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PREDICTION OF CRUDE FAT CONTENT BY IMAGE ANALYSIS

Prediction of Crude Fat Content of Longissimus Muscle of Beef Using the Ratio of Fat Area Calculated by Computer Image Analysis: Comparison of Regression Equations for Prediction using Different Input Devices at Different Stations

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ABSTRACT Crude fat content of Longissimus dorsi (ribeye) muscle of beef cattle was predicted from a ratio of fat area (RFA) to area of ribeye muscle calculated by computer image analysis (CIA). Cross sections of 64 ribeyes taken from the 6-7th rib from cattle at experiment station A and cross sections of 94 ribeyes taken from the 6-7th rib from cattle at experiment station B were used in this study. Slices (1 to 1.5 cm thickness) of just the Longissimus dorsi were homogenized and sampled for chemical estimation of crude fat content using petroleum ether. Crude fat content was estimated from each muscle sample using petroleum ether and was used as the true estimate of fat content. A CCD (Charge-Coupled Devices) camera was used as the input device at experiment station A, while a single-lens reflex camera was used at experiment station B to take photographs of ribeyes for CIA. The contour comparison method, that assigns a threshold value for each marbling particle, was used to obtain accurate binarization in this study. Minimum and maximum of chemical measurements of crude fat were 2.1 and 39.8%, and for CIA calculation of the RFA were 6.1 and 56.8%, respectively. This range covered almost complete range of the Beef Marbling Standard which is used in carcass grading in Japan. The equation for the regression of the crude fat content (Y) on RFA (X) calculated by CIA for all of the data was $Y=.793X-3.04$ with $r^2=.96$. Regression equations for prediction of crude fat percentage from RFA taking into consideration the effect of experiment station were $Y=.741X-2.22$ with $r^2=.91$ for experiment station A, and $Y=.782X-2.54$ with $r^2=.91$ for experiment station B. Analysis of covariance showed that the effects of experiment stations on intercepts and slopes were not significant ($p>.10$). The ranges of differences between actual and predicted crude fat content from the prediction equation, which was calculated without consideration of the effect of station were -6.4 to 4.0%. CIA of cross sections of the ribeye muscle seems to have potential for prediction of crude fat content.

Key words Computer image analysis, Fat area, Crude fat, Carcass

Introduction

Generally, marbling is evaluated macroscopically by a qualified grader at the time of grading. Crude fat content in beef is often measured in order to evaluate marbling more objectively in the case of feeding trials and progeny testing (Savell et al., 1986; Herring et al., 1998). However, sampling of meat for chemical analysis reduces the carcass value and requires a great deal of labor for processing.

There are some reports on prediction of crude fat in beef using non-destructive methods such as near-infrared reflectance spectroscopy. High accuracy of predicted crude fat content by near-infrared reflectance spectroscopy was reported for minced meat and cut meat by Roberts et al. (1987) and Mitsumoto et al. (1991), respectively.

A digital image with high resolution can be used for computer analysis with the development of information processing equipment in recent years. This development has created an environment that allows accurate image analysis. The most important step in image analysis is to obtain a correct threshold value, which divides lean and marbling. Kuchida et al. (1997a) developed software for image analysis using the contour comparison method. This software automatically draws contours of marbling particles for a specified area on the computer screen that displays the original true color image of the ribeye area. If the contours are judged to be wrong, it is possible to adjust the contours until they agree with those on the true color image. Kuchida et al. (1998) reported that the ratio of fat area (RFA) to area of ribeye muscle obtained with this program could be used as a linear covariate to predict crude fat percentage in ribeye muscle with high precision (r^2=.91) and accuracy (error of prediction within ±3%). However, the image data used in their study were taken by CCD camera as an input device at only one laboratory. They also did not examine prediction error when using an optical camera that is widely used to take photographs of meat. Moreover, the range of crude fat percentage of their material was quite low compared to that of most Wagyu cattle. The purposes of this study were 1) to analyze image data taken from two input devices; i.e., one was a CCD camera (using micro electronics devices) and the other was an optical camera, and 2) to investigate the ability of regression equations to predict crude fat percentage from RFA in the cross section of the ribeye.

Materials and Methods

Materials and photographing method at experiment station A

The materials were 64 Longissimus muscles (ribeye) and their cross sections from a cut at the 6-7th rib which is the standard location for measuring marbling in Japan. These were obtained from 35 Japanese Black, 6 Angus and 23 F_1 crossbred of Japanese Black sires and foreign breed dams. After slaughter, the materials (about 0.5 kg) were vacuum packaged and transported to Ouu Station, National Livestock Breeding Center (Shichinohe-machi, Japan) under low temperature storage (at 0 °C, not frozen). A CCD camera (SONY: DXC930) was used to photograph the cross-section at the 6-7th rib after the sample was kept for at lease 12 h in a refrigerator at 0 °C. Care was taken to ensure temperature of the meat surface did not increase during photographing. The CCD camera was mounted perpendicular to the meat surface. A zoom lens (SONY: VCL712BXEA) was used to take as large an image as possible.

