Animal Science Journal (2007) **78,** 189–194 doi: 10.1111/j.1740-0929.2007.00424.x

ORIGINAL ARTICLE

Effects of intramuscular fat deposition on the beef traits of Japanese Black steers (Wagyu)

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ABSTRACT

Effects of intramuscular fat deposition on the chemical composition, tenderness, and free amino acids (FAA) concentration of beef were studied using various classified carcasses of 21 Japanese Black (Wagyu) steers. The Beef Marbling Standard (BMS) No., judged on the market in 1998, and fat content, ranged from 2 to 10 and 4.8 to 39.0% in the M. longissimus thoracis, respectively. Moisture content was negatively correlated with the fat content ($r = -0.98$, $P < 0.01$). In contrast, protein content was relatively constant up to approximately 23% fat, corresponding to BMS No.4, and decreased as the fat content increased. Cooking loss was also constant up to approximately 28% fat, corresponding to BMS No.4, and markedly decreased with fat content increase. The beef in these cases were, however, classified into BMS No. around 9 and 11, respectively, according to the BMS model in 1988. A negative correlation was found between the shear-force value and the fat content ($r = -0.83$, $P < 0.05$). Most FAA concentrations on the wet weight of meat were correlated negatively with the fat content, except glutamine, and this negative relationship was still observed when the concentrations were recalculated on the basis of protein. These results indicate that higher marbling Wagyu beef (above approx. 23% fat) would have an extremely lower content of protein, which would partly explain the lower cooking loss and FAA with fat increase. In addition, some other reasons not relating to protein content (e.g. high fat content preventing the breakdown of protein to FAA) seem to explain the negative correlation between fat and FAA.

Key words: *BMS, free amino acids, intramuscular fat, Japanese Black steer, protein content.*

INTRODUCTION

Beef carcasses in Japan have been classified both by their estimated yield score and meat quality score. The latter is determined on the basis of beef marbling, meat color and fat color. The marbling score is the most important factor in carcass classification (JMGA 1988). Japanese Black Cattle, known as 'Wagyu', are characterized by their unique ability to deposit a large amount of intramuscular fat during the fatting period (Zembayashi *et al*. 1988). Mitsumoto *et al*. (1992) reported that muscles containing a large amount of intramuscular fat had a lower shear value and lower cooking loss than those containing a small amount of intramuscular fat. Matsuishi *et al*. (2001) reported that one of the main reasons why Japanese people preferred high marbling Wagyu beef was its unique sweet and fatty aroma, and a higher price is therefore paid in Japan for carcasses with more marbling. Recently, the production of beef with significant marbling has been encouraged, and this seems to have occurred after the liberalization of beef imports in 1990 because of the need to distinguish domestic and imported beef. Little is known about

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Received 20 February 2006; accepted for publication 4 July 2006.

the effects of increased fat content caused by high marbling on other beef qualities.

The purpose of the present study was to clarify the relationship between fat content and meat quality, such as chemical composition and tenderness, and to determine any new trends or characteristics of recent Wagyu meat.

MATERIALS AND METHODS

Sample preparation

Twenty-one Japanese Black steers, from 15 to 30 months of age, were slaughtered at a regular public slaughterhouse and carcass data collected. These carcasses were graded as A2, A3, A4 and A5 on the market in 1998. A portion of the M. longissimus thoracis (LT muscle), from the 6th rib area was taken from each carcass and aged at 0°C for 15 days. These samples were then prepared for chemical composition and free amino acid (FAA) analyses and to determine tenderness and cooking loss.

Moisture, protein and fat content

Samples of LT muscle were minced with plate openings of 3 mm. Moisture content was calculated by the weight difference before and after freeze-drying. After grinding the dried samples, crude protein was determined by Kjeldahl crude nitrogen analysis and crude fat by Soxhlet extraction according to AOAC (1990). The data are expressed as a percentage of fresh matter.

