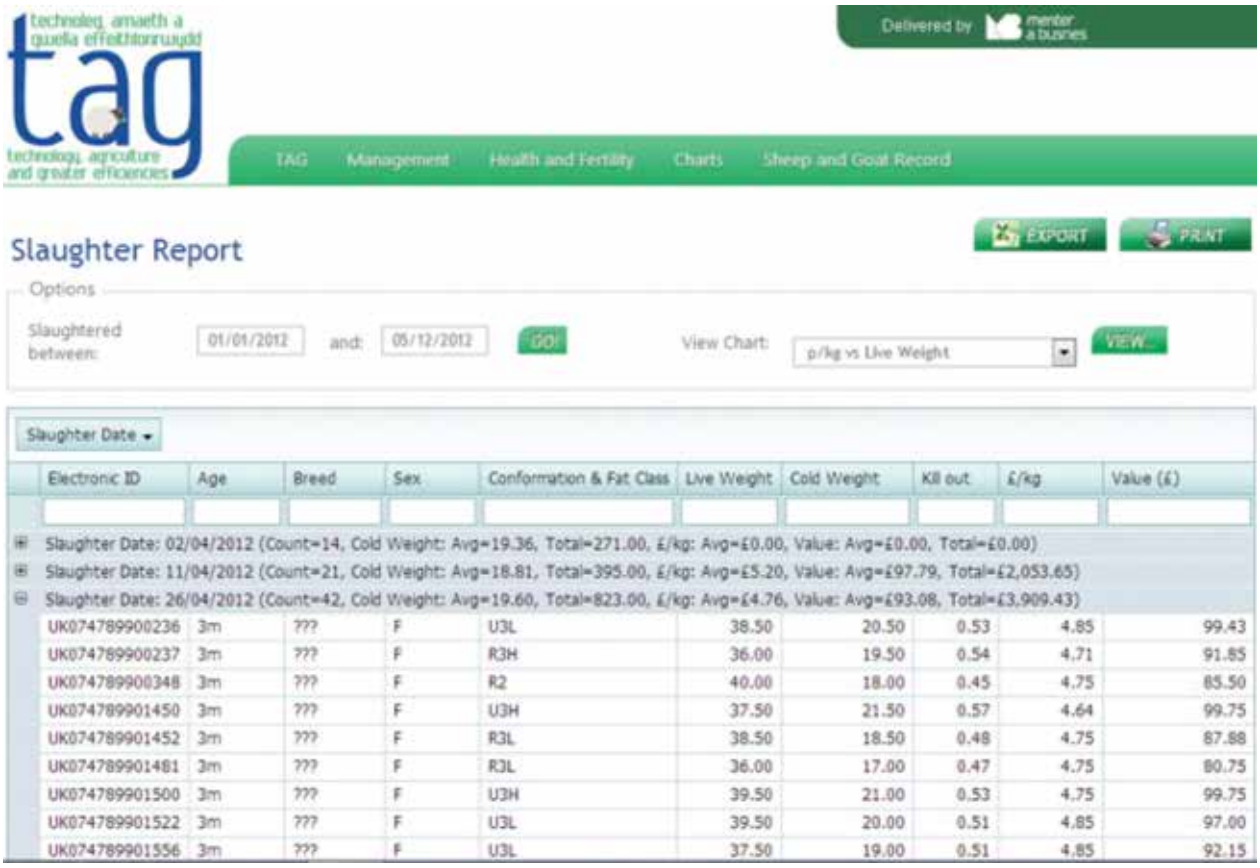
Tier 2

This group were given access to a hand held EID reader with a third party recorder downloading the collected data to the TAG database (Figure 4).

Tier 3

This group was identified due to their engagement being primarily to improve their lamb production to meet market demands. In general they were not inclined to using new technologies to gather their own results. They therefore used the facility of a service provider who visited the farm on a regular basis to collect the relevant data. The collected data was then processed on behalf of the farmer and the results communicated back to the farmer so that he/she could act upon the outcomes (Figures 2 and 3).

Key reports



Electronic ID	Age	Breed	Sex	Conformation & Fat Class	Live Weight	Cold Weight	Kill out	£/kg	Value (£)
Slaughter Date: 02/04/2012 (Count=14, Cold Weight: Avg=19.36, Total=271.00, £/kg: Avg=£0.00, Value: Avg=£0.00, Total=£0.00)									
Slaughter Date: 11/04/2012 (Count=21, Cold Weight: Avg=18.81, Total=395.00, £/kg: Avg=£5.20, Value: Avg=£97.79, Total=£2,053.65)									
Slaughter Date: 26/04/2012 (Count=42, Cold Weight: Avg=19.60, Total=823.00, £/kg: Avg=£4.76, Value: Avg=£93.08, Total=£3,909.43)									
UK074789900236	3m	???	F	U3L	38.50	20.50	0.53	4.85	99.43
UK074789900237	3m	???	F	R3H	36.00	19.50	0.54	4.71	91.85
UK074789900348	3m	???	F	R2	40.00	18.00	0.45	4.75	85.50
UK074789901450	3m	???	F	U3H	37.50	21.50	0.57	4.64	99.75
UK074789901452	3m	???	F	R3L	38.50	18.50	0.48	4.75	87.88
UK074789901481	3m	???	F	R3L	36.00	17.00	0.47	4.75	80.75
UK074789901500	3m	???	F	U3H	39.50	21.00	0.53	4.75	99.75
UK074789901522	3m	???	F	U3L	39.50	20.00	0.51	4.85	97.00
UK074789901556	3m	???	F	U3L	37.50	19.00	0.51	4.85	92.15

Figure 2. Slaughter report.

Year	Start of Lambing	End of Lambing	Ewes put to Ram	Yearlings put to Ram	Flock Replace Rate	Barren Rate	Ewes Lambed	Lambs Born Linked to Ewe	Lambs Born not Linked to Ewe	Pre-Wean Death Rate	Lambs Weaned	% of Scanned Lambs Weaned	Avg Wt of Lambs Weaned	No. Wean/ Ewe to Ram	Wt. Wean/ Ewe to Ram	Wt. Wean/ 100kg Ewe to Ram
2010	17/03/2010	20/05/2010	0	0			288	418	9	0	422		21			
2011	21/03/2011	20/05/2011	244	0	0	0	284	392	3	0	391	1	26	2	42	69
2012	11/03/2012	04/05/2012	269	78	0	0	303	441	3	0	431	1	30	2	48	84

Figure 3. KPI report.

Project highlights to date

- Assisted partner commercial companies to develop robust technical equipment for use on farm to record individual sheep information.
- Assisted our partner abattoir to develop and establish a practical operating system that can provide individual slaughter data back to the producers.
- Facilitated the development of systems that can collate data from different collection points (farm/livestock auction/abattoir).
- Developed a unique data base with a user friendly interface for recording key sheep performance data and generating key performance indicators which can be used by farmers to improve the sustainability of their sheep production enterprises.
- Established synergistic links between a selection of operators from different points within the sheep supply chain.
- Provided an independent source of information on practical issues relating to EID implementation in sheep supply chain for stakeholders including Government.

Project additionality

There are more farmers involved in using EID to monitor the performance of their sheep than would have occurred otherwise – i.e. SCALE additionality.

That a number of farmers have started recording their sheep/using EID for performance monitoring earlier than they would otherwise – TIME additionality.

That a number of farmers have started improving the quality of their stock as a result of TAG – QUALITY additionality.

+ That a degree of the stigma surrounding sheep EID has been removed – Acceptance factor.

Next steps

The TAG project has recently secured additional funding to facilitate the use of the platform developed in the project to date by other supply chains and producers.

Figure 4. Sheep EID demonstration.

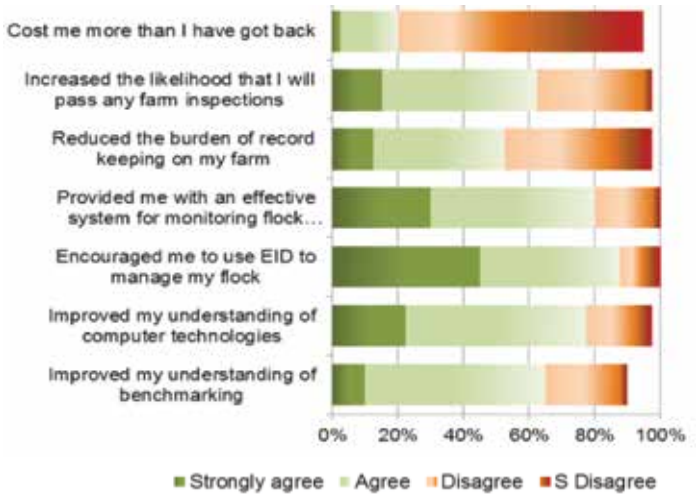


Figure 5. Farmer response (cost/benefits of TAG).

Traceability - from food safety demand to business intelligence

J. Siles

FACCSA, Estación de Cártama, Málaga, Spain

Value for industry

- Given the variability of pig carcass characteristics, there is large economic benefit to be gained by optimizing carcass usage in the slaughterhouse. The optimization of the carcasses requires a comprehensive traceability system where the information for every animal is available at any point at the line.
- The combination of an ultrasound carcass measurement system (Autofom) and the Radio Frequency Identification (RFID) technology provides the basis for a practical approach to this optimization.

Background

Faccsa is a pork meat business located in Malaga, Spain. It includes a slaughterhouse, three cutting rooms, two packing rooms and a cold storage, processes 25,000 pigs per week and supplies a wide variety of customers, from local butchers to big supermarket chains and processed food plants in third countries (55% domestic, 45% export).

The company uses a hot-deboning system, which means that the carcasses are cut the slaughter day. On average, a carcass is cut 4.5 hours after the slaughtering. The carcasses enter the cutting room at a temperature of 14°C at the centre of the loin.

The requirements for the orders in the domestic market is known the same day as the pigs are being slaughtered and cut. Therefore, the in-line decision about how to cut every single carcass has to be taken on the basis of sales forecasts.

There are many feasible ways of cutting a carcass to produce saleable items, and having all the possible combinations and the market prices for every item, the average price for each option can be calculated and ordered from the best to the worst. Typically, a difference of 0.12 €/kg carcass weight is found, which is roughly double the net margin of the company. Therefore, the optimization of the use of the carcasses is essential for the business profitability.

One optimization example

A population of 650 representative pigs (in terms of breeding, sex, conformation and weight) was used. For every pig, the primary cut for the skin-on chop is deboned in two ways: cutting pattern 1, bone-in loin with collar and, cutting pattern 2, boneless loin.

The weight of the pieces produced for each cutting pattern and pig are multiplied by their representative prices at the market, and divided by the carcass weight resulting in the average price. An average difference of 0.03 €/kg is found between both cutting patterns (minimum: 0.00 €/kg; maximum 0.05 €/kg).

The average price for each cutting pattern is represented in a scatter plot against the loin percentage (loin weight/carcass weight). There is a good correlation between the variables for both cutting patterns, so the loin percentage is an appropriate parameter for the optimization (bone-in loin R^2 = 0.7788; boneless loin R^2 = 0.9059)

Two different scenarios are simulated. The starting point is the real distribution of pigs in one slaughtering day (3,241 pigs, 6,482 primary chops), where 4,000 boneless loins, cutting pattern 2, have to be produced (the rest will be bone-in loin with collar, cutting pattern 1). In the first case, the decision is taken randomly until the quantity of desired boneless loins is achieved; in the second one, the boneless loins are produced from the pigs with the highest loin percentage. A 926 € difference is found which represents 277,800 € once extrapolated to a whole year of production (300 working days).

