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Author	Hye-Jin Kim[a,b], Hee-Jin Kim[c], Kwan-Woo Kim[d], Jinwook Lee[d], Sang-Hoon Lee[e], Sung-Soo Lee[d], Bong-Hwan Choi[d], Dong-Jin Shin[a], Ki-Hong Jeon[f], Jin-Young Choi[g], Aera Jang[a,*]
Affiliation	[a] Department of Applied Animal Science, Kangwon National University, Chuncheon 24341, Korea [b] Department of Agricultural Biotechnology, Center for Food and Bioconvergence, and Research Institute of Agriculture and Life Science, Seoul National University, Seoul 08826, Korea [c] Poultry Research Institute, National Institute of Animal Science, Pyeongchang 25342, Republic of Korea [d] Animal Genetic Resources Research Center, National Institute of Animal Science, Rural Development Administration, Hamyang 50000, Korea [e] Grassland and Forages Division, National Institute of Animal Science, Rural Development Administration, Cheonan 30801, Korea [f] Research Group of Food Processing, Korea Food Research Institute, 55365, Republic of Korea [g] Dept. of Division of Food Science and Culinary Arts, Shinhan University, 11644, Republic of Korea
Special remarks – if authors have additional information to inform the editorial office	-
ORCID (All authors must have ORCID) https://orcid.org	Hye-Jin Kim https://orcid.org/0000-0002-9384-6720 Hee-Jin Kim https://orcid.org/0000-0002-6959-9790 Kwan-Woo Kim https://orcid.org/0000-0003-2729-2181 Jinwook Lee https://orcid.org/0000-0001-9019-1653 Sang-Hoon Lee https://orcid.org/0000-0003-4836-7402 Sung-Soo Lee https://orcid.org/0000-0003-1255-9509 Bong-Hwan Choi https://orcid.org/0000-0002-4795-3285 Dong-Jin Shin https://orcid.org/0000-0003-3315-667X Ki-Hong Jeon https://orcid.org/0000-0002-0525-5075 Jin-Young Choi https://orcid.org/0000-0002-9836-1522 Aera Jang https://orcid.org/0000-0003-1789-8956

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7 **CORRESPONDING AUTHOR CONTACT INFORMATION**

For the <u>corresponding</u> author (responsible for correspondence, proofreading, and reprints)	**Fill in information in each box below**
First name, middle initial, last name	Aera Jang
Email address – this is where your proofs will be sent	ajang@kangwon.ac.kr
Secondary Email address	-
Postal address	Department of Applied Animal Science, Kangwon National University, Chuncheon 24341, Korea
Cell phone number	+82-10-9017-8643
Office phone number	+82-33-250-8643
Fax number	+82-33-251-7719

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Effect of Feeding Alfalfa and Concentrate on Meat Quality and Bioactive Compounds in Korean Native Black Goat Loin during Storage at 4°C

Abstract

The primary aim of this study was to evaluate the effect of feeding alfalfa: concentrate at different ratios (8:2 or 2:8) to Korean native black goats (KNBG) for 90 days on meat quality and bioactive compound content. Feeding KNBG alfalfa and concentrate at different ratios did not impact meat pH, color, microorganism composition, volatile basic nitrogen levels, or lipid oxidation. The low alfalfa (KLA) group exhibited increased oleic acid and monosaturated fatty acid levels, both of which impact the palatability traits of meat. The abundance of bioactive compounds increased in the loin meat of the KLA group, leading to an increase in antioxidant activities. Our results suggest that feeding alfalfa and concentrate at a 2:8 ratio to KNBG can increase taste-related fatty acids and bioactive compounds in loin meat, relative to that achieved by feeding at an 8:2 ratio. Further investigation is required to evaluate the quality and the metabolites of bioactive compounds in KNBG meat and the effect of the different dietary ratios of forage and concentrate.

Keywords alfalfa, antioxidant activity, concentrate, carnosine, Korean native black goat

Introduction

Korean native black goats (KNBG; *Capra hircus coreanae*) are an indigenous breed in Korea, ~80% of which are predominantly black (Son, 1999). KNBG have been raised as domestic livestock in Korea for more than 2,000 years (Kim et al., 2014). However, with its perceived health benefits in the elderly, children, and pregnant persons (Kim et al., 2019; Kim

et al., 2014; Son, 1999), consumption of KNBG meat has been increasing (Kim et al., 2019). In Korea, there are 14,664 farms which have raised 542,744 heads of crossbreed black goat (MAFRA, 2019). Although the production size of KNBG is significantly lower than that of crossbreed black goat, research on the production of high-quality meat from KNBG is crucial to meet the increasing demands for KNBG meat.

KNBG farming has transitioned from multiple farming systems into one intensive farming system (Kim et al., 2014), making standardized treatment more attainable. Diet is a major factor influencing meat production and quality (Marinova et al., 2001). In particular, adjusting energy levels can improve meat production and quality. Concentrate feeding is an effective dietary method to breed goats on a large scale. However, large-scale concentrate feeding may hinder the growth of goats by increasing feed costs and the risk of developing metabolic diseases (Jung et al., 2008). Hence, alfalfa hay has been used as a forage source in finishing diets to supply crude protein to ruminants (Hwang et al., 2008). The mixture of pasture and concentrates can stabilize the ruminating environment and improve feed intake, and nutrient utilization efficiency has been attracting attention recently (Lee et al., 2021). Lee et al. (2019) have reported that feeding a high forage or a high concentrate diet (8:2 or 2:8) altered ruminal fermentation and the bacterial community structure in KNBG; however a limitation on the meat quality of KNBG existed. Generally high-concentrate feeding increases the producibility and palatability (texture and flavour) of meat (French et al., 2000); thus, concentrate abundance and grass quality have increased (Kim et al., 2012). Moreover, fodders high in concentrate can increase the fat content of meat (Steen & Kilpatrick, 2000). Meanwhile, certain studies have reported that meat from grass-fed animals has better antioxidant activity compared to that from concentrate-fed animals (Gatellier et al., 2004; Yang et al., 2002; Descalzo et al., 2007). However, few studies have investigated the effect of

concentrate and grass-feeding on the physicochemical characteristics of KNBG meat (Hwang et al., 2018; Kim et al., 2014).

Meat is a significant source of bioactive compounds or nutraceuticals, such as minerals, fatty acids, vitamins, and peptides (Pogorzelska-Nowicka et al., 2018). Specifically, consumption of KNBG can provide various compounds, including carnosine, coenzyme Q10, anserine, and L-carnitine. However, storage of KNBG meat in cold rooms in markets or extended refrigeration can cause degradation of meat quality and loss of bioactive contents. Indeed, we have previously found that the abundance of certain bioactive compounds and antioxidant activity decreases in beef during storage (Kim & Jang, 2021), whereas antioxidants in meat reportedly improve meat shelf-life and quality (Velasco & Williams, 2011).

Therefore, in the current study, we aimed to evaluate the effect of feeding KNBG with alfalfa and concentrate at different ratios on loin meat quality, bioactive compound abundance, and antioxidant activity during cold storage.