The image resolution from this equipment was 512×480 pixels (about 740 K bytes for bitmap file).

To determine crude fat percentage, the whole ribeye of each sample was separated and trimmed from the intermusclar fat, then each ribeye was sliced to 1 cm in thickness and minced for analysis. Chemical measurement of the crude fat percentage was performed by ether extraction method (AOAC; 1990).

Materials and photographing method in experiment station B

The materials used were 94 Longissimus muscles and their cross sections from a cut at the 6-7th rib from Japanese Black beef bought at retail markets. Photographs of the ribeye were taken using a single-lens reflex camera (Minolta: α707si) with as large an image as possible of the ribeye area at Hiroshima Prefectural Animal Experiment Station (Shoubara-shi, Japan). The sample was kept for at least 3 h in a refrigerator at 4 °C. A strobe (Minolta: Program Flash 5400HS) with soft lighting (Minolta: Soft Lighting Set) was used from an angle of 45 ° to the surface to avoid irregular reflection on the surface of ribeye. Photographs were taken within 5 minutes after removing from the refrigerator to ensure temperature of the meat surface did not increase much during photographing. The camera was mounted perpendicular to the meat surface. Images were printed on photographic printing papers (12 by 8 cm) and were scanned using a color image scanner (Epson: GT-8500). Resolution from this equipment was about 800×600 pixels (about 1.6 M bytes for a bitmap file). Crude fat was determined by the same method as previously described.

Image analysis

The program for the computer image analysis (CIA) was written in Visual C++ (Microsoft) which is the 32 bit application development language under the Windows NT operating system.

The greatest influence on the precision of calculation of marbling percentage is the process of converting color image to binary image (0 or 1). This process divides the color image into two values (i.e., 0 or 1 to indicate lean and fat, respectively). Discriminant analysis (Otsu, 1980), which is generally used for automatically converting the color image to a binary image, may result in over- or underestimation due to the lack of uniformity of lighting, if only one threshold value is used in the conversion for the whole ribeye area. To avoid this error, an adaptive converting method (Takagi and Shimoda, 1991) has been proposed, which mechanically divides the whole image into several partitions, with calculation of threshold values for each partition. However, the brightness of the marbling particle depends not only on the illumination by reflected light, but also on size of marbling particle. Thus, it is impossible to obtain accurate RFA if the calculation is done separately for each partition.

The contour comparing method (Kuchida et al., 1997a), which assigned a threshold value for each marbling particle (if the particle was very large with irregular contours, the particle was divided into several areas), was used to obtain accurate binarization in this study. Contours of marbling particles are automatically drawn for the specified area on the computer screen that was displaying the original true color image of the ribeye area. If the contours are judged to be wrong, it is possible to adjust them until they agree with those on the true color image. The coincidence between drawn contours and contours seen on the true color image is judged macroscopically. Each pixel has 0 to 255 signals for Red (R), Blue (B) and Green (G) components in this system. The G component is used for binarization because the variance of the G component was the largest for this photographing situation.

The subject of the image analysis of this study was the inside of Longissimus muscle. A contour line of Longissimus muscle was manually drawn by operator using drawing software (Adobe Photoshop, Adobe Systems Inc., Seattle, WA) before image analysis process. Particles with small areas of less than .01cm^2 were excluded in the analysis for the purpose of reducing noise caused by binarization.

Statistical analysis

The mathematical model used to predict crude fat content from RFA calculated by CIA is:

$$Y = aX + b \qquad \text{[Model 1]}$$

where Y is crude fat content, a is the slope of the linear regression equation of crude fat content on RFA, X is RFA of each sample, and b is the intercept.

Homogeneity of slopes and intercepts of the regression equations by station was examined using the following model:

$$Y_i = a_i X_i + b_i \qquad \text{[Model 2]}$$

where, Y_i is crude fat content of the i^{th} experiment station, a_i is the partial regression coefficient for the i^{th} experiment station, X_i is RFA from the i^{th} experiment station, and b_i is the intercept of the i^{th} experiment station.

The effects of breed groups were not included in the mathematical models, although several breed groups were used at station A. Effects due to breed groups have been shown to be not significant (Kuchida et al., 1998). The GLM procedure of SAS (1989) was used for statistical analysis.

Results and discussion

Table 1 contains unadjusted statistics for ribeye area, chemical measurements of crude fat and CIA calculations of the RFA for each station. Marbling scores in Japan are assigned by comparison to the Beef Marbling Standard (BMS), which has 12 marbling levels. Kuchida et al. (1997b) reported RFA for BMS No.1 and No.12 were about 0 and 50%, respectively. The range of RFAs for beef sample from experiment stations A and B covered the range of RFA for all levels of BMS.

The size of the image data from experiment station A (760K bytes; bitmap file) was different from the size of the image data file from experiment station B (1.6M bytes; bitmap file). Kuchida et al. (1997a) examined the difference in calculated RFA due to resolution using image data with three different resolutions which were processed from one original image. They found no differences among the RFAs calculated from image data files of three sizes: 170 K bytes, 980 K bytes and 2.5 M bytes (bitmap file).