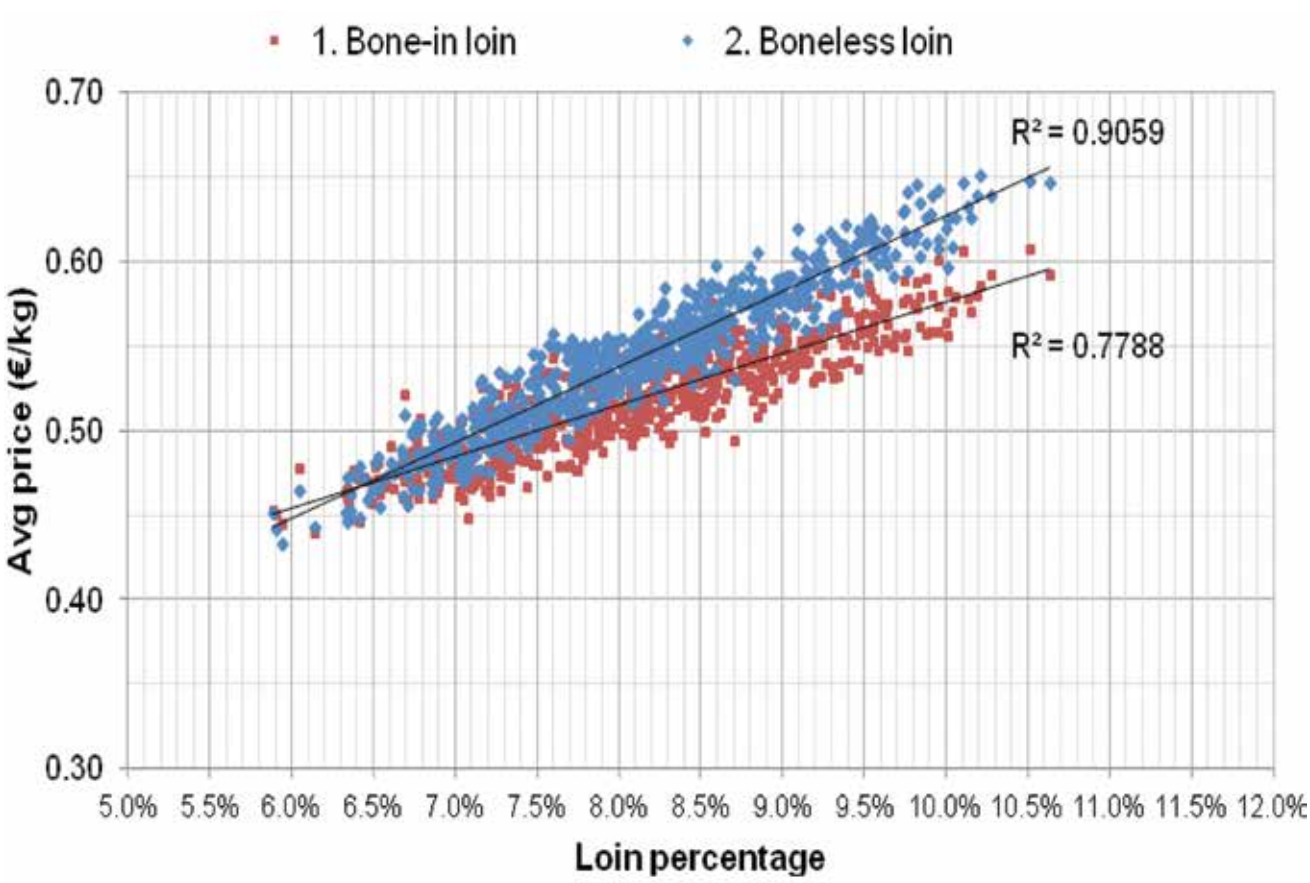


Figure 1. Average price vs cutting pattern

The optimization of the carcasses

1. Production programming

The base for the calculation is one production week. Given a certain day within the week, the potential for all the primary cuts is calculated from the pig purchase agenda (pigs to be slaughtered) and the available stock for the different articles corresponding to each primary cut. Regarding the requirements, the historical sales and current data provide the base for the sales forecast. For every primary cut, the balance between the potential and the actual needs permits the calculation of the pieces to be produced and the destination for the existing stock, which forms the daily production program.

2. Yield models

For every primary article (cutting pattern), yield data are needed for a representative number of pigs. These data include the percentage of the different articles that are produced as a result (primary and secondary) and the technical parameters that define the carcass (breeding, weight, sex, lean meat percentage, loin meat percentage...) that can be directly obtained at the line trough the different measuring systems (Autofom). The current prices at the market for every article are included at the model, so using a linear correlation analysis it is possible to express the average price for each primary article in terms of the technical parameters.

3. Carcass forecasting

The pig purchase agenda (scheduled suppliers, farms and quantities), together with the historical data for the technical parameters, allows the calculation of the expected distribution of the parameters.

4. Optimization model

The combination of the three previous elements determines the sorting criteria for the carcasses. These criteria are based on ranges for the different technical parameters which define regions in a multivariable space, each one of them corresponding to a certain cutting pattern (cutting group).

The online measuring of the carcasses gives the real value for the parameters during the slaughtering so it is possible to classify them into cutting groups. The processing of the carcasses in accordance to these cutting groups permits the production of what the market demands, so maximizing the total income.

The traceability system

The presented optimization model is put into practice via the use of a RFID based traceability system with several additional devices, all of them connected through the ERP:

1. Livestock entry. All the information for the pigs on every batch is uploaded into the system on their arrival at the lairage: day and time, transport, supplier, farm, number of pigs, food chain information, ante mortem inspection and animal welfare control.

2. RFID hooks. The use of RFID tags permits the use of all the information in the decision making. There are different RFID antennas all through the line which allow both the reading and writing of information for the carcasses in the database.

3. Autofom. The Autofom I scanner from Carometec is the core of the optimization process. Using our own calibration equations, it gives a prediction pig by pig of the parameters that are used in the classification: lean meat percentage, loin percentage, fat thickness between the 3rd and 4th ribs and the fat thickness at the *gluteus medius*.

4. Allocation assignment. For certain articles, the destination for the carcass is manually allocated. The screen shows the pending pieces for the article, the ones already allocated, and the values for the parameter limits.

5. Scale. At the scale the weight and the sex of the carcasses is incorporated to the data base. It is also the point where the slaughtering batch (supplier and farm) is correlated to the pigs.

6. Classification groups. The cutting groups are defined by two kinds of limits: a) variable limits imposed by the optimization model and b) fixed limits forced by the customer specifications.

7. Carcass marker. The carcasses are marked at the ham with the traceability codes which allow the data tracking for any of them and inform the workers about the carcass quality and cutting group.

8. Carcass sorting. The carcasses are automatically sorted into groups at the classification chilling room using a rail system.

9. Carcass control monitor. All the information for the carcasses at the classification chilling room is available at the touchable screen: number of pigs, rail, group and time. The different groups are picked and put into a queue for the automatic unloading. Therefore, the production is organized in homogeneous groups of pigs to be cut in a certain way.

10. Traffic lights. The traffic lights are a simple way to inform the workers at different places about how to cut the carcass depending on the cutting group.

11. Informative displays. Complementary to the traffic lights, there are 3 displays (loin line, belly line and shoulder line) which inform the workers about the cutting group in progress, in terms of each primary cut.

12. ID points. At the ID points, the articles are weighed and the traceability labels printed. Each label contains a bar code with an ID code.

13. Ham sorting. The hams are sorted using the same system as for the carcasses, but with their own groups and criteria.

13. Ham control monitor. The same approach as for the carcasses is used.

The next steps

The sales forecasts, the control of available stocks of fresh meat and the production program are managed in Excel files but the information has to be manually fed into the optimization tool. It is necessary to integrate them into the ERP.

The available stocks recount is done visually. It is needed to acquire the information using a comprehensive warehouse management tool based on bar codes.

The limits for the optimization of the bellies and the hams have to be adjusted manually, taking into account the production program (pieces to produce) and the specifications. It could be implemented a model, similar to the one for the loins, where the limits are automatically calculated; this would be beneficial.

The allocation assignment for certain articles is done manually and pencil marks are still used. The process needs to be fully automated.

At present the order in which the carcasses are unloaded from the sorting roomis decided by the person in charge. The unloading order determines the order in which the articles are produced and the total cooling time for the carcasses at the sorting room. This process could be optimized and fully automated.

Acknowledgements

All the application software involved in the traceability system described above is been developed by Faccsa's own IT team headed by Mr. F. Requena and Mr. O. Rodríguez.

The actual system in use is rooted in the ideas and previous work of Faccsa's former Technical and Production Director, Mr. P. Costa.

*Winner of the poster competition

Poster Competition: The winner

H. Einarsdottir

Segmentation Toolbox for Tomographic Image Data

Hildur Einarsdóttir, Bjarne Kjær Ersbøll, Rasmus Larsen

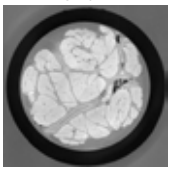
Technical University of Denmark, Richard Petersens Plads , Building 324, 2800 Kgs. Lyngby, Denmark

Introduction

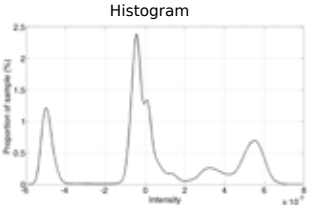
Motivation: Image acquisition has vastly improved over the past years, introducing techniques such as X-ray computed tomography (CT). CT images provide the means to probe a sample non-invasively to investigate its inner structure. Given the wide usage of this technique and massive data amounts, techniques to automatically analyze such data becomes ever more important. Most segmentation methods for large datasets, such as CT images, deal with simple thresholding techniques, where intensity values cut offs are predetermined and hard coded. For data where the intensity difference is not sufficient, and partial volume voxels occur frequently, thresholding methods do not suffice and more advanced methods are required.

Contribution: To meet these requirements a toolbox has been developed, combining well known methods within the image analysis field. The toolbox includes cluster-based methods to automatically determine parameters of the different classes present in the data, and edge weighted smoothing of the final segmentation based on Markov Random Fields (MRF). The toolbox is developed for Matlab users and requires only minimal background knowledge of Matlab..

Tomographic data



Histogram



Methods

1. Given the large amount of data in tomographic images, we first sample at random a portion of the voxels.
2. Next we fit a Gaussian mixture model (GMM) to the sampled data using the Expectation-Maximization algorithm. Here the user must specify the number of Gaussian desired.
3. To determine the predominante structure direction and spatial coherence at each voxel, the structure tensor is calculated.
4. Either an isotropic- or anisotropic MRF is used to incorporate spatial information in the segmentation process by modeling the *a priori* probability of neighbor dependencies.

Example

Three dimensional structure tensor

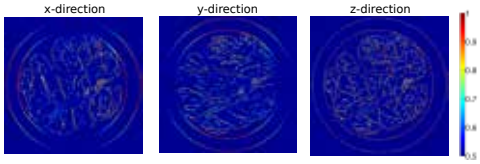
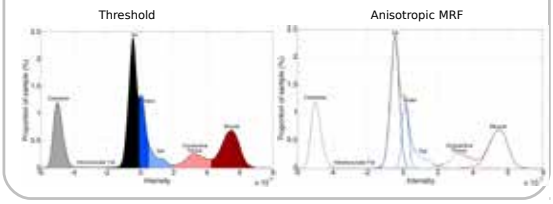
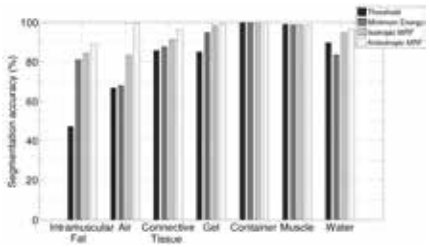


Image histograms

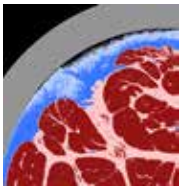


Results

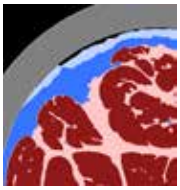
- Significant gain is acheived in segmentation accuracy when considering the spatial context of the data.
- The toolbox provides a simple MATLAB framework to implement and visualize each step of the segmentation algorithm and requires only minimal background knowledge in MATLAB.
- Code will be published in near future along with a detailed description of the entire algorithm.



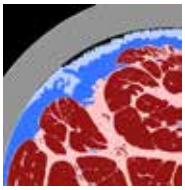
Threshold

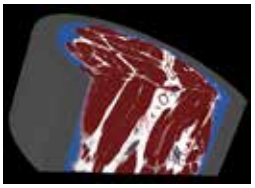


Isotropic MRF



Anisotropic MRF





DTU Compute
Department of Applied Mathematics and Computer Science

Poster Competition

S.R. Silva VIA

FAIM III
Taastrup, Denmark 24-26 September 2014

The ability of video image analysis (VIA) to predict carcass composition and cut yields of light lamb carcasses

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Background and objective

There is a growing interest in obtaining objective information about the quality of sheep carcasses. In EU the evaluation of sheep carcasses has been performed by applying two different classification schemes: the SEUROP for carcasses above 13 kg and other for light carcasses. The latter scheme is mainly utilized for carcasses from Mediterranean breeds which exhibit poor conformation and low subcutaneous fat level. These attributes make grading more difficult than for heavier carcass lamb. Currently, an objective method for estimating light lamb carcass composition is lacking. The objective of this study was to evaluate a video image analysis (VIA) system to predict carcass composition and yields in light lamb carcasses.

Material and Methods

- The trial was conducted with 30 carcasses (6.3±1.3 kg, cold carcass weight - CCW) of the Mirandesa breed (Figure 1**a**).
- Images from carcass side view were captured using a digital camera.
- The images were analyzed using ImageJ software and 30 morphometric measurements (areas, perimeters, lengths, widths and angles) were determined (Figure 1**b** to **e**).
- The carcasses were divided into leg, loin, rib, shoulder, breast and neck cuts. The cuts were dissected into muscle, fat (subcutaneous fat plus intermuscular fat) and bone.
- Carcass and cuts yields were calculated in relation to live weight and CCW, respectively.
- Stepwise regression analyses were performed to predict carcass and cut composition and yields using VIA measurements and CCW as independent variables.