Material and Methods

Sample preparation and storage conditions

Ten KNBG (48.6 ± 1.4 kg body weight; 4.8 ± 1.2 years old, castrated male) were randomly divided into two groups and were fed with the experimental diet for 90 days of finishing period. The high alfalfa (KHA) group (n = 5) was fed alfalfa and concentrate daily at an 8:2 ratio; the low alfalfa (KLA) group (n = 5) was fed alfalfa and concentrate daily at a 2:8 ratio. Animals had free access to water, and experimental feed was provided *ad libitum* twice daily. The alfalfa composition comprised 7.42% moisture, 15.0% crude protein, 1.49%

crude fat, 6.59% crude ash, 56.1% neutral detergent fibre, 44.2% acid detergent fiber, and 2.16 Mcal/kg metabolizable energy. The conventional concentrate (EE0SL0132, TS Rainbow Feed CO., LTD, Seoul, Korea) comprised 8.52% moisture, 18.8% crude protein, 3.80% crude fat, 6.75% crude ash, 23.0% acid detergent fibre, 9.03% neutral detergent fiber, and 2.77 Mcal/kg metabolizable energy. The detailed ingredients and the chemical composition of the experimental diets are shown in Table 1. The KNBG were slaughtered after 90 days on the experimental diet. After slaughtering, the carcasses were chilled for 24 h at 2℃, and loin muscles (*longissimus dorsi*) were sampled for analysis. The animal care and use protocols were followed under approval of the Institutional Animal Care and Use Committee of the NIAS, RDA, Republic of Korea (NIAS-2019-1545).

Samples were cut into 1.5 cm thick slices, placed on a polystyrene tray with low-density polyethylene (LDPE) film, and stored in an aerobic environment at 4 ± 2℃, which reflects the common storage condition in homes. Samples were selected on day 1, 5, 10, and 15 and evaluated in terms of quality, bioactive compounds, and antioxidant activities.

Proximate composition

Proximate composition was determined using methods established by the Association of Official Agricultural Chemists (AOAC, 1997). The moisture content was calculated using weight difference after drying at 105℃ for 12 h. The Kjeldahl method was employed to analyse crude protein content, and the ether extraction method was used to determine crude fat content. The crude ash content was determined based on the weight difference after burning at 550℃ in a furnace.

pH and meat color

The pH was determined using a homogenate prepared with 10 g of KNBG loin meat sample and 90 mL of distilled water using a pH meter (Orion 230 A; Thermo Fisher Scientific, Waltham, MA, USA).

To analyze meat color, the Commission Internationale de l'Eclairage (CIE) lightness (L*), redness (a*), and yellowness (b*) of KNBG was defined 10 min after removal of the LDPE film using a CR-400 Minolta colorimeter (Minolta Co., Osaka, Japan) with a C illuminant and 8-mm aperture size.

Total aerobic bacteria and Escherichia coli/coliform

Ten grams of meat sample was homogenized with 90 mL of saline. One milliliter of the homogenate was placed on Petrifilms to quantify the total aerobic bacteria and *E. coli*/coliform (3M Microbiology, St. Paul, MN, USA). The Petrifilms were aerobically incubated for 48 h at 37℃, and typical colonies were counted according to the manufacturer's protocol.

Volatile basic nitrogen (VBN) assay

VBN content in meat samples was determined using the micro-diffusion method (Kim et al., 2020). A 10-g meat sample was mixed with 50 mL distilled water for 30 min and passed through a filter paper. Next, one milliliter of the filtrate and 1 mL of saturated K_2CO_3 were loaded to the outer cell of a Conway dish. To the inner cell, 0.01 N H_2SO_4 was loaded. After 1 h incubation at 25°C, 20 μL of Brunswick reagent was mixed with and titrated with 0.01 NaOH. The VBN content was presented as mg/100 g of meat.

2-Thiobarbituric acid reactive substance (TBARS) assay

TBARS was estimated using the methods described by Kim et al. (2020). Briefly, 5 g of meat was added to 50 μL of tert-butyl-4-hydroxyanisole and homogenized with 15 mL of distilled water. One milliliter of the meat homogenate was mixed with 2 mL of 20 mM thiobarbituric acid (in 15% trichloroacetic acid). After boiling at 90℃ for 15 min and subsequent centrifugation at 2,000×g for 10 min, the absorbance of the supernatant was measured at 531 nm using a microplate reader (Molecular Devices, Sunnyvale, CA, USA). The TBARS content was expressed as mg malondialdehyde (MDA) per kg of meat.

Fatty acid composition

The method described by Kim and Jang (2021) was used to estimate fatty acid composition. Two grams of meat sample was homogenized with Folch reagent (15 mL) with 0.3% BHA (40 μL) and then filtered. The bottom layer was collected by shaking vigorously with 0.88% KCl and dried under nitrogen gas. Fatty acid methyl ester derivatives were generated by adding 13% boron trifluoride in methanol by boiling at 90℃ for 1 h and used for gas chromatography analysis (6890N; Agilent Technologies, Santa Clara, CA, USA) with a capillary column (100 m × 0.25 mm id × 0.20 μm film thickness; CP7489, Agilent Technologies) and a flame ionization detector. The temperatures of the injector and detector were 260℃ and 280℃, respectively. The initial temperature of the oven was 150℃, which was increased to 200℃ at a rate of 7℃/min, held constant at 200℃ for 20 min, increased to 250℃ at a rate of 3℃/min, and held constant at 250℃ for 5 min. The carrier gas was helium, and its flow rate was 1 mL/min. The sample was injected at 1 μL with a splitting ratio of 1:100. Each fatty acid was identified using a standard (PUFA No. 2-Animal Source; Supelco, Bellefonte, PA, USA).

Bioactive compounds

Coenzyme Q_{10} (CoQ_{10})

CoQ_{10} quantitation was performed using a method described by Kim and Jang (2021) with slight modifications to the protocol of No et al. (2011). Briefly, 10 g meat samples were homogenized with 90 mL of ethanol, shaken using a magnetic stirrer for 1 h, and adjusted to 100 mL with ethanol. The supernatant was filtered and used for liquid chromatography (Agilent 1260 Infinity, Agilent Technologies) equipped with a C18 column (ZORBAX Eclipse XDB-C18, 4.6 × 150 mm, 3.5 μm, Agilent Technologies). The column was isocratically eluted at 1.5 mL/min at 40℃ with mobile phase solution (methanol: ethanol mixture = 40: 60, v/v), and CoQ_{10} was detected at 275 nm. The CoQ_{10} content was calculated using a standard curve and expressed as mg per 100 g of meat.

