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Prediction Method of Beef Marbling Standard Number Using Parameters Obtained from Image Analysis for Beef Ribeye

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Abstract Factors affecting the difference between the Beef Marbling Standard (BMS) number assigned by examiners (BMSSUB) and the BMS number estimated from marbling percentage by image analysis (BMSFAT) were investigated. Pictures of ribeye area of 106 Japanese Black steers with BMSSUB were used. Marbling percentage in ribeye area, means and standard deviations of the area and of the form score for marbling particles classified into 5 levels (over 0.01, 0.05, 0.1, 0.5, and 1.0 cm^2), and standard deviations of marbling percentages in small areas which were obtained by dividing the ribeye into 4, 9, 25, and 100 partitions were calculated by image analysis. Multiple regression equations with the difference between BMSSUB and BMSFAT as the dependent variable were obtained by a stepwise method starting with 25 independent covariates for image analysis traits and ribeye area. The final number of independent covariates used in the equation was limited to three. The range of the difference between BMSFAT and BMSSUB was from -3 to $+4$ and the percentage of the differences within ± 1 was 67.0%, while the range of the difference between BMSSUB and the BMS number which was calculated from a multiple regression equation was from -2 to $+2$ and percentage of the differences within ± 1 was 91.5%. These results show that the accuracy of prediction for BMS number has improved by using not only the ratio of fat area but also other image analysis traits.

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Key words : Image analysis, BMS number, Japanese Black

Marbling is one of the most important characteristics to improve for "Wagyu" in Japan. Therefore, methods to evaluate marbling objectively and to predict genetic parameters from these evaluations are necessary for more efficient improvement of "Wagyu". Generally, marbling is evaluated macroscopically by a qualified examiner at the time of carcass grading. Meat quality is graded mainly from the marbling percentage in ribeye area. The size, form and dispersion of marbling particles in the ribeye area also are comparatively important factors.

Umekita *et al.*[6] analyzed crude fat content in ribeye and pointed out the high correlation between crude fat content and BMS number. They also reported that BMS numbers ranged over several levels even for crude fat content of the same level. Kuchida *et al.*[4] reported that Beef Marbling Standard (BMS) numbers assigned by examiners differed by -1 to $+2$ from the BMS number based on the marbling percentage estimated by image analysis, and that this difference

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might have been affected by the form of large marbling particles (over 0.1 cm^2). The conclusion was that prediction of BMS numbers from only the marbling percentage in ribeye area might be difficult.

The goals of this study were to investigate causes of the difference between BMS numbers assigned by examiners and those estimated from marbling percentage by image analysis, and to examine the possibility of predicting BMS numbers using several parameters obtained from image analysis.

Materials and Methods

Pictures of the ribeye area from the 6th to 7th rib cross-section of Japanese Black steers with BMS numbers assigned by examiners of Wagyu Registry Association were used in this study. A single-lens reflex camera was used to photograph the cross-section of the 6th to 7th rib, and the image with a size of about 1 MB was taken into the computer using a film scanner (Nikon ; CoolScan II). The number of pictures of ribeye areas for Japanese Black steers was 106 after excluding blurred photographs.

The greatest influence on the precision of calculation of marbling percentage was the process of converting color image into 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). In this study, the contour comparison method developed by Kuchida *et al.*[3] was used for the conversion. Contours of marblings were automatically drawn for the specified area on the computer screen which was displaying the original true color image of the ribeye area. If the contours are judged to be wrong, it is possible to make adjustments until the contours agree with those of marbling on the true color image.

Ratio of marbling area to ribeye area (defined as marbling percentage), averages and standard deviations of the area and of the form scores of marbling particles, and dispersion of the marbling in the ribeye were calculated by the image analysis. Form score of each marbling particle was calculated as :

$$\text{Form score} = \text{Circumference length}^2/\text{Area}.$$

The form score tends to increase as the circumference of marbling particles become more complicated regardless of the area of the marbling particle. Kuchida *et al.*[4] reported that the form scores for marbling particles with comparatively large areas had a significant influence on the BMS number assigned by examiners. Five averages and five standard deviations of areas and form scores of marbling particles (for those that were over 0.01, 0.05, 0.1, 0.5 and 1.0 cm^2 in area), were calculated. For example, the averages and the standard deviations for area of marbling particles being over 0.1 cm^2 were defined as AA 01 and SA 01, respectively. The averages and the standard deviations for form score of marbling particles being over 0.1 cm^2 were defined as AF 01 and SF 01, respectively.