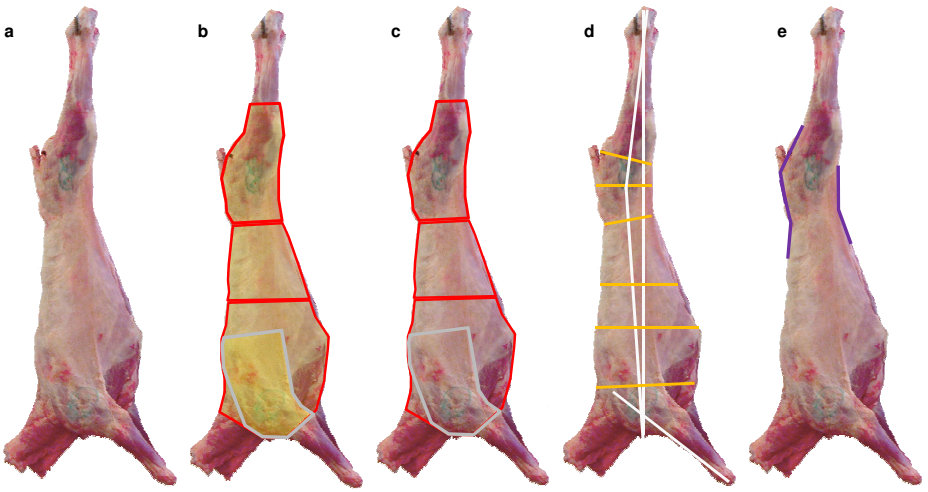

Figure 1: Miradesa light carcass (**a**); and areas (**b**); perimeters (**c**); lengths and widths (**d**) and angles (**e**) carcass measurements.

Results

For cut weight the best fit was obtained after 2 to 4 steps with CCW and VIA measurements (R^2 ranged between 0.70 and 0.97, P<0.01). The best prediction equation explained 98 % of the carcass muscle variation, whereas for carcass fat the best fit was R^2 = 0.64 (P<0.01). The prediction of cut yields was less accurate (R^2 ranged between 0.32 and 0.48, P<0.01). The same trend was observed for muscle yield (R^2 = 0.15, P = 0.02) and fat yield (R^2 = 0.43, P<0.01).

Conclusions

The CCW and VIA measurements show ability to predict cut and carcass tissue weights. The results show a need for further refinement to predict the carcass composition yield.

Poster Competition

L. Bünger

The effect of using coronal or sagittal CT topograms to quantify spine characteristics in sheep

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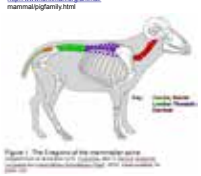
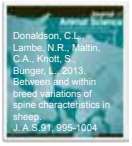
Introduction

http://www.funkman.org/animal/mammal/pigfamily.html

- Spine traits have potential to increase meat production, particularly from the highly valuable loin.
- There is substantial knowledge about the genetics of spine characteristics in pigs
- Commercial pigs can have up to 4 more vertebrae than the ancestral 19
- Little information on sheep spine traits (length, vertebrae number) until recently





Recent knowledge is based on coronal topograms obtained from computed X-ray tomography (CT). This view ignores the curvature of the spine, which could affect individual or breed differences.

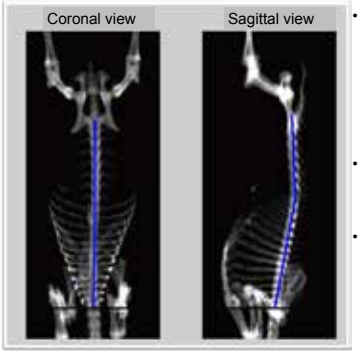
Objective

This study aims to investigate variation in spine length measures between ram lambs of three different terminal sire breeds using both coronal and sagittal topograms.

Materials & methods

Mobile CT unit (Burgess Diagnostics) with a GE LightSpeed (16 slice)

Figure 2: 2D topograms reconstructed from spiral CT images



- Both 2D topograms taken from ~50 ram lambs of each of three breeds:
  - Charollais, CHA
  - Suffolk, SUF
  - Texel, TEX
- Analysed using STAR software (Mann *et al.*, 2013).
- Lumbar and thoracic spine lengths (straight lines) measured and summed for total length
- Data analysed using GLM in SAS (SAS Institute Inc., Cary, NC, USA) with breed as a fixed effect and live weight at CT scan as a covariate (for length traits, not count traits)

Results

	CHA	SUF	TEX	avSE	ANOVA P
Vertebrae number					
Lumber	6.75[a]	6.81[a]	6.36[b]	0.063	<.0001
Thoracic	13.23[b]	13.57[a]	13.66[a]	0.065	<.0001
Thoracolumbar	19.98[b]	20.38[a]	20.02[b]	0.088	0.0024
Spine length (C)					
Lumber	227.20[a]	233.15[a]	210.65[b]	2.242	<0.0001
Thoracic	311.74[c]	339.20[a]	329.79[b]	2.920	<0.0001
Thoracolumbar	538.93[b]	572.34[a]	540.45[b]	3.420	<0.0001
Spine length (S)					
Lumber	234.47[a]	232.65[a]	210.26[b]	1.984	<0.0001
Thoracic	333.44[b]	343.89[a]	336.49[b]	2.766	0.013
Thoracolumbar	567.82[a]	576.55[a]	546.77[b]	3.431	<0.0001
Length difference (S-C)					
Lumber	7.28[a]	-0.51[b]	-0.39[b]	1.961	0.0391
Thoracic	21.71[a]	4.70[b]	6.70[b]	2.701	0.0005
Thoracolumbar	28.89[a]	4.20[b]	6.32[b]	3.174	<.0001

Significant breed effects on all 3 count traits:
- SUF mostly highest

Significant breed effects on spine length traits:
- SUF mostly longer

Table 1: Least-square means (average standard error, avSE) for CT-measured spine traits in different breeds based on the coronal (C) and sagittal (S) topograms

Breed differences can be affected by the type of topogram used to measure the spine lengths. The comparison coronal vs. sagittal measures showed that:
- Sagittal measures are usually longer, especially in the thoracic region, also affecting total (thoracolumbar) length
- There are breed effects on this difference - CHA lambs affected to a greater extent than SUF and TEX in this study.

Discussion

Breed effects on the comparison of length measurements taken from coronal and sagittal topograms indicate different curvatures. SUF and TEX animals seem to have little curve to their spines, as the lengths measures are very similar and differ by a maximum of only 2% on average. Differences in CHA are larger: 3, 7 and 5% when measured from the sagittal view.
Although the sagittal measurement allows the curvature of the spine to be accounted for (in this study just by measuring 2 straight lines), there seems to be a larger error associated with this method. In parts of the image in this view, several bones overlap, making it difficult to position the measurement points accurately. The topograms in Fig. 2 are reconstructed, so they don't show this effect (very white areas).
Topogram reconstruction using all images obtained via spiral CT would be an alternative way to avoid this, but then the slice distance directly affects the measurement error. The slice distance in this study is 7.5mm, which might be too large to improve accuracy.
WG01T07 shows low to moderate heritabilities for spine length traits measured on coronal topograms. This indicates that they are sufficient to be used in breeding. However, the use of sagittal views could possibly increase the accuracies and therefore heritabilities, but it requires the use of reconstructed topograms. This method needs to be evaluated regarding costs and benefits.

Conclusions

- Breed comparisons can be affected by differences in spine curvature that are not accounted for by the sole use of coronal topograms
- Although measurements based on sagittal topograms allow the curvature of the spine to be accounted for, there seems to be a larger error associated with this method due to difficulty taking measurements where bones overlap.
- The use of reconstructed topograms based on images obtained from spiral scanning could remove this cause of error but requires a short image interval to avoid the introduction of another error source. The use of reconstructed topograms needs to be evaluated regarding accuracy and cost-benefit.

Acknowledgements We thank the Scottish Government (RESAS), Capes-BR (funding A.M.M) and COST Action FAIM for their support of these studies.

Poster Competition

S. Schwanitz

LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

Boar taint and body composition – evaluated in boars, immunological castrated pigs and barrows

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[1]Livestock Center Oberschleissheim, Veterinary Faculty, Ludwig-Maximilians-University, Munich, Germany
[2]University of Applied Sciences, Weihenstephan-Triesdorf, Germany

Aim of this study

Evaluation of **body composition** and **boar taint in boars (B), immunological castrated (I) pigs and barrows (C)** by dual-energy X-ray absorptiometry (DXA), organoleptic test (OT) and stable-isotope-dilution-assay (SIDA).

Material & methods

- **70 crossbred pigs** (Piétrain x German Landrace)
- raised in **2 different groups.** In each group: **B, I, and C**, housed together in one pen (Gr.1 born in early July and Gr.2 born end of September)
- **C** were **castrated five days after birth**
- **I** received **two subcutaneous injections** of a **commercial gonadotropin releasing factor analogon** (injection at the age of 77 ± 1 days and 4 weeks before slaughter)
- **GE Lunar *i*DXA** was used for DXA examination, receiving: **bone mineral density** (BMD (g/cm²)), **bone mineral content** (BMC (g)), **fat content** (fat (kg)), **fat percentage** (fat (%)) and **lean tissue** (lean (kg))
- **3 scans during fattening period:** Scan 1 (est. weight: **30 kg**), Scan 2 (est. weight: **60 kg**), Scan 3 (est. weight: **90 kg**); **measurements were analysed** for each pig **individually**

- **statistic analysis: SAS software 9.3., GLM, fixed effect: gender (B/I/C), co-variable: weight**
- **backfat samples** taken after slaughter and **analysed via SIDA**, receiving Skatole, Androstenone and Indole concentration levels
- **cheek and salivary samples** were also taken and **analysed by OT**: proceeding in Step 1: microwave cooking test, Step 2: boiling test, Step 3: melting test

Results

I show **lower boar taint than B** in OT. In **Gr.1** appeared **less boar taint** than in **Gr.2**.
I and B have **higher** value of **lean tissue** and **lower fat content than C**.

Only significant differences (different superscripts) are displayed, showing the estimated mean and SEE.

DXA:

Gender	BMD (g/cm²) Scan 2	BMD (g/cm²) Scan 3	
B	0,73 ± 0,01[a]	0,96 ± 0,01[a]	
I	0,73 ± 0,01[a]	0,96 ± 0,01[a]	
C	0,75 ± 0,01[b]	1,09 ± 0,01[b]	
Gender	**fat (%) Scan 3**	**fat (kg) Scan 3**	**lean (kg) Scan 3**
B	11,62 ± 0,34[a]	10,64 ± 0,32[a]	79,70 ± 0,32[a]
I	12,41 ± 0,35[a]	11,40 ± 0,32[a]	78,83 ± 0,32[a]
C	15,26 ± 0,35[b]	13,94 ± 0,32[b]	76,33 ± 0,32[b]

SIDA:

Gender	Skatole (ng/g)	Androstenone (ng/g)	Indole (ng/g)
B	35,75 ± 3,71[a]	353,31 ± 48,81[a]	19,74 ± 1,38[a]
I	23,95 ± 3,80[b]	177,02 ± 49,82[b]	18,83 ± 1,41[a]
C	18,40 ± 3,80[b]	157,97 ± 52,14[b]	14,24 ± 1,41[b]
Group	**Skatole (ng/g)**	**Androstenone (ng/g)**	**Indole (ng/g)**
1	20,86 ± 2,98[a]	139,05 ± 40,48[a]	15,20 ± 1,11[a]
2	31,20 ± 3,17[b]	319,82 ± 41,56[b]	20,00 ± 1,19[b]

OT:

Group 1:	B (n=13)	I (n=12)	C (n=12)
Step 1	4 = 30,77%	2 = 16,67%	2 = 16,67%
Step 2	1 = 7,68%	1 = 8,33%	0
Step 3	0	1 = 8,33%	0
Group 2:	**B (n=11)**	**I (n=12)**	**C (n=10)**
Step 1	7 = 63,64%	1 = 8,32%	1 = 10%
Step 2	4 = 36,35%	0	1 = 10%
Step 3	4 = 36,35%	0	1 = 10%
Total	**B (n=24)**	**I (n=24)**	**C (n=22)**
Step 1	11 = 45,82%	3 = 12,50%	3 = 13,64%
Step 2	5 = 20,82%	1 = 4,17%	1 = 4,55%
Step 3	4 = 16,67%	1 = 4,17%	1 = 4,55%

Conclusion

Referred to body composition and boar taint, **immunological castration** seems to be an **alternative to boar fattening**. In this study, the **appearance of boar taint** showed **seasonally** caused **differences for the two groups**.

