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An Evaluation of Equipment and Procedures for the Prediction of Intramuscular Fat in Live Swine

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Summary and Implications

Real-time ultrasound is a valuable tool for measurement of loin intramuscular fat (IMF) in the pig and is being utilized by genetics companies. If IMF becomes a component in pork carcass pricing in the United States, producers of commercial market pigs may demand terminal line genetics with the ability to generate higher levels of IMF in their commercial progeny. Thus, swine genetics companies will need to utilize the most time-efficient and accurate methods available for predicting IMF content in live animals to stay competitive.

Results can be used as a decision making guide for equipment and procedures used for the prediction of IMF. Useful information is provided for those interested in enhancing their IMF prediction techniques.

Introduction

A decrease in intramuscular fat (IMF) over time may be partially responsible for the overall decline in pork quality that has been well documented. Research has shown that IMF is influential in determining taste, juiciness, and flavor of the pork loin, and in overall consumer acceptance and willingness to purchase pork instead of chicken (NPPC, 1995). Reports suggest that a minimum level of IMF (2.0 to 3.0%) is necessary for acceptable eating quality (Bejerholm and Barton-Gade, 1986; DeVol et al., 1988; Barton-Gade, 1990).

Regardless of the role IMF plays in the determination of pork quality, it is the only pork quality trait that has been successfully measured in live animals, allowing for identification of superior animals without sibling or progeny testing. Intramuscular fat has been reported to be moderately heritable and to be genetically associated with other indicators of meat quality (Schwab et al., 2010).

Previous research on the prediction of IMF in swine using real-time ultrasound has focused on proof of the concept (Ragland, 1998) and on refinement of prediction models (Newcom et al., 2002). Prior to this study, the accuracy of different types of equipment and procedures used for the prediction of IMF in swine has not been investigated. With the possibility of IMF becoming a trait of interest in genetic selection programs, there is a need to explore available technologies and procedures for prediction of IMF. The objectives of this study were to compare accuracy of: 1) 2 commercially available ultrasound scanners, 2) 2 image capturing devices, 3) 2 image collection methods, and 4) 3 region of interest box options.

Materials and Methods

Data description

Animals utilized for this project were from the 2008 National Barrow Show Progeny Test held at the Iowa Swine Testing Station and from the Lauren Christian Swine Research Center, Iowa State University. The population was comprised of barrows and gilts of 6 pure breeds and crossbreds (n = 454) that were scanned at a mean live weight (LW) of 115.9 kg. Ultrasound image collection was completed during 8 sessions from June through October of 2008.

Ultrasound image collection procedure

Animals were restrained in a weigh scale to facilitate image collection and soybean oil was used as a couplant between the ultrasound transducer and the skin. Scanning was accomplished by a National Swine Improvement Federation certified technician that was experienced in collection of longitudinal images. The transducer was positioned on the right side of the animal, parallel to and approximately 7 cm from the dorsal midline. The transducer was positioned by the technician to collect images that included the posterior tip of the trapezius muscle and the 10th through 13th ribs. Real-time ultrasound images were collected using 5 different combinations of ultrasound scanner, image capturing device, and image collection method.

Ultrasound scanners

Pigs were scanned with an Aloka SSD 500V (**AL**) real-time ultrasound scanner fitted with a 3.5 MHz, 12.5 cm linear array transducer (Corometrics Medical Systems, Wallingford, CT) and an Aquila Vet (**AQ**) real-time ultrasound scanner fitted with a 3.5 MHz, 18 cm linear array transducer (Esaote Europe, B.V., The Netherlands). Gain settings were: Overall, 90; Near, -25; Far, 2.1 for the AL and Overall, 255; Near, 80; Far, 1 for the AQ. The AL was set to 1.5x magnification, and focus 1 and 2 were enabled. Magnification for the AQ was set at 26 frames per second.

Image capturing devices and collection methods

A splitter device connected to the output port of the AL allowed for the attachment of 2 image capturing devices. Images were captured with a VCE Model B5A01 (Imperx, Inc., Boca Raton, FL) (**VCE**) and a Sensoray Model 2255 (Sensoray, Inc., Tigard, OR) (**SEN**). A laptop computer equipped with an image capturing and processing software package (Biotronics, Inc., Ames, IA) was connected to each capturing device and a minimum of 6 AL images were captured simultaneously with both devices. The SEN and its associated software program had video recording capabilities. After the 6 individual images were captured, the SEN was used to record a live video stream of images for 2 to 4 seconds at a rate of 15 images per second. Only the SEN capturing device and a single laptop computer were connected to the AQ scanner. The AQ and SEN combination was also used to collect a minimum of 6 individual images and 2 to 4 seconds of live video.

