

The effect of boiled feed on trace elements of *longissimus dorsi* muscle in Hanwoo steers

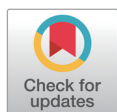
Jaeyoung Kim¹, Meyungok Jung^{1,2}, Sangkeun Jin³, Hyunseok Seo¹, Jungheun Ha^{4*} and Jungseok Choi^{1*}

¹Department of Animal Science, Chungbuk National University, Cheongju 28644, Korea

²Foundation of Agriculture Technology Commercialization & Transfer, Jeonju 54667, Korea

³Department of Animal Resources Technology, Gyeongnam National University of Science and Technology, Jinju 52725, Korea

⁴Department of Food Science and Nutrition, Dankook University, Cheonan 31116, Korea



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*Corresponding author

Jungheun Ha

Department of Food Science and Nutrition, Dankook University, Cheonan 31116, Korea.

Tel: +82-41-550-3479

E-mail: ha@dankook.ac.kr

Jungseok Choi

Department of Animal Science, Chungbuk National University, Cheongju 28644, Korea

Tel: +82-43-261-2551

E-mail: jchoi@chungbuk.ac.kr

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ORCID

Jaeyoung Kim

<https://orcid.org/0000-0002-2847-1731>

Meyungok Jung

<https://orcid.org/0000-0003-4578-4569>

Sangkeun Jin

<https://orcid.org/0000-0002-8983-5607>

Hyunseok Seo

<https://orcid.org/0000-0003-0962-2255>

Jungheun Ha

<https://orcid.org/0000-0001-5282-531X>

Abstract

Boiled feed is obtained by mixing and boiling agricultural by-products such as rice straw, rice bran, and bean curd with grains. The study explored the change in fatty acid, free amino acid, nucleotide, mineral, cholesterol, myoglobin and collagen of *longissimus dorsi* muscle in Hanwoo steers fed with boiled feed. Forty steers, 20 heads per group, were divided into two groups: a control group and a boiled feed group. The steers were raised for 10 months. The boiled feed group was enriched with palmitoleic acid, oleic acid, arachidonic acid and unsaturated fatty acids compared with the control group. There were no significant differences in amino acid and nucleic acid composition between the two groups. The boiled feed group contained higher levels of iron and manganese in the boiled feed group compared with the control group. The total cholesterol level was significantly increased, whereas calorie levels, myoglobin and collagen composition showed no differences. As the supply of boiled feed increases the content of fatty acids, unsaturated fatty acids and minerals related to flavor, it should be a feed that leads to the production of high-quality beef.

Keywords: Boiled feed, *Longissimus dorsi* muscle, Fatty acids, Amino acids, Nucleic acids, Trace substance

INTRODUCTION

The cost of cattle feed exceeds 70% of the total production cost in the Korean beef industry [1,2]. International grain prices have an important influence on the cost of Korean livestock industry because most feed is imported. The recent increase in feed cost has been attributed to the use of feed materials in bioenergy production and reduced export of feedstock by the major grain-producing countries. Higher import prices of feedstock will weaken the competitive position of the Korean beef industry in the global market [3]. To minimize the feed expenditure and enhance productivity, the cattle are provided with the boiled feed, which is used traditionally in Korea.

The boiled feed was provided to Hanwoo steers in Korea before it was used to improve beef cattle. The boiled feed comprises a mixture of rice straw, by-products such as rice bran, corn husk, bean-curd,

Jungseok Choi
<https://orcid.org/0000-0001-8033-0410>

Competing interests

No potential conflict of interest relevant to this article was reported.

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Not applicable.

Availability of data and material

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions

Conceptualization: Jung M, Choi J.
 Data curation: Kim J.
 Formal analysis: Kim J, Jung M.
 Methodology: Kim J, Seo H.
 Software: Jin S, Ha J.
 Validation: Jin S.
 Investigation: Jung M.
 Writing - original draft: Kim J, Jung M.
 Writing - review & editing: Jin S, Ha J, Choi J.