Cooking loss and shear-force value

To evaluate the cooking loss and tenderness of LT muscle, samples were prepared according to Graafhuis *et al*. (1991). Samples were cubed, approximately 40 mm thick and weighing about 100 g, and placed in a polyethylene bag. These samples were cooked in a water bath at 80°C until the internal temperature reached 75°C, and then immediately removed and placed on ice. Sample cubes were weighed before and after cooking, the difference being the rate of cooking loss (expressed as a percentage of the original weight). The Warner–Bratzlar shear-force value was determined from 10 mm × 10 mm specimens of each cooked sample.

Free amino acid analysis

Approximately 10 g of muscle was precisely weighed and homogenized with an equivalent weight of 5% sulfosalicylic acid and 1 mL of 0.2% s-(2-aminoethyl)-L-cysteine (Sigma, Tokyo, Japan) as an internal standard in a centrifuge tube. This homogenate was centrifuged at 10 000 *g* for 15 min. The supernatant was decanted into a volumetric flask through filter paper (5A), and diluted to 50 mL with distilled water. The solution was then used to analyze FAA using an auto amino acid analyzer (JLC-300; Jeol, Tokyo, Japan) equipped with a lithium-type ion exchange column, and amino acid derivatives. The value of each FAA concentration was calculated on the wet weight of tissue and the value was also recalculated on the weight of protein.

Statistical analysis

The correlation between fat content and moisture, tenderness, and each FAA was determined using the SAS system (software release 6.11; SAS Institute, Cary, NC, USA). A linear, piecewise regression model was used to determine the relationship between fat content and protein content, and fat content and cooking loss, with reference to Marsh *et al*. (1991).

RESULTS AND DISCUSSION

Moisture, protein, BMS No. and fat content

The chemical components are shown as a percentage of moisture and protein to fat content in Figures 1 and 2, respectively. The relation between BMS No. and fat content are shown in Figure 3. As the fat content

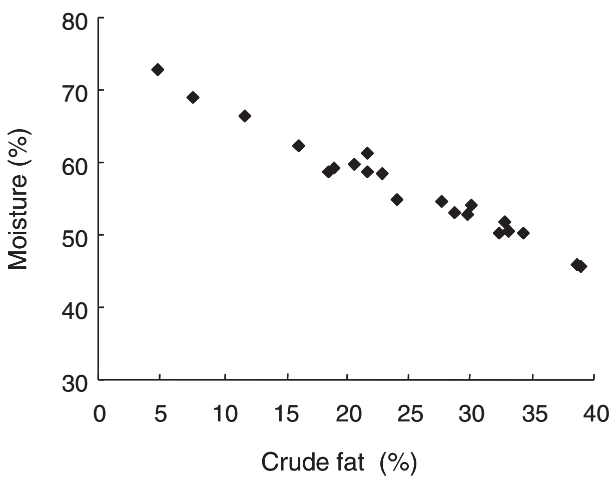

Figure 1 Correlation between fat content and moisture content in the M. longissimus thoracis from Japanese Black steers. $y = -0.743x + 74.933$ ($P < 0.01$, $r = -0.97$).

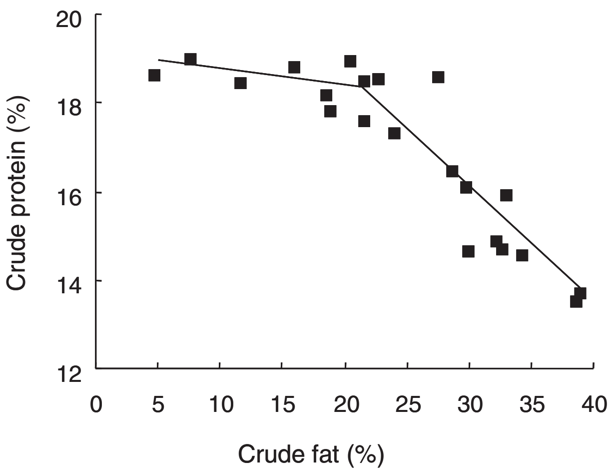

Figure 2 Correlation between fat content and protein content in the M. longissimus thoracis from Japanese Black steers.