Poster Competition

G. Kušec

Backfat and muscle surface measurements as predictors of lean meat percentage in pig carcasses

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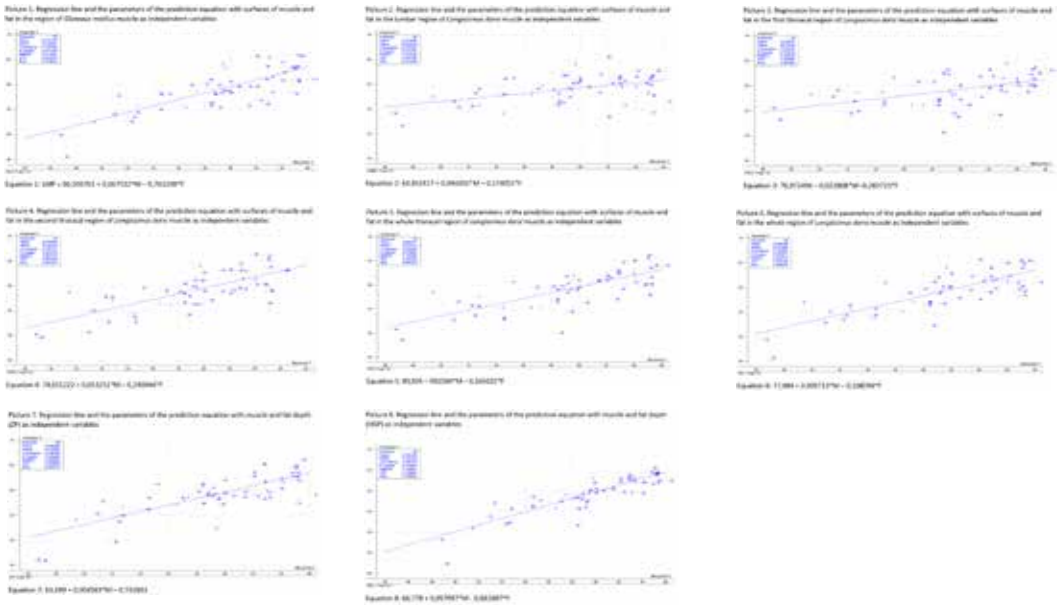
Abstract

Historically, lean meat percentage (LMP) has been assessed by use of subcutaneous fat measurements at various positions along the dorsal midline. Later on, muscle thickness was included as an independent variable and used together with backfat thickness in predicting formulae in EU countries. This is still the basis for most of the methods of LMP prediction. High correlation of these measurements with the actual lean meat content in the pig carcass is crucial for their use. However, there are a lot of investigations on new methods for the prediction of LMP using different measurements with the aim of improving the accuracy. Most of the measurements in use are thickens or depths of subcutaneous fat and muscle expressed in centimetres or millimetres. The aim of this paper is to investigate the possibility of using backfat and muscle surfaces instead. The dissection experiment was conducted on 56 pig carcasses. The measurements of backfat and muscle surfaces were taken at the split line using transparent paper on which the margins of measurement areas were drawn manually. Later on, the drawn areas were measured by geometric procedure using digital planimeter. The areas were split into several parts involving two toracal regions, lumbar region and the region of *m. gluteus medius* with belonging fat, similar as in the ZP method. These measurements will be compared with HGP and ZP measurements in their ability of LMP prediction of Croatian pigs.

Table 1. Descriptive statistics for the EU referent LMP and independent variables used in its prediction

	EU ref	tot M	tot F	glu M	glu F	lum M	lum F	tor1 M	tor1 F	tor2 M	tor2 F	tor M	tor F	zp M	zp F	hgp M	hgp F
mean	58.96	530.44	202.71	84.82	17.43	116.76	57.74	143.42	51.95	185.44	75.59	328.87	127.54	73.41	14.09	70.55	13.50
st.dev.	5.00	62.41	37.32	20.19	4.86	21.97	14.78	19.97	10.28	28.87	16.51	42.06	23.74	5.56	5.36	7.03	4.90
min.	46.29	416.50	141.30	48.30	8.70	78.80	28.00	98.40	35.30	124.10	45.00	252.20	84.90	59.00	5.00	50.00	6.00
max.	66.09	720.20	320.30	123.60	33.40	171.20	95.10	194.80	75.50	276.40	116.70	461.60	191.80	87.00	31.00	82.00	30.00
CV %	8.49	11.76	18.41	23.80	27.87	18.82	25.59	13.93	19.78	15.57	21.84	12.79	18.61	7.57	38.05	9.97	36.29



Conclusion

The results of this investigation showed that certain backfat and muscle surface measurements can be utilised as the predictors of LMP in pig carcasses. Naimely, when surfaces of muscle and fat in the region of *Gluteaus medius* muscle was used as prediction variables, RMSEP (3.01) was lower than one obtained for the equation which used usual ZP measurements (3.03). When surfaces of muscle and fat in the whole region of *Longissimus dorsi* muscle were used as the predictors of LMP, RMSEP (3.03) was quite similar to the one obtained for ZP equation. However, the lowest RMSEP (2.31) was still found for HGP equation for LMP prediction in pig carcasses.

Poster Competition

A. Bauer

Department of Safety and Quality of Meat

Supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme

Carcass composition of boars compared to gilts and barrows

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Department of Safety and Quality of Meat, Kulmbach, Germany

Conclusion

Our study quantifies gender related differences in carcass composition between boars, gilts and barrows for the present slaughter pig population in Germany. This database forms the reference for the analysis of images from X-ray computed tomography at the MRI.

Keywords – Body composition, boars, barrows, gilts, lean meat, main cuts

Introduction

The forthcoming 2018 ban of surgical castration of male piglets without anaesthesia triggered many projects on the fattening of boars as an alternative. It is established that boars have a different shape and carcass tissue composition compared to gilts and barrows. In particular, boars have a higher percentage of lean meat at the expense of fat. But some aspects are unclear in detail, e.g. the quantitative ratio between lean meat and fat which is important for carcass value. Therefore, we compared the carcass composition of boars, gilts and barrows.

Results

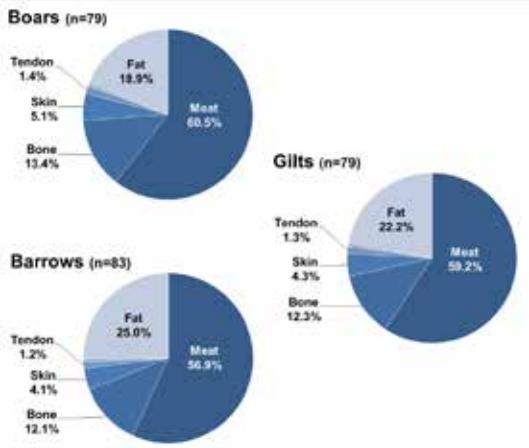


Figure 1: Average tissue composition of carcasses for boars (Bauer and Judas, 2014), and for gilts and barrows (Judas et al., 2012), as a result from full manual dissection (0.7% other tissue unlabelled).

Table 1: Mean weight percentage of carcass main cuts by gender (standard deviation in brackets, differences tested with generalized linear models, SAS 9.3).

	Shoulder	Neck with neck fat	Loin with back fat	Belly	Tenderloin	Ham	Total
Boars n=79	14.2^{a} (0.5)	9.2^{a} (0.7)	16.6^{b} (1.0)	9.9^{ab} (0.8)	1.7^{a} (0.1)	24.6^{b} (1.3)	76.2^{b} (1.1)
Gilts n=79	13.8^{b} (0.5)	9.1^{a} (0.5)	16.9^{ab} (0.9)	9.7^{b} (0.8)	1.7^{a} (0.1)	25.4^{a} (1.0)	76.6^{a} (1.0)
Barrows n=83	13.9^{b} (0.6)	9.1^{a} (0.5)	17.0^{a} (0.7)	10.1^{a} (0.7)	1.6^{b} (0.1)	25.2^{a} (1.0)	76.9^{a} (1.0)

$^{a, b, c}$ Different indices within columns denote significant differences between genders ($p<0.05$)

- As expected, highest amount of lean meat (61%) and lowest amount of fat tissue (19%) for boar carcasses (Fig. 1)
- In contrast, lowest lean meat (57%) and highest fat percentage (25%) for barrow carcasses
- Carcass composition of gilts ranged in between (lean meat 59%, fat 22%)
- Relatively larger shoulders and smaller hams in boar carcasses compared to gilts and barrows (Table 1)
- Same trend in tissue composition for main cuts as for the whole carcass, e.g. highest lean meat and lowest fat percentages in cuts from boars (for details, see Bauer and Judas, 2014)
- Boar carcasses also with highest percentages of bone, skin and (except tenderloin) tendon (for details, see Bauer and Judas, 2014)

Material and Methods

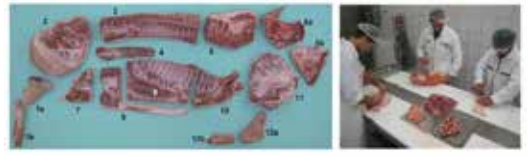


Figure 2: Cuts of a left half pig carcass according to the DLG method (left) and example of manual full dissection (right). 1a hind shank, 1b hind foot, 2 ham, 3 loin with back fat, 4 tenderloin, 5 neck with neck fat, 6a head, 6b cheek, 7 to ventral part of the belly, 8 ventral part of the belly, 9 belly, 10 jawl, 11 shoulder, 12a front shank, 12b front foot (from Judas et al., 2012).

- Dissection into main cuts and manual full dissection of tissues for overall 241 carcasses of boars (in 2012/2013, n=79), and of gilts and barrows (in 2009/2010, n=79 and n=83, resp.) (Fig. 2)

Table 2: Samples analysed by full manual dissection (n=241), stratified by gender, weight and morphological type.

	Du*DK	Pi*FR	Pi*Nord	Pi*Süd	Yk*NL
< 90 kg[1]	5:6:6	4:6:6	4:5:5	4:5:6	5:5:5
90<100 kg[1]	6:5:5	7:4:6	6:5:5	6:6:5	6:6:5
≥ 100 kg[1]	5:5:6	5:5:5	6:6:5	5:5:6	5:6:6
Boars:Gilts:Barrows	16:16:17	16:15:17	16:16:15	15:17:16	16:16:17

[1] Warm carcass weight, incl. 250 g correction for eye and ear cutouts for gilts and barrows

- Stratification by 5 morphological types in 3 weight groups representative for German slaughter pigs (Table 2)
- Differentiation of tissues: meat, fat, bone, skin, tendon, other tissue

Bauer A., Judas M., 2014. Schlachtkörperqualität von Mastebern im Vergleich zu Sauen und Börgen. Züchtungskunde 86 (5/6), 374-389.
Judas M., Branscheid W., Höreth R., 2012. Neue Ergebnisse zur Variabilität der Gewebeanteile beim Schwein. Mitteilungsblatt Fleischforschung Kulmbach 51 (195), 1-16.

Poster Competition

D. Lucas

IRTA RESEARCH & TECHNOLOGY FOOD & AGRICULTURE

Generalitat de Catalunya Government of Catalonia

Relationship between fat and muscle thickness measured in live pigs with ultrasounds and computed tomography

Daniel Lucas[1,2], Marina Gispert[1], Joaquim Soler[3], Mateu Tulsà Vert[3], Joan Tibau[3], Maria Font-i-Furnols[1]

[1] IRTA- Product Quality. Finca Camps i Armet. 17121 Monells (Girona). Catalonia. Spain ; [2] UFF-Facultade de Veteninaria. Niterói. RJ. Brasil; [3] IRTA- Animal Breeding and Genetics. Veïnat de Sies. E-17121 Monells (Girona). Catalonia. Spain

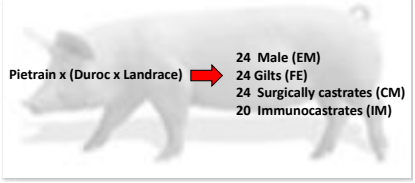
cost F A I M

FAIM III Conference. 24th – 25th September. Taastrup. Denmark

INTRODUCTION & OBJECTIVES

The most non invasive technique used in farms to evaluate live animals is based on ultrasounds (US). Nowadays there it is also possible to use computed tomography (CT). a non invasive technology based on X-ray emission. X-rays are attenuated in their way through the body depending on the density of the tissues. producing images of the interior part of the body. The aim of this experiment was to find correlations between CT and US measures of pigs from different sexes during their growth.

MATERIAL & METHODS



Pietrain x (Duroc x Landrace) → 24 Male (EM), 24 Gilts (FE), 24 Surgically castrates (CM), 20 Immunocastrates (IM)

Number of EM / FE / CM / IM	30 kg	70 kg	100 kg	120 kg
12 / 12 / 12 / 12	▲	▲	▲	▲
4 / 4 / 4 / 0	●			
4 / 4 / 4 / 4		●		
4 / 4 / 4 / 4			●	

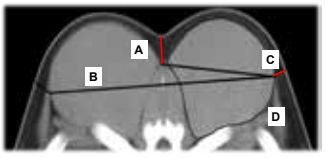
▲ Measured *in vivo* at different weights during their growht and slaughtered at 120 kg
● Measured *in vivo* and slaughered

General Electric HiSpeed Zx/i 140 kV. 145 mA. 512x512 matrix. Axial 10 mm-thick slices every 10 mm (7 mm-thick every 7 mm in 30 kg pigs).

Piglog 105 A-mode ultrasound device (Carometec. DK)

X

CT Image analysis



A: Dorsal fat thickness.; B: Maximum loin depth; C: Lateral fat thickness perpendicular to the skin; D: Loin area and perimeter. All this measures were performed at last rib level.

