Effects of Mixed Bone and Brisket Meat on Physico-Chemical Characteristics of Shank Bone and Rib Extracts from Hanwoo

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Abstract
This study was conducted to investigate the effects of mixed bone and brisket meat on the quality characteristics and nutritional components of shank bone extract and rib extract from Hanwoo. The pH values were influenced by the raw bones, mixed bone, brisket meat and their interactions (p<0.05). The salinity, sugar content, turbidity, and essential amino acid values increased significantly with addition of mixed bone and brisket meat. All attributes of sensory evaluation score were the highest in T6 (Rib 500 g + Mixed bone 500 g + Brisket meat 400 g) (p<0.05). The mixed bone significantly increased the saturated fatty acids of shank bone extract (p<0.001). Thus, the addition of mixed bone and brisket meat had a positive effect on the quality and nutritional components in shank and rib extracts of Hanwoo cattle.

Keywords: Hanwoo, shank bone, rib, mixed bone, brisket meat, nutritional components

Introduction
Recently, with the increase in national income, the interest of consumers in the safety and hygiene of animal food has greatly increased. In particular, meat-processing companies are trying to produce high-quality and eco-friendly premium meat products to compete with imported meat products (Korea Meat Industries Association, 2009). Koreans traditionally consume soups using extracts that have been obtained from the shank bone, tail, rib, knee cartilage, and mixed bone of cattle and have been boiled for a long time. Until now, the soup “Gomtang” was mainly produced using extracts from legs and other portions of Hanwoo cattle (Kim et al., 2014). The soup “Galbitang” is prepared from extracts from ribs and other portions of Hanwoo cattle in Korea.

Bone mainly consists of the following: 1/3, organic substances; 2/3, inorganic substances such as collagenous fiber and bone minerals (Malmberg and Nygren, 2008). In addition, collagen is a key building block of cells of bones, cartilage, ligaments, and the brain (Poundarik et al., 2015). Kim et al. (2007) reported that collagen protein and chondroitin sulfate were abundant in the Hanwoo shank bone. Gel obtained from bone extraction is also formed by the bonding of salt-soluble proteins from meat, such as myosin, actin, and actomyosin, and gelatinization of collagen in bone (Chen et al., 2014; Comfort and Howell, 2003).

Recently, the nutritional importance of microelements in the diet has gained attention; among these, calcium is one of the most nutritive components whose deficiency is often seen in human diets. Lack of calcium intake is closely related to growth and bone maintenance, osteoporosis, fractures, and bone diseases (Lupsa and Insogna, 2015). In particular, as aging progresses, calcium intake becomes more important for the prevention and cure of skeletal disorders (Chapuy et al., 1992). Cattle bone-derived calcium is an effective calcium source because of higher calcium bioavailability than other calcium sources. High phosphorus content in the bone is reported to inhibit calcium absorption in human body (Spencer et al., 1978).

Therefore, this study was conducted to compare the effects of mixed bone and brisket meat on quality characteristics and nutritional components of shank bone and rib extracts from Hanwoo.
Materials and Methods

Preparation of Hanwoo bone extracts

Commercial Hanwoo shank, rib, mixed bone, and brisket meat were purchased from a local market. Visible impurities, subcutaneous debris, and excessive connective tissues were removed from bones and meat. The bones were washed thrice with water, and this water was discarded. The extraction process was performed by adding 2.5 L distilled water to the bones and boiling them over medium heat for 8 h. The experiment design was as follows: T1 (Shank bone 1 kg), T2 (Shank bone 500 g + Mixed bone 500 g), T3 (Shank bone 500 g + Mixed bone 500 g + Brisket meat 400 g), T4 (Rib 1 kg) T5 (Rib 500 g + Mixed bone 500 g), T6 (Rib 500 g + Mixed bone 500 g + Brisket meat 400 g). After extraction, the extracted liquid was adjusted to 2 liter, and filtered using cotton stuff, and cooled at 4°C for 6 h in a cold chamber, subsequently, the supernatant fat was removed. After 18 h, the final liquid extracts were continuously analyzed according to the experimental items including pH, salinity, sugar content, turbidity, sensory evaluation, TBARS, VBN, free amino acids and fatty acid composition.

