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# NON-DESTRUCTIVE DETERMINATION OF INTRAMUSCULAR FAT (IMF) IN PIGS BY MEANS OF ACOUSTIC PARAMETERS DERIVED FROM A CLINICAL B-MODE ULTRASOUND DEVICE

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## Introduction

The intramuscular fat content (IMF) of the m. longissimus (MLD) of pigs is widely regarded as an important parameter influencing sensory characteristics of porcine meat (Fernandez et al. 1999a,b; Kipfmüller et al. 2000; Eggert et al. 2002). Several authors consider an IMF content of 2 – 2.5 % (at 13th/14th lumbal vertebra / with acid pre-treatment extraction) as the optimal level in terms of sensory properties of porcine meat. (Fernandez et al. 1999) reported positive relationships up to 3,5 % IMF. Both the implementation of IMF in breeding programs in order to raise the intramuscular fat content and payment systems that also focus on quality still require an objective and reliable real-time procedure to non-destructively determine IMF in living pigs and carcasses, respectively.

Ultrasound has increasingly been investigated as a non destructive method to analyse tissue for medical applications. Compared to image analysis, spectral analysis of ultrasound echo signals provides more extensive information. (Lizzi et al. 2003) Thus, it has become one of the prominent tools for quantitative tissue characterization and differentiation of soft tissue in medical research, e.g. to classify cancer or atherosclerotic plaque (Watson et al. 2000; Raju and Srinivasan 2001; Scheipers et al. 2003).

## Objectives

The aim of this study was to investigate the use of acoustic parameters obtained by spectral analysis of ultrasound echo signals to non-destructively predict intramuscular fat content of porcine longissimus muscle.

## Methodology

The ultrasound data acquisition system consists of a clinical B-mode-device (KONTRON SIGMA 44 HVCD, C.K. Medical GmbH, D-84030 Ergolding) that is equipped with a mechanical sector scanner (KONTRON type Wobbler AA5A). The centre frequency of the probe is 3.5 MHz. The corresponding -6dB bandwidth ranges from 2.8 to 3.8 MHz. The B-mode device is equipped with an interface to obtain the backscatter data (voltages) prior to digitalisation by means of a SPECTRUM PCI.212 A/D converter-card (Spectrum Systementwicklung, D-22962 Siek) with a sampling frequency of 20 MHz.

Ultrasound data acquisition was performed about 45 min post mortem after carcass classification at hot carcasses and about 20 hours p.m. at chilled loins (with and without back fat, respectively). Table 1 illustrates the measurement conditions.

Table 1: Survey of the measurement conditions of ultrasound data acquisition.

| time | object | temperature [°C] |
|---|---|---|
| 45 min post mortem | carcass warm | 37.2 ± 2.5 |
| 20 h post mortem | loin w/backfat | 4.5 ± 1.4 |
| 20 h post mortem | loin w/o backfat | 4.5 ± 1.4 |

Scanning localization at m. longissimus was chosen with respect to the official site of carcass classification at slaughter ($13^{th}/14^{th}$ rib). The ultrasound transducer was placed about 6 cm from the middle line of the pig back towards ventral. As for scanning direction, 3 measurements each were conducted both perpendicular and parallel to the muscle fibre direction. This is with respect to reported anisotropy of acoustic parameters. Sonogel was used as coupling medium. All measurements were performed by the same person experienced in diagnostic ultrasound.

Parameter calculation was done off-line from the raw data that have been stored. Therefore, regions of interest (ROI) were selected covering approximately 8,4 cm² of longissimus muscle. The corresponding ultrasound echo signals were then transformed into spectra by means of Fast Fourier Transform (FFT). From the spectra, 60 parameters were derived that mainly describe attenuation and backscattering of the ultrasound. (Mörlein, Rosner et al. 2005) Further laboratory work aimed at both the compensation of system specific effects and user settings, e.g. time gain compensation (TGC). That included measurements on tissue mimicking phantoms and sound pressure distribution measurements by means of the thin-wire method as reported in detail in. (Brand 2004)

In total, 115 carcasses of the same genetic origin were investigated. At the site of ultrasound data acquisition one chop was removed from the longissimus muscle for chemical determination of intramuscular fat content in duplicate by means of petrol ether using Soxtherm-apparatus with acid pre-treatment. The IMF content is related to fresh matter.

Statistical analysis was done using SAS (The SAS institute, version 8e), STATISTICA (STATSOFT, version 6.1) and The Unscrambler (Camo, version 9.0) software packages. Regression and discriminant analyses were performed with stepwise procedures. The obtained acoustic parameters were averaged, i.e. 3 measurements per scanning direction each, and a total average over both scanning directions.

**Results & Discussion**

Table 2 displays descriptive statistics of the carcasses including mean and range. The chemically determined intramuscular fat content of the carcasses used here ranged from 0.71 to 3.18 %.

