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ORIGINAL ARTICLE

# Effects of high protein levels in concentrate feed during the early fattening stage on physico-chemical composition and sensory characteristics of M. longissimus in Japanese Black heifers

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## ABSTRUCT

The effects that high levels of protein in concentrate feed has during the early fattening stage on physico-chemical composition and sensory characteristics of M. longissimus in Japanese Black heifers were investigated. Four sets (8 head) of identical twins of Japanese Black heifers were divided into two groups: a group fed high levels of protein in concentrate feed in the early fattening stage (HCP); and a control group. Moisture, fat, protein, cooking loss, Warner-Bratzler shear force and fatty acid composition of M. longissimus were similar in both groups. Levels of free amino acid (FAA), aspartic acid, glutamine, taurine and histidine were lower in the HCP group than in the control group ($P < 0.05$). Levels of glutamic acid, serine, asparagine, threonine and total FAA in the HCP group tended to be lower than in the control group ($P < 0.1$). There were no significant differences between the sensory characteristics (juiciness, tenderness, fattiness, flavor and overall acceptability) of the two groups. These results suggest that the use of high levels of protein in concentrate feed during the early fattening stage does not affect the sensory characteristics of Japanese Black beef, but does alter the FAA content.

***Key words:*** *early fattening stage, free amino acid, high protein, physico-chemical composition, sensory evaluation.*

## INTRODUCTION

Meat yield and quality are above all important in beef cattle production, but palatability is becoming increasingly important. Thus, using technology to produce a carcass that is high in yield, quality and palatability, at low cost, has become important in Japan. With the aim of improving growth and feed efficiency, many fattening tests have previously been performed. Some of these tests have investigated the use of high levels of protein in beef cattle feed (Dartt *et al*. 1978; Hanson & Klopfenstein 1979; Byers & Moxon 1980; Perry *et al*. 1983; Okumura *et al*. 2005).

Sensory characteristics are generally influenced by physico-chemical composition. Physico-chemical composition of beef is affected by sex (Field & Chang 1969; Clemens *et al*. 1973), sire (Inoue *et al*. 2002; Oka *et al*. 2002), age (Lengyel *et al*. 2003), feed (Westerling & Hedrick 1979; Melton *et al*. 1982; Kimura *et al*. 1996; Mandell *et al*. 1998). However, the relationship between sensory characteristics and feed has rarely been researched in the case of Japanese Black beef cattle. The effects of high levels of protein in concentrate feed during the early fattening stage on sensory characteristics of M. longissimus have not been reported.

The objectives of the present study are to determine the effects of high levels of protein in concentrate feed

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during the early fattening stage on the physico-chemical composition and sensory characteristics of M. longissimus in Japanese Black heifers.

## MATERIALS AND METHODS

### Animals

Four sets (8 head) of identical twins of Japanese Black heifer were used in this study. These animals were derived from bisected embryos, collected 7 days after artificial insemination with superstimulated Japanese Black donors. The two resultant demi-embryos were transferred to the crossbred heifers.

Animals were divided into the following two groups for each pair of identical twins: a group fed high levels of protein in concentrate feed during the early fattening stage (HCP); and a control group (control). The animals were fattened from 7 to 24 month of age. In the fattening stages, the early stage, the middle stage and the final stage were from 7–12, 13–21, and 22–24 month of age, respectively. Animals were fed concentrate feed and roughage, Timothy hay and Italian ryegrass hay to 12 month, rice straw from 13–24 months (Table 1). The animals had free access to their feeds.

Animal care and use adhered to the protocol approved by the National Livestock Breeding Center's Animal Care and Use Committee.

### Feed intake and physico-chemical analyzes

Concentrate feed and roughage intake was measured daily for both groups. Total digestible nutrients (TDN) and crude protein (CP) intake from concentrate feed were calculated for each fattening stage.

**Table 1** Composition of concentrate feed (as fed-basis) for HCP (fed high levels of protein in concentrate feed in the early fattening stage) and control groups

| Feed (%) | Early fattening stage | | Middle and final fattening stages† |
|---|---|---|---|
| | HCP | control | |
| Corn | 18 | 20 | 30 |
| Barley | 45 | 50 | 45 |
| Wheat bran | 16 | 20 | 20 |
| Soybean meal | 20 | 9 | 4 |
| Calcium carbonate | 1 | 1 | 1 |
| TDN | 73.1 | 72.5 | 73.0 |
| CP | 18.0 | 14.3 | 12.3 |

†HCP and control fed the same for the middle and final fattening stages. CP, crude protein; TDN, total digestible nutrients.