Physico-chemical analysis methods

pH

pH was measured in triplicate using a digital pH meter (8603, Metrohm, Swiss). Approximately 10 g of the sample was cut into small pieces to which 90 mL of distilled water was added, and a homogenate was prepared using a homogenizer (T25B, IKA Sdn, Bhd., Malaysia); then, the pH was measured using a pH meter. The pH meter was calibrated daily with standard buffers of pH 4.0 (9863 pH buffer solution; Mettler Toledo Swiss) and 7.0 (9865 pH buffer solution; Mettler Toledo Swiss) at 25°C.

Salinity and sugar content

The sample was filtered using Whatman No. 1 filter paper, and the salinity and sugar content of filtered samples were determined using a digital salimeter (PAL-03S, ATAGO, Japan) and saccharometer (PAL-3, ATAGO, Japan) respectively.

Turbidity

A double beam spectrophotometer (Optizen 3220UV, Mecasys, Korea) was used for measuring turbidity. The bone extract sample was filtered using Whatman No. 1 filter paper. The turbidity of the filtered sample was measured at 590 nm; the blank was distilled water. Turbidity has been represented as % transmittance.

Sensory evaluation

Sensory evaluation was performed by a panel of 15 semi trained tasters. The panel consisted of 10 researchers and 5 technicians at the Chungbuk National University (male: female: 1:1; age range, 25-45 years). All samples were given random numbers and were served randomly. The samples had warmed up at microwave for 1 min, and served in paper cups per 50 mL. The panel evaluated each treatment within each replication in triplicate, and the evaluation was performed with the samples at room temperature. The panelists rinsed their mouths with water and some neutral crackers between the samples. The color, aroma, flavor, viscosity, and overall acceptability (1 = extremely slight or poor, 5 = extremely intense or superb) of the extracts were evaluated using a five-point scale.

2-thiobarbituric acid reactive substance (TBARS)

Five milliliters of the sample was added into a 50 mL test tube and homogenized with 15 mL of de-ionized distilled water using a homogenizer (T25; IKA Werke GmbH & Co., KG, Germany) for 10 s at 3,000 rpm. Then, 1 mL sample homogenate was transferred to a disposable test tube (3×100 mm), and butylated hydroxyanisole (50 µL, 10%) and thiobarbituric acid/trichloroacetic acid (TBA/TCA) (2 mL) were added. The mixture was vortexed and then incubated in a boiling water bath for 15 min for color development. The sample was cooled in cold water for 10 min, vortexed again, and centrifuged for 15 min at 2,000 g. The absorbance of the resulting supernatant solution was determined at 531 nm against a blank containing 1 mL distilled water and 2 mL TBA/TCA solution. The TBARS concentration has been expressed as milligrams of malondialdehyde per kilogram of sample.

Volatile basic nitrogen (VBN)

VBN, a measure of protein degradation, was measured as described previously, with some modifications (Pearson, 1976). Briefly, 10 mL of the sample and a few drops of phenolphthalein indicator (0.5 wt% solution in 50 wt% ethanol) were placed in a distillation flask; then 3.5 mL of 20% sodium hydroxide solution was added. The apparatus was then sealed immediately, and the end of the steam distillate was collected in a flask containing 20 mL 4% boric acid and a few drops of Tashiro indicator (methyl red-methylene blue = 2:1). The steam distillation procedure was continued until 250 mL of distillate had been
collected. Next, the basic solution obtained was titrated against 0.01 M hydrochloric acid to the end point, which was indicated by a green-to-gray color change. The VBN content was measured after a blank correction determined by steam distillation of 6% perchloric acid.

**Free amino acids analysis**

Using the method of Aristoy and Toldra (1991), free amino acids were extracted with 0.01 N HCl. The, 300 µL extracted sample was mixed with 10 µL internal standard (l-citrulline) and 690 µL acetonitril, and the mixture was incubated for 30 min at 4°C; subsequently, it was centrifuged at 10,000×g for 15 min at 4°C. The supernatant was filtered through a 0.45-µm membrane filter. The filtered sample and external standard (amino acid standard: 0.25 nM, Agilent Technologies, USA; glutamine, Sigma) was analyzed with O-phthalaldehyde (OPA) and 9-fluorenylmethyl chloroformate (FMOC) derivatization using HPLC (Agilent, USA), following the method of Herbert et al. (2000). Analysis condition was following a method (DAD detector, 262 nm, 338 nm; Column, Zorbax Eclipse AAA, 4.6×60 mm, 5 µm; column temp, 40°C; Mobile phase A, acetonitril: methanol: water, 45:45:10, v:v:v) of Henderson et al. (2000).