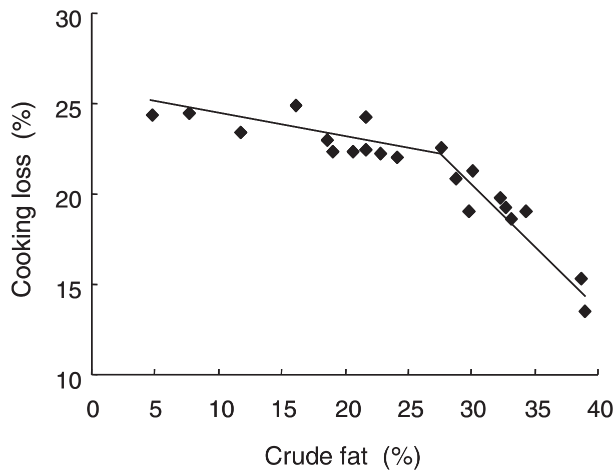

Figure 4 Correlation between fat content and cooking loss in the M. longissimus thoracis from Japanese Black steers.

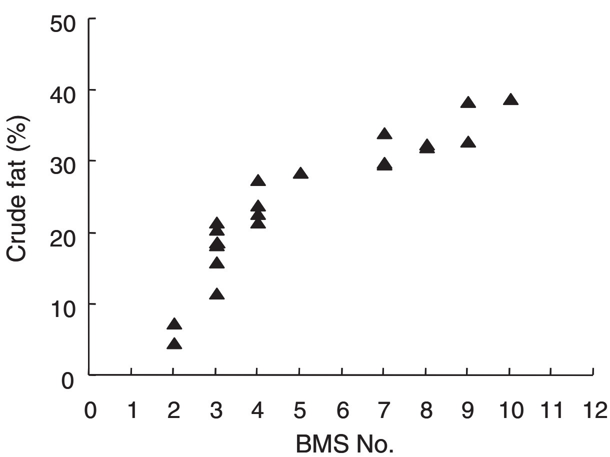

Figure 3 Correlation between fat content and BMS No. in the M. longissimus thoracis from Japanese Black steers. $y = 3.339 \times + 7.379$ ($P < 0.01$, r = 0.91). (BMS No. was judged on commercial slaughter house in 1998.)

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 ($P < 0.01$). In contrast, the protein level was relatively fixed on approximately 18% in the lower fat content range and a marked decrease was observed in the higher fat content range (Fig. 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 $y = -0.048x + 19.082$ ($4.8 \leq \times \leq 22.867$) and $y = -0.288x + 24.578$ ($22.867 \leq \times \leq 39.0$). 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 (Savell *et al*. 1986; Park *et al*. 2000; Kim & Lee 2003). However, those studies were carried out with a low fat content (<10.4%). The present protein content result with lower fat agreed with these reports, but markedly decreased protein has not been reported previously. It is known that moisture is replaced by fat as fat increases in meat, but this explanation cannot be adapted to the observation of protein decrease.

We could show there are two phases of protein changes on the series of fat content, and marked decrease commences above the point of approximately 23% fat. Beef with this level of fat was classified into BMS no. 4 on the market in 1998 (Fig. 3). But it should be noted that the judgment is affected by the market. Beef with approximately 23% fat in the present study would be classified into BMS no. 9 according to the beef Marbling Standard of Japan Meat Grading Association, established in 1988, or to the report by Kuchida *et al.* (2000). This inconsistency seems to increase with time.