For individual image collection, the technician used a freeze switch to momentarily lock the frame on the scanner console monitor to be saved. For live video image collection, the technician maintained the transducer position on the animal during the recording period. The initiation and termination of the recording period was conveyed verbally from the technician to the laptop computer operator.

Meat sample collection and chemical IMF determination

Animals were harvested at Hormel Foods, Austin, MN. At 24 hours postmortem, a section of the longissimus muscle containing the 10th through 13th ribs was excised from the right side of carcasses by Iowa State University personnel. Rib sections were identified, wrapped in plastic bags, and packed in ice for transportation to the Iowa State University Meat Laboratory. At 48 hours postmortem, the spinal process, ribs, and subcutaneous fat were removed from the longissimus muscle. A 1.25 cm longissimus slice from the 10th rib end was completely trimmed of subcutaneous fat, vacuum packaged, and frozen, to be used for determination of chemical IMF percentage.

Longissimus samples were thawed, homogenized with a blender, and sampled in triplicate for the determination of total lipid content. The total lipid extraction procedure was performed on 3 homogenized samples weighing 1.95 to 2.05 g using methanol and chloroform as described by Bligh and Dyer (1959). If the coefficient of variation among the triplicate samples was greater than 10%, the procedure was repeated.

Statistical analysis

Chemical IMF percentage was used as the objective measurement of IMF to determine accuracy. Systems were evaluated for accuracy using **Bias**, standard error of prediction (**SEP**), and the absolute difference between predicted and chemical IMF percentage (**ABSDiff**).

Bias for each combination of ultrasound scanner, image capturing device, image collection method, and ROI box option was determined first:

$$Bias = \frac{\sum (P - C)}{n}$$

where P is the predicted percentage of IMF, C is the percentage of IMF determined by chemical extraction, and n is the number of observations. Bias was determined as the mean difference between predicted and chemical IMF percentage for each combination of equipment and procedures.

Bias was then used to calculate the SEP for each combination of scanner, image capturing device, image collection method, and ROI box option:

$$SEP = \sqrt{\frac{\sum (P - C - Bias)^2}{n - 1}}$$

where P is the predicted percentage of IMF, C is the percentage of IMF determined by chemical extraction, and n is the number of observations.

In order to evaluate the accuracy of the scanners, image capturing devices, image collection methods, and ROI box options while accounting for other sources of variation, 2 linear models were used to analyze the dependent variable ABSDiff:

ABSDiff = | PIMF – CIMF | .

The first model was used to compare scanners, image collection methods, ROI box options, and different system combinations that used the SEN image capturing device (Table 1).

The second model was used to compare image capturing devices, ROI box options, and different system combinations that used the AL scanner and individual image collection (Table 2).

Results and Discussion

Accuracy of the AL and AQ was similar within image collection methods and ROI box combinations, as evaluated by the SEP statistic. Standard error of prediction ranged from 1.07 to 1.33% for the AL and from 1.06 to 1.34% for the AQ. The AQ consistently overestimated IMF to a greater degree than the AL. Though the AQ generally exhibited a larger bias, the bias was beneficial to the SEP, meaning that it was consistent in direction. The Aloka 500 was more accurate ($P < 0.0001$) when the absolute value difference between predicted and chemical intramuscular fat was analyzed.

Standard error of prediction ranged from 1.07 to 1.15% for the SEN and from 1.15 to 1.24% for the VCE. When compared using 1 or 2 ROI boxes, the SEN overestimated IMF to a greater degree than the VCE, evidenced by a larger bias. The SEN had a consistently lower SEP than the VCE, indicating that the bias was consistent in direction and thus, beneficial. The Sensoray image capturing device was more accurate and offers more versatility than the VCE. The Sensoray was connected to the notebook computer through a USB port; whereas, the VCE was inserted into the video card slot. Sensoray images were digitized and displayed larger and clearer than VCE images, as assessed by the trained interpreter.

Across scanners, individual image collection had a lower bias and SEP than live video image collection. The live video image collection method restricted the scan technician's ability to control image quality. An immobilization device for restraining the pig might allow the technician to better control image quality, which could make live video collection practical. Further development and research is needed before this method will produce accuracy similar to that of the traditional individual image collection method.