L-carnitine

L-carnitine content was estimated using a method reported by Kim et al. (2019) with a slight modification of the protocol described by Shimada et al. (2004). Briefly, 5 g of samples were homogenized with 0.3 M perchloric acid (PCA) and filtered using a glass microfiber filter. The supernatant was collected after neutralizing it with 1.2 M K_2CO_3. The standard or the supernatant sample (50 μL) was then reacted with 50 μL of a working solution (0.55 mM acetyl-CoA, 0.93 mM 5,5′-dithiobis-(2-nitrobenzoic acid), 3.05 mM ethylenediaminetetraacetic acid, and 610 mM Tris–HCl; pH 7.5) in a 96-well microplate. After incubation at 37℃ for 10 min, the absorbance of the reactant was measured at 415 nm using a spectrophotometer as a blank control. The final absorbance was measured by adding 25 μL of 0.5 U carnitine acetyltransferase (EC 2.3.1.7; Sigma-Aldrich) and incubating the

177 mixture at 37℃ for 30 min. The absorbance difference between blank and final was
178 calculated, and L-carnitine content was expressed as μM per gram of meat compared to the
179 standard.
180
181 Creatine, creatinine, carnosine, and anserine
182 Creatine, creatinine, carnosine, and anserine were estimated using the method reported
183 by Kim et al. (2019). Briefly, meat samples (2.5 g) were homogenized with 0.01 N HCl (7.5
184 mL) and filtered. The filtrate (250 μL) was mixed with 750 μL of acetonitrile at 4°C for 20
185 min to remove the protein. The supernatant was filtered after centrifugation at 10,000 ×g for
186 10 min and assessed via liquid chromatography (1260 Infinity; Agilent Technologies),
187 equipped with an HILIC column (4.6 × 150 mm × 3 μm; Waters, Milford, MA, USA). The
188 column temperature was 35℃, and mobile phase B was flowed at 1.4 mL/min using a linear
189 gradient method increasing the flow rate from 0–100% for 13 min. Mobile phase A and B
190 were 0.65 mM ammonium acetate in water/acetonitrile (pH 5.5; 25:75, v/v) and 4.55 mM
191 ammonium acetate in water/acetonitrile (pH 5.5; 70:30, v/v), respectively. Creatine,
192 carnosine, and anserine were detected at 214 nm, while creatinine was detected at 236 nm
193 and expressed as mg per 100 g of meat.
194
195 **Antioxidant activities**
196 Preparation of meat samples for evaluating antioxidant activities
197 Four grams of meat was homogenized with 20 mL of distilled water and passed through
198 filter paper No. 4. Subsequently, 4 mL of chloroform was added to the filtrate and vortexed.
199 The upper layer was collected and lyophilized at -20℃ before analysis.
200

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

A colorimetric method was used to evaluate DPPH radical scavenging activity of the samples (Kim & Jang, 2021). The prepared meat sample was reacted with 0.2 mM DPPH solution (100 μL) and incubated for 30 min in the dark at 25°C; absorbance at 517 nm was obtained. The final DPPH radical scavenging activity was expressed as μmol Trolox equivalents (TE)/g dry matter.

2,2-Azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity

To assess ABTS radical scavenging activity, the protocol set out by Kim et al. (2019) was followed. The $ABTS^{+}$ radical solution was diluted with distilled water to an absorbance of 0.700 ± 0.002 at 735 nm at 30℃. A 50-μL sample was reacted with 950 μL of the $ABTS^{+}$ radical solution at 30℃ for 30 min, and absorbance was measured at 735 nm. The results were expressed as μmol TE/g dry matter.

Ferric reducing antioxidant power (FRAP) activity

The FRAP assay was carried out according to the method described by Kim et al. (2019). Each sample (25 μL) was incubated with the FRAP working solution (175 μL) at 37℃ for 30 min in the dark, and absorbance was determined at 590 nm. The results were expressed as μmol TE/g dry matter.

Multivariate statistical analysis

To identify the difference in bioactive compounds and antioxidant activity, multivariate statistical analysis was performed using principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA). Analyses were performed with log-

transformed and auto-scaled data using Metaboanalyst 5.0 (https://www.metaboanalyst.ca/) according to Lee et al. (2021). The validity of the PLS-DA model was verified using correlation coefficients (R2) and cross-validation correlation coefficients (Q2).

Statistical analysis

The SAS software v.9.4 (SAS Institute, Cary, NC, USA) was used for statistical analysis. Means among treatment groups were compared by one-way analysis of variance (ANOVA) with a general linear model. Significant differences among means were defined using Tukey's test at $p < 0.05$. All data were expressed as mean values and standard error of the mean.

Results and Discussion

Proximate composition

Significant differences were not observed in the proximate composition of loin meat obtained from the two experimental groups (Table 2). Moreover, the moisture, crude protein, and crude ash composition were similar to those in other previous studies (Kim et al., 2019; Sebsibe, 2008). According to Kim et al. (2019), black goat loin contains 75.00% moisture, 21.60% crude protein, and 1.41% ash. In another study investigating different goat breeds, the meats were composed of 67.0–75.2% moisture, 18.9–24.8% crude protein, and 0.95–1.19% crude ash (Sebsibe, 2008). However, crude fat content of black goat meat ranged from 1.48 to 12.6% (Kim et al., 2019; Sebsibe, 2008). Previous studies reported that increased supplementation of concentrate decreases moisture content, while increasing fat content (Kim et al. 2014). However, in this study, no significant differences occurred in the fat contents of loin from the KHA and KLA groups. These findings agree with those of Hwang et al. (2018),

who reported that KNBG loin meat fat content is not significantly affected by alfalfa and concentrate supplementation.

Meat pH and color

The initial pH of loin meat from the KHA and KLA groups was 6.00 and 5.92, respectively (Table 3). During storage, the pH of both groups increased ($p < 0.05$). Generally, the pH of meat increases due to degradation of proteins and growth of spoilage microorganisms, which causes formation and accumulation of amines and ammonia (Kim & Jang, 2021). However, no significant differences were detected in these parameters between the two groups. The lightness (CIE L*) of loin from both groups decreased after day 5 ($p < 0.05$), while redness (CIE a*) and yellowness (CIE b*) significantly decreased during the storage of meat from both groups. No significant differences were detected between groups in the color of KNBG loin meat during storage except for the lightness at day 10. In a study by Realini et al. (2004), a darker meat color of pasture-fed steers was reported compared to that of concentrate-fed steers. However, meat color varies with breed and is not dependent on diet (Kadim et al., 2004). Moreover, feeding alfalfa and concentrate does not reportedly impact KNBG meat color (Hwang et al., 2018), which agrees with our findings. Thus, altering the ratio of alfalfa and concentrate in KNBG feed for 90 days does not impact KNBG meat pH or color.

Microorganisms

The total abundance of aerobic bacteria in fresh loin (day 1) from both groups was 2.35 (KHA) and 2.17 (KLA) log CFU/g, respectively, and increased during storage ($p < 0.05$; Table 4). With the exception of day 1, no significant differences were observed in total

aerobic bacteria of KNBG loin meat between groups. On day 15, total aerobic bacteria in loin from the KHA and KLA groups was 6.77 and 7.16 Log CFU/g, respectively. The International Commission on Microbiological Specifications for Foods has recommended the number of total aerobic bacteria to be < 7 Log CFU/g (ICMSF, 1986). However, in 2018, the Ministry of Food and Drug Safety (MFDS) in Korea revised the guideline for fresh meat (beef, pork, and chicken) distributed in meat packing centers and meat shops from 7 Log CFU/g (1×10^7 CFU/g) to 6.70 Log CFU/g (5×10^6 CFU/g) (MFDS, 2018). Although few studies have monitored total aerobic bacteria in fresh KNBG loin, total aerobic bacteria in Hanwoo beef loin (grade 1) increased from 2.30 Log CFU/g to 6.87 Log CFU/g after 12 days of storage (Sujiwo et al., 2019), which is similar to our results.