Cooking loss

Figure 4 shows the relationship between fat content and cooking loss. Cooking loss was negatively correlated with fat content and the slope of the relationship was weak in the low fat content range, but a steep slope was observed in high fat meat. This tendency was similar to the relationship between fat and protein. A piecewise linear regression model was also applied and the relationship was expressed by $y = -0.116x + 25.247$ $(4.8 \leq x \leq 28.02)$ and $y = -0.662x + 40.555$ $(28.02 \leq x \leq 39.0)$ and the intercept of the two line segments was $x = 28.02$ ($r^2 = 0.97$). This indicates that cooking loss dramatically decreases in beef with ≥28% fat content. Beef with this fat content was classified into BMS No.4 (Fig. 3), but the beef corresponded to around BMS No. 11 by the BMS model as aforementioned.

Mitsumoto *et al*. (1992) reported that muscles containing a large amount of intramuscular fat had lower cooking loss using beef containing 4.9–20.8% fat. A non-significant negative correlation ($r = -0.32$) was reported between fat content and cooking loss using beef with fat under 20% (Mitsumoto *et al*. 1995). The present result of a negative correlation between fat content and cooking loss was in agreement with that report in the low fat content range, and the results of the present study show how the increase in cooking loss increased in beef with ≥28% fat content. This relationship between fat content and cooking loss was similar to the relationship between fat content and protein content described here. Cooking loss mainly occurs by the release of hydration water bound to proteins (Hamm 1960). This statement supports the hypothesis that marked decrease of cooking loss is affected by marked decrease of protein. But the change of protein to fat content was not completely consistent with those of cooking loss to fat. We could not explain this inconsistency clearly, but cooking loss also depends on the amount of connective tissue, and fat will melt and drip out in cooking.

Tenderness

Tenderness is one of the most important traits in beef quality, and Wagyu is famous for its high-quality tenderness. Figure 5 shows the relationship between fat level and tenderness expressed as a Warner–Bratzlar shear-force value (SFV). SFV ranged from 2.08 to 4.56 kg/cm^2 and a significant negative correlation was observed between the SFV and fat level, which ranged from 4.8 to 39.0% ($r^2 = 0.62$, $P < 0.05$). This observation was not as strong as the relationship between protein content or cooking loss to fat content (Figs 2,3), which was expressed by a piecewise linear regression model consisting of two equations. Armbruster *et al*. (1983) reported that intramuscular fat content contributed very little to sensory tenderness

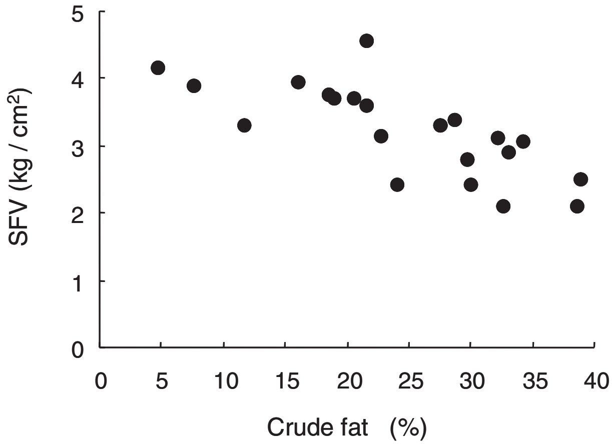

Figure 5 Correlation between fat content and Warner–Bratzlar shear-force value (SFV) in the M. longissimus thoracis from Japanese Black steers. $y = -0.053x + 4.519$ ($P < 0.05$, $r = -0.83$).

using beef with fat ranging from 2 to 18%, which was lower than that in the present study, while the marbling score of Limousin steers was negatively correlated with calpastatin activity and positively correlated with panel tenderness (Wulf *et al*. 1996). Other researchers have also reported the effect of fat tissue on tenderness: adipose tissue deposits in the longissimus muscle appeared to disrupt the structure of intramuscular connective tissue (Nishimura *et al*. 1999) and matrix metalloproteinases (MMP), while the expression increased during adipocyte differentiation (Bouloumie *et al*. 2001), improving meat tenderness (Phillips *et al*. 2000). These reports suggest that meat tenderness was affected by not only the chemical composition but also by protease activity, resulting in piecewise linear regression that was difficult to identify, as shown in Fig. 5. In the present study the SFV decreased when the fat level increased (Fig. 5), although there was little change in the protein content in beef containing fat <23% (Fig. 2). The SFV decrease was caused by the structural breakdown of muscle fibers or connective tissue affected by calpain or MMP as described here.