**Fatty acids analysis**

Total lipids of samples were extracted by using chloroform-methanol (2:1, v/v) according to the procedure of Folch et al. (1957). An aliquot of the total lipid extract was methylated as described by Morrison and Smith (1964). Fatty acid methyl esters were analyzed using a gas chromatograph (Varian 3800) fitted with a fused silica capillary column, Omegawax 205 (30 m×0.32 mm i·d., 0.25-µm film thickness). The injection port was at 250°C and the detector was maintained at 300°C. Results have been expressed as percentages based on the total peak area.

**Statistical analysis**

The entire experiment was replicated three times at different times in the same place, and a completely randomized design was used. The data of physico-chemical properties of extracts were analyzed by General Linear Model procedure of SAS program. The statistical model included raw bones, mixed bone, brisket meat and interactions of them, as well as the panelist in sensory evaluation, as fixed errors, with individual extract defined as the error term (experimental unit). Effects of the bones, mixed bone, brisket meat and their interactions with \( p<0.05 \), \( p<0.001 \) and \( p<0.0001 \) were judged as ‘significance’ and ‘tendency,’ respectively. All data analysis was performed using SAS for Windows, version 9.1.3 (SAS, 2003).

**Result and Discussion**

**Physico-chemical properties of bone extracts**

The effects of mixed bone and brisket meat on physico-chemical properties of shank bone and rib extracts are presented in Table 1. The pH values of extracts significantly were influenced by the raw bones, mixed bone, brisket meat and their interactions \( (p<0.05) \). The highest values of salinity and sugar contents were observed in shank bone extract added with mixed bone and brisket meat (T3), all effects excluding raw bones and brisket meat interaction affected the values in salinity and sugar contents \( (p<0.05) \). Also, the turbidity values significantly were influenced by all raw materials. All bones contain minerals such as calcium, phosphorous, magnesium, and sodium. These minerals can be extracted by high-temperature or boiling water extraction. In these bone extracts, the minerals exist as calcium carbonate, calcium phosphate, and magnesium phosphate (Lorprayoon, 1986). According to Kim (2006), shank bone and rib bone extracts of cattle extracted for eight hours contained 0.39 and 0.45 mg/100 mL calcium, 0.05 and 0.08 mg/100 mL magnesium, 0.19 and 1.44 mg/100 mL phosphorous.

**Table 1. Effects of mixed bone and brisket meat on physico-chemical characteristics of shank and rib extracts in Hanwoo**

| Items \(^1\) | pH | Salinity (%) | Sugar contents (%) | Turbidity (%) |
|-------------|----|--------------|--------------------|--------------|
| T1          | 8.24 | 2.57 | 3.17 | 2.28 |
| T2          | 8.12 | 1.63 | 1.97 | 2.32 |
| T3          | 6.56 | 3.03 | 3.53 | 2.80 |
| T4          | 6.31 | 0.80 | 1.23 | 2.14 |
| T5          | 6.85 | 1.27 | 1.63 | 2.89 |
| T6          | 6.36 | 2.63 | 3.30 | 2.69 |
| SEM         | 0.19 | 0.19 | 0.21 | 0.04 |

\(^1\)T1 (Shank bone 1 kg), T2 (Shank bone 500 g + Mixed bone 500 g), T3 (Shank bone 500 g + Mixed bone 500 g + Brisket meat 400 g), T4 (Rib 1 kg) T5 (Rib 500 g + Mixed bone 500 g), T6 (Rib 500 g + Mixed bone 500 g + Brisket meat 400 g).

\(^2\)R: Raw bones, \(^3\)M: Mixed bone addition, \(^4\)B: Brisket meat addition, \(^5\)R×M: Interaction between raw bones and mixed bone, \(^6\)B×M: Interaction between bones and brisket meat.

\(*p<0.05, \**p<0.001, \***p<0.0001\)
100 mL phosphorous, respectively. Furthermore, calcium, magnesium, and phosphorous contents in shank bone extract increased significantly as the cooking time increased from 2 to 12 h (Seol and Jang, 1990). Therefore, it was determined that the pH of bone extracts increases because ionized minerals have the capacity to exchange hydrogen ions. The pH of bone extracts with brisket meat was significantly lower, due to the influence of lower pH meat (general pH range of Hanwoo meat, 5.4-5.6) (Cho et al., 2008), than that of the bone extract with rib bone meat attached. Kim et al. (2014) showed that sodium content (211.77, 254.40, 134.93 mg/kg) was the highest in shank bone extract, feet extract, and tail extract, respectively. These results were consistent with those of Park (1986).