M. longissimus at the 7–8th thoracic vertebrae was analyzed to determine its moisture, fat (as ether extract)and protein content, cooking loss, Warner-Bratzler shear force (WBSF), water holding capacity (WHC), fatty acid composition and free amino acid (FAA) content. M. longissimus at the 9–10th thoracic vertebrae was used for the sensory evaluation. Samples for FAA content analysis and sensory evaluation were aged 9 days postmortem, 2°C. Aged muscles were frozen at −30°C and stored until analyzed. Moisture content was determined in duplicate by drying for 24 h at 105°C. Fat content was determined by Soxhlet extraction of the dried samples with diethyl ether for 16 h. Protein content was determined by the Kjeldahl method using a nitrogen distillation titration device (2400 Kjeltec Auto Sampler System; FOSS, Hillerod, Denmark).

Cooking loss (%) was determined by placing approximately 50 g meat in a sealed plastic bag in a water bath at 70°C for 1 h.

WBSF was analyzed using samples of at least 4 cakes of muscles that had already been analyzed for cooking loss and were cut (vertical cross section $1 \times 1$ cm$^2$) parallel to the long axis of the muscle fibers (SALTAR; Zenken, Tokyo, Japan). WHC was analyzed by following the Wierbicki and Deatherage (1958) method. Approximately 500 mg triplicate samples of muscle were placed in a filter-press device and compressed at 35 kgf/cm$^2$ for 1 min.

In analyzing fatty acid composition, intramuscular fat was extracted by means of a modification of the Folch *et al*. (1957) method using chloroform/methanol (2/1; v/v). Fatty acid composition was determined as methyl esters with a gas chromatograph (Detector FID, model GC380; GL Science, Tokyo, Japan) using a capillary column CP-Sil 88 W-cot 0.25 mm × 50 mol/L (GL Science).

For FAA content, the minced samples, to which ultra pure water was added, were homogenized with norleucine as an internal standard. Samples of FAA were obtained after removal of fat and protein using hexane and acetonitrile, respectively. The samples were analyzed by a HPLC (Waters 2487, detector UV; Waters, Milford, MA, USA), using a column for FAA (picotag 3.9 i.d. × 300 mm; Waters).

### Sensory evaluation

Sensory characteristics were evaluated by a panel of 8–13 panelists (average 10.0 panelists) who passed the

discrimination test of basic taste and hardness (JMISC 2005). The panel was trained, inhouse, in evaluating beef under the direction of the panel leader. A scale of 1–8 was used in the sensory evaluation. Sensory evaluation was conducted four times for each pair of twins. Aged muscles were thawed at 2°C, one day prior to cooking. The muscles were roasted at 165°C in a drying oven to an internal temperature of 70°C and were cut to size ($1 \times 1 \times 2$ cm$^3$). The samples were served at 60°C. The panelists were asked to assess the following attributes:

- Juiciness (scale 1–8; 1, extremely dry – 8, extremely juicy)
- Tenderness (scale 1–8; 1, extremely tough – 8, extremely tender)
- Fattiness (scale 1–8; 1, not fatty – 8, extremely fatty)
- Flavor I, II (scale 1–8; 1, extremely bland – 8, extremely intense)
- Overall acceptability (scale 1–8; 1, not acceptable – 8, extremely acceptable)

Juiciness, tenderness and fattiness were defined as the feeling from the first moment of the sample to the last swallow. Flavor was defined as the feeling of retronasal aroma and the taste through the oral cavity. Flavor was divided into flavor I, defined as sweet flavor and taste, and flavor II, defined as acid and animal flavor and taste. Overall acceptability was defined as the general feeling on the basis of each attribute.

Sensory evaluation was conducted under red lights, in order to eliminate the appearance influencing ratings.

## Statistical analysis

All data are presented as the mean ± standard error of the mean. The statistical significance of the data between the two groups was analyzed using two-sides paired *t*-test.