The relationship between chemically measured crude fat percentage and RFA calculated by CIA is plotted in Figure 1. The regression equation (model 1) obtained for prediction of crude fat percentage from RFA without and with accounting for the effect of experiment station were:

$Y=.793X-3.04$ with $r^2=.96$ for overall,

$Y=.741X-2.22$ with $r^2=.91$ for experiment station A, and

$Y=.782X-2.54$ with $r^2=.91$ for experiment station B.

These prediction equations indicated the relationship between chemically analyzed crude fat content and RFA by CIA was linear, as quadratic and cubic terms were not significant. Analysis of covariance using Model 2 showed effects of experiment stations on intercepts (Station A;-2.22, Station B;-2.54) and on slopes (Station A;.741, Station B;.782) were not significant ($p>0.10$).

Prediction errors were obtained subtracting actual crude fat content from predicted crude fat content using the prediction equations (Model 1 or Model 2) and are summarized in Table 2. The range of prediction error from Model 1, which did not consider of the effect of station, were from -2.2 to 3.0% for experiment station A, and from -6.4 to 4.0% for experiment station B, respectively. Ranges from Model 2, which considered the effect of station, were -2.5 to 2.8% for experiment station A, and -6.4 to 4.0% for experiment station B.

The method of predicting crude fat content described in this study was not influenced by joint effect of experiment station and input devices, as differences between intercepts and slopes due to stations with Model 2 were not significant ($P>.10$). Ranges of prediction errors from Model 1 and Model 2 also were similar.

The RFA increases about 3% for each level of the standard scale from BMS No.1 to No.10 and increases about 10% for each level from BMS

No.10 to No.12 (Kuchida et al,; 1997b). The proportion of prediction errors from Model 1 that were within ±3.0% was .930.

Possible causes of prediction error were examined for the ribeyes (n=11) with prediction errors from Model 1 larger than 3%. These cross sections were found to contain large marbling particles with these areas greater than 4.0cm^2 and to be in contact with the periphery of ribeye for eight of the 11 samples.

For chemical analysis for crude fat, samples were sliced 1 cm (1 to 1.5 cm for experiment station B) thick from a cross section of the ribeye area and then were minced. For prediction of crude fat from the RFA calculated by CIA, it is assumed that RFA on the surface of the ribeye is the constant through 1 cm (or 1 to 1.5 cm) thickness, in reality, the ratio is not constant. Masses of fat which could not be seen on the surface of the photographed ribeye could be seen when the material was ground, although these data were not recorded. Violation of this assumption might be one of the primary causes of prediction error. Accuracy of estimation might be improved if more thinly sliced meat was used. This factor might be one of the greatest causes of prediction error.

Kuchida et al.(1999) attempted to evaluate marbling score by CIA. Japanese Marbling Standard was highly correlated to RFA by image analysis with r^2=.47. They succeeded to predict Japanese Marbling

Standard using several parameters from CIA (fineness, distribution of marbling within Longissimus muscle, etc.). According to their results, RFA is a main effect for Japanese MS, although Japanese MS would be affected by other image analysis traits.

Implications

This study showed that two combinations of input devices, photographing techniques or size of image data file were not different for prediction of fat content from ratio of fat area to total area of a cross section of the Longissimus muscle. With this method, the cross section of the carcass must be photographed in a perpendicular direction. This drawback could be solved by improvement in input devices for photographing the cross section of the ribeye. Advantages of this method are that no special device is needed and a photograph taken of the ribeye area in a past examination can be used. Differences among the results due to human carcass graders can not be removed, because marbling score is evaluated macroscopically. If CIA could be used to gather data for marbling evaluations from progeny testing and feeding trials, the crude fat content predicted by CIA could be a reference standard for level of marbling.

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Table 1. Unadjusted means and standard deviations for rib-eye area, crude fat content and ratio of fat area by experiment stations

	Station A (n=64)			Station B (n=94)		
	Mean±SD	Minimum	Maximum	Mean±SD	Minimum	Maximum
Rib-eye area (cm^2)	41.3±8.7	21.8	69.4	50.1±6.1	37.3	66.7
Crude fat (%)	11.8±4.4	2.1	27.1	24.7±6.7	9.6	39.8
Ratio of fat area (%)	19.0±5.7	6.1	36.1	34.9±8.1	13.0	56.8

Table 2. Summary of basic statistics of errors[a] of prediction of crude fat content from ratio of fat area using two prediction equations

	Model 1[b]		Model 2[c]	
	Station A	Station B	Station A	Station B
Mean (%)	-.17	.11	.01	-.01
Standard deviation (%)	1.34	2.05	1.31	2.05
Minimum (%)	-3.02	-3.96	-2.81	-4.04
Maximum (%)	2.18	6.44	2.53	6.43

[a]Prediction error is the difference between predicted and actual crude fat content.

[b]Model 1 did not account for the effect of station.

[c]Model 2 was calculated taking into consideration the effects of stations.

Figure 1. Relationship between crude fat content measured by ether extraction method and fat area ratio calculated by computer image analysis of the rib-eye image from two different experiment stations