US Image analysis

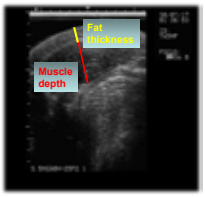


Image of Longissimus dorsi. at the the last rib level at 4- 6 cm from the spine.

RESULTS

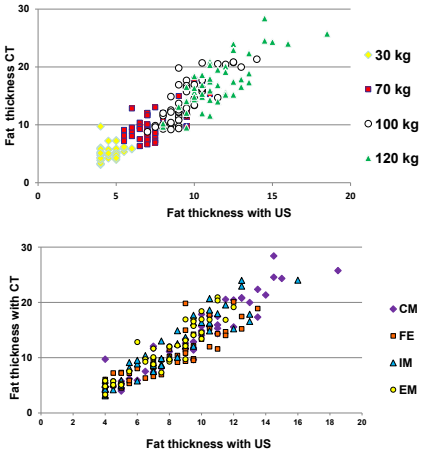


Figure 1: Fat thickness at the last rib level obtained with US vs. CT presented by weight group (upper plot) and sex type (lower plot)

Table 1. Significant (P<0.05) correlation values between CT and US fat thickness and loin area measures

Thick measures	Animals	Weight	Pearson correlation
Lateral fat thickness (C)	188	30 – 120	0.88
Dorsal fat thickness (A)	188	30 – 120	0.92
Lateral fat thickness (C)	47	70	0.68
Lateral fat thickness (C)	47	100	0.82
Lateral fat thickness (C)	47	120	0.79
US loin area (D)	141*	70 – 120	0.82

* there was not possible to measure area of the 30 kg animals

Measurements performed for muscle depth between US and CT presented low and not significant (P>0.05) correlations.
The correlation by sexes presented results higher than 0.88. The higher correlation was found for CM and IM (r= 0.95 and 0.94. respectively). Although the results of US fat and CT lateral fat thickness for females presented correlation of only 0.89.

CONCLUSIONS

The present work shows that, except for 30kg pigs, there is an important correlation between ultrasound and the CT fat thickness measures. The results highlight the usefulness of US technology for animals growing evaluation of fat thickness. More evaluations should be carried out considering different CT measures and anatomical regions and estimating carcass lean meat content in live pigs.

ACKNOWLEDGEMENTS

The work has been financed by INIA (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria) through the project RTA2010-00014-00-00. The authors thank Anna Carabús. Albert Brun. Albert Rossell. Agustí Quintana and Pedro Rodríguez for their technical assistance.

Poster Competition

S.R. Silva IMF

FAIM III
Taastrup, Denmark 24-26 September 2014

Using high resolution images to predict intramuscular fat in lean beef meat

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Background and Objective

Consumer demand in relation to beef meat has been increasingly shifting towards products that are safe, nutritious, of good eating quality and produced through sustainable farming practices. It is recognized that intramuscular fat (IMF) plays an important role in the eating quality of beef meat. Although large variation among countries, 3 to 4% of IMF represents a target for optimal taste in *Longissimus thoracis et lumborum* muscle (LM). However, very often the beef meat in EU market shows IMF values below 2%. Additionally several reports pointed that the prediction of IMF in lean cattle (IMF < 2%) represents a challenging task either in vivo or at slaughter. To include information about IMF in a quality based marketing system a simple, non-destructive and inexpensive method is necessary. The objective of the current work is to study a system based on high resolution digital images (Figure 1) to predict IMF in lean beef samples.

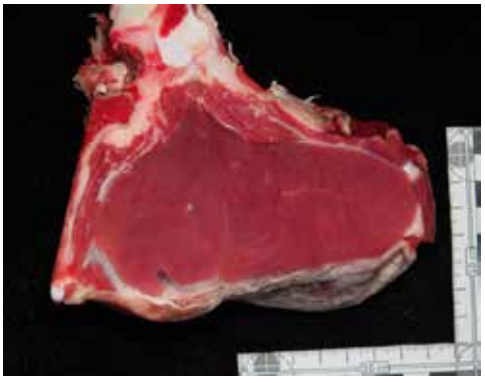

Figure 1: Example of a LM sample photo.

Results

Correlation and regression analysis were established between the chemical IMF and IMF features obtained after image analysis. A large variation was found for the IMF features (CV 7 to 90 %) and for chemical IMF (CV = 53%). It was possible to predict accurately IMF from IMF features obtained after image analysis (R^2Adjusted=0.92, RMSE=0.19%).

Material and Methods

- Twenty two LM samples were obtained from rib cuts of crossbred beef lean carcasses.
- Photos of LM samples were taken using an Olympus EM-5 with 16 Megapixel sensors mounted on a stand. The camera was equipped with an M. Zuiko ED 12-50mm f3.5-6.3 EZ lens at 24 mm and open at f8 with a circular polarizer filter.
- For illumination it was used an Olympus OM T28 Macro Twin Flash with polarizer filters in both heads.
- All LM samples were placed over a black opaque cloth.
- The image analysis was performed with ImageJ software (Figure 2).
- For image analysis a threshold value for IMF was determined from the histogram gray level and was then used to numerical data extract of 18 variables related with IMF.
- The IMF chemical content of LM samples was obtained in triplicates after ether-extraction.
- Regression analysis were established between the chemical IMF and IMF features obtained after image analysis

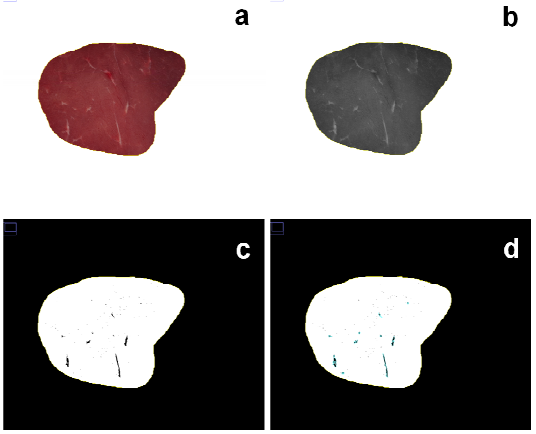

Figure 2: Steps of image analysis using ImageJ for the determination of IMF features. **a-** freehand LM segmentation; **b-** 8-bit image; **c-** image after threshold application and **d-** numerical data extract.

Conclusions

This result encourages the use of image analysis to predict IMF however further research is necessary for full automatic LM segmentation. Further investigation is needed to apply this approach to a carcass cutting room.

Poster Competition

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FAIM III: Third Annual Conference on Body and Carcass Evaluation, Meat Quality, Software and Traceability
Danish Technological Institute, Gregersensvej 1, 2630 Taastrup, Denmark 25th and 26th of September 2014

Vis-NIRS to predict the cooking yield of loins

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INTRODUCTION

Despite beeing well documented in a fresh meat context, the meat quality of the loin and its suitability for meat processing have been rarely studied. In a previous study (Vautier et al., 2011), visible spectroscopy appeared to be an alternative of ultimate pH for the prediction of the cooking yield. The aim of this study is to confirm determinant meat quality parameters for processed loins, including conductance and pH at early post mortem stage or after 24 hours, and to test the accuracy of a visible+NIR spectroscopy calibration for cooking yield prediction.

MATERIALS AND METHODS

- 80 loins from Piétrain sire pigs, DNA test practiced for Halothane genotype determination.
- Early post mortem measurments (30 min.) on carcass at the last thoracic vertebrae level: pH1, conductance (Cond1) and core temperature (T30).
- On bacon-style debonned loins (24 H. post mortem): 18 ultimate pH measurements practiced every 5 cm in two rows (medial and lateral), conductance measured at the last thoracic vertebrae level (Cond24), visible+NIR spectroscopy (350-1800 nm) performed on 9 sites every 5cm (one central row) with a two way optic fiber probe inserted in the muscle core.
- Loins were individually processed into « Rôti Cuit Supérieur » (no phosphate or carraghenan allowed) by a meat processing company according to Vautier et al. (2011) protocol. Cooking yield and slicing yields (paste-like defect and cohesion defect rates) were individually recorded.

Figure 1: paste-like defect (left) and cohesion defect (right) on processed loin

RESULTS

- The relationship between ultimate pH and the cooking yield is very close with a correlation level from r=0.60 to r=0.69 depending on the measurement site (table 2)
- Conductance did not appear to be an accurate predictor for the cooking yield
- External validation results for NIRS prediction of the cooking yield showed a satisfying accuracy (r=0.65, figure 2)
- Cohesion defect is more frequent in the caudal part of the loin
- Paste-like defect showed its highest rate in both caudal and especially cranial end of the loin
- Halothane genotype is not considered as a major risk factor for both defects (table 1)

Table 1: slicing results by halothane genotype

	Halothane genotype		p.=
	NN	Nn	
n=	22	58	
Paste-like defect (%)	23	28	ns
Cohesion defect (%)	54	66	ns

Table 2: ultimate pH mapping of the loin and correlation with cooking yield (n=80)

m	Corr./cooking yield	Sites :	m	Corr./cooking yield
5.53	0.69	1 2	5.58	0.30
5.51	0.69	3 4	5.52	0.62
5.48	0.68	5 6	5.52	0.66
5.47	0.64	7 8	5.53	0.67
5.46	0.69	9 10	5.53	0.63
5.46	0.66	11 12	5.54	0.61
5.48	0.61	13 14	5.54	0.60
5.52	0.60	15 16	5.56	0.65
5.55	0.61	17 18	5.55	0.61
Lateral side			Medial side	

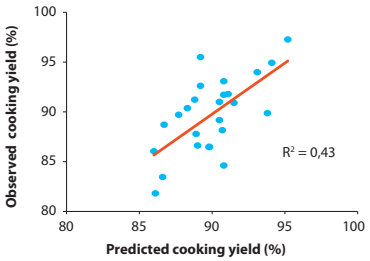

Figure 2: Longissimus external validation results for the prediction of the cooking yield by visible+NIRS (site J)

Table 3: PLS regression results for visible+NIRS prediction of the cooking yield

Site	Calibration (n=56)		Cross validation (n=56/3)	External validation (n=24)	
	R²	Nb. pls factors	Rmsec mini	r	Rmsep
C	0.26	3	3.8	0.28	3.7
D	0.09	1	3.8	-	-
E	0.66	6	3.5	0.26	3.7
F	0.05	1	3.8	-	-
G	0.08	1	3.8	-	-
H	0.02	1	3.9	-	-
I	0.29	3	3.9	0.31	3.6
J	0.78	9	3.8	0.65	2.9
K	0.15	4	3.9	0.49	3.3

Conclusions

This study confirms that the ultimate pH can be considered as the best predictor of the cooking yield of the processed loin (r=0.69). Early post mortem meat quality parameters (pH1, T30, Cond1) showed lower correlation level (r=0.01 to r=0.39). External validation results for NIRS prediction of the cooking yield (r=0.65) let us consider this technique as a reliable alternative to ultimate pH for the cooking yield prediction, if applied at the caudal end of the *Longissimus*. The paste-like defect is not linked with the halothane genotype.

Authors thanks Fleury Michon meat processing company (Pouzauges, France) for their help in this work

Poster Competition

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Estimate of intramuscular fat content in pig muscles using ultrasound and video image analysis

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BACKGROUND

It is well known that optimal level of intramuscular fat (2.0 – 3.05 %) has a positive influence on the sensory qualities of pork (Fernandez et al., 1999). However, present slaughter hybrids have in the many cases IMF under this optimal level reflecting low values in parent generations. Breeding programmes for improving meat quality including IMF require the collection of measurements on the carcass. Therefore, direct selection could not be performed recently. New alternative methods for prediction of IMF ammount in the muscles of pigs in vivo have been investigated (van Oeckel et al., 1999; Leroy et al., 2003). One of them is real-time ultrasound method (Newcom et al., 2002; Leaflet et al., 2006; Bahelka et al., 2007) that is noninvasive, easy to use and has low cost.

AIM

The aim of this study was to determine the prediction ability of real-time ultrasound device ALOKA 2 Prosound for estimation of intramuscular fat content in pig muscles.

MATERIAL and METHODS

Totally, eighty hybrid pigs (castrates and gilts) were included in the trial. All pigs were measured using ultrasound device ALOKA 2 Prosound for one – three days prior to slaughter. The point of measure was on the right side of the back. Longitudinal images of longissimus dorsi muscle in the last rib area (approx. 10 cm laterally from the backbone) were stored in the device memory and later analysed using video image analysis - VIA (software LUCIA, Laboratory Imaging, Prague, Czech Republic). The method of „peaks detection" for prediction of intramuscular fat content in muscle was used. Different variables were taking into account: the number of algorithm repeats marked as „x3" or „x6", intensity of thresholding (70 or 80) and place for evaluation of IMF („r" – ROI, region of interest, square 100x100 pixels over last rib or „c" – whole visible area).

Day after slaughter, dissection of right half carcass was done and samples of longissimus dorsi muscle (150 g) were taken (at the same place as ultrasonic images were made) for laboratory analysis of intramuscular fat content (LAIMF). The results were calculated using package SAS/STAT, version 9.1.3. (2002-03).