Ethics approval and consent to participate

This article does not require IRB/ACUC approval because there are no human and animal participants.

dried radish leaves, sesame dregs with grains, which are boiled in a big cauldron [4]. The boiled feed is inexpensive with readily available raw materials compared with the general feed blend. Therefore, the supply of the boiled feed is expected to shelve the production cost. The equipment that automatically facilitates throwing, agitating, heating and discharging of different ingredients is readily available commercially in Korea [5].

In the recent studies, it was reported that the boiled feed was faster degraded and absorbed in the rumen than a general feed, and the cattle fed with the boiled feed grew rapidly in lean meat content [4,6]. Different feed ingredients are thought to have effects on meat quality as well as meat quantity. However, there was very rare about the research of the boiled feed in spite that it was widely supplied to beef cattle in Korea. The objective of this work is to investigate the effect of boiled feed on the composition of minor substances in Hanwoo steer's *longissimus dorsi* muscle.

MATERIALS AND METHODS

Animal management and feeds

Forty Hanwoo steers (22 months old) were purchased from Hanwoo farms in Chungnam Korea and raised for 11 months from February 2016 to December 2016 including the acclimation period for a month. The experimental animals were divided into two groups of 20 each: control and boiled feed. The control group was fed on non-boiled feed, and the boiled feed group was treated with boiled feed. The formula of feed mixture is shown in Table 1. Each group was randomly allotted 4 heads in a 790 cm × 800 cm square of cattle pen and repeated 5 times. The water and minerals were provided arbitrarily, and the feed was given twice a day at 8 and 16 o'clock. The feed was purchased from Easy Farms (Cheonan, Korea), and the by-products including rice bran, rice straw and barley straw was utilized as roughage (Table 1). To prepare the boiled feed, raw feed was inserted to cauldron and the same weight of water was added to a cauldron. The feed was heated at 135 °C for 6 h, whereas the control feed was unheated. The ingredients of the two types of feed were listed in Table 2. The feed ingredient was measured by AOAC method [7]. The water content of feed was analyzed based on the difference in weight before and after drying at 105 °C for 8 h. The sample was burnt to ash at 550 °C for 12 h and the mineral content was estimated. The crude protein was measured with Kjeldahl method, and the crude fat content was estimated with soxhlet method. The contents of neutral detergent fiber (NDF) and acid detergent fiber were measured using Van Soest's methods [8].

Trace substances of *longissimus dorsi* muscle

The animals were sacrificed and cooled at 0 °C for 18 to 24 h. The *longissimus dorsi* muscle was deboned, separated, shaped, vacuum-sealed, and stored at 4 °C.

To measure the fatty acid composition of *longissimus dorsi* muscle, a 50 g sample was

Table 1. Formulation of feed mixture

Items	Formulation		Intake yield (kg/h/d) ¹⁾
	Weight (kg)	Ration (%)	
Total mixed ratio	1,500	51	4.29
Rice bran	300	10	0.86
Rice straw	1,050	36	3.00
Barley straw	100	3	0.29
Total	2,950	100	8.44

¹⁾ (kg/head/day): daily intake per a head.

Table 2. Chemical composition of experimental feeds

Items	Control	Boiled feed
Moisture (%)	11.6	35.9
Crude fat (%)	3.0	3.8
Crude protein (%)	8.5	8.5
Crude fiber (%)	18.1	10.0
Ash (%)	7.2	7.0
Calcium (%)	0.4	0.5
Phosphorus (%)	0.3	0.5
NDF	44.6	27.1
ADF	24.2	15.2

NDF, neutral detergent fiber; ADF, acid detergent fiber.

homogenized in 150 mL organic solvent (chloroform : methanol = 2 : 1 vol/vol) and the lipids were isolated. The lipid extract was concentrated at 50°C–55°C after removing moisture with anhydrous sodium sulfate, followed by the addition of 1 mL of 0.5N NaOH to the concentrated lipid extract and heating at 100°C for 20 min. The mixture was treated with 2 mL of BF₃-methanol and NaCl-heptane solution [9]. Finally, the fatty acids were measured with gas chromatography (Agilent Technologies, Santa Clara, CA, USA). The gas chromatography conditions for analysis of fatty acids were as follows; the column was an Omegawax 205 fused-silica bond capillary column (30 m, 0.3 × 2 mm I.D., 0.25µm film thickness), the detector was a flame ionization detector, the mobile phase was 99.99% nitrogen gas and 1mL/min column influx rat.