Moreover, *E. coli* and coliform were not detected until day 10, with coliform abundance on day 15 found to be only 0.20–0.51 Log CFU/g. Thus, feeding different ratios of alfalfa and concentrate to finishing KNBG for 90 days did not impact microorganism levels in loin meat; however, meat from both groups may be spoiled by day 15 in aerobic storage at 4℃.

VBN and TBARS value

Initial VBN contents of loin meat from both groups were 6.68–6.82 mg/100 g and increased during storage ($p < 0.05$; Table 5). On day 5 and 10, the VBN value in loin meat from the KLA group was higher than that from the KHA group ($p < 0.05$). However, on day 15, VBN values from both groups were not significantly different. It was reported that feeding antioxidants, such as vitamin E, to animals can lower VBN values in meat (Kang et al., 2012). In this study, feeding different ratios of alfalfa and concentrate to finishing KNBG for 90 days did not impact the initial VBN value in loin meat; however, the interaction between feeding ratio and cold storage may have affected the VBN contents of loin meat

297 from both groups on day 5 and 10. According to the Food Code in Korea, meat with a VBN
298 value > 20 mg/100 g is considered spoiled (MFDS, 2020). In this study, VBN values of loin
299 meat from KHA and KLA groups were 19.87 and 18.80 mg/100 g, respectively. Although
300 this could be considered fresh according to the criteria of spoiled meat in Korea, it is
301 necessary to also consider the microorganism abundance. Therefore, we propose that loin
302 meat from the KHA and KLA remains safe for consumption until 10 days of storage, under
303 the conditions described herein.

304 The initial TBARS value for loin meat from both groups was 0.17 mg MDA/kg and
305 increased during storage to 1.03–1.04 mg MDA/kg on day 15 ($p < 0.05$). However, we
306 observed no significant differences in TBARS values between the two groups. The TBARS
307 value indicates the degree of lipid oxidation, which can be measured based on the intensity of
308 the red color generated by the reaction between malondialdehyde and thiobarbituric acid.
309 According to Filgueras et al. (2010), the extent of lipid oxidative processes in meat is
310 dependent on the balance between muscle antioxidant molecules such as vitamin E and
311 antioxidant enzymes (catalase, superoxide dismutase, and glutathione peroxidase) and lipidic
312 substances sensible to peroxidation (i.e. polyunsaturated fatty acids or PUFA). In this study,
313 the higher level of antioxidant substances in loin meat such as L-carnitine, carnosine, and
314 anserine which are relatively abundant in lean meat may be responsible for the surprisingly
315 lower anti-lipid oxidation effect. Rancidity of meat, as determined based on lipid oxidation,
316 can lead to an unpleasant flavour. In particular, Prommachart et al. (2020) reported that the
317 threshold of TBARS values, as assessed by panelists, for oxidized flavor varied greatly from
318 0.6–2.0 mg MDA/kg meat. In our study, TBARS values of loin from both groups reached 1
319 mg MDA/kg meat on day 15 of storage. A previous study had reported that meat from grass-
320 fed bulls exhibited significantly higher oxidative stability than that from concentrate-fed bulls

(Nuernberg et al., 2005). This was postulated to be due to high concentrations of vitamin E in the muscles of grass-fed bulls (Nuernberg et al., 2005). However, in this study, feeding different ratios of alfalfa and concentrate to finishing KNBG for 90 days did not impact lipid oxidation of loin meat during storage.

Fatty acid composition

The predominate fatty acids detected in loin meat from the KHA and KLA groups were palmitic acid, linoleic acid, oleic acid, arachidonic acid, and stearic acid (Table 6). These results agree with those of other studies on goat meat (Kim et al., 2019; Hwang et al., 2018; Kim et al., 2014). Loin meat from the KLA group had higher oleic acid and monounsaturated fatty acids (MUFAs) compared to that from the KHA group during storage ($p < 0.05$). Meanwhile, the meat from the KHA group had higher polyunsaturated fatty acid (PUFA) composition, including arachidonic acid, linolenic acid, and linoleic acid, compared to that from the KLA group ($p < 0.05$). The levels of saturated fatty acids (SFAs) in loin meat from the KHA and KLA groups were 38.72–42.05% and 39.85–41.59% during storage, respectively. Similarly, a previous study reported that the composition of SFAs and unsaturated fatty acids (UFAs) in goat meat was 40–50% and 50–60%, respectively (Marino et al., 2006). Moreover, according to Marino et al. (2006), the pasture-fed meat contains lower MUFA, higher PUFA, and similar SFA levels compared with concentrate-fed meat. Increasing concentrate in the fodder could alter the microbiota composition in the rumen, which occurs in meat with higher MUFA content (Mateescu et al., 2012). Moreover, the oleic acid and MUFA contents in meat can serve as positive factors for organoleptic properties (Kim & Jang, 2021). In particular, oleic acid is related to fat softness due to the lower melting point of oleic acid, which contributes to the umami taste of beef (Jung et al., 2016). Indeed,

345 the oleic acid composition in goat meat reportedly increases from 43.51% to 48.90%, with an
346 increase in diet concentrate levels (Kim et al., 2014). Therefore, the higher oleic acid content
347 and MUFA in the KLA group than that in the KLA group may have impacted the palatability
348 traits of loin meat.
349
350 **Bioactive compounds**
351 Meat is a good source of bioactive compounds, including vitamins, minerals, CoQ_{10}, L-
352 carnitine, creatinine, creatine, carnosine, and anserine, all of which exert beneficial effects on
353 human health (Pogorzelska-Nowicka et al., 2018). However, few studies have evaluated the
354 effect of feeding systems and storage on the bioactive compounds in goat meat. Hence, we
355 have evaluated bioactive compound (CoQ_{10}, L-carnitine, creatinine, creatine, carnosine, and
356 anserine) content in loin meat from the KHA and KLA groups during storage (Table 7).
357 CoQ_{10} is a fat-soluble vitamin-like compound that can be supplied externally or
358 produced endogenously (Ercan & El, 2011). CoQ_{10} content in meat samples ranges from 1.38
359 to 19.2 mg/100 g depending on the animal breed and cut (Kubo et al., 2008). Our results
360 revealed the CoQ_{10} levels in the loin meat of the KHA group to be 1.30–1.70 mg/100 g,
361 which was significantly higher than that in the KLA group on day 1 and 5 ($p < 0.05$). During
362 storage for 15 days, the CoQ_{10} levels in the loin meat of both groups did not change.
363 Similarly, Purchas and Busboom (2005) reported that high-pasture-fed New Zealand cattle
364 had higher CoQ_{10} content than high-concentrate-fed US cattle. CoQ_{10} can be endogenously
365 synthesized by a process including the synthesis of the benzoquinone ring from tyrosine as a
366 precursor (Bank et al., 2011). Although we did not perform a complete amino acid
367 composition analysis for alfalfa and concentrate, alfalfa contains 3.3–4.17% tyrosine
368 (Giner-Chavez et al., 1997; Kaldy et al., 1980; Brito et al., 2014), while concentrate for goat

feed has 2.87% tyrosine (Zhang et al., 2020). We, therefore, postulated that the high tyrosine content in alfalfa might be related to CoQ_{10} synthesis in the loin meat of the KHA group.