Table 1. Basic statistics for ultrasound and laboratory analysed IMF

Variable	*n*	*mean (%)*	*sd.*	*min*	*max*
x6_70r	62	1.24	1.06	0.07	5.05
x6_70c	62	1.04	0.90	0	5.10
x6_80r	62	0.55	0.56	0	2.70
x6_80c	62	0.45	0.48	0	2.76
x3_70r	62	0.42	0.42	0	2.10
x3_70c	62	0.35	0.36	0	2.03
x3_80r	62	0.17	0.20	0	1.08
x3_80c	62	0.14	0.18	0	1.04
LAIMF	62	1.61	0.71	0.60	3.90

Table 2. Correlations between ultrasound and laboratory analysed IMF

Variable	*LAIMF*
x6_70r	0.307*
x6_70c	0.209
x6_80r	0.306*
x6_80c	0.206
x3_70r	0.284*
x3_70c	0.189
x3_80r	0.247
x3_80c	0.186

* P<0.05

Table 3. Regression analysis of dependence of laboratory analysed IMF

Variable	regression formula	SEP for		RMSE	R^2
		b_0	b_1		
x6_70r	*y = 1.87191 – 0.20601x*	0.136	0.083	0.689	0.09

CONCLUSION

The results document the ability to measure IMF in live pigs using ultrasound method. In combination with VIA it could by used for selection of breeding pigs, especially young boars provided IMF will be introduced as selection criterion in M-BLUP AM.

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Poster Competition

E. Fulladosa

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RESEARCH & TECHNOLOGY FOOD & AGRICULTURE

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Estimation of global salt content of dry-cured ham at the end of process using computed tomography

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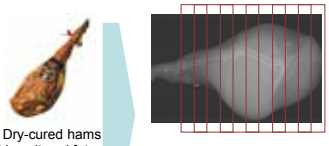
INTRODUCTION

Last years, computed tomography (CT) has received attention by the meat science community because it can give information of the composition and quality of meat and meat products in a non-destructive way. Until now. the major drawback was the price and the safety and working requirements to be used online at industry. Recently. several companies have resolved to develop industrial CT devises adapted to the meat industry but fast measurements are still required.

The **objective** of this study was to evaluate the feasibility of CT to determine the total salt content of bone-in dry-cured hams using a partial scanning of the hams which will allow a faster measurement and to determine the position/s of the tomograms to achieve accurate predictions.

MATERIAL AND METHODS



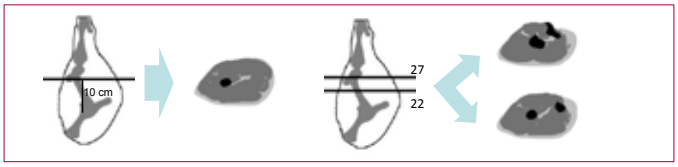
40 Dry-cured hams (wide salt and fat contents variation)

- Scanning using medical CT
- Every 20 mm
- 80, 120 and 140 kV.

- Hams were boned and soft tissues (fat and lean) were minced together. Salt content was chemically determined.
- Standardization of the number of slices per ham is carried out to account for different ham length. Best predicting slices would refer to the same anatomical region of the ham, regardless of the length.
- For each slice, bone and fat tissues were removed. The sum of the voxels attenuation was computed for the lean tissue.
- Using Multilinear regression analysis, predictive models were developed using this value from the tomograms acquired at different energies and positions.
- Predictive errors when using one or several tomograms/energies were determined (Table 1).
- Residual predictive deviation (RPD) were determined taking into account a variation of 2% of salt in the commercialized products (Conzen, 2006).
  RPD >3, the model is considered reliable for rough prediction
  RPD> 5, the model is considered good enough for quality control

RESULTS

Figure 1. Position and tomograms the most represenative for the predicttion of the global salt content in dry-cured ham using only one energy (80 kV) and 1 or 2 tomograms.



- The most representative tomogram to determine salt content is about 10 cm from the aitch bone in the distal direction (Figure 1).
- Only a tomogram taken at 80 kV at the central part of the ham was enough to predict the total salt content of a ham with an error of 0.3%.
- Combination of various tomograms or energies did not significantly decrease the predictive error.
- Errors are similar to those found previously for specific areas of the dry-cured ham (Santos-Garcés et al (2010) Meat Sci. 101, 187-192)

Table 1 Cross-validation error, coefficient of determination (R^2) and RPD, considering an standard deviation in the market of 2% of salt. Values obtained using 1, 2 o 3 tomograms and the combination of the information obtained at different X-ray energies (80. 120 y 140 kV) in dry-cured hams of different breeds (LW and DU) at the end of the elaboration process.

Used energies			1 Tomogram			2 Tomograms			3 Tomograms		
80 kV	120 kV	140 kV	R^2	RMSECV (%)	RPD	R^2	RMSECV (%)	RPD	R^2	RMSECV (%)	RPD
x	x	x	0.86	0.32	6.3	0.88	0.33	6.1	0.89	0.33	6.1
x	x		0.86	0.32	6.3	0.87	0.33	6.1	0.88	0.32	6.3
x		x	0.82	0.31	6.5	0.84	0.32	6.3	0.85	0.32	6.3
	x	x	0.80	0.33	6.1	0.80	0.34	5.9	0.81	0.35	5.7
x			0.82	0.31	6.5	0.84	0.32	6.3	0.85	0.32	6.3
	x		0.80	0.32	6.3	0.80	0.33	6.1	0.81	0.34	5.9
		x	0.65	0.49	4.1	0.68	0.52	3.8	0.68	0.55	3.6

CONCLUSION

It can be concluded that industrial CT could be useful to categorize dry-cured ham online according to the salt content.

ACKNOWLEDGEMENTS

This work was supported by INIA (contract n. RTA2010-00029-CO4-01/02)

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Poster Competition

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Use of Raman spectroscopy to predict cooking temperature of cooked meat after storage

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FAIM-III, Taastrup, Denmark September 25-26,2014 WG2P05

Objective:
➢ To use Raman spectroscopy to predict cooking temperature of cooked meat after storage

Background
Endpoint temperature (EPT) of heat treated meat products is determinant factor to control food-borne illness. Hence, meat processing industries need to monitor EPT of a product in order to ensure inactivation of the food-borne illness causing microorganisms. However, the meat industries and food inspection authorities are lacking a reliable, fast and non-destructive technique for inspection. Raman spectroscopy is a useful technique for monitoring changes in protein structure[1,2] and provides information at the molecular level. This technique can, therefore, be used to predict the cooking temperature of a cooked product.

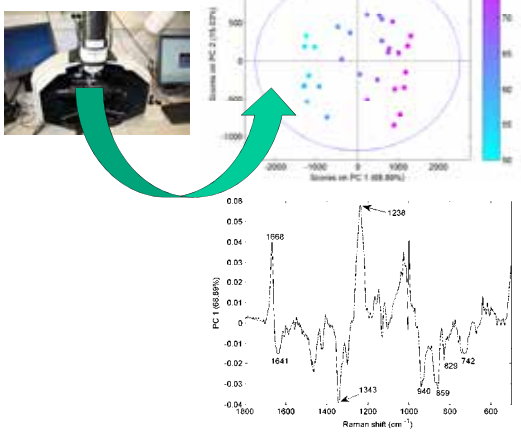

Figure 1. PCA of Raman spectra from *longissimus thoracis* muscle cooked at 50 – 80 °C for 2h then stored at 4 °C for 4 days. PC-score plot coloured according to cooking temperature (top) and PC-loadings plot for PC-1 (bottom). (n=32)

MATERIALS AND METHODS
Longissimus dorsi (LD) muscle of pigs was sampled and cooked at different cooking temperatures (50 - 80 °C with 2 °C interval) for 2 h. Cooking loss was calculated. Raman spectra were acquired using RamanRxn1 instrument using an average spectrum of 2 scans each with an exposure time of 10 s. They were stored as Raman shifts in the range 1800 - 200 cm $^{-1}$. Raman spectra were preprocessed using Extended Multiplicative Scatter Correction (EMSC). Prediction models were cross validated using contiguous blocks.

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CONCLUSION
It was possible to predict cooking temperature with a very good correlation independent of storage time up to 4 days. The present study showed that Raman spectroscopy can be applied for controlling cooking temperature even after storage.

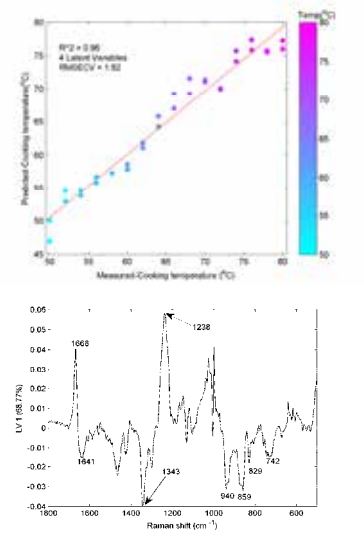

Figure 2. Prediction of cooking temperature using Raman spectra (1800-500 cm^{-1}) from *longissimus thoracis* of pork cooked at different cooking temperature (50 °C-80 °C with 2 °C interval) for 2 h then stored at 4 °C for 4 days. Measured vs predicted cooking temperature coloured according to cooking temperature (°C) (Top) and LV-1 (Bottom). (n=32)

RESULTS and DISCUSSION
PC-1 and PC-2 discriminated the samples based on the cooking temperature (Fig.1 top). The spectra are from day 4 measurements. Similar PCA-results were obtained from day 0 measurements. Loadings plot for PC-1 showed that Raman bands assigned to amide-I, amide-III and aromatic amino acids were the main contributors (Fig. 1 bottom). Cooking loss was predicted with R^2 = 0.96 and RMSECV = 2.80 % (Fig. not shown). Cooking temperature was predicted with a very good correlation both in day 0 (R^2 = 0.97, RMSECV = 1.63 °C) and day 4 (R^2 = 0.96, RMSECV = 1.82 °C) using 4 latent variables. Interestingly, Raman bands which contributed for the PCA-calculation were also exactly the same as the bands contributed for the prediction of cooking loss and cooking temperature (Fig. 1 bottom) indicating the interrelationship among the cooking temperature, structural changes and cooking loss.

Reference
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Poster Competition

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USE OF DIELECTRIC SPECTROSCOPY TO STUDY MICROSTRUCTURAL CHANGES IN DRY-CURED HAM SUBJECTED TO DIFFERENT HIGH PRESSURE TREATMENTS

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INTRODUCTION

Dielectric spectroscopy determines the **dielectric properties** of the sample as a function of frequency and can be used to describe physicochemical aspects, components interactions and structural changes in foodstuffs. High pressure processing (HPP), industrially used to increase safety in food, produces rupture of non-covalent interactions and thus a re-formation of molecular bonds within protein molecules that changes water distribution of the sample. The **aim** of this study was to study **dielectric and microstructural changes** on dry-cured ham subjected to 3 different high pressure levels (200, 400 and 600 MPa) by means of dielectric spectra combined with Cryo-SEM microscopy.

MATERIALS AND METHODS

10 dry-cured hams — HHP — Dielectric spectra — Cryo-SEM

From the dielectric spectra we obtained the complex permittivity (ε_r) which is the dielectric property that describes the behavior of the food when it is subjected to an electromagnetic field (Eq. 1).

$$\varepsilon_r = \varepsilon' - j \cdot \varepsilon'' \qquad \text{(Eq. 1)}$$

ε' → dielectric constant → related with the material ability to store energy
ε'' → dielectric loss factor → related to the absorption and dissipation of the electromagnetic energy in other kinds of energy
$j = \sqrt{-1}$

The permittivity decreases in steps called dispersions along the electromagnetic spectrum: **α** (due to charges with mobility), **β** (due to fixed charges) and γ (due to the orientation of dipolar molecules).

In this study a mathematical model which consists in three Gompertz equations is proposed. This mathematical model provides the information about the dispersions in radiofrequency and microwave ranges.

RESULTS AND DISCUSSION

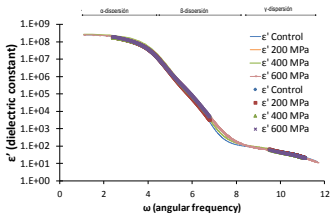

Figure 1. Prediction of Gompertz model. Where: (—) are the values of the mathematical model and (♦) are the data obtained at 25 ºC.

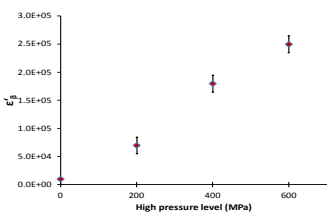

Figure 2. Dielectric constant in the β-dispersion obtained from the Gompertz model regarding the high pressure treatment.

Figure 1 demonstrates the effect of the HPP on the α and β relaxations. Following the decrease of permittivity modulus at β dispersion, and the increase at α dispersion, it is possible to conform an algorithm to follow the **effect of HPP** on dry-cured ham subjected to different pressures.

Figure 2 shows the dielectric constant of the permittivity in the β-dispersion increases with the increase of pressure. This is explained by the clear **tissue breakdown** caused by the HPP treatment mainly due to protein denaturation process. This fact produces an increase of the available charges from the proteins. The β-dispersion represents the orientation of the available charges from the solid structure, in this case the proteins.