## RESULTS AND DISCUSSION

Concentrate, TDN and CP intake from animals for each fattening stage are shown in Table 2. Concentrate intake during the early fattening stage of HCP and control were 913.7 kg and 1088.4 kg, respectively ($P < 0.05$), and TDN intake from concentrate of HCP was lower than that of control ($P < 0.05$). There was no significant difference in CP intake from concentrate between the two groups because concentrate feed of HCP has higher levels of CP than control. Roughage

**Table 2** Concentrate feed intake in the early, middle and final fattening stages for HCP (fed high levels of protein in concentrate feed in the early fattening stage) and control groups

| Intake (kg) | Early stage | | Middle and final stages | | Total | | *P*-values | | |
|---|---|---|---|---|---|---|---|---|---|
| | HCP | control | HCP | control | HCP | control | Early stage | Middle and final stages | Total |
| Concentrate | 913.7 ± 23.3† | 1088.4 ± 26.5 | 2837.1 ± 47.0 | 2885.5 ± 25.4 | 3750.8 ± 57.4 | 3973.9 ± 35.9 | 0.031 | 0.488 | 0.051 |
| TDN‡ | 667.9 ± 17.0 | 789.1 ± 19.2 | 2071.1 ± 34.3 | 2106.4 ± 18.6 | 2739.0 ± 41.9 | 2895.5 ± 26.1 | 0.035 | 0.488 | 0.056 |
| CP‡ | 164.5 ± 4.2 | 155.6 ± 3.8 | 349.0 ± 5.8 | 354.9 ± 3.1 | 513.4 ± 7.9 | 510.6 ± 4.8 | 0.310 | 0.488 | 0.788 |

†Means ± standard error. ‡Intake from concentrate feed. CP, crude protein; TDN, total digestible nutrients.

intake of HCP and control was similar in the two groups (Hay: 259.0 kg and 260.8 kg, rice straw: 365.5 kg and 333.5 kg, respectively).

The physico-chemical analysis of M. longissimus are shown in Table 3. Fat content for HCP and control were 34.9% and 33.2%, respectively, which did not show a significant difference. Physical traits of cooking loss, WBSF and WHC were similar between the two groups.

Fatty acid composition of M. longissimus is shown in Table 4. The proportions of saturated fatty acid did not show significant differences between the two groups. The proportions of oleic acid, which makes up the greatest proportion of unsaturated fatty acids, in M. longissimus were 46.3% and 45.4% for HCP and control, respectively, which did not show a significant difference between the two groups. The proportions of other unsaturated fatty acids were similar between the two groups as well. Furthermore, total unsaturated fatty acid per total saturated fatty acid (US/S) of the two groups were similar.

The feed given to HCP did not affect the fatty acid composition of M. longissimus. Generally, it is known that fatty acid composition of beef is affected by feed. There are numerous studies examining the effects of different feeding regiems during the fattening period, including the final fattening stage, on fatty acid composition of beef (Westerling & Hedrick 1979; Kimura *et al*. 1996; Mandell *et al*. 1998). In the present study, different feeds were given to the two groups only during the early fattening stage; all animals received the same feed during the middle and final fattening stages. It was hypothesized that the feeding method used in this study would not affect fatty acid composition of M. longissimus.

FAA content of M. longissimus are shown in Table 5. The levels of FAA, aspartic acid, glutamine, taurine and histidine of HCP were lower than that of control ($P < 0.05$). Glutamic acid, serine, asparagine and threonine of HCP tended to be lower than that of control ($P < 0.10$). Almost all FAAs analyzed in this

**Table 3** Physico-chemical composition of *M. longissimus* for HCP (fed high levels of protein in concentrate feed in the early fattening stage) and control groups

| | HCP | control | *P*-values |
|---|---|---|---|
| Moisture (%) | 49.3 ± 2.7† | 50.7 ± 2.0 | 0.161 |
| Fat (%) | 34.9 ± 3.7 | 33.2 ± 2.3 | 0.324 |
| Protein (%) | 15.3 ± 0.9 | 15.4 ± 0.3 | 0.878 |
| Cooking loss (%) | 21.5 ± 1.0 | 21.9 ± 1.1 | 0.594 |
| WBSF (*n*) | 10.4 ± 2.0 | 10.3 ± 1.6 | 0.948 |
| WHC | 82.4 ± 0.8 | 84.6 ± 2.5 | 0.316 |

†Means ± standard error. WBSF, Warner-Bratzler shear force; WHC, water holding capacity.