L-carnitine contributes to energy production in mitochondria by shuttling long-chain fatty acids and is abundant in red meat (Kim et al., 2019). L-carnitine contents in loin meat from the KHA and the KLA groups were 2.80 and 3.29 μmol/g, respectively, with loin meat from the KLA group having a higher L-carnitine content than that from the KHA group on day 1, 5, and 10 ($p < 0.05$). During storage, L-carnitine content increased on day 5 ($p < 0.05$) and continuously increased until day 15. These results agree with those of Kim and Jang (2021), who reported higher L-carnitine levels in KNBG loin meat compared to that in crossbred black goats (1.37 μmol/g) (Kim et al., 2019), which were similar to those reported in beef round meat (2.64 μmol/g) (Kim & Jang, 2021). L-carnitine is endogenously synthesized in organs by using lysine and methionine as precursors (Sarica et al., 2007). According to previous research, alfalfa contains 6.1–6.6% lysine and 1.8–1.9% methionine (Kaldy et al., 1980; Brito et al., 2014). Meanwhile, concentrate in goat feed contains 5.63% and 1.09%, respectively (Zhang et al., 2020). We, therefore, postulated that the high lysine and methionine content in alfalfa may have contributed to the synthesis of L-carnitine in the loin meat from the KHA group.

Creatine and creatinine have important roles in energy metabolism, with creatine non-enzymatically converted to creatinine via dehydration and formation of a ring structure in muscles (Kim & Jang, 2021). In this study, the levels of creatine and creatinine in loin meat from the KHA and KLA groups were 171.39–192.63 and 1.67–3.96 mg/100 g, respectively, which was similar to previous results in black goat loin meat (187.87 mg/100 g creatine and 3.13 mg/100 g creatinine) (Kim et al., 2019). Creatinine content in loin meat from the KLA group was higher than that from the KHA group ($p < 0.05$). However, creatine content did

not differ significantly between groups. During storage, creatinine content increased in the meat from both groups, while creatine content decreased ($p < 0.05$). Creatine is enzymatically synthesized from glycine and arginine in the kidneys and liver (Kreider et al., 2017). According to previous studies, concentrate contains significantly higher glycine and arginine contents at 4.9% and 7.6%, respectively (Zhang et al., 2020) compared to alfalfa at 3.7% and 5.3%, respectively (Giner-Chavez et al., 1997). We, therefore, postulated that the high glycine and arginine content in concentrate may have contributed to creatine synthesis in the loin meat from the KLA group, which was then non-enzymatically transformed into creatinine.

Carnosine exhibits antioxidant and anti-glycation activities via chelating metal ions and scavenging reactive oxygen species (Mateescu et al., 2012). Carnosine content in the loin meat from the KLA group was 54.12 mg/100 g on day 1, which was higher than that in the KHA group ($p < 0.05$). During storage, the carnosine content in meat from the KLA group decreased on day 15 ($p < 0.05$), while that in the KHA group remained unchanged. Moreover, the content of anserine, the methylated form of carnosine, in meat from the KHA and the KLA groups was 52.98–54.99 mg/100 g on day 1, with no significant differences between the two groups during storage. Meanwhile, the anserine content decreased in the meat from the KLA group on day 15 ($p < 0.05$), while that from the KHA group did not change. In comparison, carnosine content has been reported as 462 mg/100 g in pork loin (Kubo et al., 2008), 372 mg/100 g in beef (Mateescu et al., 2012), 65.25 mg/100 g in black goat loin (Kim et al., 2019), and 63.16 mg/100 g in chicken breast (Kim et al., 2019). Meanwhile, the anserine content in pork loin is 10.76 mg/100 g (Mora et al., 2007), 67 mg/100 g in beef (Mateescu et al., 2012), 81.93 mg/100 g in black goat loin (Kim et al., 2019), and 92.60 mg/100 g in chicken breast (Kim et al., 2020).

As carnosine (β-alanyl-l-histidine) and anserine (β-alanyl-l-methyl-l-histidine) are histidine-dipeptides, carnosine is synthesized from β-alanine and L-histidine (Qi et al., 2018). Increased histidine: lysine ratios (0.64) and histidine contents in diet increased the carnosine and anserine contents in chicken muscle and blood (Lackner et al., 2021). Although goats (ruminant) have a different amino acid metabolism than chickens (monogastric) (Teleni, 1993; Vernon, 1980), we postulated that the high histidine: lysine ratio in the concentrate (0.51; Zhang et al., 2020) helps improve the histidine-dipeptide contents in loin meat from the KLA group, compared to that in alfalfa (0.36; Kaldy et al., 1980). However, further analysis is required to evaluate the effect of histidine supplementation on carnosine and anserine contents, as well as the underlying metabolic mechanism in goat meat.

Antioxidative activities

With an increase in storage time, antioxidant activities (DPPH and ABTS radical scavenging activities, FRAP activity) of loin meat from both groups decreased ($p < 0.05$; Table 8), similar to the results of Kim and Jang (2021). Previously, the DPPH, FRAP, and ABTS activities of boiled pork were determined to be 10.58–13.65, 3.66–5.31, and 26.60–39.43 μmol TE/g dry matter, respectively (Gil et al., 2016), while the ABTS and FRAP activities of black goat loin were obtained as 12.90 and 15.92 μmol TE/g dry matter, respectively (Kim et al., 2019). These results imply that loin meat from the KLA group had higher ABTS radical scavenging activity than boiled pork and black goat meat, similar to beef loin.

In our study, loin from the KLA group had higher ABTS radical scavenging and FRAP activities compared to that from the KHA group on days 1 and 5 ($p < 0.05$). In particular, loin meat from the KLA group had 1.5 times higher FRAP activity than that from the KHA group,

throughout storage ($p < 0.05$). It was reported that beef from pasture-fed cows in Argentina has a higher level of FRAP than that from grain-fed cows; however, $ABTS^{+}$ radical scavenging activity did not significantly differ between the cows (Wu et al., 2008). However, vitamin E content and superoxide dismutase activity in loin meat from pasture-fed steers were higher than in those fed a mixed diet composed of silage, cattle-cake, and cereals mixture; however, glutathione peroxidase and OH radical scavenging activities were higher in loin meat from steers fed a mixed diet (Gatellier et al., 2004). Generally, grass and pasture contain many phytochemicals with high antioxidant activity (Gatellier et al., 2004). However, in this study, loin from the KLA group showed higher ABTS radical scavenging and FRAP activities than that from the KHA group. This activity was highly correlated with high carnosine, L-carnitine, and creatinine contents in the loin meat from the KLA group. Moreover, this phenomenon can be explained by the fact that concentrate diet is rich in polyphenols, such as phytic acid and pro-anthocyanidins, which can also trap OH radicals (Yang et al., 2002).

