



## EFFECTS OF INTRAMUSCULAR FAT DEPOSITION ON THE BEEF TRAITS OF JAPANESE BLACK STEERS

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### Background

Marbling score is the most important factor for the carcass classification in Japanese market. Japanese Black cattle, known as “WAGYU”, are characterized for their unique ability to deposit a large amount of intramuscular fat in muscles during the fattening period (Zembayashi et al., 1988). Matsuishi et al. (2001) reported that one of the main reasons why Japanese people preferred Wagyu beef was the preferable, sweet and fatty aroma that comes only from high marbling WAGYU. A higher price is therefore paid in Japan for carcasses with more marbling than for those with less marbling. Recently the production of beef containing significant marbling has been more strongly encouraged than previously, which seems to have occurred after the liberalization of beef imports in 1990 because of the need to distinguish domestic beef from imported beef.

### Objectives

Beef quality traits as seen in the market place appear to have changed in the last decade due to this trend. Little is known about the effects of increased marbling on beef quality such as chemical composition and free amino acid content. The purpose of this study was to clarify the relationship between fat content and meat quality, and to determine any new trends or characteristics of recent Wagyu meat.

### Materials and methods

Twenty-one Japanese Black steers were slaughtered, and a portion of *M. Longissimus thoracicus* (*LT* muscle) was taken from the carcasses and aged at 0°C for 15 days. The sample of *LT* muscle was minced. The moisture content was calculated by the weight difference between before and after freeze-drying. Crude protein was determined by Kjeldahl crude nitrogen analysis and crude fat by Soxhlet extraction. To evaluate the cooking loss and toughness, samples were cut into cubes (ca. 100g each). These samples were weighed before and after cooking, the difference being the rate of cooking loss. Then, the Warner-Bratzler shear force value of cooked sample was measured. Approximately 10 g of fresh muscle was homogenized with an equivalent weight of 5% sulfosalicylic acid and the solution was then used for the analysis of free amino acids by an auto amino acid analyzer.

### Results and discussion

#### Moisture, protein and fat content

As the fat content increased from 4.8% to 39.0%, the moisture decreased linearly from 72.9% to 45.6%, and the coefficient of this correlation was 0.97 ( $p < 0.01$ , Figure 1). In contrast, the protein level was relatively constant with ca. 18% to low fat content. A marked decrease was observed in the region of higher fat content (Figure 2). A linear, piecewise regression model was used to describe the relationship between fat content ( $x$ ) and protein content ( $y$ ) and equations were expressed by  $y = -0.048x + 19.082$  and  $y = -0.288x + 24.578$ . The intersection point was  $x = 22.867$  ( $r^2 = 0.91$ ). It is generally accepted that the moisture content of bovine muscle has a negative correlation with fat content, and protein is relatively constant irrespective of fat content. Our result of the protein content with lower fat agreed with this statement, but markedly decreased protein has not been reported before. Gotoh et al. (1999) suggested that type I fibers were replaced by type IIB fibers in *LT* muscles during the fattening period. Type I fibers had the ability to accumulate intramuscular fat (Suzuki et al., 1978) and the diameter of type I fibers was shorter than that of type IIB fibers (Calkins et al., 1981). From these reports, *LT* muscles containing higher fat in this study would have a higher percentage of type I fibers, and fat would be present where fibers (protein) should be. The protein content



per unit mass consequently decreased and fat developed in the endomysium. This finding is applicable to beef containing 23% or more fat.

#### Cooking loss

Cooking loss was negatively correlated with fat content and the slope of the relation was weak in the area of low fat content, but a steep slope was observed in the high fat meat. This tendency was similar to the relation between fat and protein. A piecewise linear regression model was also applied and the relationship was expressed by  $y = -0.116x + 25.247$  and  $y = -0.662x + 40.555$  and the intercept of the two line segments was 28.02 ( $r^2 = 0.97$ ). This indicates that the cooking loss remarkably decreases in meat including 28% of fat or more. A not significant but negative correlation ( $r = -0.32$ ) was reported between fat content and cooking loss using beef with fat below 20% (Mitsumoto et al., 1995). Our result of a weak correlation in the region of low fat agreed with their report. However, a strong correlation was observed with fat of 28% or above in this study. Cooking loss mainly occurs by keeping the meat juice out of the contracted muscle fibers by heating, and the loss of holding capacity by protein denaturation. Therefore, cooking loss is lower in meat with lower moisture, and in addition, moisture release is lower with lower protein content. Markedly decreased cooking loss was consequently observed in meat with 28% fat content or above.