In addition, the contents of sodium and potassium in Hanwoo beef cuts such as rib, sirloin, chuck roll, tender loin, and fore shank were shown to be 51.49-68.21 mg/100 g and 144.57-338.81 mg/100 g, respectively (Kim et al., 2010). In addition, the contents of nucleotides and their related compounds such as AMP, IMP, inosine, and hypoxanthine were significantly higher in brisket soup than in shank bone soup (Cho and Jung, 1999). Therefore, the salinity and sugar contents were increased following the addition of mixed bone and meat. Kim (2006) reported that turbidity of shank bone extract was lower than that of rib bone extract, which was consistent with the observations of this study. However, turbidity did not show a clear tendency in the extracts with mixed bone and brisket. This could be because mixed bone contains unequal proportions of different parts.

Sensory evaluation

The results of sensory evaluation of bone extracts added with mixed bone and brisket meat were presented in Table 2. The brisket meat significantly affected the color values of bone extracts, and interaction between raw bones and mixed bone was found in the bone extracts. The aroma values of extracts were only influenced by the raw bones (p<0.05). The values in flavor and total acceptability significantly were influenced by the brisket meat addition, and the highest values were observed in rib extract. According to Kim (2006), rib bone extract showed significantly higher sensory evaluation scores for color, flavor, taste, and overall acceptability than did shank bone extract. These reasons can be inferred as the following results of Kim (2006). The contents of solids, proteins, free-amino acids, and collagen — flavor components — were significantly higher in rib bone extracts than in shank bone extracts. This finding suggested that sensory evaluation scores could be influenced by flavor components in meats.

TBARS and VBN

The effects of mixed bone and brisket meat on TBARS and VBN of bone extracts in Hanwoo were presented in Table 3. The TBARS value of extract from rib added with mixed bone and brisket meat was the highest among the treatment groups. In addition, shank bone groups tended to show lower TBARS values compared to those of rib groups. The VBN values were significantly increased by the addition of brisket meat (p<0.0001). The TBARS content represents fat oxidation in meat products. Excessive fat oxidation results in malodor and rancidity of the meat. Fat oxidation could be promoted by high fat content and the presence of high amounts of free fatty acids in the meat, sunlight, irradiation, thermal treatment, and transition metal ions such as iron, copper, zinc, and magnesium (Dominguez et al., 2014; Falowo et al., 2014; Lorenzo and Pateiro, 2013; Nam et al., 2003; Pelser et al., 2007). Kim (2006) demonstrated that metal ion of the rib extract was higher than that of the shank bone extract, which explains the higher TBARS content in rib extracts. Furthermore, the VBN content was found to be derived from meat rather than bone, because VBN values were higher in the extracts containing brisket meat and rib. Park (1986) reported that shank bone contained approximately 20%
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Collagen. Only 20% of the total collagen content was extracted during 20 h of hot water extraction.

Free amino acids

The free amino acid contents in bone extracts are presented in Table 5. Glycine, glutamic acid, alanine, and aspartic acid were the main free amino acids in shank bone and rib extracts with mixed bone and brisket meat. The contents of glutamic acid, a flavor component, were higher in extracts with brisket meat than in other groups. The contents of sweet amino acids, such as threonine, serine, glycine, and alanine, were the highest in shank bone extract with mixed bone and brisket meat. Further, the sum of essential amino acids methionine, threonine, valine, isoleucine, leucine, phenylalanine, lysine, histidine, and arginine were higher in T1, T3, and T6 extracts. These groups also showed total amino acid contents of more than 2.1 mg% (data not shown). According to the report of Kim et al. (2014), the content of glycine, which is the main amino acid in animal proteins, was the most abundant amino acid in shank bone extract, followed by proline, alanine, glutamate, and arginine. The results of this study were consistent with those of Kim et al. (2014), regardless of the additions. Cho and Jung (1999) reported that the quantity of total amino acids was far higher in shank bone extract with brisket than in shank bone extract. In addition, Park (1986) reported that bone extract contained all essential amino acids, except tryptophan, but their quantity was very small. Therefore, he suggested