The free amino acids were analyzed by homogenizing 0.5 g *longissimus dorsi* muscle in 1.5 mL distilled water, followed by centrifugation at 15,000×g and 4°C for 15 min. The supernatant was diluted 10-fold with ethanol and centrifuged at 13,000×g and 4°C for 10 min. Free amino acid content was analyzed with LC/MS/MS (Xevi TQ-S, Waters, Milford, MA, USA).

For the analysis of nucleic acids, 0.3 g *longissimus dorsi* muscle was added to 5 mL 0.5M perchloric acid and centrifuged at 9,200×g and 4°C for 5 min. After the addition of 0.25 mL of 2.1M KHCO₃ to 1 mL supernatant, it was centrifuged at 9,200×g and 4°C for 5 min and filtered with a 0.45 µm syringe filter. Standard reference materials including hypoxanthine, uridine, inosine, guanosine monophosphate, adenosine monophosphate, adenosine diphosphate, and inosine monophosphate (IMP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Nucleic acids were analyzed with High Performance Liquid Chromatography (HPLC, Shiseido, Tokyo, Japan).

Mineral contents of *longissimus dorsi* muscle were estimated by AOAC method [7]. An appropriate amount of beef sample was burnt to ashes with an electric muffle furnace (JSMF-270T, JS Research, Gongju, Korea) at 600°C for 12 h. The ashes were dissolved in an acidic solution (HCl : H₂O = 1 : 1) overnight and the solution was filtered with a Whatman No. 6 filter paper. The absorbance was measured via atomic absorption spectrometry (ICP Spectrophotometer, Spectroflame, Spectro, Germany) and the mineral content was calculated using the following formula.

$$\text{Mineral content(mg/kg)} = \frac{\text{Sample absorbance} / 1 \text{ ppm standard absorbance} \times \text{dilution rate}}{\text{Sample weight (g)} \times 10^6} \times 100$$

The cholesterol content of the *longissimus dorsi* muscle was measured via the following procedure. Five grams of beef sample were added to the solution mixture containing 8 mL of 60% KOH and 40 mL of alcohol solution (ethanol : methanol : isopropyl alcohol = 90 : 5 : 5), and was saponified

at 100 °C for 1 h. The saponified matter was concentrated with benzene and KOH in a separating funnel and was mixed with 1,000 ppm squalene. The cholesterol content was analyzed via gas chromatography (HP-7890A, Agilent Technologies). The column that was made of a fused-silica bond capillary (30 m, 0.22 × 0.32 mm, nonpolar 5% diphenyl-95% dimethylsiloxane) was used for cholesterol analysis.

The number of calories required to utilize 1 g of beef was measured in a calorimeter (6400, Parr Instrument, St, Moline, IL, USA).

To analyze the myoglobin content, 2 g of ground beef was homogenized with 40 mM phosphate buffer (pH 6.8) and centrifuged at 5,200×g for 10 min. The supernatant was filtered with a Whatman No. 2 filter paper. The absorbance of the filtrate was estimated at 525 nm and 700 nm.

To investigate the collagen content of *longissimus dorsi* muscle, 4 g beef sample was transferred into 30 mL sulfuric acid solution and heated in a dry oven at 105 °C for 16 h. It was diluted with distilled water, homogenized and filtered with a filter paper (Whatman No.2), followed by the addition of 1 mL of oxidant solution to the filtrate diluted 20-fold and left at room temperature for 20 min. It was mixed with a color reagent (Chloramin-T, Sigma-Aldrich), heated at 60 °C for 15 min, and cooled down at room temperature. The absorbance of the final reactant was measured at 558 nm with a spectrophotometer (Optizen-3220UV, Mecasys, Daejeon, Korea).

Statistical analyses were carried out with the Student's t-test using SAS statistical package 9.4. Values of $p < 0.05$ indicated significant differences.

