Accuracy of real-time ultrasound and image processing parameters to predict percentage intramuscular fat in beef cattle. M. Izquierdo<sup>1</sup>, V. Amin<sup>2</sup>, D. E. Wilson<sup>2</sup>, G. H. Rouse<sup>2</sup>. <u>S. García<sup>1</sup></u>. <sup>1</sup>Servicio de Investigación y Desarrollo Tecnológico, Junta de Extremadura, Apdo. 22. 06080 Badajoz, Spain <sup>2</sup>Department of Animal Science, 109 Kildee hall, Iowa State University, Ames, IA.

## Background

A primary factor in determining beef quality grades and, thus, market value is the amount of intramuscular fat or marbling. Currently, in the United States, marbling levels are determined by visual inspection for the amount and distribution of fat in a crosssectional area of the logissimus dorsi (ld) muscle at the 12-13th rib. Moreover, the beef industry needs an accurate and objective method to measure the actual amount of intramuscular fat in beef carcasses because quality grade is important in determining beef carcass value. Additionally, seed stock breeders need a reliable tool for selecting young bulls for carcass quality merit. Research has been undertaken to develop a tool for sorting live animals according to marbling levels. For example, Cross et al. (1983) used a video image analyzer (VIA) for beef grading. Using computer-aided tomography (CAT), Thompson (1991) applied an X-ray technique to quantify intramuscular fat content in beef, and Forrest et al. (1989) investigated the possibility of combining the technologies of ultrasound imaging, electromagnetic scanning, and the use of optical electronic fat-lean probes for on-line carcass composition measurements. After evaluation of these technologies, not all were found suitable for measuring marbling in the live animal. Ultrasound showed promise of a further development because of the following properties: 1) it has the ability to reflect fatty-tissue (Park, 1991; Whittaker et al., 1992), 2) it is completely noninvasive and easy to use in the live animal, and 3) the technology is relatively inexpensive. However, in the beginning, inconsistent results were obtained when relating ultrasound parameters and the corresponding carcass marbling score (Berlow et al., 1989; Thane et al., 1989). More recent work that combined image processing parameters (histogram, texture, and Fourier transformation) in multiple regression models or neuronal networks showed a good potential for real-time ultrasound technology to predict intramuscular fat (Wilson et al., 1992; Amin et al., 1993; McCauley et al., 1994; Brethour, 1994).

The objective of this research was to develop a robust methodology to predict the percentage of intramuscular fat (PIFAT) in the live animals.

## Methods

*Description of the data.* The *longissimus dorsi (ld)* of 820 yearling bulls and steers was scanned by using a Real-time ultrasound (RTU) machine. The average slaughter age was 440 days. After a 24-hr. chilling period, marbling was scored by a grader, and a rib facing from across the *ld* muscle at the 12th rib was obtained to determine the PIFAT.

*Equipment used.* An ALOKA 500V machine (Corometrics Medical System, Inc., Wallingford, CT) equipped with a 3.5-Mhz 17-cm linear array transducer, developed specifically for animal applications, was used to collect the images. This machine and the 17-cm transducer made it possible to scan the entire *ld* cross-sectional area. In the begining, a video home system (VHS) using a standard 1/2-inch videotape cassette recorder was used to capture and store images for the scanning performed. Lately, a portable personal computer equipped with a frame grabber board (Cortex from Image Nation, Inc., Beaverton, OR) was used to digitize the images directly at the time of scanning. Digitizing at the time of scanning saves time, enhances the quality of the image data by reducing the sources of error in the video recording process, and maintains a higher possible resolution as indicated by Zhang et al. (1993) and Brethour (1994).

*Scanning procedure*. The animals were restrained in a squeeze chute. The scanning site was determined by physical palpation of the 13th rib. Once the area across the 11th, 12th, and 13th ribs was located, the animal was clipped, oiled, and curried. Vegetable oil was used as the acoustic couplant. Two different scans of the *ld* were collected on the right side of the animal by an experienced RTU technician. The longitudinal scan was collected from across the 11th, 12th, and 13th ribs approximately 15 cm from the animal midline. A second scan was collected between the 12th and 13th ribs by using a Superflab (Nicks Radio-Nuclear Instruments, Inc., Bronx, NY) transducer guide that conforms to the general shape of curvature between the 12th and 13th ribs. This image was used to measure the ultrasound fat thickness (UFAT). The frozen image collected from the ALOKA machine, containing the date and the animal identification number, was digitized. Digitized images were preprocessed by using custom software developed for the image processing routines of PV-Wave (Precision Visuals, Boulder, CO). Image processing parameters were determined for a selected region of interest (ROI).

*Image parameters*. From the selected ROI, image processing parameters were calculated by using histogram analysis, texture analysis, and Fourier transformation, for a better description of the parameters see Izquierdo (1996).