The ribeye area was divided into small partitions. The standard deviation of marbling percentages within these partitions would be small if the marbling particles were arranged uniformly in the ribeye area. Thus, the dispersion of marbling in the ribeye area can be estimated using this parameter. The major axis is the longest line that connects two points on the periphery of the ribeye. The minor axis is the longest line that connects two points on the periphery of the ribeye at right angles to the major axis. The major axis and the minor axis were divided into 2, 3, 5 and 10 equal parts, respectively. The marbling percentages in the 4, 9, 25 and 100 rectangular areas that were made by intersections of these lines were calculated. The marbling percentage was not calculated when the rectangular area was out of the ribeye or on the periphery of the ribeye and when the number of pixels in this area was less than half of the maximum pixels in the other areas of the same image. The standard deviations of marbling percentages in the 4, 9, 25, and 100 rectangular areas were defined as STD 4, STD 9, STD 25, and STD 100, respectively.

The relationship between the marbling percentage which was calculated by the image analysis and BMSSUB is shown in Fig. 1 from the preliminary analysis. The average of the marbling percentage was calculated for each BMS number. The regression equation of BMSSUB on the average marbling percentage was obtained as :

$$\text{BMSSUB} = 0.462 \times (\text{Marbling percentage}) - 1.26$$
$$\text{with } r^2 = 0.98,\ P < 0.01.$$

The value which was calculated from this equation was rounded off to the decimal point above the original value and was defined as BMS number based on the marbling percentage (BMSFAT). Values of BMS number 13 and 14 calculated from substituting the data used in this study into the regression equations were used exactly as they were, although such BMS numbers are not in the Beef Marbling Standard.

The value obtained by subtracting BMS number predicted by image analysis from BMSSUB was

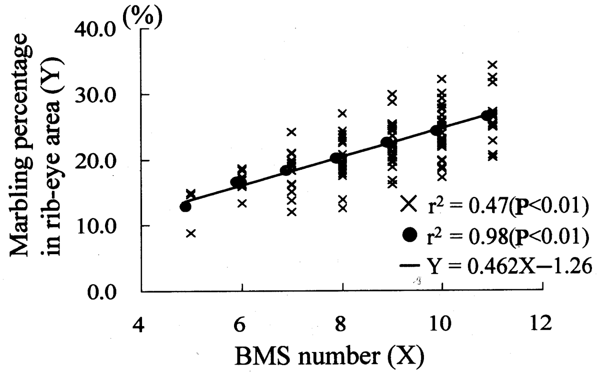

Fig. 1. Relationships between subjectively evaluated BMS number and marbling percentage in ribeye area (× ; n=106), and between BMS number and average marbling percentage of each BMS number (● ; m=7) in Japanese Black steers.

defined as the difference (DIFBMS) to be analyzed the dependent variable.

DIFBMS = BMSSUB − BMS by image analysis

Multiple regression equations with the DIFBMS as dependent variable were obtained by the stepwise method using 25 covariates associated with image analysis traits and ribeye area (Table 1). The number of independent covariates in the final equation was limited to three. If any of the correlation coefficients among the selected three independent covariates were significant, the stepwise method was performed once again after removing selected independent covariates except for the one variable with the highest F value. A significantly negative correlation ($r = -0.69$, $P < 0.01$) was found between DIFBMS and BMSFAT in the preliminary analysis. The BMSFAT were divided into six groups. The multiple regression equations to predict DIFBMS were calculated by the stepwise method using data of each group and also using all of data. The BMSFAT were classified into six marbling levels as follows, BMSFAT 6 or less (marbling level 1), 7 (2), 8 (3), 9 (4), 10 (5) and 11 or over (6).

The degree of equivalence between BMSSUB and BMS number predicted by image analysis could be a

Table 1. Candidate independent covariates to determine the multiple regression equation by stepwise method for prediction of difference (DIFBMS) between Beef Marbling Standard number assigned by examiner and by image analysis

Category of covariates	Candidate independent covariates				
Average area of marbling	AA001[a]	AA005	AA01	AA05	AA1
Standard deviation of marbling area	SA001	SA005	SA01	SA05	SA1
Avarage form score of marbling	AF001	AF005	AF01	AF05	AF1
Standard deviation of form score of marbling	SF001	SF005	SF01	SF05	SF1
Dispersion of marbling	STD4[b]	STD9	STD25	STD100	
Ribeye area	RIBAREA				

[a] AA(XXX), SA(XXX), AF(XXX), and SF(XXX) are the average area, standard deviation of area, average of form score and standard deviation of form score of marbling particles, respectively, with areas greater than 0.01, 0.05, 0.1, 0.5 and 1.0 cm^2.