### Table 3. Effects of mixed bone and brisket meat on TBARS, and VBN characteristics of shank and rib extracts in Hanwoo at day 1

| Items  | TBARS  | VBN  |
|--------|--------|------|
|        | (mg malonaldehyde/ 1,000g) | (mg%) |
| T1     | 0.12   | 2.33 |
| T2     | 0.17   | 1.69 |
| T3     | 0.13   | 8.20 |
| T4     | 0.19   | 6.18 |
| T5     | 0.21   | 3.71 |
| T6     | 0.24   | 8.83 |
| SEM    | 0.01   | 0.77 |

| p-value |     |     |
|---------|-----|-----|
| R²      | **  |     |
| M(5)    | -   |     |
| B(6)    |     | *** |

1) 2-thiobarbituric acid reactive substance, 2) Volatile basic nitrogen, 3) T1 (Shank bone 1 kg), T2 (Shank bone 500 g + Mixed bone 500 g), T3 (Shank bone 500 g + Mixed bone 500 g + Brisket meat 400 g), T4 (Rib 1 kg) T5 (Rib 500 g + Mixed bone 500 g), T6 (Rib 500 g + Mixed bone 500 g + Brisket meat 400 g). 4) R: Raw bones, 5) M: Mixed bone addition, 6) B: Brisket meat addition, 7) R×M: Interaction between raw bones and mixed bone, 8) R×B: Interaction between raw bones and brisket meat. *p<0.05, **p<0.001, ***p<0.0001.

### Table 4. Effect of mixed bone and brisket meat on free amino acids of shank and rib extracts in Hanwoo (mg%)

| Items  | T1  | T2  | T3  | T4  | T5  | T6  | SEM  | R²  | M(5) | B(6) | R×M  | R×B  |
|--------|-----|-----|-----|-----|-----|-----|------|-----|------|------|------|------|
| Cys    | 0.003 | 0.002 | 0.006 | 0.002 | 0.003 | 0.006 | 0.000 | -   | **   | ***  | **   | ***  |
| Met    | 0.020 | 0.011 | 0.020 | 0.003 | 0.007 | 0.016 | 0.001 | *** | ***  | ***  | ***  | ***  |
| Asp    | 0.147 | 0.082 | 0.166 | 0.038 | 0.062 | 0.166 | 0.012 | *** | ***  | ***  | ***  | ***  |
| Thr    | 0.051 | 0.029 | 0.059 | 0.014 | 0.023 | 0.058 | 0.004 | *** | ***  | ***  | ***  | ***  |
| Ser    | 0.083 | 0.046 | 0.090 | 0.022 | 0.034 | 0.088 | 0.006 | *** | ***  | ***  | ***  | ***  |
| Glu    | 0.273 | 0.154 | 0.332 | 0.085 | 0.125 | 0.343 | 0.024 | *** | ***  | ***  | ***  | ***  |
| Gly    | 0.536 | 0.275 | 0.550 | 0.117 | 0.182 | 0.510 | 0.042 | *** | ***  | ***  | ***  | ***  |
| Ala    | 0.211 | 0.111 | 0.227 | 0.051 | 0.079 | 0.215 | 0.017 | *** | ***  | ***  | ***  | ***  |
| Val    | 0.054 | 0.030 | 0.059 | 0.013 | 0.023 | 0.059 | 0.004 | *** | ***  | ***  | ***  | ***  |
| Iso    | 0.038 | 0.022 | 0.044 | 0.011 | 0.017 | 0.043 | 0.003 | *** | ***  | ***  | ***  | ***  |
| Leu    | 0.090 | 0.053 | 0.105 | 0.026 | 0.042 | 0.105 | 0.007 | *** | ***  | ***  | ***  | ***  |
| Tyr    | 0.026 | 0.015 | 0.024 | 0.009 | 0.013 | 0.026 | 0.001 | *** | ***  | ***  | ***  | ***  |
| Phe    | 0.055 | 0.031 | 0.058 | 0.015 | 0.023 | 0.055 | 0.004 | *** | ***  | ***  | ***  | ***  |
| Lys    | 0.084 | 0.048 | 0.104 | 0.025 | 0.040 | 0.113 | 0.008 | *** | ***  | ***  | ***  | ***  |
| His    | 0.022 | 0.015 | 0.062 | 0.019 | 0.019 | 0.067 | 0.005 | *   | ***  | ***  | ***  | ***  |
| Arg    | 0.187 | 0.097 | 0.195 | 0.040 | 0.065 | 0.184 | 0.015 | *** | ***  | ***  | ***  | ***  |
| Pro    | 0.220 | 0.120 | 0.225 | 0.060 | 0.084 | 0.205 | 0.016 | *** | ***  | ***  | ***  | ***  |

