Food Structure

Volume 11 | Number 1 Article 6

1992

Image Analysis to Determine Intramuscular Fat in Muscle

T. Ishii

R. G. Cassens

K. K. Scheller

S. C. Arp

D. M. Schaefer

FOOD STRUCTURE, Vol. 11 (1992), pp. 55-60
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

1046-705X/92$3.00+.00

IMAGE ANALYSIS TO DETERMINE INTRAMUSCULAR FAT IN MUSCLE

T. Ishii[1], R.G. Cassens[2], K.K. Scheller[2], S.C. Arp[2] and D.M. Schaefer[2]

[1]Dept. of Animal Science, Faculty of Agriculture, Kyoto Univ. Kyoto, Japan

[2]Meat and Animal Science Dept., Muscle Biology Laboratory, 1805 Linden Drive
University of Wisconsin, Madison WI, 53706

Abstract

The area of intramuscular fat in Holstein steer longissimus was determined using an image analyzing system. Slaughter weights of 500, 636 and 773 kg differed ($p < 0.05$) for intramuscular fat area, marbling score, and ether extractable lipid. Repeated measurements of intramuscular fat area in a given section showed high accuracy. However, comparing two sections from the same sample, there was often a large difference in fat content between the sections. Fat content determined by the imaging system was correlated significantly with marbling score ($r = 0.49$) and ether extractable lipid ($r = 0.34$). Sampling is critical, and in order to obtain a high correlation several samples would be required from each muscle.

Key Words: Image analysis, Intramuscular fat, Marbling score, Slaughter weight.

Initial paper received July 18, 1991
Manuscript received December 9, 1991
Direct inquiries to R.G. Cassens
Telephone number: 608 262 1792
Fax number : 608 262 5157

Introduction

Image analyzing systems provide a means for quantitative evaluation of microscopically viewed sections. Data can be obtained and processed rather rapidly. Therefore, such systems have been used for studies in which the size distribution of either adipocytes or muscle fibers was determined morphometrically (Hermansson, 1987; Ishii *et al.*, 1990).

In beef muscle, there has been a continuing interest in quantifying visible fat (marbling) because it is an important factor in determining the USDA grade (Orme *et al.*, 1958; Blumer and Fleming, 1959; Moody and Cassens, 1968; Melton *et al.*, 1974; Dikeman *et al.*, 1986; Renk *et al.*, 1986). The USDA grading standards, however, still contain factors that are never measured objectively. Hence, more objective means of determining grades have been sought. Image analyzing systems have been tested by Cross *et al.* (1983) and Wassenberg *et al.* (1986) who reported that those systems had considerable potential as a yield-grading device.

Our objective was to establish the accuracy of an image analyzing system in determining intramuscular fat from histological sections, and to determine if such histologically determined fat was correlated to either visually assessed marbling score or ether extractable lipid.

Materials and Methods

Twenty Holstein steers in each of three different slaughter weight classifications (500, 636 and 773 kg) were used. Following normal slaughter, chilling and processing procedures, carcasses were evaluated by an experienced grader according to current USDA quality and yield grading standards. Subcutaneous fat thickness; percentage of kidney, heart and pelvic fat; marbling score; yield; and quality grades, were determined in each carcass (see Table 1).

Image analysis: A single 7 mm diameter core was taken from the central area of the Longissimus dorsi

