# Repeatability of Ultrasound-Predicted Percentage Intramuscular Fat

## Leaflet R1435

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### Summary

Repeatability of ultrasound-predicted percentage intramuscular fat was studied using observations from 144 head of cattle. Animals were scanned at an average age of 433-d by a certified technician. Each animal was scanned five times using two Aloka 500V machines. Percent intramuscular fat was then predicted by placing a box at two different positions within an image. Overall standard deviation of observations within an image was .5% and the within animal standard deviation of observations was estimated at .9%. The overall repeatability was .69. There were slight differences in repeatability when data were analyzed by machine (probe). Prediction of percentage intramuscular fat was more repeatable when boxes were placed in a "best" position rather than specifically between the 12<sup>th</sup> and 13<sup>th</sup> ribs. Evaluation of the effect of repeated measurements indicates that increasing the number of images per animal plays a significant role in reducing the standard error of prediction more so than taking more measurements within a single image.

## Introduction

Real-time ultrasound imaging has been reported to give an accurate and repeatable measure of external fat cover and ribeye area. For several years, Iowa State University has been a pioneer in the application of this technology to measure carcass merit in live cattle. The primary endeavor in this area has been the use of ultrasound for predicting the percentage of intramuscular fat in live cattle. Over the years several studies and technical measures have been made to improve accuracy of prediction. Therefore, this study is one of the trials designed to provide additional information to technicians involved in the prediction of percentage intramuscular fat. The specific objectives of the study include:

(a) Determining the repeatability of ultrasound predicted percentage intramuscular fat, and

(b) Evaluating the effect of repeated measurements on the accuracy of prediction.

## **Materials and Methods**

In this analysis data from 144 Simmental and Angus sired progeny (bulls and steers) were used. These cattle were part of a serial scan and serial slaughter project designed to evaluate sex, age, and frame size differences on carcass composition.

Cattle were ultrasonically scanned at an average age of 433 days by a certified technician using two separate Aloka 500V machines (Corometrics Medical Systems, Inc, Wallingford, Connecticut ). Each was equipped with a 3.5 Mhz, 17.2 cm linear array transducer. After entering a chute, each animal was scanned five times by each machine. Images were taken longitudinally without a wave guide across the 11<sup>th</sup> to 13<sup>th</sup> ribs of the animal at a position three fourth of the distance from the chine end of the ribeye area. Images were digitized and saved on a personal computer for latter processing. In the ultrasound laboratory, images were processed using ISU developed software. For each image percentage intramuscular fat was predicted by placing a region of interest box at two different positions: (a) the  $12^{th}$ - $13^{th}$  rib, and

(b) the "best" position, defined as an area within an image with the most uniform texture.

#### Data analysis

Initial evaluation of data was made using descriptive statistical tools. In further evaluations, components of variances were estimated based on a subset and the overall data. For the overall data the model used was,

# $Y = \mu + P + A + M + E$

Where,

Y = predicted percentage intramuscular fat

 $\mu = overall mean$ 

P = fixed effect of probe

A = random effects of animal

M = random effect of image within a probe and animal, and

E = random error

Estimation of variance components was made using the REML procedure (SAS, 1989). In all cases percentage data were used without any form of transformation.

#### Results

The overall mean predicted percentage intramuscular fat was 4.79%, with a coefficient of variation of .34 (Table 1). The mode and the median values were closer to the mean at 4.2 % and 4.5%, respectively. When rounded to the nearest

tenth of a percent, there was no difference in mean and spread of observations between probes and box positions.

Variances of random effects used in our modes are depicted in Table 2. Regardless of the model or part of the data set used, all variance estimates were significantly (p < .01) different from zero. According to the model fitted to the overall data, standard deviation of observation within an image was 0.5%. The standard deviation of observations within an image was less for probe A (.45%) than probe B (.53%). For measurements of an animal, the variance is the sum of the image and error variance. This gave an overall within animal standard deviation of 0.9%. The within animal standard deviation of observations was less for probe A (.84) than for probe B (.95). When evaluated by box position, the standard deviation of observations within animal was similar at 0.9%.