#### Tenderness

The results of SFV ranged from 2.08 to 4.56 kg/cm<sup>2</sup> and a significant negative correlation ( $r = -0.83$ ,  $p < 0.05$ ) was observed between the SFV and fat level (Figure 4). This observation was different from the relation of protein content or cooking loss to fat content which was expressed by piecewise regression model (Figures 2 and 3). Adipose tissues in *LT* muscle appeared to disrupt the structure of intramuscular connective tissue (Nishimura et al., 1999). And matrix metalloproteinases (MMPs) which increased during adipocyte differentiation (Bouloumine et al., 2001) could improve the meat tenderness (Phillips et al., 2000). In addition to the above factors, the decreased diameter of muscle fibers would lead to the production of unique tender beef, due to the replacement of type IIB with type I in beef with significant marbling (>23% of fat) in the present study.

#### Free amino acid content

Table 1 shows the correlation coefficient between fat content and each free amino acid and peptide, expressed as both wet meat and protein base. In the wet meat base, almost all free amino acids and Ans and Car content except Glutamine (Gln) had a significantly negative correlation with fat content ( $p < 0.001 - 0.05$ ). In the protein base, although there was no significant relationship in some free amino acids, a significant negative correlation was still observed in several amino acids (Threonin, Serine, Alanin, Valine, Methionin, IsoLeucine, Leucine, Arginin). The same trend was found in Car. It can be seen that these negative correlations were due to a lower protein content to higher fat beef as mentioned above (Figure 2). However, this does not apply to meat with low fat (<23%), because of a relatively constant protein content (Figure 2). Lower levels of free amino acids were observed in muscles at 35 months than 25 months in cattle (Watanabe et al., 2004), and this suggested that the results of lower concentration would be due to a lower muscle growth rate. Therefore, the reason for the negative correlation between the fat content and most free amino acids can be explained by not only protein content but also animal maturity. On the other hand, Gln has a positive relationship to fat content in both wet and protein bases. Gln has the ability to stimulate the formation of lipids (Yatzidis, 2002) or lipogenesis (Lavoine et al., 1987). Cornet and Bousset (1998) reported that red oxidative muscle had higher levels of Gln, and increased fat content lead to increased type I fibers (red oxidative muscle) as mentioned above. These reports could explain why Gln increases as fat increases.

### **Conclusions**

The effects of intramuscular fat deposition on the chemical composition, tenderness, and free amino acid concentration in beef were studied using various classified carcasses of 21 Japanese Black (Wagyu) steers. Fat content ranged from 4.8 to 39.0% in *M. Longissimus thoracicus*, respectively. The protein content was relatively constant until there was ca. 23% of fat, and then decreased as the fat increased. Cooking loss was also constant until there was ca. 28% of fat, and markedly decreased with the fat increase. A weak correlation was found between the shear-force value and the fat content ( $r = -0.83$ ,  $P < 0.05$ ). Most of the amino acids had a negative correlation to the fat content except glutamine, and this observation continued even when calculated by protein base. It is concluded that too much amount of fat in beef would be a possibility of leading to the protein content decrease. And consequently, it would influence the level of cooking loss and free amino acid content.



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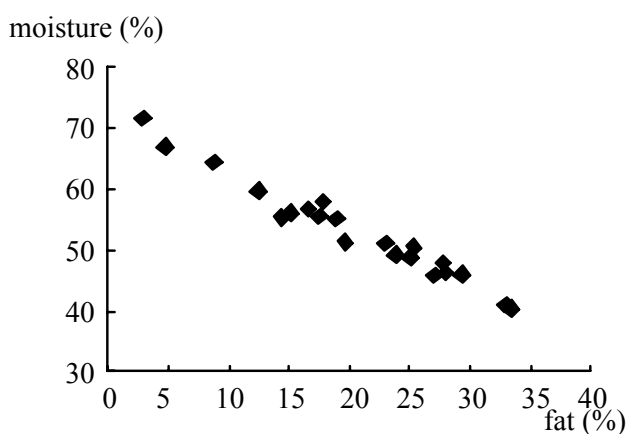