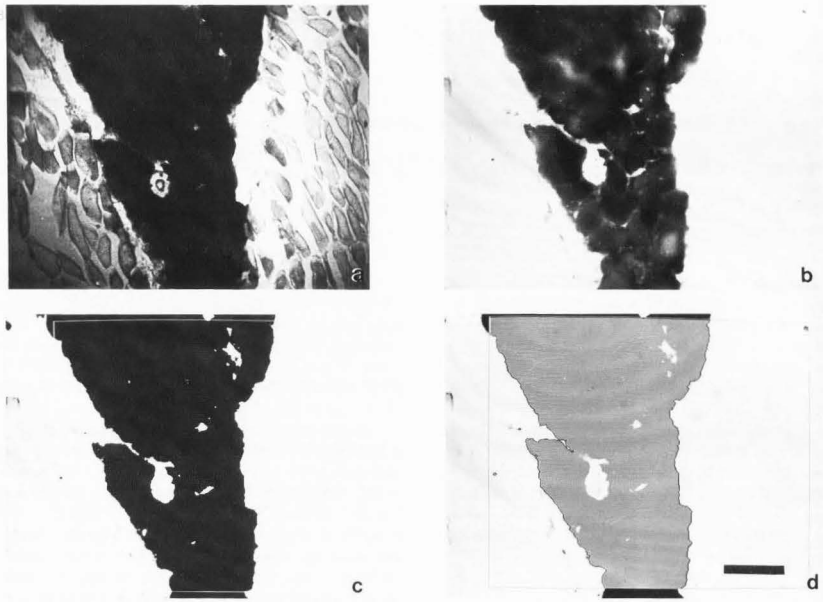

Figure 1. Images on the monitor screen of the imaging system: **a**) a live image as a source image (black area is fat); **b**) a digital image with enhanced contrast by adjusting digital illumination (almost all of cross-sectioned muscle fibers become invisible); **c**) a binary image (an object image of interest to measure) before measurement; **d**) the binary image after measurement. A box on the screen shows a window. Measured area inside a window has changed its color. Bar = 200 μm.

Table 1. The averages of the area of intramuscular fat, marbling score, ether extractable lipid, and other variables.

Variable	Slaughter weight (kg)			Standard Error (S.E.)
	500	636	773	
Number of cattle	20	18	20	
Fat area (%)	4.7^{a}	$9.5^{a,b}$	11.0^{b}	1.1
Marbling score	11.1^{a}	15.6^{b}	18.4^{c}	0.6
Ether extractable lipid (%)	4.2^{a}	8.2^{b}	9.9^{c}	0.5
Subcutaneous fat thickness (cm)	1.5^{a}	2.2^{b}	2.7^{c}	0.1
Kidney, Heart and Pelvic fat (%)	3.1^{a}	3.6^{b}	6.0^{c}	0.2
USDA Yield grade	2.8^{a}	3.6^{b}	5.1^{c}	0.2
USDA Quality grade	13.0^{a}	14.6^{b}	15.5^{c}	0.2

^{a,b,c} : Means with different superscripts in the same row are significantly different ($p < 0.05$).

Table 2. Repeatability of measurements and comparison of area value between two sections in the same sample.

Animal	1		2		3		4		5		6	
Section	1	2	1	2	1	2	1	2	1	2	1	2
1[a]	13.4	13.2	2.5	6.3	1.2	6.2	25.1	52.2	8.0	67.9	6.6	3.5
2	11.7	13.3	2.6	6.6	1.0	5.9	26.7	49.8	8.3	63.3	6.6	3.2
3	14.5	13.5	2.7	6.6	1.1	7.3	27.6	50.9	8.7	63.3	5.9	3.7
4	13.4	13.0	2.5	6.9	1.1	7.3	26.3	52.1	9.0	61.3	6.1	3.6
5	14.4	13.7	2.8	6.5	1.5	5.9	26.9	53.3	9.1	63.1	6.5	3.4
Mean	13.5	13.3	2.6	6.6	1.2	6.5	26.5	51.7	8.6	63.8	6.3	3.5
S.D.	1.1	0.3	0.1	0.2	0.2	0.7	0.9	1.3	0.5	2.5	0.3	0.2
C.V.	0.08	0.02	0.05	0.03	0.16	0.11	0.03	0.03	0.05	0.04	0.05	0.06

[a] : Each section measured five times.

muscle located caudally from the 12-13 rib junction. The core was cylindrical and its longitudinal axis almost paralleled the run of muscle fibers. Cores from all samples were fixed in 10% formalin, frozen-sectioned, and stained with Sudan Black B. The area of intramuscular fat was determined using a Universal Imaging Corporation system with Image-1 software. Digital fat images were made from source images obtained through a microscope equipped with a high sensitive video camera. The image analyzing system processed the digital images into binary images (object images of interest to measure) and then measured area of the binary images (Fig. 1). The measuring window size of 1059 μm x 849.4 μm occupied 72% of the monitor screen. The full size of the screen was not used because there was image distortion at the periphery. Entire sections were scanned in a predetermined pattern (Fig. 2). The number of fields measured per section ranged from 19 to 40. Measurements were repeated five times on each of two sections from a given sample in an attempt to assess repeatability of measurement.