RESULTS AND DISCUSSION

The formulation of the boiled feed that based on our previous study [4] is in Table 1. Agricultural by-products including rice straw that is the commonest agricultural side product in Korea was added to total mixed ration fodder. The boiled feed was prepared by addition of the same weight of water with the raw feed mixture.

There is the chemical composition of experimental feeds in Table 2. The boiled feed contained more moisture than the control feed did. The higher moisture of the boiled feed in comparison with the control feed was due to add water for it to be prepared. The fiber percentage was lower in the boiled feed than in the control feed. The fiber is sorted out as soluble and insoluble [10]. The boiled feed contained lower fiber because the soluble fiber was dissolved in water and the insoluble one was likely to be released easily into water while the feed was boiled. The advantage of the boiled feed is to make the digestion of coarse fodder easy by rumen microbes. The NDF of the boiled feed was dissolved more than twice of the control feed in rumen [6]. It seems to cause the silica-lignin-cellulose bond of rice straw to be loosen and/or break that the boiled feed was prepared to boil it for hours.

The fatty acid composition of beef affects the meat quality and determines its nutritional value, flavor, and expiration date [11]. We investigated the effect of boiled feed on the fatty acids composition of Hanwoo steer's *longissimus dorsi* muscle. The boiled feed group contained higher levels of palmitoleic acid, oleic acid, and arachidonic acid than the control group but lower levels of stearic acid, linoleic acid, and eicosenoic acid ($p < 0.05$; Table 3). Hanwoo meat contains higher levels of oleic acid compared with the other cattles [12] and Hanwoo steer meat contains higher levels of oleic acid, stearic acid, and palmitoleic acid compared with different species of cattle [12–15]. Intake of boiled feed increased the levels of oleic acid and palmitoleic acids in the steers' *longissimus dorsi* and improved the Hanwoo steer's characteristics. The supply of the boiled feed is the higher levels of unsaturated fatty acids including monounsaturated fatty acids in Hanwoo steer's *longissimus dorsi* in comparison of the control feed ($p < 0.05$). The higher the beef grade, the higher

Table 3. Fatty acids composition of *longissimus dorsi* muscle of Hanwoo steers by boiled feed

Items	Control (%)	Boiled feed (%)	p-value
Myristic acid (C _{14:0})	2.86 ± 0.08	3.01 ± 0.09	0.249
Palmitic acid (C _{16:0})	27.61 ± 0.12	26.86 ± 0.31	0.090
Palmitoleic acid (C _{16:1n7})	3.60 ± 0.30 ^b	4.47 ± 0.21 ^a	0.031
Stearic acid (C _{18:0})	13.04 ± 0.70 ^a	11.15 ± 0.32 ^b	0.015
Oleic acid (C _{18:1n9})	49.93 ± 0.57 ^b	52.11 ± 0.46 ^a	0.011
Vaccenic acid (C _{18:1n7})	ND	ND	-
Linoleic acid (C _{18:2n6})	2.11 ± 0.17 ^a	1.64 ± 0.11 ^b	0.030
γ-Linolenic acid (C _{18:3n6})	0.04 ± 0.00	0.05 ± 0.00	0.810
Linolenic acid (C _{18:3n3})	0.07 ± 0.01	0.07 ± 0.01	0.582
Eicosenoic acid (C _{20:1n9})	0.63 ± 0.04 ^a	0.51 ± 0.02 ^b	0.011
Arachidonic acid (C _{20:4n6})	0.11 ± 0.01 ^b	0.14 ± 0.01 ^a	0.042
Saturated fatty acids (SFA)	43.5 ± 0.69 ^a	41.0 ± 0.51 ^b	0.011
Unsaturated fatty acids (UFA)	56.5 ± 0.69 ^b	59.0 ± 0.51 ^a	0.011
Monounsaturated fatty acid (MUFA)	54.2 ± 0.85 ^b	57.1 ± 0.52 ^a	0.008
Polyunsaturated fatty acids (PUFA)	2.33 ± 0.18 ^a	1.89 ± 0.12 ^b	0.052
n3	0.07 ± 0.01	0.07 ± 0.01	0.582
n6	2.26 ± 0.18 ^a	1.83 ± 0.11 ^b	0.047
MUFA/SFA	1.25 ± 0.04 ^b	1.40 ± 0.03 ^a	0.011
PUFA/SFA	0.05 ± 0.00	0.05 ± 0.00	0.144

Means ± SEM.