Figure 1. Correlation between fat content and moisture content in *M. Longissimus thoracicus* from Japanese Black steer.

$$y = -0.743x + 74.933 \quad (p < 0.01, r = -0.97)$$

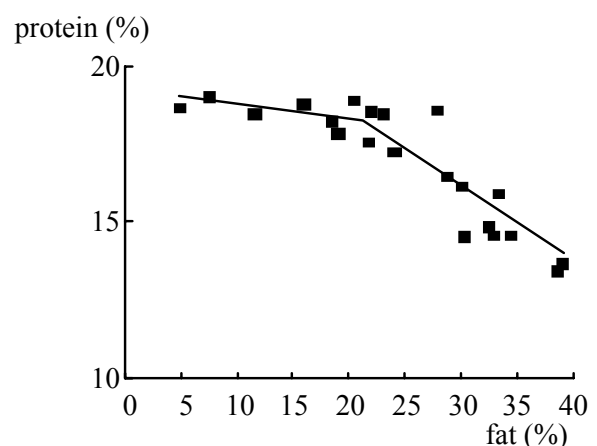


Figure 2. Correlation between fat content and protein content in *M. Longissimus thoracicus* from Japanese Black steer

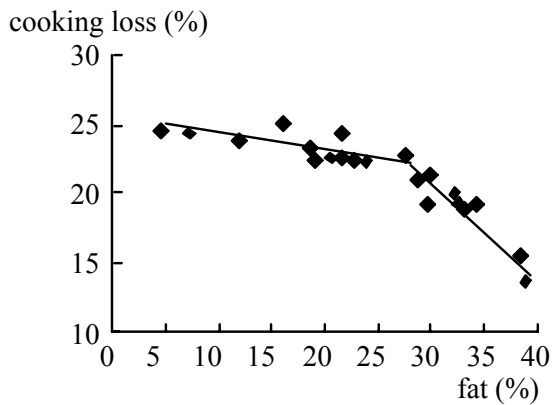


Figure 3. Correlation between fat content and cooking loss in *M. Longissimus thoracicus* from Japanese Black steer

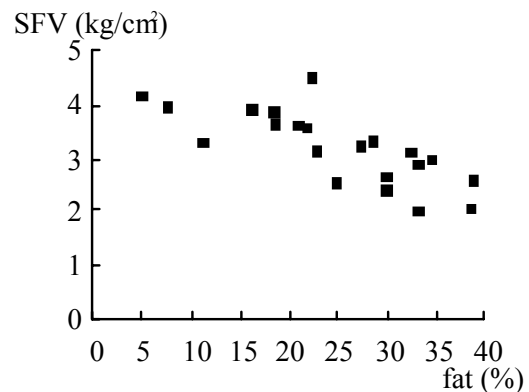


Figure 4. Correlation between fat content and SFV in *Longissimus thoracicus* from Japanese Black cattle.  
 $y = -0.053x + 4.519$  ( $p < 0.05$ ,  $r = -0.83$ )

Table 1. Correlation coefficients between fat content and free amino acids

	wet meat base	protein base
Asp	-0.76 *	-0.60
Thr	-0.78 **	-0.72 *
Ser	-0.78 **	-0.72 *
Glu	-0.70 *	-0.64
Gln	0.69 *	0.67 *
Gly	-0.81 ***	-0.72
Ala	-0.88 ***	-0.74 *
Val	-0.77 **	-0.72 *
Met	-0.81 **	-0.75 *
Ile	-0.78 **	-0.73 *
Leu	-0.79 **	-0.73 *
Tyr	-0.78 **	-0.72
Phe	-0.78 **	-0.72
His	-0.78 ***	-0.70
Lys	-0.74 *	-0.69
Ans	-0.46	-0.08
Car	-0.87 ***	-0.61
Arg	-0.76 **	-0.69 *

\*;  $p < 0.05$ , \*\*;  $p < 0.01$ , \*\*\*;  $p < 0.001$