Within individual image collection, the addition of a second and third ROI box reduced the bias and SEP for both scanners. Regardless of the ultrasound scanner or capturing device used, addition of a second and third ROI box resulted in incremental improvements in accuracy. Textural properties can vary within an image. Increasing the amount of information gathered per image by increasing the number of ROI boxes was a benefit to prediction accuracy in this study.

Table 1. Least squares means (± SE) of absolute difference between predicted and chemical intramuscular fat percentage by scanner, image collection method, region of interest box option, and system combination.

Effect	Least Squares Means
Scanner [1]	
Aloka 500	0.92 ± 0.032 [a]
Aquila Vet	1.03 ± 0.032 [b]
Image Collection Method [2]	
Individual	0.94 ± 0.034 [a]
Live Video	1.38 ± 0.034 [b]
# of ROI Boxes [3]	
1	0.94 ± 0.034 [c]
2	0.82 ± 0.034 [b]
3	0.75 ± 0.034 [a]
System Combination [4]	
ALISEN1	0.85 ± 0.039 [b]
ALISEN2	0.78 ± 0.039 [a]
ALISEN3	0.75 ± 0.039 [a]
AQISEN1	1.03 ± 0.039 [c]
AQISEN2	0.86 ± 0.039 [b]
AQISEN3	0.74 ± 0.039 [a]
ALVSEN1	1.28 ± 0.039 [d]
AQVSEN1	1.48 ± 0.039 [e]

Least squares means within a column and within scanner, image collection method, ROI box option, and system combination without a common superscript differ ($P < 0.05$).

[1] Comparsions were made across image collection methods and ROI box options.

[2] Comparisons were made across scanners using a single region of interest box per image.

[3] Comparisions were made across scanners using individual image collection.

[4] Abbreviations: AL=Aloka 500, AQ=Aquila Vet, I=individual image collection, V=live video image collection, SEN=Sensoray Model 2255, 1=1 ROI box, 2=2 ROI boxes, 3=3 ROI boxes.

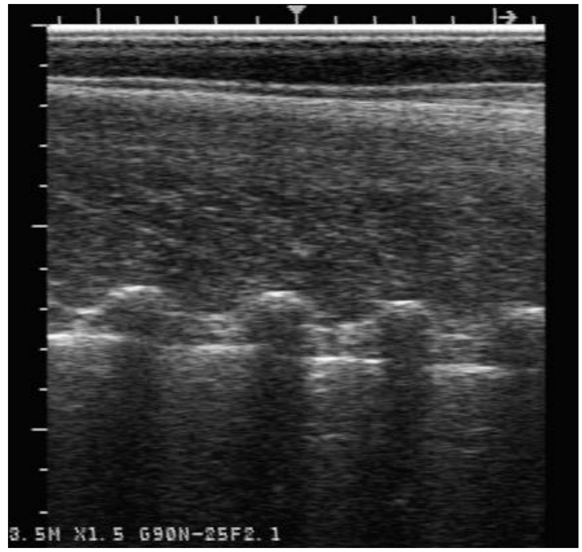

Figure 1. Typical longitudinal image taken over the 10th through 13th ribs with an Aloka SSD 500V real-time ultrasound scanner.

Table 2. Least squares means (± SE) of absolute difference between predicted and chemical intramuscular fat percentage by image capturing device, region of interest box option, and system combination

Effect	Least Squares Means
Image Capturing Device [1]	
VCE	0.84 ± 0.036 [b]
Sensoray	0.80 ± 0.036 [a]
# of ROI Boxes [2]	
1	0.87 ± 0.036 [b]
2	0.81 ± 0.036 [a]
3	0.78 ± 0.036 [a]
System Combination [3]	
ALIVCE1	0.89 ± 0.038 [d]
ALIVCE2	0.83 ± 0.038 [bc]
ALIVCE3	0.82 ± 0.038 [bc]
ALISEN1	0.85 ± 0.038 [cd]
ALISEN2	0.79 ± 0.038 [ab]
ALISEN3	0.75 ± 0.038 [a]

Least squares means within a column and within image capturing device, ROI box option, and system combination without a common superscript differ ($P < 0.01$).

[1] Comparisons were made across region of interest box options.

[2] Comparisons were made across image capturing devices.

[3] Abbreviations: AL=Aloka 500, I=Individual image collection, VCE=VCE, SEN=Sensoray Model 2255, 1=1 ROI box, 2=2 ROI boxes, 3=3 ROI boxes.

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