Chemical analysis were performed in triplicate similarly to those described by Faustman *et al.* (1989). Approximately 3 grams of meat was placed on a preweighed piece of Whatman no. 1 filter paper which had been previously dried in a 60 °C oven. The paper was folded over the meat, and this sample was then dried to a constant weight in a 110 °C oven for 15-18 hours. Samples were then removed, cooled in a desiccator for 3 hours and weighed. Dried samples were then loaded into a Soxhlet extraction apparatus filled with fresh ethyl ether. Batches of dried samples were each extracted for 48 hours. Following extraction, samples were dried in

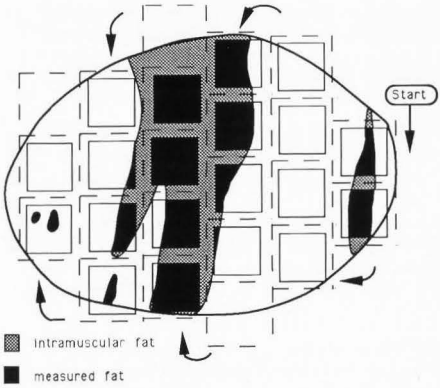

Figure 2. Typical scanning pattern. The size of the measuring window was much smaller than that of tissue sections. Measuring was always started from the right side at the top of tissue sections. If the window was one-half or more filled, it was measured. Solid line denotes perimeter of a tissue section. A solid line box describes the border of a measuring window. A dashed line box outside the measuring window indicates the full size of monitor screen of the imaging system. Arrows indicate a scanning sequence.

the 110 °C oven overnight and placed in a desiccator to cool for 3 hours. Samples were reweighed and percent ether-extractable lipid calculated on a fresh weight basis.

Intramuscular fat area, ether extractable lipid, and carcass grading results were analyzed with the SAS general linear models procedure.

Results

Repeated measurements of a given section showed that measurements could be made accurately (Table 2), and the coefficient of variance was small, ranging from 0.02 to 0.16. There usually was, however, a large difference in area of intramuscular fat between two sections from the same sample. The difference of the means of five repeated measurements between two sections ranged from 2 to 7 times.

There were significant differences ($p < 0.05$) among slaughter weights for intramuscular fat area, marbling score, ether extractable lipid, and other carcass characteristics (Table 1). This shows that fat content in muscles increased with heavier slaughter weights. With the exception of subcutaneous fat thickness, the area of intramuscular fat determined by the imaging system was correlated significantly ($p < 0.01$) with marbling score ($r = 0.49$), ether extractable lipid ($r = 0.34$) and other carcass characteristics (Table 3), but accounted for only 10 to 24 % of the observed variation in these variables.

Discussion

Fat content determined by the imaging system was correlated significantly with marbling score and ether extractable lipid although the correlations were not great. Comparing two sections from the same sample, it was shown that fat content was quite different (Table 2). This suggested that fat content would vary in the same sample from location to location. Therefore, it was thought that this variation in location made the correlation low. Measurement of only one location could not estimate accurately the fat content in muscle. Thus, sampling is critical.

The effect of slaughter weight was significant for intramuscular fat area, marbling score and ether extractable lipid because the number of animals measured was large enough to reveal the different amounts of intramuscular fat among slaughter weights.

Either trapped bubbles or non-specific stain, which could not be eliminated, hindered resolution in the binary image and also caused errors in measurement. This made the error of repeated measurements larger in some sections. The coefficient of variance was, however, less than 0.05 in many sections. Therefore, it was thought that measurements could be made accurately.

Table 3. Correlations between the area of intramuscular fat and other variables.

Variable	Correlation
Slaughter Weight (kg)	0.33*
Marbling score	0.49**
Ether extractable lipid (%)	0.34**
Subcutaneous fat thickness (cm)	0.13
Kidney, Heart and Pelvic fat (%)	0.42**
USDA Yield grade	0.31*
USDA Quality grade	0.49**

* $P < 0.05$; ** $P < 0.01$.