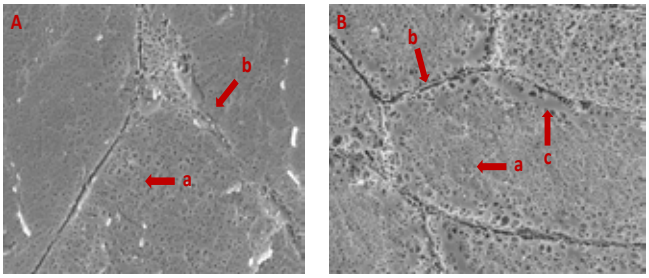

Figure 3. Micrograph (1000x) of control (**A**) and treated at 200 MPa (**B**) dry-cured ham.

Figure 3 shows that the myofibrillar bundles (**a**) of the treated ham were more compacted than in the control ham, and that the layout of the structural elements of the sarcomere have been noticeably altered. Treated samples present a reduction of interstitial space (**b**) between bundles of muscle fibres and muscles fibres, and a disturbance (**c**) of the internal **ultrastructure** of myofibrils. These changes can be related to the variations of the dielectric parameters explained in Figure 2.

ACKNOWLEDGEMENTS

This work has been partially funded by the project RTA2010-00029-CO4-01 and RTA 2013-00030-CO3-01 of the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) of Spain.

CONCLUSIONS

- Gompertz model adjusts the dielectric constant fitting the parameters of each dispersion.
- Dielectric constant in the β-dispersion changes significantly when increase the pressure throughout the treatment.
- HPP produced damages to the myofibril ultrastructure and denaturation of muscle proteins in dry-cured ham.

Poster Competition

M. Gondekova

NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

COMPARISON OF NUTRITIONAL, PHYSICAL-TECHNOLOGICAL AND ORGANOLEPTIC QUALITY OF COWS MEAT TWO AGE CATEGORIES

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INTRODUCTION

•Currently cows are slaughtered at slaughterhouses in Slovakia in growing amount (50 – 55%) and the age of cows is younger than in the past

OBJECTIVE

The aim of the study was to comparison of nutritional, physical-technological and organoleptic quality of beef (cow's meat) two age categories

MATERIAL & METHODS

•323 cows various breeds and crosses of Slovakia, slaughtered in 2006-2012
•According to the age of cows the group was divided into two categories: category A - cows under 4 years (n=79), category B - cows over 4 years (n=244)
•The samples (approx. 800 g) of *musculus longissimus lumborum et thoracis* between 8th to 9th rib from the right side of the carcass taken 24 h pm
• The analysis of the basic chemical composition of meat, measurement of colour, pH_{48} and the content of water holding capacity were performed within 48 hours pm
•7 days after slaughter a consumer test for determination of sensory traits (odour, taste, tenderness, juiciness) evaluated by 5-point scale (1 -the worst, 5 - the best), 4 minutes grilled 2.0 cm thick steaks analyzed and shear force was measured

indicator	A (n=79)		B (n=244)		T-test
	Mean	Std	Mean	Std	
odour	3,62	0,65	3,57	0,60	-
taste	3,53	0,69	3,40	0,66	-
juiciness	3,43	0,72	3,32	0,69	-
tenderness	3,29	0,85	3,15	0,79	-

indicator	A (n=79)		B (n=244)		T-test
	Mean	Std	Mean	Std	
total water, g.100g-1	75,69	2,73	74,79	3,17	+
protein content g.100g-1	20,07	1,08	20,69	1,13	+++
fat content g.100g-1	3,24	2,45	3,53	3,15	-
energetic value KJ.100g-1	458,4	95,06	478,9	117,3	-

+P<0,05; +++P<0,001

CONCLUSIONS

•The content of intramuscular fat was expectedly determined higher in older cows (B)
•Significant results were noticed in total water (75.69 % vs. 74.79 %, P<0.05) and protein content (20.07 % vs. 20.69 %, P<0.001)
•The statistically significant differences in physical-technological parameters were found in colour lightness L (30.38 vs. 29.44, P<0.05) and in shear force (13.9 kg vs. 18.99 kg, P<0.001)
•Older cows had a tendency toward darker meat color and had a higher value of shear force
• The consumer evaluation of the organoleptic qualities didn´t find any statistical significancy with regard to the age of cows, but more favourable results in the group of younger cows were determined

indicator	A (n=79)		B (n=244)		T-test
	Mean	Std	Mean	Std	
colour, L*	30,38	3,61	29,44	3,41	+
pH48	5,85	0,34	5,77	0,39	-
water holding capacity g.100g-1	27,79	5,91	27,89	6,08	-
shear force, kg	13,9	8,67	18,99	10,47	+++
marbling	7,82	1,5	7,79	1,49	-

+P<0,05; +++P<0,001

ACKNOWLEDGEMENT
This study was performed during realization of the project "LAGEZ No.26220120051" and „CEGEZ No. 26220120042" supported by the Operational Programme Research and Development funded from the European Regional Development Fund.

Poster Competition

C. Xavier

Modelling the pH and temperature decline early post-mortem of beef carcasses

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Objectives

1. Model the pH and temperature decline early post-mortem on beef carcasses
2. Study the effect of gender, genotype and weight class on the pH and temperature decline patterns

Materials and Methods

Animals

- A total of 35 beef animals slaughtered in a local abattoir were sampled
- Gender: 18 males and 6 females
- Breed: 17 crossbred and 7 Mirandesa
- Carcasses had an average hot carcass weight of 203.4 ± 52.61 kg

Temperature and pH measurements

- *Longissimus thoracis* muscle at 4^{th} rib level
- Data was recorded at intervals of 10 minutes during 24 hours
- OMEGA wireless receiver/host (UWTC-REC1) were used

Modelling of pH and temperature decline

The temperature and pH decline were modelled by the Exponential Decay (ED) function (Hwang and Thompson, 2001):

$$Y_{(t)} = A_{(u)} + (A_{(i)} - A_{(u)}) \times e^{-k \times t}$$

where:

- $Y_{(t)}$is the pH or temperature at time t
- $A_{(u)}$ is the final pH or temperature
- $A_{(i)}$ is the initial pH or temperature
- k is the exponential constant of decay for pH or temperature
- t is the time in hours after slaughtering

Data analysis

- The ED parameters ($A_{(u)}$, $A_{(i)}$ and K) were estimated by non-linear least squares (**nls**) function
- Regression analysis was used to study the relationship between $pH_{3.0}$ and pH_{24}

References

R Core Team, 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Hwang, I. H. and J. M. Thompson, 2001. The interaction between pH and temperature decline early post-mortem on the calpain system and objective tenderness in electrically stimulated beef longissimus dorsi muscle. Meat Science, 58, 167-174.

Conclusions

1. The exponential decay model showed a good fit to the pH and temperature data, and parameters K_{pH} and K_T were found to be independent.
2. This model can be used to predict the meat quality indicators $pH_{3.0}$, pH_{24}, T_{pH6}.
3. The predicted pH at three hours after slaughtering ($pH_{3.0}$) seems to be a good predictor of the final pH (pH_{24}) in beef meat.

Results

The k_{pH} varied between 0.092 and 1.149, and the results clearly indicated that large differences in pH decline are likely to occur among carcasses (Figure 1).

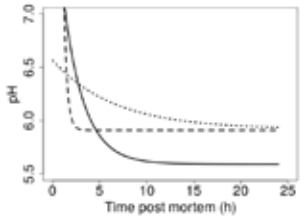

Figure 1 : pH decline of three carcasses during cooling showing different patterns

The k_T varied between 0.074 and 0.235, showing that also the temperature decline was highly variable among carcasses, and this variability was associated with high (> 35 °C) and low (< 15 °C) temperatures when pH reached the critical value of 6.0 (Figure 2).

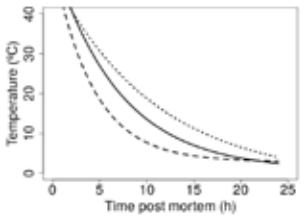

Figure 2 : Temperature decline of three carcasses during cooling showing different patterns

The K_{pH} and K_T parameters presented a low and non-significant correlation (0.35, $p < 0.05$). The high (0.930, $P < 0.01$) correlation between the $pH_{3.0}$ and pH_{24} shows that $pH_{3.0}$ can be used to predict the final pH (pH_{24}) of beef meat (Figure 3).

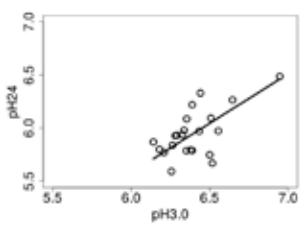

Figure 3 : Relationship between $pH_{3.0}$ and pH_{24}

Poster Competition

J. Gonzalez

IRTA RESEARCH & TECHNOLOGY FOOD & AGRICULTURE

Nofima

The use of Near Infrared Spectroscopy as a tool to predict the composition of fatty acids in pigs

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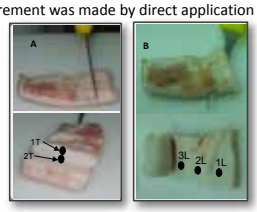
COST FAIM III 24th – 25th September Taastrup, Denmark

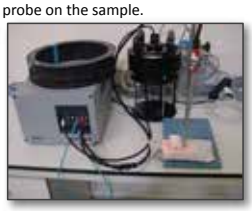
INTRODUCTION & OBJECTIVES

- ✓ **Near Infrared Spectroscopy (NIRS)** has been successfully used as a substitute of chemical analysis, due to its recognized advantages as a **rapid, non-destructive and accurate tool** to predict meat composition and fatty acids, requiring little or no sample preparation, also **no reagents** are required and **no waste** is produced.
- ✓ The **aim** is to use NIRS technology to **predict the fatty acid profile in pig fat**, and depending on the results obtained to discuss its **potential use** to be applied **at-line or on-line** in slaughterhouses and cutting plants.

MATERIAL & METHODS

- ✓ **Animals and diets:** 153 pigs fed three different diets, according to fat composition.
- ✓ **Sample preparation:** subcutaneous fat samples were obtained from the ham from each animal, over the *Gluteus medius* muscle. Spectra were obtained in two locations along a transversal cut of the fat sample (1T, 2T) and in three along a longitudinal cut (1L, 2L, 3L).
- ✓ **NIRS equipment and spectra acquisition:** FT-NIRS BRUKER Optics Matrix-F duplex spectrometer equipped with a fibre optics probe (IN-268-2), over the range of 11,000–4,000 cm^{-1}. The spectral resolution was set at 8 cm^{-1} and the spectra were recorded performing 24 scans for both reference and samples. The measurement was made by direct application of a fibre optic probe on the sample.





- ✓ **Reference method:** the fatty acid profile was analyzed using gas chromatography following the method ISO 5509-1978.
- ✓ **Chemometrics:** spectral data was obtained and preprocessed by using OPUS software (BRUKER) and for the multivariate analysis and model construction The Unscrambler (version 9.8, CAMO PROCESS AS, Oslo, Norway) software was used, relating them to the reference method data using PLS.

RESULTS

Range, mean, SD and CV of relative percentage of fatty acid groups by gas chromatography.

	Calibration set (N=115)				Validation set (N=38)			
Fatty acid	Range (%)	Mean	SD	CV	Range (%)	Mean	SD	CV
SFA	24.77-41.04	33.07	3.70	11	26.78-43.27	33.44	3.96	12
MUFA	32.99-48.91	40.92	4.79	12	33.02-47.81	40.78	4.79	12
PUFA	18.57-32.16	26.01	2.49	10	18.27-33.02	25.78	3.10	12

Statistical parameters of the NIR calibration models for fatty acid groups (% weight) of subcutaneous pig ham fat for longitudinal and transversal cuts.

	Longitudinal cuts					Transversal cuts				
	Calibration set (N=115)			Test set (N=38)		Calibration set (N=115)			Test set (N=38)	
Fatty acid	Fact	R^2	RMSECV	R^2	RMSEP	Fact	R^2	RMSECV	R^2	RMSEP
SFA	7	0.85	1.4	0.81	1.7	7	0.80	1.6	0.77	1.9
MUFA	7	0.92	1.3	0.94	1.2	8	0.88	1.7	0.93	1.2
PUFA	7	0.77	1.2	0.73	1.6	9	0.74	1.3	0.79	1.4

SD: *standard deviation.*
CV: *coefficient of variation.*
R^2: *coefficient of determination.*
RMSECV: *root mean square error of cross-validation.*
RMSEP: *root mean square error of prediction.*
Fact: *number of factors included in the model.*
SFA: *saturated fatty acids.*
MUFA: *monounsaturated fatty acids.*
PUFA: *polyunsaturated fatty acids.*

CONCLUSIONS

- ✓ **These results indicated that NIRS technology has potential as a rapid tool to discriminate carcasses from animals fed diets with different fatty acid composition by a direct measurement on the fat from the ham.**
- ✓ **The application of NIRS technology in slaughterhouses, cutting plants or meat processors would be highly profitable when selecting the raw meat according to fat quality.**

Poster Competition

Tong Qiao

Singular spectrum analysis for hyperspectral imaging based beef eating quality evaluation: a new pre-processing technique

Tong Qiao[1], Jinchang Ren[1], Jaime Zabalza[1], Cameron Craigie[2,3]
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3- AgResearch Ruakura, Hamilton, 3240, New Zealand.
4- Biomics Ltd, Inverurie, AB51 0LE, UK.