[b] STD 4, STD 9, STD 25, and STD 100 are the standard deviations of marbling percentage for the 4, 9, 25, and 100 partitions, respectively.

Table 2. Summary of basic statistics for ribeye area, marbling percentage by image analysis, BMS numbers by subjective and image analysis methods and difference (DIFBMS) between Beef Marbling Standard number assigned by examiner and by image analysis in Japanese Black steers (n=106)

Traits	Means±S.D.	Minimum	Maximum
Ribeye area (cm^2)	45.5±5.1	35.0	65.0
Marbling percentage in ribeye area (%)	21.6±4.2	8.9	34.2
BMS number by subjective method	8.7±1.6	5	11
BMS number by image analysis[a]	8.2±2.2	2	14
DIFBMS[b]	0.4±1.6	−3	4

[a] BMS number by image analysis=integer (0.462×marbling percentage−1.26)
[b] DIFBMS=BMS by subjective method−BMS by image analysis

Table 3. Coefficient of determination (R^2) for multiple regression equations for prediction of the difference (DIFBMS) between Beef Marbling Standard number assigned by examiner and by image analysis and selected covariates with signs of their regression coefficient (in parenthesis) using the stepwise method for each marbling level

Marbling level[a]	n	R^2	Selected variables		
All (2–14)	106	0.35**	RIBAREA (+)	AA001 (−)	STD100 (−)
Level 1 (2–6)	21	0.34†	RIBAREA (−)	SA001 (+)	STD9 (+)
Level 2 (7)	22	0.41**	RIBAREA (+)	AF001 (+)	
Level 3 (8)	17	0.50*	RIBAREA (+)	STD4 (−)	STD9 (+)
Level 4 (9)	16	0.32*	STD4 (−)		
Level 5 (10)	14	0.80**	RIBAREA (+)	AF01 (+)	
Level 6 (11–14)	16	0.44†	SF001 (−)	AF01 (+)	STD25 (−)

†; $P<0.10$, *; $P<0.05$, **; $P<0.01$
[a] BMS number by image analysis are in parentheses.
BMS number by image analysis=integer (0.462×marbling percentage−1.26)

guide to an accurate BMS number prediction by image analysis. The degree of equivalence (root mean squared error : RMSE) was calculated as :

$$RMSE = \{(\Sigma\,(\text{BMS by image analysis} - \text{BMSSUB})^2)/n\}^{0.5}$$

where n was the number of observations[2)]. Not only the bias of the prediction but the degree of accuracy of prediction could be explained by RMSE[1)]. The STEPWISE procedure of SAS[5)] was used for statistical analysis.

Results and Discussion

The basic statistics for ribeye area, marbling percentage in the ribeye, BMSSUB, BMSFAT calculated by the image analysis and the DIFBMS are shown in Table 2. The range of BMSFAT (2 to 14) was greater than the range of BMSSUB (5 to 11). The DIFBMS was the index used to express the difference in the BMS number among samples with the same level of marbling percentage. For example, BMSSUB for seven samples with marbling percentage of 17.5 to 18.5% ranged from 6 to 10. This range indicates that it is difficult to predict BMS number using only marbling percentage.

The plot of marbling percentage against BMSSUB is shown in Fig. 1. The average of marbling percentage for each BMS number was also plotted against BMSSUB. Linear regression was used to measure the relationship between average marbling percentage for each BMSSUB and BMSSUB. Ushigaki *et al.*[7] examined the validity of using BMS number (1, 2, 3, ……, 11, 12) or marbling score (0, 0 +, 1 −, ……, 4, 5) in genetic evaluation. They found that the relation between BMS number and marbling percentage was linear whereas that between marbling score and marbling percentage was quadratic. They concluded that the BMS number was appropriate for genetic evaluation. Linearity would be decreased if marbling score was used in this analysis, because there were two grades (marbling score 3 + and 4 −) between BMS number 10 (marbling score 3) and 11 (marbling score 4). These results indicate that BMSSUB is a linear measure of marbling percentage for records assigned by examiners of Wagyu Registry Association, although these data did not include BMS of numbers 1 to 4 or number 12.

The coefficients of determination for the multiple regression equations to predict DIFBMS and the independent covariates with the sign of their regression coefficients are shown in Table 3. Ribeye area, AA 001 and STD 100 were selected for the multiple regression equation using all the data ($n=106$). While the sign for the regression coefficient for ribeye area was positive, those for AA 001 and STD 100 were negative. This equation indicates that large ribeye area, small average of area of marbling particles over $0.01\,cm^2$ and small variation of marbling percentage in small areas obtained by dividing the ribeye into 100 areas tended to improve BMSSUB for a constant marbling percentage.