Each sample repeated 3 times with triple.

^{a,b}Means with different superscripts in the same row differ significantly ($p < 0.05$).

ND, not detected.

was the level of oleic acid and unsaturated fatty acids including monounsaturated fatty acids in the beef. Conversely, the lower the beef grade, the higher was the level of linoleic acid, polyunsaturated fatty acids and saturated fatty acids in the beef [16–18]. The supply of the boiled feed is expected to promote safe beef production with reduced content of saturated fatty acids in addition to enhanced beef quality.

Meat is a nutritionally important source of essential amino acids and protein. Also, specific amino acids enhance the palatability and flavor of meat. The free amino acid content of the steer's *longissimus dorsi* muscle following supply of boiled feed showed no difference between control and boiled feed groups (Table 4). The boiled feed did not affect the free amino acid content of the steer's *longissimus dorsi* muscle. According to the previous study, muscle mass of Hanwoo fed with boiled feed increased [4], but muscle's major proteins are composed of skeletal muscle proteins and sarcoplasmic proteins [19]. Thus, unlike the fatty acid composition, which is directly affected by the feeding effect of feed [20], the amino acid composition of the same muscle is considered to have little effect from feeding.

Inosine 5'-monophosphate (IMP), inosine and hypoxanthine are nucleic acid-related compounds, which are generated by adenosine triphosphate dissolution in the muscle after slaughter [21]. These nucleic acids affect the palatability of meat [22–24]. The nucleic acid content of the steer's *longissimus dorsi* muscle following consumption of boiled feed showed no difference between the control and the boiled feed groups (Table 5). The boiled feed did not affect the nucleic acid content of the *longissimus dorsi* muscle.

Beef contains an abundance of minerals as well as protein [25,26]. We investigated the effects of boiled feed on the mineral contents of Hanwoo steer's *longissimus dorsi* muscle. The boiled feed group showed higher levels of Fe²⁺ and Mn²⁺ ions in the *longissimus dorsi* muscle than the control

Table 4. Free amino acids (FAA) compositions of *longissimus dorsi* muscle of Hanwoo steers by boiled feed

Items	Control (mg/100 g)	Boiled feed (mg/100 g)	p-value
Glycine (Gly)	0.25 ± 0.06	0.18 ± 0.02	0.221
Alanine (Ala)	20.62 ± 1.50	21.63 ± 0.71	0.503
Serine (Ser)	2.21 ± 0.18	2.31 ± 0.09	0.574
Proline (Pro)	1.76 ± 0.17	2.12 ± 0.16	0.170
Valine (Val)	3.36 ± 0.38	3.96 ± 0.21	0.150
Threonine (Thr)	1.98 ± 0.16	2.05 ± 0.11	0.733
Leucine (Leu)	2.84 ± 0.38	3.64 ± 0.40	0.201
Isoleucine (Ile)	0.57 ± 0.16	0.68 ± 0.13	0.593
Aspartic acid (Asp)	0.53 ± 0.00	0.53 ± 0.00	-
Lysine (Lys)	3.21 ± 0.31	3.27 ± 0.14	0.851
Glutamic acid (Glu)	1.62 ± 0.20	2.26 ± 0.27	0.111
Methionine (Met)	0.45 ± 0.03	0.53 ± 0.06	0.294
Histidine (His)	2.17 ± 0.11	2.14 ± 0.10	0.843
Phenylalanine (Phe)	0.99 ± 0.09	1.25 ± 0.15	0.212
Arginine (Arg)	4.35 ± 0.47	4.00 ± 0.15	0.408
Tyrosine (Tyr)	2.35 ± 0.08	2.57 ± 0.10	0.156
Cystine (Cys-cys)	0.73 ± 0.00	0.73 ± 0.00	-

Means ± SEM.

Each sample repeated 3 times with triple.