In order to obtain a high correlation more than three histological samples would be required from each muscle. Measurement of fat content using the imaging system was useful for revealing the increasing intramuscular fat with heavier slaughter weight. For effective use of this imaging system, one of the most important things was to prepare sections in which intramuscular fat optically contrasts with muscle fibers. Sudan Black B staining was enough to provide a contrast to fat against background. Therefore, it was possible to acquire easily a clear-cut image of intramuscular fat from samples, and thus, to quickly measure the area of intramuscular fat. Measurement times for each field and section were about 20 seconds and 15 minutes respectively. Thus, this system would be valid to determine visible fat in muscle. In the future, ability to analyze a wider range of intramuscular fat content and distribution pattern of marbling is expected.

Acknowledgments

This work was supported by the College of Agricultural and Life Sciences of the University of Wisconsin-Madison, the Cattlemen's Beef Promotion and Research Board in cooperation with the Beef Industry Council of NLMB, the Wisconsin Beef Council, and Packerland Packing Co., Green Bay, WI.

References

Blumer TN, Fleming HP. (1959). A method for the quantitative estimation of marbling in the beef rib eye muscle. J. Anim. Sci. **18**, 959-963.

Cross HR, Gilliland DA, Durland PR, Seideman S. (1983). Beef carcass evaluation by use of a video image analysis system. J. Anim. Sci. **57**, 908-917.

Dikeman ME, Reddy GB, Arthaud VH, Tuma HJ, Koch RM, Mandigo RW, Axe JB. (1986). Longissimus muscle quality, palatability and connective tissue histological characteristics of bulls and steers fed different energy levels and slaughtered at four ages. J. Anim. Sci. **63**, 92-101.

Faustman C, Cassens RG, Schaefer DM, Buege DR, Williams SN, Scheller KK. (1989). Improvement of pigment and lipid stability in Holstein steer beef by dietary supplementation with vitamin E. J. Food Sci. **54**, 858-862.

Hermansson AM. (1987). Meat raw materials in comminuted meat products. 33rd Internatl. Cong. Meat Sci. Tech. Proc. **2**, 290-293.

Ishii T, Ishibashi T, Mitani K. (1990). Variation in cross-sectional areas of muscle fibers of three body weight groups in skeletal muscles of cattle. Jpn. J. Zootech. Sci. **61**, 695-700.

Melton CC, Dikeman ME, Tuma HJ, Schalles RR. (1974). Histological relationships of muscle biopsies to bovine meat quality and carcass composition. J. Anim. Sci. **38**, 24-31.

Moody WG, Cassens RG. (1968). A quantitative and morphological study of bovine longissimus fat cells. J. Food Sci. **33**, 47-52.

Orme LE, Pearson AM, Bratzler LJ, Magee WT. (1958). Specific gravity as an objective measure of marbling. J. Anim. Sci. **17**, 693-698.

Renk BZ, Schaefer DM, Kaufman RG. (1986). Age and deposition of intramuscular fat in the longissimus of Holstein steers. Univ. Wisconsin - Madison, College Agric. Life Sci., Res. Report R3389.

Wassenberg RL, Allen DM, Kemp KE. (1986). Video image analysis prediction of total kilograms and percent primal lean and fat yield of beef carcasses. J. Anim. Sci. **62**, 1609-1616.

Discussion with Reviewers

H.W. Swatland: More information is needed on the orientation of the cores used as samples since, from earlier work, it is known that orientation is important. In the Figures, muscle fibers appear to have been sectioned obliquely. In general, it is likely that transverse sections will be superior to longitudinal sections, hence the need to know the orientation of the sections examined in this study.

Authors: In our study, measurement of intramuscular fat, but not muscle fibers, was the goal. We believe, therefore, that even sections in which muscle fibers have been sectioned obliquely are still suitable to determine the fat.

H.W. Swatland: How was the field size used in the study chosen? Does field size affect the results? Is there any way to improve on the correlations reported here?

Authors: We indicated in the Materials and Methods section that the distorted peripheral image must be excluded from the field. Although the image analyzing system used measures any field size, it is limited by the condition that the ratio of field area to that of the monitor screen is an integer and ranges from 60 to 80%. Of the 4 fields from which to chose the size of 1059 μm x 847.4 μm (the ratio was 72%) was selected.

The field size of 1059 μm x 847.4 μm was compared with the size of 800 μm x 500 μm (the ratio was 39%) to determine the effect of field size. The same sections from 30 samples were measured using the different field sizes. The difference of fat area between two fields in the same section ranged from 0 to 2.4%. There were no marked differences between this range and the variation in repeated measurements of the same section shown in Table 2. Therefore, it was thought that the effect of field size on the result was small.