Multivariate statistical analysis

To elucidate the relationship between bioactive compounds and antioxidant activities in the KHA and the KLA groups, PCA and PLS-DA analyses were performed. The PCA plot showed a clear separation between the KHA and the KLA groups. Two principal components (PC1 = 57%, PC2 = 16.1%) accounted for 73.1% of the total variation (Fig. 1A). The KLA group was highly correlated with anserine, L-carnitine, carnosine, and creatinine contents and antioxidant activities (Fig. 1B). The PLS-DA score plot also showed a clear separation between the KHA and the KLA groups with a correlation coefficient (R2) of 0.97, an accuracy of 1.0, and a cross-validation correlation coefficient (Q2) of 0.92 (Fig. 1C). FRAP,

creatinine, ABTS radical scavenging activity, L-carnitine, and carnosine were evaluated with high VIP scores in PLS-DA and showed high abundance in the KLA group (Fig. 1D). These results supported the correlation between high creatinine, L-carnitine, and carnosine contents and FRAP and ABTS radical scavenging activity in the loin meat from the KLA group. Research on the relationship between bioactive compounds and antioxidant activities in goat meat is associated with a limitation. However, in our previous study, bioactive compounds (carnosine and anserine) and the antioxidant activity of beef loin and round showed a significant positive correlation ($0.572 \leq r \leq 0.931$), which is consistent with our present study results.

Conclusion

In this study, we found that loin from the KLA group had high L-carnitine, creatinine, and carnosine content, suggesting that supplementation with high alfalfa for 90 days to finishing KNBG does not induce an increase in bioactive compounds within loin meat. Rather, supplementation with alfalfa and concentrate at a ratio of 2:8 increased the bioactive compounds in loin meat. We postulate that the various nutrients and certain amino acids contained in concentrates might contribute to the metabolism of bioactive compounds in KNBG; however, further research is needed to evaluate the quality and metabolites of bioactive compounds in KNBG meat and determine whether it is affected by the different dietary ratios of forage and concentrate.

References

AOAC. 1997. Official Methods of Analysis of AOAC International, 16th ed.; AOAC International: Gaithersburg, MD, USA.

Brito AF, Tremblay GF, Bertrand A, Castonguay Y, Bélanger G, Michaud R, Lafrenière C, Martineau R, Berthiaume R. 2014. Alfalfa baleage with increased concentration of nonstructural carbohydrates supplemented with a corn-based concentrate did not improve production and nitrogen utilization in early lactation dairy cows. Int J Dairy Sci 97:6970-6990.

Descalzo AM, Rossetti L, Grigioni G, Irurueta M, Sancho AM, Carrete J, Pensel NA. 2007. Antioxidant status and odour profile in fresh beef from pasture or grain-fed cattle. Meat Sci 75:299-307.

Ercan P, El SN. 2011. Changes in content of coenzyme Q10 in beef muscle, beef liver and beef heart with cooking and in vitro digestion. J Food Compos Anal 24:1136-1140.

Filgueras RS, Gatellier P, Aubry L, Thomas A, Bauchart D, Durand D, Sante-Lhoutellier V. 2010. Colour, lipid and protein stability of Rhea americana meat during air-and vacuum-packaged storage: Influence of muscle on oxidative processes. Meat Sci 86:665-673.

French P, O'riordan EG, Monahan FJ, Caffrey PJ, Vidal M, Mooney MT, Troy DJ, Moloney AP. 2000. Meat quality of steers finished on autumn grass, grass silage or concentrate-based diets. Meat Sci 56:173-180.

Gatellier P, Mercier Y, Renerre M. 2004. Effect of diet finishing mode (pasture or mixed diet) on antioxidant status of Charolais bovine meat. Meat Sci 67:385-394.

Gil J, Kim D, Yoon SK, Ham JS, Jang A. 2016. Anti-oxidative and anti-inflammation activities of pork extracts. Korean J Food Sci Anim Resour 36: 275-282.

Hwang YH, Bakhsh A, Ismail I, Lee JG, Joo ST. 2018. Effects of intensive alfalfa feeding on meat quality and fatty acid profile of Korean native black goats. Korean J Food Sci Anim Resour 38:1092-1100.

International Commission on Microbiological Specifications for Foods (ICMSF). 1986.

512 Sampling for Microbiological Analysis: Principles and Scientific Applications. 2nd ed.
513 Toronto: University of Toronto Press.
514 Lee D, Lee HJ, Yoon JW, Ryu M, Jo C. 2021. Effects of cooking conditions on the
515 physicochemical and sensory characteristics of dry-and wet-aged beef. Anim Biosci 34:
516 1705-1716.
517 Jung EY, Hwang YH, Joo ST. 2016. The relationship between chemical compositions, meat
518 quality, and palatability of the 10 primal cuts from Hanwoo steer. Korean J Food Sci Anim
519 Resour 36:145-151.
520 Jung GW, Jo IH, HwangBo S, Lee SH, Song HB. 2008. Effects of different feeding systems
521 on nutrient availability, nitrogen retention and blood characteristics in native or crossbred
522 Korean black goats. J Kor Grassl Forage Sci 28:341-350.
523 Kadim IT, Mahgoub O, Al-Ajmi DS, Al-Maqbaly RS, Al-Saqri NM, Ritchie A. 2004. An
524 evaluation of the growth, carcass and meat quality characteristics of Omani goat
525 breeds. Meat Sci 66:203-210.
526 Kang SN, Chu GM, Song YM, Jin SK, Hwang IH, Kim IS. 2012. The effects of replacement
527 of antibiotics with by-products of oriental medicinal plants on growth performance and
528 meat qualities in fattening pigs. Anim Sci J 83:245-251.
529 Kim HJ, Jang A. 2021. Correlations between the levels of the bioactive compounds and
530 quality traits in beef loin and round during cold storage. Food Control 120:107491.
531 Kim HJ, Kim HJ, Jang, A. 2019. Nutritional and antioxidative properties of black goat meat
532 cuts. Asian-australas J Anim Sci 32:1423-1429.
533 Kim HJ, Kim HJ, Jeon J, Nam KC, Shim KS, Jung JH, Kim KS, Choi Y, Kim SH, Jang, A.
534 2020. Comparison of the quality characteristics of chicken breast meat from conventional
535 and animal welfare farms under refrigerated storage. Poult Sci 99:1788-1796.