The overall repeatability of predicted percentage intramuscular fat was 0.69. Repeatability of measurements for probe A was better than probe B. These two probes are of the same make and there was no preferential procedure to account for their difference. Prediction of percentage intramuscular fat was more repeatable when boxes were placed in the "best" position rather than between the 12<sup>th</sup> and 13<sup>th</sup> rib.

In the prediction of percentage intramuscular fat using ultrasound technology, the main concern is improving accuracy of prediction for an individual animal. Hence, one of the practical implications of this analysis is that for moderately repeatable measures like ultrasound-predicted intramuscular fat, taking repeated measurements per animal can improve accuracy of prediction. Accuracy in this case refers to the standard error of animal mean measure (SEM).

If one considers results in Table 2, when the variance of observations from an animal is partitioned into components attributable to image and box position within an image (error), the image variance contributed to almost 70% of the variation, and the rest was due to a random error. Based on this data structure, there are two options to increase number of observations per animal. One can either increase the number of images per animal or make multiple measurements on an image, or both. Assuming balanced data, the standard error of an animal mean is calculated as:

SEM = 
$$\sqrt{(MS_{image})/nm}$$
  
=  $\sqrt{[\sigma_e^2 + n\sigma_{im}^2]/nm}$ 

$$= \sqrt{(\sigma_e^2 / nm) + (\sigma_{im}^2 / m)}$$

where,

 $MS_{image} = Image mean square,$   $\sigma_e^2 = error variance, and$   $\sigma_{im}^2 = image variance, and where$ n = number of observations per image, and

m = number of images per animal

Since  $\sigma_{im}^2$  is larger, increasing the number of observations per animal through multiple images can bring a much faster reduction in SEM than making more predictions within an image. Based on the estimated components of variances, the relationship between SEM and number of observation per animal is shown in Figure 1. The different lines represent the number of images (one through six). The trend within each line is due to increased sampling within an image. As expected, increased sampling within an image did not cause an appreciable reduction in SEM. On the other hand, as depicted in Figure 2, SEM reduces faster as the number of images per animal increases. However, the rate of reduction in SEM declines after a maximum reduction (29%) when the number of images increased from 1 to 2 images per animal.

Routine evaluation and updating of the prediction model is the key to further improvement in the accuracy of prediction. In addition to technician skill and choice of machine types, efforts like increasing the number of observation per head could bring a substantial contribution.

Taking repeated measures can not compensate for a faulty machine or inadequate technician training and experience. In taking repeated images per animal technicians need to follow the specific guidelines, and any improper practice may still lead to a biased estimate. After taking repeated measures, the standard deviation of observations of an individual animal needs to be looked at before calculating means. It is suggested that individual animal measures with a within animal standard deviation of one or less can be averaged to provide animal means.

## Implications

Technicians using percentage intramuscular fat developed by Iowa State University should take 3 or 4 images per animal and average the resulting predicted percentage intramuscular fat values to reduce the standard error of prediction.

	n	Mean	Sd	Min	Max
Overall	2901	4.79	1.62	1.38	13.22
Probe A B	1467 1434	4.81 4.78	1.60 1.64	1.38 1.51	11.14 13.22
Position Best 12 <sup>th</sup> -13 <sup>th</sup>	1451 1450	4.81 4.78	1.64 1.59	1.38 1.38	12.78 13.22

Table 1. Descriptive statistics for ultrasound-predicted percentage intramuscular fat data.

Table 2. Components of variances for ultrasound-predicted percentage intramuscular fat.

	Components of variances								
	$\sigma_a^2$	$\sigma^{2}_{im}$	$\sigma_{e}^{2}$	$\sigma^2_{total}$	t				
Overall									
	1.855±.23**	.579±.03**	.245±.01**	2.679	.69				
probe									
А	1.833±.23**	.506±.04**	.205±.01**	2.544	.72				
В	1.91±.25**	.609±.05**	.286±.02**	2.805	.68				
position									
Best	1.932±.24**		.807±.032**	2.739	.71				
12 <sup>th</sup> -13 <sup>th</sup>	1.772±.22**		.824±.032**	2.569	.68				

\*\* P < .01