**Table 4** Fatty acid composition of *M. longissimus* for HCP (fed high levels of protein in concentrate feed in the early fattening stage) and control groups

| | HCP | control | *P*-values |
|---|---|---|---|
| C12:0 (%) | 0.1 ± 0.0† | 0.1 ± 0.0 | 0.696 |
| C14:0 (%) | 3.4 ± 0.4 | 3.5 ± 0.5 | 0.475 |
| C14:1 (%) | 0.9 ± 0.1 | 1.0 ± 0.1 | 0.147 |
| C15:0 (%) | 0.5 ± 0.1 | 0.5 ± 0.0 | 0.690 |
| C16:0 (%) | 29.2 ± 0.9 | 29.6 ± 1.8 | 0.832 |
| C16:1 (%) | 3.9 ± 0.2 | 4.0 ± 0.2 | 0.326 |
| C17:0 (%) | 1.2 ± 0.2 | 1.2 ± 0.1 | 0.892 |
| C17:1 (%) | 1.0 ± 0.1 | 1.1 ± 0.1 | 0.598 |
| C18:0 (%) | 9.9 ± 0.5 | 10.0 ± 0.8 | 0.919 |
| C18:1 (%) | 46.3 ± 1.7 | 45.4 ± 2.9 | 0.672 |
| C18:2 (%) | 3.0 ± 0.3 | 3.0 ± 0.4 | 0.757 |
| C18:3 (%) | 0.3 ± 0.0 | 0.3 ± 0.1 | 0.949 |
| C20:1 (%) | 0.3 ± 0.1 | 0.3 ± 0.1 | 0.129 |
| US/S | 1.3 ± 0.1 | 1.3 ± 0.1 | 0.928 |

†Means ± standard error. US/S, total unsaturated fatty acid per total saturated fatty acid.

**Table 5** Free amino acid (FAA) content of *M. longissimus* for HCP (fed high levels of protein in concentrate feed in the early fattening stage) and control groups

| FAA | HCP | control | (μmol/g) *P*-values |
|---|---|---|---|
| Aspartic acid | 0.08 ± 0.01† | 0.10 ± 0.01 | 0.020 |
| Glutamic acid | 0.36 ± 0.05 | 0.45 ± 0.08 | 0.054 |
| Hydroxyproline | 0.03 ± 0.01 | 0.03 ± 0.02 | 0.910 |
| Serine | 0.47 ± 0.02 | 0.55 ± 0.03 | 0.064 |
| Asparagine | 0.19 ± 0.01 | 0.23 ± 0.01 | 0.063 |
| Glycine | 0.86 ± 0.05 | 0.93 ± 0.06 | 0.302 |
| Glutamine | 1.12 ± 0.18 | 1.50 ± 0.29 | 0.044 |
| β-Alanine | 0.08 ± 0.01 | 0.10 ± 0.02 | 0.479 |
| Taurine | 1.67 ± 0.05 | 1.96 ± 0.11 | 0.034 |
| Histidine | 0.14 ± 0.01 | 0.17 ± 0.01 | 0.022 |
| Threonine | 0.38 ± 0.01 | 0.48 ± 0.03 | 0.054 |
| Alanine | 2.74 ± 0.26 | 3.06 ± 0.13 | 0.308 |
| Arginine | 0.33 ± 0.02 | 0.36 ± 0.04 | 0.149 |
| Proline | 0.24 ± 0.00 | 0.28 ± 0.02 | 0.119 |
| Tyrosine | 0.27 ± 0.01 | 0.30 ± 0.02 | 0.116 |
| Valine | 0.45 ± 0.03 | 0.50 ± 0.03 | 0.313 |
| Methionine | 0.24 ± 0.03 | 0.27 ± 0.02 | 0.207 |
| Isoleucine | 0.32 ± 0.03 | 0.35 ± 0.02 | 0.275 |
| Leucine | 0.64 ± 0.05 | 0.70 ± 0.04 | 0.302 |
| Phenylalanine | 0.31 ± 0.03 | 0.35 ± 0.02 | 0.170 |
| Tryptophan | 0.09 ± 0.01 | 0.10 ± 0.01 | 0.108 |
| Lysine | 0.39 ± 0.04 | 0.43 ± 0.05 | 0.397 |
| Total | 11.40 ± 0.53 | 13.22 ± 0.63 | 0.057 |