536 Kim SW, Park SB, Kim MJ, Kim DH, Yim DG. 2014. Effects of different levels of
537 concentrate in the diet on physicochemical traits of Korean native black goat
538 meats. Korean J Food Sci Anim Resour 34:457-463.
539 Kim SW, Yoon SH, Kim JH, Ko YG, Kim DH, Kang GH, Kim YS, Lee SM, Suh SW. 2012.
540 Effects of feeding levels of concentrate on the growth, carcass characteristics and
541 economic evaluation in feeds based on rice-straw of Korean black goats. J Kor Grassl
542 Forage Sci 32:429-436.
543 Kubo H, Fujii K, Kawabe T, Matsumoto S, Kishida H, Hosoe K. 2008. Food content of
544 ubiquinol-10 and ubiquinone-10 in the Japanese diet. J Food Compos Anal 21:199-210.
545 Lackner J, Albrecht A, Mittler M, Marx A, Kreyenschmidt J, Hess V, Sauerwein H. 2021.
546 Effect of feeding histidine and β-alanine on carnosine concentration, growth performance,
547 and meat quality of broiler chickens. Poult Sci 100:101393.
548 Lee J, Lee SS, Kim CL, Choi BH, Lee SH, Kim DK, Lee ED, Kim KW, Ryu CH. 2021.
549 Effect of forage sources in total mixed ration (TMR) on in vitro rumen fermentation of
550 goat. J Kor Grassl Forage Sci 41:102-109.
551 Marino R, Albenzio M, Girolami A, Muscio A, Sevi A, Braghieri A. 2006. Effect of forage to
552 concentrate ratio on growth performance, and on carcass and meat quality of Podolian
553 young bulls. Meat Sci 72:415-424.
554 Marinova P, Banskalieva V, Alexandrov S, Tzvetkova V, Stanchev H. 2001. Carcass
555 composition and meat quality of kids fed sunflower oil supplemented diet. Small Rumin
556 Res 42:217-225.
557 Mateescu RG, Garmyn AJ, O'neil MA, Tait Jr RG, Abuzaid A, Mayes MS, Garrick DJ, Van
558 Eenennaam AL, VanOverbeke DL, Hilton GG, Beitz DC, Reecy JM. 2012. Genetic
559 parameters for carnitine, creatine, creatinine, carnosine, and anserine concentration in

560 longissimus muscle and their association with palatability traits in Angus cattle. J Anim
561 Sci 90:4248-4255.
562 Ministry of Agriculture, Food and Rural Affairs (MAFRA). 2019. Statistical yearbook of
563 agriculture, food and rural affairs.
564 Ministry of Food and Drug Safety (MFDS). 2018. [cited 2022 Feb 3]. Available from:
565 https://www.law.go.kr/LSW/admRulLsInfoP.do?admRulSeq=2100000109889.
566 Ministry of Food and Drug Safety (MFDS). 2020. Food Code, Ministry of Food and Drug
567 Safety.
568 Mora L, Sentandreu MA, Toldrá F. 2007. Hydrophilic chromatographic determination of
569 carnosine, anserine, balenine, creatine, and creatinine. J Agric Food Chem 55:4664-4669.
570 No KM, Leem DG, Kim MG, Park KS, Yoon TH, Hong J, Park SY, Jeong JY. 2011. Analysis
571 of coenzyme Q 10 in dietary supplement by HPLC. J Food Hyg Saf 26:12–15.
572 Nuernberg K, Dannenberger D, Nuernberg G, Ender K, Voigt J, Scollan ND, Wood WD, Nute
573 GR, Richardson RI. 2005. Effect of a grass-based and a concentrate feeding system on
574 meat quality characteristics and fatty acid composition of longissimus muscle in different
575 cattle breeds. Livest Prod Sci 94:137-147.
576 Pogorzelska-Nowicka E, Atanasov AG, Horbańczuk J, Wierzbicka A. 2018. Bioactive
577 compounds in functional meat products. Molecules 23:307.
578 Prommachart R, Belem TS, Uriyapongson S, Rayas-Duarte P, Uriyapongson J, Ramanathan
579 R. 2020. The effect of black rice water extract on surface color, lipid oxidation, microbial
580 growth, and antioxidant activity of beef patties during chilled storage. Meat
581 Sci 164:108091.
582 Purchas RW, Busboom JR. 2005. The effect of production system and age on levels of iron,
583 taurine, carnosine, coenzyme Q10, and creatine in beef muscles and liver. Meat Sci 70:

584 589-596.
585 Qi B, Wang J, Ma YB, Wu SG, Qi GH, Zhang HJ. 2018. Effect of dietary β-alanine
586 supplementation on growth performance, meat quality, carnosine content, and gene
587 expression of carnosine-related enzymes in broilers. Poult Sci 97:1220-1228.
588 Realini CE, Duckett SK, Brito GW, Dalla Rizza M, De Mattos D. 2004. Effect of pasture vs.
589 concentrate feeding with or without antioxidants on carcass characteristics, fatty acid
590 composition, and quality of Uruguayan beef. Meat Sci 66:567-577.
591 Sarica S, Corduk M, Ensoy U, Basmacioglu H, Karatas U. 2007. Effects of dietary
592 supplementation of L-carnitine on performance, carcass and meat characteristics of quails.
593 S Afr J Anim Sci 37:189-201.
594 Sebsibe A. 2008. Sheep and goat meat characteristics and quality. Sheep and Goat Production
595 Handbook for Ethiopia. Ethiopian Sheep and Goats Productivity Improvement Program
596 (ESGPIP), Addis Ababa, Ethiopia. pp.323-328.
597 Shimada K, Sakuma Y, Wakamatsu J, Fukushima M, Sekikawa M, Kuchida K, Mikami M.
598 2004. Species and muscle differences in L-carnitine levels in skeletal muscles based on a
599 new simple assay. Meat Sci 68:357–362.
600 Son YS. 1999. Production and uses of Korean native black goat. Small Rumin Res 34:303-
601 308.
602 Steen RWJ, Kilpatrick DJ. 2000. The effects of the ratio of grass silage to concentrate in the
603 diet and restricted dry matter intake on the performance and carcass composition of beef
604 cattle. Livest Prod Sci 62:181-192.
605 Sujiwo J, Kim HJ, Song SO, Jang A. 2019. Relationship between quality and freshness traits
606 and torrymeter value of beef loin during cold storage. Meat Sci 149:120-125.
607 Teleni E. 1993. Catabolism and synthesis of amino acids in skeletal muscle: their significance

608 in monogastric mammals and ruminants. Australian Journal of Agricultural Research,
609 44:443-461.
610 Velasco V, Williams P. 2011. Improving meat quality through natural antioxidants. Chil J
611 Agric Res 71:313-322.
612 Vernon RG. 1980. Comparative aspects of lipid metabolism in monogastric, pre-ruminant and
613 ruminating animals. Biochem Soc Trans 8:291–292
614 Wu CHERRY, Duckett SK, Neel JPS, Fontenot JP, Clapham WM. 2008. Influence of
615 finishing systems on hydrophilic and lipophilic oxygen radical absorbance capacity
616 (ORAC) in beef. Meat Sci 80: 662-667.
617 Yang A, Lanari MC, Brewster M, Tume RK. 2002. Lipid stability and meat colour of beef
618 from pasture-and grain-fed cattle with or without vitamin E supplement. Meat Sci 60:41-50.
619 Yang A, Lanari MC, Brewster M, Tume RK. 2002. Lipid stability and meat color of beef
620 from pasture-and grain-fed cattle with or without vitamin E supplement. Meat Sci 60:41-50.
621 Zhang XX, Li YX, Tang ZR, Sun WZ, Wu LT, An R, Chen HY, Wan K. Sun ZH. 2020.
622 Reducing protein content in the diet of growing goats: implications for nitrogen balance,
623 intestinal nutrient digestion and absorption, and rumen microbiota. Animal 14:2063-2073.
624