Free amino acids concentrations

FAA concentrations are affected by meat aging, breed type, animal age (Watanabe *et al*. 2004), pH, and the activity of proteases (Nishimura *et al*. 1988a), and some of these FAA contribute to meat taste (Nishimura *et al*. 1988b). There are, however, few reports about the relationship between FAA concentrations and intramuscular fat.

Table 1 Correlation coefficients between fat content and FAA concentration

	Fresh meat basis	Protein basis
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*
Tvr	−0.78**	−0.72
Phe	−0.78**	−0.72
His	−0.78***	−0.70
Lys	−0.74*	−0.69
Arg	−0.76**	−0.69*
Ans	−0.46	−0.08
Car	−0.87***	−0.69*

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
Concentration of each free amino acid (FAA) was calculated on the wet weight of meat (fresh meat basis) and on the weight of protein (protein basis).

Sixteen FAA and two peptides, anserine (Ans) and carnosine (Car), were detected in FAA analysis. Table 1 shows the correlation coefficient between fat content and each FAA and peptide, expressed as both wet weight of meat base and weight of protein. On the wet weight of meat, almost all FAA and Car concentrations were significantly and negatively correlated and only glutamine (Gln) was positively correlated with fat content ($P < 0.001$–0.05). On the weight of protein, significant negative correlations were still observed but weaker in several FAA.

It can be seen that this negative correlation between fat content and each FAA concentration was due to lower protein content, similar to the correlation between protein content and higher fat content in the beef (Fig. 2). However, this correlation was observed even in low-fat meat (<23%) where the protein content was relatively constant (Fig. 2). In addition, a negative correlation was still observed when the concentrations were recalculated on the basis of protein. From these results, the decreased FFA level as fat content increased cannot be explained only by the protein content.

It is generally known that cattle accumulate significant fat and the muscle growth rate is lower at the end of the fattening period. Watanabe *et al.* (2004) observed that almost all FAA were lower in 35-month-old steers compared with younger steers, suggesting that well-fattened steers had lower FAA because of the lower muscle growth at the end of fattening. The increase in amino acids in beef during storage is lower than that of pork and chicken (Nishimura *et al.* 1988b). In addition, Sekikawa *et al.* (1999) reported that protein degradation with some enzymes, which act in living muscle cells, is one of the primary factors affecting meat conditioning in the immediate post-mortem period. Therefore, the negative correlation between fat content and most FAA in the present study can be explained by the decreased rate of protein synthesis and degradation at maturity, and fat deposition in the muscle of steers, which affect the decrease of FAA generation. This suggests that high fat accumulated in the muscle may decrease FAA in beef.

In contrast, Gln has a positive relationship with fat content on both a wet and protein basis. Gln is known as an energy source (Yatzidis 2002), indicating that a higher energy diet possibly accumulates a higher content of Gln in muscles. In addition, Gln can stimulate the formation of lipids (Yatzidis 2002) or lipogenesis (Lavoinne *et al.* 1987). Cornet and Bousset (1999) reported that red oxidative muscles had higher levels of glutamine, and that increased fat content led to increased type I myofibers (red oxidative myofiber) as mentioned here. Increase of type I myofiber has been found in high marbling beef (Calkins *et al.* 1981). These reports could explain why Gln increases with fat accumulation.

In conclusion, the higher marbling beef (above approx. 23% fat content) of Japanese Black steers has a dramatic decrease of protein content, which is partly responsible for the decrease of cooking loss and FAA concentration. In addition, some other reasons, such as muscle metabolism, explain the negative correlation between fat and FAA.

ACKNOWLEDGMENT

The authors thank Dr Shigeo Takahashi, National Institute of Livestock and Grassland Science, for his helpful suggestions for the statistical analysis.

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