†Means ± standard error.

study were lower in HCP than in control. Total FAA of HCP and control were 11.40 μmol/g and 13.22 μmol/g, respectively ($P < 0.10$). Each FAA as a percentage of total FAA content of M. longissimus is shown in Table 6. The histidine percentage of HCP was lower than that of control ($P < 0.05$), and the tyrosine percentage of HCP was higher than that of control ($P < 0.01$).

**Table 6** Each free amino acid (FAA) to total free amino acid content of *M. longissimus* for HCP (fed high levels of protein in concentrate feed in the early fattening stage) and control groups

| FAA (%) | HCP | control | *P*-values (%) |
|---|---|---|---|
| Aspartic acid | 0.70 ± 0.10† | 0.79 ± 0.11 | 0.271 |
| Glutamic acid | 3.16 ± 0.47 | 3.37 ± 0.46 | 0.314 |
| Hydroxyproline | 0.29 ± 0.10 | 0.25 ± 0.13 | 0.868 |
| Serine | 4.13 ± 0.27 | 4.17 ± 0.12 | 0.824 |
| Asparagine | 1.67 ± 0.02 | 1.73 ± 0.05 | 0.169 |
| Glycine | 7.49 ± 0.11 | 7.05 ± 0.21 | 0.139 |
| Glutamine | 9.90 ± 1.65 | 11.26 ± 1.85 | 0.132 |
| β-Alanine | 0.75 ± 0.09 | 0.73 ± 0.15 | 0.919 |
| Taurine | 14.65 ± 0.34 | 14.82 ± 0.59 | 0.823 |
| Histidine | 1.24 ± 0.04 | 1.32 ± 0.04 | 0.032 |
| Threonine | 3.38 ± 0.26 | 3.63 ± 0.18 | 0.194 |
| Alanine | 23.88 ± 1.14 | 23.20 ± 0.25 | 0.655 |
| Arginine | 2.87 ± 0.21 | 2.71 ± 0.19 | 0.398 |
| Proline | 2.09 ± 0.08 | 2.15 ± 0.22 | 0.707 |
| Tyrosine | 2.41 ± 0.13 | 2.28 ± 0.13 | 0.003 |
| Valine | 3.96 ± 0.11 | 3.78 ± 0.05 | 0.272 |
| Methionine | 2.14 ± 0.20 | 2.09 ± 0.12 | 0.622 |
| Isoleucine | 2.76 ± 0.14 | 2.64 ± 0.12 | 0.264 |
| Leucine | 5.62 ± 0.38 | 5.28 ± 0.30 | 0.095 |
| Phenylalanine | 2.75 ± 0.19 | 2.67 ± 0.18 | 0.230 |
| Tryptophan | 0.75 ± 0.04 | 0.79 ± 0.06 | 0.556 |
| Lysine | 3.41 ± 0.20 | 3.26 ± 0.29 | 0.472 |

†Means ± standard error.

Meat FAA content is affected by factors such as sex (Field & Chang 1969), type of muscle (Field & Chang 1969; Mikami *et al*. 1995), aging (Field *et al*. 1971; Nishimura *et al*. 1988; Mikami *et al*. 1995) and aminopeptidase (JMISC 2005). As the samples used in this study were from identical twins, changes in FAA content were not influences by the type of muscle, aging or genotype. Tsuneishi *et al*. (2006) report that FAA content in beef can be differentiated on the basis of grazing and fattening cattle. Fujimura *et al*. (2001) reported that FAA content of chicken meat is affected by restricted feeding.

In the present study, protein content of the two groups was found to be similar, but most FAAs were lower in HCP than in control. Weight gain was similar in both groups across all fattening stages, but muscle weight in M. longissimus of HCP tended to be greater than that of control ($P < 0.10$). Muscular growth might affect the size of muscular fiber, and it might cause changes of FAA content in M. longissimus in both groups. On the other hand, concentrate feed intake and TDN intake of HCP was lower than that of control. Although the factor which affected FAA content in M. longissimus could not be demonstrated in this study, these results suggest that changes of FAA content in M. longissimus are affected by the feeding and management of the animals.