The standard deviation of the particle area and the average of form score for the marbling particles over $0.01\,cm^2$ were selected as the independent covariates in the multiple regression equations for marbling levels 1 and 2, respectively. This result indicates that small marbling particles affect DIFBMS for samples with low marbling percentage. The AF 01 was selected in

Table 4. Frequencies and root mean square errors (RMSE) for the difference (DIFBMS) between Beef Marbling Standard numbers assigned by examiner and by image analysis for three models

DIFBMS	Model 1[a]	Model 2[b]	Model 3[c]
−3	4	2	0
−2	6	11	3
−1	20	22	24
0	25	35	50
+1	26	23	23
+2	12	8	6
+3	11	5	0
+4	2	0	0
RMSE	1.63	1.32	0.88

[a] BMS number by image analysis was based on the marbling percentage in ribeye area.
[b] BMS by image analysis was calculated from the regression equation for all data (see Table 3).
[c] BMS by image analysis was calculated by the regression equation for partial data classified by marbling levels (see Table 3).

the multiple regression with positive regression coefficient for marbling levels 5 and 6. This result showed that BMSSUB tended to be highly evaluated when the shapes of marbling particles (over $0.1\,cm^2$) were complex. Kuchida *et al.*[4] examined the causes for DIFBMS using different materials ($n=16$). They reported a significant positive correlation ($r=0.64$, $P<0.01$) between the average form score of comparatively large particles (over $0.1\,cm^2$) and DIFBMS in agreement with the present study.

For marbling levels 3 and 4, STD 4, which is an index of dispersion of marbling in the ribeye ; for marbling levels 1 and 3, STD 9 ; and for marbling level 6, STD 25 ; were selected into the multiple regression equations. The signs of the regression coefficients for STD 4 and STD 25 were negative, which indicates that small standard deviation of fat area percentage in small areas lead to high BMSSUB for these marbling levels. However, the reason for the positive sign of the regression coefficient for STD9 is not known.

The frequencies and RMSE for DIFBMS between

BMSSUB and the BMS number predicted only from marbling percentage (Model 1), BMS number by the multiple regression equation using all the data (Model 2), and BMS number from the multiple regression equations for each marbling level (Model 3) are shown in Table 4. Here, the BMS number of Model 1 was equal to BMSFAT. According to the RMSE, the accuracy of prediction of BMS number was highest for Model 3 and lowest for Model 1. While the range of DIFBMS for Model 1 was from -3 to $+4$, the range of DIFBMS for Model 3 was from -2 to $+2$. The percentage of DIFBMS for Models 2 and 3 being within ± 1 was 75.5 and 91.5%, respectively, whereas the percentage of the difference between BMSFAT and BMSSUB being within ± 1 was 67.0%. Model 3 was the most appropriate prediction method for BMSSUB according to RMSE and the frequency of DIFBMS. This result indicates that calculation of the multiple regression equation separately for each marbling level would be desirable.

Analyses of variance were performed assuming DIFBMS as a fixed effect for the 9 samples with Model 3 that were -2 or $+2$ in Table 4. The dependent variables are shown in Table 1. The effect of DIFBMS was significant for only ribeye area and AF 001 out of a total of 25 traits. The means for ribeye area and AF 001 for samples with DIFBMS being $+2$ were 45.2 cm^2 and 62.4, respectively. The means for samples with DIFBMS being -2 were 42.3 cm^2 and 48.4, respectively. These means indicate that these effects were not completely explained by the multiple regression equation used to predict DIFBMS, even though ribeye area and AF 001 were included in the model as independent covariates. Adding the square and square root of ribeye area and AF 001 as covariates, the multiple regression analysis was conducted again with the stepwise method. Then SA 001, $RIBAREA^{0.5}$, and $AF001^2$ were selected for the multiple regression equation for marbling level 2, and the coefficient of determination increased from 0.41 to 0.52. The DIFBMS changed $+1$ from $+2$ for only one sample. However, the added covariates did not influence the multiple regression equations for other marbling levels and for all of the data.

Predicting DIFBMS is equivalent to predicting BMS number. In other words, it is equivalent to assigning the BMS number using image analysis. With information from image analysis and ribeye area, over 90% of BMS numbers samples were assigned with high precision (DIFBMS being within ± 1). The differences for samples with DIFBMS being $+2$ and -2 were explained. We have plans to improve accuracy of BMS prediction by devising a way to classify marbling levels and by using many combinations of values calculated from the image analysis software after accumulating more image data.

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