Abstract

- Hyperspectral imaging (HSI) is an emerging platform technology that integrates conventional imaging and spectroscopy to attain both spatial and spectral information from an object.
- In recent years, HSI has rapidly matured into one of the most powerful tools for food quality analysis and control.
- In the project, HSI has been applied for beef eating quality evaluation.
- Pre-processing of HSI spectral profiles is needed, in order to eliminate undesired noises.
- Singular spectrum analysis (SSA) will be demonstrated to be an effective pre-processing step in de-noising HSI spectra.

Data collection

- 211 beef samples (2.5 cm thick) of the *M. longissimus thoracis* (11th rib) were imaged at 2 days post-mortem using Gilden photonics HSI system (Fig.1).
- HSI system wavelength range: 400 – 863 nm.
- Beef eating quality is related to tenderness, juiciness and flavour.
- Slice shear force (SSF) was measured at 7 days and 14 days post-mortem using Tenderscot meat tester (Fig.2a) as the tenderness reference.
- Ultimate pH was measured at 7 days and 14 days post-mortem using Hanna meat pH meter (Fig.2b) as the flavour reference.
- Data was split into calibration set (75%) and validation set (25%) for each quality trait.

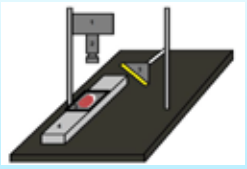


Fig.1. Schematic diagram of the HSI system.
[1] CCD camera [2] Spectrograph and lens [3] Halogen lamp [4] Sliding track [5] Scanning tray

(a) **(b)**

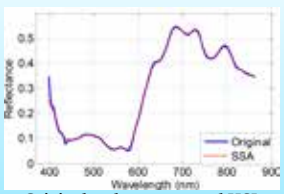
Fig.2. Instruments for measuring beef eating quality.
(a) Meat tester (b) pH meter

Table 1. Summary statistics of studied beef quality traits.

Trait		SSF7	SSF14	pH7	pH14
Calibration set	n	159	159	154	154
	Min	46.97	63.35	5.44	5.46
	Max	299.54	291.56	6.37	6.46
	Mean	131.46	132.23	5.63	5.69
	SD	48.18	42.91	0.13	0.14
Validation set	n	52	52	51	51
	Min	69.41	73.61	5.46	5.48
	Max	285.62	239.82	6.34	6.41
	Mean	130.73	131.32	5.63	5.69
	SD	45.69	39.91	0.14	0.14

Data pre-processing

- SSA is a new technique commonly used for time series analysis and forecasting.
- SSA is based on the singular value decomposition (SVD), which is able to decompose the original vector into a few independent components, including the 'clean' vector, oscillations and noise.
- Usually the 'clean' vector is located in the biggest eigenvalue (corresponding to the 1st component), so reconstruction can be done using the 1st component.
- Parameter to tune: window size L.



Window size
L = 5

Fig.3. Original and reconstructed HSI spectral profile.

Experiments and results

- Principal component analysis (PCA) was applied on the SSA treated spectra to reduce the dimensionality to 30.
- Support vector machine (SVM) was used to construct the regression model on the calibration set.
- The model performance was assessed on the validation set using coefficient of determination (R^2) and ratio of performance deviation (*RPD*).

Table 2. Performance comparison of original HSI spectra and SSA treated spectra for predicting beef eating quality attributes on the validation set.

Trait	Original spectra		SSA treated spectra		
	R^2	RPD	L	R^2	RPD
SSF7	0.1938	1.1019	2	0.3288	1.2082
SSF14	0.1001	1.0264	2	0.1104	1.0249
pH7	0.4227	1.2490	3	0.4511	1.2822
pH14	0.2785	1.1234	7	0.3419	1.2090

- In conclusion, SSA demonstrates its ability in removing noise and improving the prediction accuracy for HSI based beef eating quality evaluation.

The project is funded by
Quality Meat Scotland (QMS)
and
Hyperspectral Imaging (HSI) Centre, University of Strathclyde.

Poster Competition

M. Gispert

Estimating and modelling the growth of fat of pigs of different sexes and genotypes scanned by Computed Tomography

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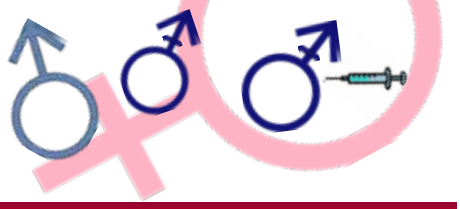
Third Annual FAIM Conference (25-26 September 2014, Denmark)

INTRODUCTION

Changes in body composition play a large role in the growth of pigs, and account for large differences among genetic types and sexes. Computer-aided tomography (CT) is a non-invasive way to study the body composition of live animals during growth, replacing serial slaughters. CT scans produce a large number of X-ray attenuation values corresponding to the various densities of the body tissues. These different densities make it possible to visualise and quantify lean mass, bone, fat and air.

OBJECTIVES

The purposes of this study were (1) to predict the amount of fat of pigs from 30 to 120 kg live weight using CT images and (2) to model the growth of fat of pigs of different genotypes (GEN) and sexes (SEX).



MATERIAL and METHODS

- Animals → Two different data sets were used. The first data set (EXP 1) included 60 gilts of three different GEN: 20 Landrace x Large White (LDxLW), 20 Pietrain x (Landrace x Large White) (PI x (LDxLW)) and 20 Duroc x (Landrace x Large White) (DU x (LDxLW)). The second data set (EXP 2) included 96 pigs of four different SEX: 12 females (F), 12 entire males (EM), 12 castrated males (CM) and 12 immunocastrated males (IM), (Improvac® was injected twice, at 12 and 18 weeks of age), all of them were Duroc x (Landrace x Pietrain).
- All the pigs were scanned at 30, 70, 100 and 120 kg, and subsets from the first experiment (15 pigs) of each genotype and target weight were slaughtered and carcass cutting and full dissection were performed to determine total fat in primal cuts (ham, loin, shoulder and belly)
- Instrumental settings: 140 kV, 145 mA ,512 x 512 matrix and 7 mm thickness (30 kg pigs) and 10 mm thickness (70, 100 and 120 kg pigs)
- Data from Exp. 1 were log transformed to normalize the variance, then subjected to stepwise elimination regression to estimate primal fat from CT measurements
- Statistical analyses were carried out in SAS (version 9.3., SAS Institute Inc, Cary, NC, USA, 2011). The final regression model included the number of pixels (NP) within the fat density range (between -149 and -1 Hounsfield values) as the range as the sole predictor, and no significant differences were observed between genotypes:

$$FAT = 0.2209 * NP^{1.0184}$$

- Primal fat was estimated for all animals and then an allometric equation was fitted using Proc Mixed model for each genotype and sex (Figure 1)
- The equation was applied to all the animals and the mean square prediction error (MSPE) was decomposed into systematic error (Table 1)

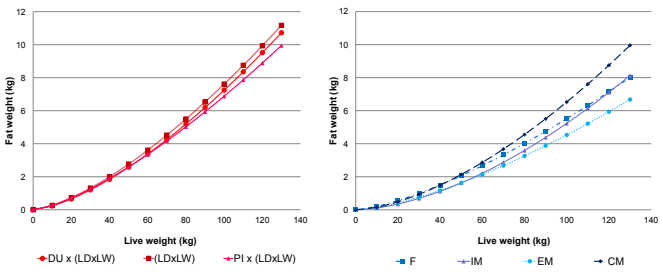
RESULTS and DISCUSSION

Table 1. Prediction error analyses from the estimated fat from GEN and SEX

Model	MSPE	T	R	RE
GEN	0.0849	0.0006	0.0017	0.9977
SEX	0.2127	0.0698	0.0006	0.9296

Errors in central tendency (T), errors due to regression (R) and errors due to random errors (RE)

Figure. 1. Allometric growth of body fat for EXP 1 (left in red) and for EXP 2 (right in blue)



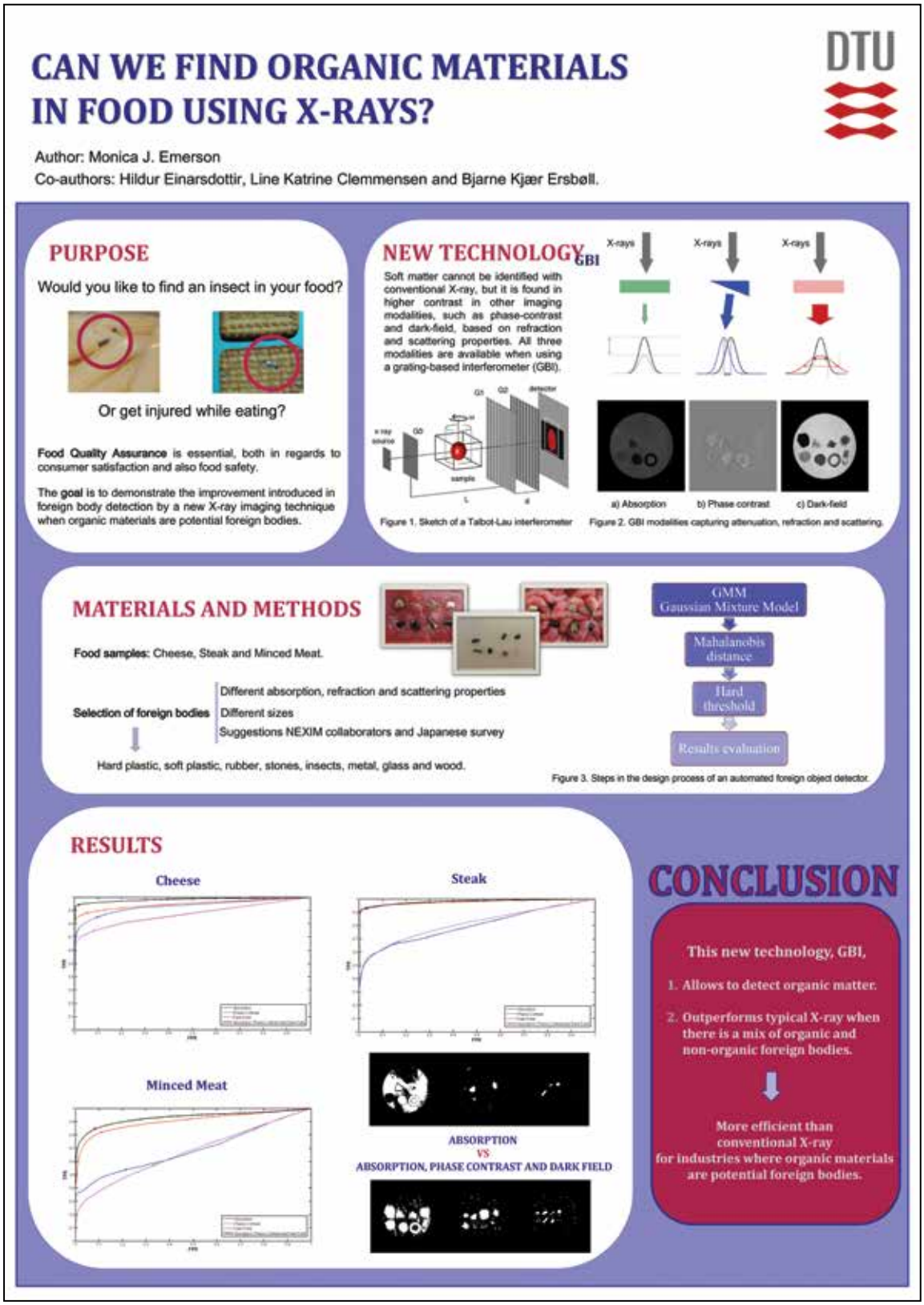
CONCLUSIONS

- Total primal fat in pigs between 30 and 120 kg body weight may be estimated from CT scan data without distinction between genotypes and sexes
- The growth of fat from birth to finishing weights is well represented using an allometric function. Further studies using different growth models are necessary

The work has been financed by INIA (Instituto Nacional de Investigación y Tecnología Agraria) through the project RTA2010-00014-00-00. INIA is also thanked for the scholarship to Anna Carabús. The authors also thank Albert Brun, Carles Francàs, Albert Rossell and Agustí Quintana for their technical assistance.

Poster Competition

M.J. Emerson



Notes

Notes

About this book

This book comprises reports of some of the activities of the COST action FAIM during 2013-2014. The papers contained in the book were presented at the FAIM meeting held in Copenhagen, Denmark in September 2014.

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EUROPEAN COOPERATION
IN SCIENCE AND TECHNOLOGY

ISBN 978-0-9931063-0-9

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FA2673_12/14