1) T1 (Shank bone 1 kg), T2 (Shank bone 500 g + Mixed bone 500 g), T3 (Shank bone 500 g + Mixed bone 500 g + Brisket meat 400 g), T4 (Rib 1 kg) T5 (Rib 500 g + Mixed bone 500 g), T6 (Rib 500 g + Mixed bone 500 g + Brisket meat 400 g). 2) R: Raw bones, 3) M: Mixed bone addition, 4) B: Brisket meat addition, 5) R×M: Interaction between raw bones and mixed bone, 6) R×B: Interaction between raw bones and brisket meat. *p<0.05, **p<0.001, ***p<0.0001.
that when preparing bone extract soup, bone should be boiled with meat. In this study, bone extracts with brisket meat showed higher contents of essential amino acids than extracts of bones alone.

Fatty acid compositions
The fatty acid compositions of bone extracts is presented in Table 5. The contents of palmitic acid (C16:0) were high in the rib extracts, whereas the contents of stearic acid (C18:0) were high in shank bone extracts significantly ($p<0.0001$). The contents of oleic acid (C18:1) in the shank bone and rib extracts were significantly decreased by the addition of mixed bone. In addition, the shank bone extract with mixed bone (T2) had the highest contents of saturated fatty acids, whereas the rib extract (T4) had the highest contents of unsaturated fatty acid. To date, the fatty acid composition of Hanwoo bone extract soup has not been reported. However, the fatty acid composition of our bone extract was similar that of Hanwoo beef (Cho et al., 2013). In Korea, when bone extract soup is consumed, most of the fat is removed after cooling. Therefore, generally, only a small amount of fat will be present in the extract.

**Conclusion**

Based on these results, it was concluded that the quality and nutritional components of bone extracts were influenced by the addition of raw materials, and given that studies on this topic have not been conducted domestically or globally, our findings could provide basic data for further research on using animal bone extract in the food industry.

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| Items | T1 | T2 | T3 | T4 | T5 | T6 | SEM | p-value |
|-------|----|----|----|----|----|----|-----|---------|
| C14:0 | 2.87 | 2.99 | 2.86 | 3.42 | 3.29 | 3.08 | 0.05 | *** |
| C16:1 | 3.59 | 3.26 | 3.82 | 4.74 | 4.25 | 4.31 | 0.12 | *** |
| C18:1 | 15.88 | 17.32 | 14.98 | 11.84 | 13.65 | 14.03 | 0.43 | *** |
| C18:2 | 48.34 | 47.32 | 48.83 | 49.50 | 48.20 | 48.15 | 0.17 | *** |
| C18:3 | 1.76 | 1.73 | 1.78 | 1.42 | 1.66 | 1.63 | 0.02 | *** |
| C20:1 | 0.12 | 0.15 | 0.11 | 0.13 | 0.14 | 0.14 | 0.00 | - |
| C20:4 | 0.07 | 0.03 | 0.08 | 0.03 | 0.04 | 0.06 | 0.00 | - |
| SFA$^3$ | 45.65 | 47.06 | 44.88 | 43.67 | 45.21 | 45.20 | 0.26 | *** |
| USFA$^4$ | 54.34 | 52.93 | 55.11 | 56.32 | 54.78 | 54.79 | 0.26 | *** |
| M-USFA$^5$ | 52.38 | 51.00 | 53.11 | 54.75 | 52.90 | 52.94 | 0.28 | *** |
| P-USFA$^6$ | 1.96 | 1.93 | 2.00 | 1.56 | 1.84 | 1.84 | 0.03 | *** |
| USFA/SFA | 1.19 | 1.12 | 1.12 | 1.28 | 1.21 | 1.21 | 0.01 | *** |

$^1$T (Shank bone 1 kg), T2 (Shank bone 500 g + Mixed bone 500 g), T3 (Shank bone 500 g + Mixed bone 500 g + Brisket meat 400 g), T4 (Rib 1 kg) T5 (Rib 500 g + Mixed bone 500 g), T6 (Rib 500 g + Mixed bone 500 g + Brisket meat 400 g).

$^2$R: Raw bones, $^3$M: Mixed bone addition, $^4$B: Brisket meat addition, $^5$R×M: Interaction between raw bones and mixed bone, $^6$R×B: Interaction between raw bones and brisket meat, $^7$Saturated fatty acid, $^8$Unsaturated fatty acid, $^9$Mono-unsaturated fatty acid, $^{10}$Poly-unsaturated fatty acid.

$p<0.05$, $**p<0.001$, $***p<0.0001$. 
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