The most effective way to improve on the correlations would be to sample more locations from the muscle.

S.H. Cohen: Why did the authors provide only 2 sections from a given sample?

Authors: We believe that measuring only two sections from a given sample would suffice to prove that fat content would vary in the same sample from location to location.

S.H. Cohen: Was rotary evaporation used as part of the extraction procedure?

Authors: No.

S.H. Cohen: What is meant by "fresh weight basis"?

Authors: Each meat sample was weighed three times during the extraction procedure giving the weights of fresh meat, dried meat before ether extraction, and redried meat after ether extraction. The formula for calculating the amount of ether extractable lipid was: Lipid = (dried meat weight - redried meat weight) / fresh meat weight. Then, "fresh weight basis" means the weight of fresh meat used as a denominator in the above relation.

S.H. Cohen: What is the SAS general linear models procedure?

Authors: SAS is short for Statistical Analysis System which is a commonly used statistical analysis program package. The SAS general linear models procedure provides analysis of variance and regression analysis. It is indispensable for analysis of multi-way layout designs

with either many factors or missing data, since a conventional statistical analysis program can not analyze data from such a design.

F.W. Comer: The results and/or problems of applying a microscopic technique to a macroscopic application are predictable. Can the technique used be modified to effectively scan a much larger carcass area, i.e., the area scanned visually by the grader? Has photography or video recording been considered or used in image analyzing? Scanning film negatives would seem to be feasible, e.g., 8 mm. Near infrared spectroscopy has been applied to the determination of fat in carcasses, and scanning heads are used on-line in several other food applications. Was this technique considered or has it been tried as a predictor of marbling score? The poor correlation of marbling score with ether extract suggests that marbling score is more complex than simply measuring fat area, and therefore any objective technique should provide pattern recognition data as well as area.
Authors: For effective use of an image analyzing system, it is important to obtain a clear-cut image from the sample. There are two ways to do this. One is to make an object stand out optically. Another is to catch the physicochemical character of an object by using a special detector and then to form an image from the detected signal of that character. When acquiring an image of intramuscular fat, in the former way, the intramuscular fat would stand out by some selective staining. This is why we used Sudan Black B staining in our study. This staining procedure is easy and reliable. Hence, it can be easy to analyze an image of a much larger carcass area if the carcass is prepared according to selective staining for fat. However, it is not easy to selectively stain fat in a large block of meat. On the other hand, application of near infrared spectroscopy may be the way to detect the physicochemical character of fat and make an image from the detected signal of the character. Unfortunately, this technique has not been tried yet as a predictor of marbling score.

Marbling score does not represent only the amount of intramuscular fat visually evaluated, but also contains complicated effects of visual factors such as shape, location and distribution pattern. We believe, therefore, that an image analyzing system would be a powerful and objective tool for quantitative and qualitative evaluation of morphologically viewed marbling in meat.

J.D. Fairing: The very large variation between sections of the same sample raises the question of the applicability of the technique. How do these differences compare with the variations in the marbling score and ether extractions?
Authors: The large variation between sections from the same sample suggested that visually determined fat content would also vary from location to location. Since marbling score was evaluated once in each sample, it is impossible to calculate a statistical variation. Therefore, the variation between sections cannot be compared with the marbling score. The variation in the ether extract calculated from the results of measurement in triplicate, contains the error in location. The differences among three results of repeated measurements in the ether extraction ranged from 0.1 to 5.1%. This variation was rather smaller than that between sections.

J.G. Sebranek: Would it be appropriate to include a wider range of intramuscular fat content by including other breeds when evaluating this system?
Authors: This is an interesting idea for another research undertaking.

J.G. Sebranek: What were the absolute quality and yield grade ranges represented in this study?
Authors: The quality and yield grades ranged from 10 to 17 and from 2.16 to 7.19, respectively.

J.G. Sebranek: Could this system be applied more consistently to products such as coarse ground meat to be used in formulation, based on fat, of sausage mixtures?
Authors: The authors believe that this system can be applied to products such a coarse ground meat.

J.G. Sebranek: Is this system fast enough and easy enough to be considered for in-plant use?
Authors: Yes, this system would be fast enough and easy enough to be considered for in-plant use if an adequate scanning input device could be developed.