Statistical analysis. Image parameters and actual PIFAT were statistically analyzed to select a set of parameters for regression model development. Most of the analyses were done using SAS (SAS Institute Inc., 1988). Stepwise regression procedures were used for the final variable selection to determine the prediction model (Snedecor and Cochran, 1967). After editing bad images, 710 images were randomly divided into two groups. One group (392 images) was used to develop a linear multiple regression model to predict PIFAT. The other set of 318 images was used to validate and test the accuracy of the developed prediction model. Models developed in the past (Wilson et al., 1992; Izquierdo et al., 1994a) although predicting PIFAT accurately, included parameters such as UFAT, sex, and age of the animals and were sensitive to variation in those parameters. Newly developed models included only image processing parameters invariant to age and sex of the animals. For the developing set, the root mean square error (RMSE) and R<sup>2</sup> from the regression of the actual on the predicted PIFAT and the correlation between the actual and the predicted PIFAT were used. In addition, the residuals were plotted against the predicted values to confirm that they were uncorrelated with a mean around zero and to identify outliers (MacNeil, 1983). To understand the nature of the outliers observed in the residual distribution, different categories of PIFAT were defined as the actual PIFAT less than 3%, between 3% and 6%, between 6% and 9%, and greater than 9%. Absolute

Automation and on-line meters



residual means and standard deviations were computed for each class of PIFAT. Cluster analysis procedures were used to classify images into two groups: low (less than 8% PIFAT, approximately) and high (more than 8% PIFAT, approximately). The variables used in the cluster procedures were those highly correlated with PIFAT. Once images were classified into two clusters, a different model was developed for each cluster. The models were developed and validated following the process described earlier.

## Results and discussion

Number of observations and data distribution, including mean, standard deviation, and maximum and minimum values of PIFAT, for both development and validation sets are summarized in Table 1. Both sets have similar PIFAT distributions. The diagnostic statistics are also summarized in Table 3. The RMSE and  $R^2$  were 1.43% and 0.59. The intercept and slope of the regression between the actual and predicted PIFAT was .47% (p > .1) and .97%. The intercept was not significantly different from 0, indicating that the model was unbiased. Slope was very close to 1, indicating a good model fit. Finally, correlation coefficients between actual and predicted PIFAT was .6. There are few studies reporting results for predicting PIFAT in live beef cattle. Amin et al. (1993) obtained a RMSE of 1.40% by using a multiple regression linear model with only texture parameters for a sample size of <sup>126</sup> animals. Liu et al. (1993) developed an autoregressive model based on the relationship between ultrasound speckle autocorrelation in the direction of the ultrasound waves and marbling. The  $R^2$  and RMSE of this model was .7 and .92, respectively, from a sample size of 60 animals. Izquierdo et al. (1994a) developed a multiple regression model including ultrasound fat thickness, Fourier, and histogram parameters with a RMSE of 1.17% and  $R^2$  of .5. Root mean square error and the  $R^2$  are not sufficient to fully explain the fit of the prediction models, particularly when development and validation data sets are used. Diagnostic statistics explain different aspects of the accuracy and fit of the model. RMSE equal to 1.43% indicates that 66% of the observations are going to be predicted with an error smaller than 1.43%. Slopes larger than one indicate overprediction, and slopes smaller than one indicate underprediction. The plot of the residuals versus the predicted values is a good indicator of the fit of the model and allows for <sup>visual</sup>izing the residual mean and outliers. Residual means for animals with low (0 to 3), medium (3 to 6), high (6 to 9), and very high (more than 9%) actual PIFAT are presented in Table 2. This table indicates an average residual mean of .85% and a maximum residual of 2.24% for class 2. A single Fourier parameter was able to separate data into two clusters. One cluster included animals with PIFAT values not larger than 7% (cluster1) and the other included both low and high values (cluster2). The prediction model for cluster1 included mainly Fourier parameters. Contrarily, model for cluster2 included more texture and less Fourier parameters. Results in Table 2 and 3, indicated that clustering will slightely decrease prediction error.

**Implications:** Image processing parameters obtained from RTU *ld* muscle images can be combined in a multiple regression model to predict the percentage of intramuscular-fat. This newly implemented model will allow the beef industry to consistently measure PIFAT in live animals. Predicted PIFAT values can be used to select superior animals in beef breeding programs for carcass quality.

able 1.	Data	distribution	of percentage	intramuscular fat	
forball	Juna	distribution	of percentage	intraintuseurar rat	
- Uoth	deve	lopment and	l validation da	ta sets	

All	No. <sup>a</sup>	Avg	$SD^{b}$	Min <sup>c</sup>	Max <sup>d</sup>
All data	710	4.98	2.12	1.1	14.68
Development set	392	5.02	2.19	1.1	14.09
Validation set	318	4.91	2.03	1.61	14.68

able	2.	Absolute	residual	means	for	four	classes	of
actual	PI	FAT						

r .

FIFAT <sup>6</sup>	No. <sup>c</sup>	Avg	$SD^d$	Min <sup>e</sup>	Max <sup>r</sup>	
0-3	57	1.03	.66	.10	2.82	
3-6	183	.85	.66	.003	2.78	
6-9	68	1.65	1.09	.06	2.24	
9-++	10	5.32	1.82	2.04	8.40	
0						

Actual percentage of intramuscular fat classes. <sup>°</sup>Number of <sup>0bservations.<sup>d</sup>Standard deviation. <sup>°</sup>Minimum value. <sup>f</sup>Maximum value.</sup>

<sup>Table 3.</sup> Diagnostic statistic results for PIFAT prediction and <sup>validation</sup>

	Predic	tion	I	alidation	
	RMSE <sup>b</sup>	R <sup>2c</sup>	Intercept <sup>d</sup>	Slope <sup>e</sup>	r
del <sup>a</sup>	1.43	587	.47	.97	.6
ster1	1.13	.44	.004	1.03	.60
ster2	1.51	.52	.57	.92	.65

<sup>Model</sup> developed. <sup>b</sup>Root Mean Squared Error of prediction. <sup>c</sup>Coefficient of determination. <sup>d</sup>Intercept of the regression between actual and <sup>predicted</sup> PIFAT values. <sup>c</sup>Slope of the regression between actual and <sup>predicted</sup> PIFAT values. <sup>f</sup>Correlation between actual and predicted <sup>PIFAT</sup> values.

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