The sensory characteristics of M. longissimus are shown in Figure 1. The sensory characteristics of juiciness, tenderness, fattiness, flavor I and II were similar in the two groups. Overall acceptability of HCP and control were 4.86 points and 5.03 points, respectively, showing no significant difference. The result of the sensory evaluation of M. longissimus, which showed no significant difference in fat content, WBSF, WHC or fatty acid composition, and which only differed

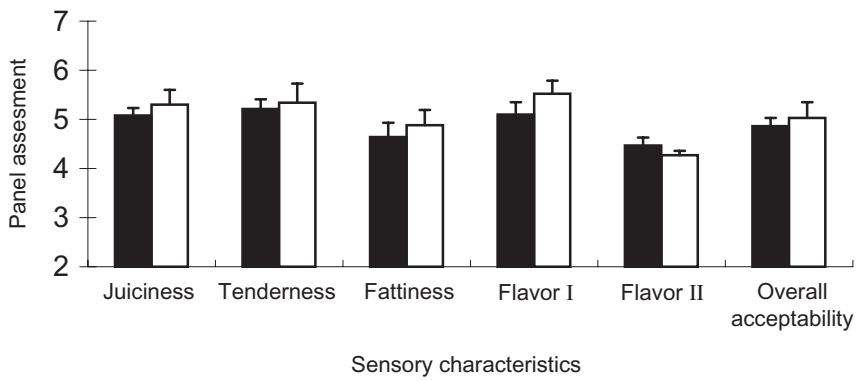


**Figure 1** Sensory characteristics of M. longissimus for HCP (■) (fed high levels of protein in concentrate feed in the early fattening stage) and control groups (□). Means ± standard error.

significantly in FAA content, indicates that FAA content did not affect the sensory characteristics of M. longissimus in this study.

Juiciness of beef is affected by intramuscular fat (Okumura *et al*. 2007) and cooking loss (Jost *et al*. 1983). In the present study, it was thought that juiciness between the two groups did not show a significant difference because the fat content and cooking losses of the two groups were not significantly different. WBSF is widely used as an index of beef tenderness. It was thought that tenderness was similar between the two groups because there was no significant difference between the groups in WBSF. Also, in general, it is thought that fattiness is affected by intramuscular fat content and quality. In this study, it was thought that fattiness between the two groups were similar because fat content and fatty acid composition between the two groups did not show any significant differences.

In this study, flavor was defined as the feeling of retronasal aroma and the taste. There is a relationship between flavor and fatty acid composition (Dryden & Marchello 1970; Westerling & Hedrick 1979; Melton *et al*. 1982). Moreover, there is a relationship between both FAA content (Kawamura *et al*. 1983; Nishimura *et al*. 1988) and oligopeptide (Nishimura *et al*. 1988) in meat and sensory characteristics. On the other hand, Field *et al*. (1971) report that a relationship between FAA content in beef and flavor is low, and Kimata *et al*. (2001) reported that FAA content and inosine 5′-monophosphate (IMP) content do not obviously contribute to flavor in pork. Since fatty acid composition in the two groups did not show a significant difference, flavor was considered to be the results of taste components. FAA content, which showed a significant difference between the two groups, did not affect flavor in this study. Although taste components affect sensory characteristics, humans have a taste threshold (Maga 1994) and difference threshold (JUSE 1973). In this study, it was thought that the differences in FAA content between the two groups were so small that panelists could not distinguish. Yamaguchi (1967) reported a synergic effect between glutamic acid and IMP and Maga (1994) reported the individual taste thresholds for various umami compounds, alone and in combination with each other. However IMP was not analyzed in this study, and the importance of IMP with regards to beef taste should be investigated in the future.

Although using high levels of protein in concentrate feed in the early fattening stage did not affect sensory characteristics of Japanese Black beef, it did affect the FAA content of Japanese Black beef. These results suggest that there is room to improve beef quality, including palatability, by changing fattening methods in feedlots.

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