Table 2: Descriptive statistics of carcass and meat quality characteristics (number of observations = 115 ; s.d. = standard deviation of the mean).

| parameter | mean | s.d. | minimum | maximum |
|---|---|---|---|---|
| carcass weight [kg] | 94.8 | 7.6 | 76.8 | 113.4 |
| lean meat [%] | 55.1 | 3.1 | 45.3 | 61.4 |
| backfat width [mm] | 17.0 | 3.4 | 10.2 | 28.5 |
| muscle width [mm] | 57.3 | 5.2 | 43.7 | 69.1 |
| intramuscular fat [%] | 1.51 | 0.55 | 0.71 | 3.18 |
| protein [%] | 23.80 | 0.57 | 21.73 | 24.97 |
| dry matter [%] | 25.72 | 0.66 | 23.80 | 27.32 |
| ash [%] | 1.22 | 0.04 | 1.08 | 1.37 |

Correlations between acoustic parameters and intramuscular fat range up to r = 0.40 dependent on measurement conditions. Among the acoustic parameters there is extensive collinearity that is also confirmed by principal component analysis (not shown in detail here).

In table 3 results of multiple linear regression analyses are shown. Best results are obtained using mean acoustic parameters that are averaged from 6 measurements in both perpendicular and parallel orientation referring to the muscle fiber orientation. Thus, 60 percent of the variation of intramuscular fat content is explained by the ultrasound information, and root mean square error of prediction (SEP) amounts 0.36 % IMF. In this case, 80 % of the residuals between ultrasound predicted IMF and chemical IMF is below 0.40 IMF %. Using averages of 3 corresponding scans per scanning direction, the predictions of IMF are worse as indicated by the coefficient of determination $R^2$ (table 3). Perpendicular scans seem to outperform parallel scans in terms of predicting the fat content.

Table 3: Coefficients of determination ($R^2$) and corresponding root mean square errors of prediction (SEP) in % IMF obtained with multiple regression analysis with relation to measurement conditions.

| | | perpendicular | parallel | both directions |
|---|---|---|---|---|
| hot carcasses | $R^2$ | 0.46 | 0.24 | 0.60 |
| | SEP [% IMF] | 0.42 | 0.49 | 0.36 |
| chilled loins with backfat | $R^2$ | 0.26 | 0.26 | 0.25 |
| | SEP [% IMF] | 0.48 | 0.48 | 0.49 |
| chilled loins w/o backfat | $R^2$ | 0.35 | 0.03 | 0.10 |
| | SEP [% IMF] | 0.46 | 0.55 | 0.53 |

Much closer relations between the intramuscular fat content and the calculated ultrasound parameters, and higher precision of prediction arise when exclusively the carcasses with an IMF content greater than 2% IMF are included in the regression analyses. This indicates a detection threshold for the estimation of the IMF by means of ultrasound scans on pigs. Whether or not this is confirmed would however have to be investigated in additional tests with a much greater number of carcasses.

Discriminant analyses were performed to discriminate carcasses according to their intramuscular fat content. Threshold for fat classes HIGH/LOW was set at 2 % IMF. As seen with regression results, discrimination was most successful for data acquisitions at hot carcasses. Results are given in table 4 as relative proportion of correctly classified carcasses. Best results are obtained by use of averaged parameters derived from repeated scans in perpendicular direction at hot carcasses. i.e. 77 % of the carcasses are correctly classified.

Table 4: Relative proportion of correctly classified carcasses into HIGH/LOW fat class (threshold level: 2.0 % IMF) by means of acoustic parameters dependent on measurement conditions.

| objects | perpendicular | parallel | both directions |
|---|---|---|---|
| hot carcasses | 77 | 73 | 70 |
| chilled loins w/ back fat | 65 | 72 | 76 |
| chilled loins w/o back fat | 58 | - | 63 |

Both regression and discriminant analyses were performed by means of stepwise procedures. Regarding to the measurement conditions, the resulting functions widely vary in terms of the significant regression variables included in the models. That is probably due to the collinearity amongst the acoustic parameters. Besides further data acquisition to verify the above findings, future work is also aimed at clarifying these relationships within the spectral parameters.

## Conclusions

For the first time, comprehensive investigations on the use of acoustic parameters derived from spectral analysis of backscattered ultrasound to estimate the intramuscular fat content in porcine longissimus muscle have been reported. The data acquisition system was developed to transfer echo signals from a clinical B-mode system to a personal computer for further signal processing. Over and above the results presented here appear promising to estimate intramuscular fat in pig carcasses with ultrasound spectral parameters. The analyses carried out, and simulations in the ultrasound laboratory provide further valuable information with respect to the ideal ultrasound system if the potential of the ultrasound echo signals for characterising tissues and predicting the fat content is to be used in full. Furthermore, our study also showed that only a small share of the marketed pigs have the - from a sensorial point of view, desired - fat content greater than 2% in the cutlet (here: 17% of the carcasses).

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