Table 5. Nucleotide-related compounds of *longissimus dorsi* muscle of Hanwoo steers by boiled feed

Items	Control (umol/g)	Boiled feed (umol/g)	p-value
Hypoxanthine	0.10 ± 0.01	0.11 ± 0.01	0.558
Uridine	0.03 ± 0.00	0.03 ± 0.00	0.633
Inosine	0.30 ± 0.02	0.28 ± 0.02	0.451
AMP	0.12 ± 0.00	0.12 ± 0.00	0.792
GMP	0.34 ± 0.00	0.32 ± 0.01	0.068
IMP	3.22 ± 0.20	3.09 ± 0.12	0.552
ADP	0.13 ± 0.00	0.13 ± 0.02	0.837

Means ± SEM.

Each sample repeated 3 times with triple.

AMP, adenosine monophosphate; GMP, guanosine monophosphate; IMP, inosine monophosphate; ADP, adenosine diphosphate.

group ($p < 0.05$), but no differences in the levels of other minerals (Table 6). Iron deficiency is widespread in the world [27]. Iron deficiency causes anemia and adversely affects tissues including the central nervous system [28]. Manganese plays an important physiological role in the central nervous system, bone and metabolic activities [29]. Minerals are required in the human diet, and beef is an excellent source of minerals [30]. The boiled feed intake increased the levels of Fe^{2+} and Mn^{2+} ions in the steer's *longissimus dorsi* muscle.

Cholesterol is a structural component of the biological membrane and maintains the fluidity of cell membrane [31]. However, the excessive intake of cholesterol triggers atherosclerosis [32]. Myoglobin contributes to the taste and color of beef, which are crucial for consumer acceptance [33]. The content of collagen, the main protein of connective tissue, affects the chewiness of meat [34–37]. The cholesterol level in the *longissimus dorsi* muscle of steers in the boiled feed group was 63.8 mg/100 g compared with 40.6 mg/100 g in the control group ($p < 0.001$; Table 7). This result

Table 6. Mineral contents of *longissimus dorsi* muscle of Hanwoo steers by boiled feed

Items	Control (Unit: mg/kg)	Boiled feed (Unit: mg/kg)	p-value
Ca	90.16 ± 4.82	91.22 ± 7.65	0.922
Mg	195.52 ± 5.62	197.27 ± 2.41	0.745
Fe	20.43 ± 0.82 ^b	29.54 ± 2.24 ^a	0.009
K	2,859.72 ± 119.37	2,964.53 ± 58.44	0.391
P	1,577.85 ± 36.31	1,562.59 ± 20.81	0.699
Na	717.14 ± 18.74	671.64 ± 17.53	0.114
Cu	1.49 ± 0.17	1.71 ± 0.19	0.436
Mn	0.21 ± 0.04 ^b	0.52 ± 0.10 ^a	0.037
Zn	41.26 ± 3.88	42.01 ± 1.70	0.084

Means ± SEM.

Each sample repeated 3 times with triple.

^{a,b}Means with different superscripts in the same row differ significantly ($p < 0.05$).**Table 7.** Total cholesterol, calorie, myoglobin and collagen contents of *longissimus dorsi* muscle of Hanwoo steers by boiled feed

Items	Control	Boiled feed	p-value
Cholesterol (mg/100 g)	40.58 ± 1.99 ^b	63.84 ± 1.27 ^a	0.001 <
Calorie (cal/g)	2,675.17 ± 88.23	2,789.00 ± 79.72	0.374
Myoglobin (mg/g)	4.94 ± 0.24	5.42 ± 0.15	0.095
Collagen (g/100 g)	0.47 ± 0.06	0.47 ± 0.04	1.000

Means ± SEM.

Each sample repeated 3 times with triple.

^{a,b}Means with different superscripts in the same row differ significantly ($p < 0.05$).

is within the range of cholesterol content in meat of approximately 30–120 mg per 100 g [38,39]. No significant differences were found in the calorie, myoglobin and collagen composition of the beef. Intake of boiled feed increased the cholesterol content but did not affect the caloric, myoglobin or collagen contents.

While the treatment with boiled feed does not influence the most of trace substance content, the levels of oleic acid, unsaturated fatty acids, Fe²⁺ and Mn²⁺ can be optimized to produce a high quality of beef as well as to cut production costs.

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