625 **Table 1. Ingredients and chemical composition of experimental diets**

Item	% of DM	
	Alfalfa	Concentrate
Ingredients		
Corn grain		12.0
Wheat grain		9.0
Brown rice		2.5
Lupin seeds		2.0
Rice bran		2.0
Wheat bran		0.7
Corn germs meal		9.0
Lupin hulls		12.0
Corn gluten feed		15.0
Cashew nut hulls		2.0
Rape seeds meal		3.0
Coconut meal		5.0
Palm kernel meal		16.0
Molasses		6.0
Limestone		0.6
Salt		0.6
Vitamin mix		0.3
Mineral mix		0.5
Sodium bicarbonate		0.8
Yeast culture		1.0
Total		100
Chemical composition		
Dry matter (DM), g/kg as fed	907	933
Neutral detergent insoluble crude protein, g/kg of DM	60	32
Acid detergent insoluble crude protein, g/kg of DM	22	7
Neural detergent fiber, g/kg of DM	547	354
Acid detergent fiber, g/kg of DM	423	182
Non fiber carbohydrates, g/kg of DM	306	439

626

627

628 **Table 2. Proximate composition of loin from Korean native black goat fed alfalfa and**

629 **concentrates**

Treatment	Proximate composition (%)			
	Moisture	Crude Protein	Crude Fat	Crude ash
KHA	73.60	20.32	5.21	1.08
KLA	74.72	20.77	5.62	1.02
SEM	0.547	0.230	0.206	0.023

630 KHA, KNBG fed high alfalfa (alfalfa: concentrate = 8:2); KLA, KNBG fed low alfalfa

631 (alfalfa: concentrate = 2:8)

632

633 **Table 3. Effect of feeding alfalfa and concentrates on pH and meat color of Korean**

634 **native black goat loin during the cold storage**

Traits	Treatment	Storage (days)				SEM
		1	5	10	15	
pH	KHA	6.00^{Ab}	6.17^{Aa}	6.23^{Aa}	6.28^{Aa}	0.040
	KLA	5.92^{Ac}	6.10^{Ab}	6.19^{Aab}	6.27^{Aa}	0.027
	SEM	0.030	0.027	0.036	0.041	
CIE L*	KHA	41.41^{Aa}	38.42^{Ab}	37.56^{Bb}	37.98^{Ab}	0.663
	KLA	40.81^{Aa}	38.79^{Ab}	38.88^{Ab}	38.07^{Ab}	0.289
	SEM	0.647	0.459	0.307	0.568	
CIE a*	KHA	24.61^{Aa}	19.55^{Ab}	15.23^{Ac}	14.04^{Ac}	0.772
	KLA	25.01^{Aa}	18.75^{Ab}	16.53^{Ac}	15.17^{Ac}	0.478
	SEM	0.395	0.807	0.646	0.651	
CIE b*	KHA	14.17^{Aa}	11.37^{Ab}	10.05^{Abc}	9.73^{Ac}	0.336
	KLA	13.94^{Aa}	10.88^{Ab}	10.26^{Ac}	10.09^{Ac}	0.152
	SEM	0.224	0.326	0.226	0.254	

635 $^{A-B}$ Means within a column with different superscript differ significantly at $p<0.05$.

636 $^{a-c}$ Means within a row with different superscript differ significantly at $p<0.05$.

637 KHA, KNBG fed high alfalfa (alfalfa: concentrate = 8:2); KLA, KNBG fed low alfalfa

638 (alfalfa: concentrate = 2:8)

639

640 **Table 4. Effect of feeding alfalfa and concentrate on microorganisms of Korean native**

641 **black goat loin during the cold storage**

Microorganisms (Log CFU/g)	Treatment	Storage (days)				SEM
		1	5	10	15	
Total aerobic bacteria	KHA	2.35^{Ac}	2.87^{Ac}	4.66^{Ab}	6.77^{Aa}	0.146
	KLA	2.17^{Bd}	2.93^{Ac}	5.00^{Ab}	7.16^{Aa}	0.158
	SEM	0.053	0.028	0.204	0.217	
E. coli	KHA	ND	ND	ND	ND	-
	KLA	ND	ND	ND	ND	-
Coliform	KHA	ND	ND	ND	0.20^{A}	-
	KLA	ND	ND	ND	0.51^{A}	-
	SEM	-	-	-	0.294	

642 $^{A-B}$ Means within a column with different superscript differ significantly at $p<0.05$.

643 $^{a-d}$ Means within a row with different superscript differ significantly at $p<0.05$.

644 KHA, KNBG fed high alfalfa (alfalfa: concentrate = 8:2); KLA, KNBG fed low alfalfa

645 (alfalfa: concentrate = 2:8)

646

Table 5. Effect of feeding alfalfa and concentrate on volatile basic nitrogen (VBN) and thiobarbituric acid reactive substance (TBARS) of Korean native black goat loin during the cold storage

Treatment		Storage (days)				SEM
		1	5	10	15	
VBN (mg/100 g)	KHA	6.68^{Ad}	8.68^{Bc}	10.62^{Bb}	19.87^{Aa}	0.119
	KLA	6.82^{Ad}	9.25^{Ac}	11.66^{Ab}	18.80^{Aa}	0.377
	SEM	0.226	0.114	0.166	0.469	
TBARS (mg MDA/kg)	KHA	0.17^{Ad}	0.43^{Ac}	0.57^{Ab}	1.03^{Aa}	0.009
	KLA	0.17^{Ad}	0.45^{Ac}	0.54^{Ab}	1.04^{Aa}	0.010
	SEM	0.006	0.009	0.013	0.008	

$^{A-B}$ Means within a column with different superscript differ significantly at $p<0.05$.

$^{a-d}$ Means within a row with different superscript differ significantly at $p<0.05$.

KHA, KNBG fed high alfalfa (alfalfa: concentrate = 8:2); KLA, KNBG fed low alfalfa (alfalfa: concentrate = 2:8)

655 **Table 6. Effect of feeding alfalfa and concentrate on fatty acid composition (%) of**

656 **Korean native black goat loin during the cold storage**

Fatty acid	Storage (days)								
	1			10			15		
	KHA	KLA	SEM	KHA	KLA	SEM	KHA	KLA	SEM
C14:0 (myristic acid)	1.75[a]	1.70[a]	0.146	2.18[a]	2.21[a]	0.098	2.02[a]	2.08[a]	0.114
C16:0 (palmitic acid)	22.84[a]	23.69[a]	0.688	24.49[a]	25.11[a]	0.620	22.72[b]	24.85[a]	0.500
C16:1n7 (palmitoleic acid)	1.53[a]	1.65[a]	0.107	1.56[a]	1.64[a]	0.132	1.29[a]	1.53[a]	0.153
C18:0 (stearic acid)	15.81[a]	14.46[a]	0.741	14.70[a]	14.22[a]	0.488	13.98[a]	14.66[a]	0.452
C18:1n9 (oleic acid)	31.82[b]	39.39[a]	0.764	36.83[b]	43.71[a]	0.907	39.12[b]	43.56[a]	0.769
C18:1n7 (vaccenic acid)	2.14[a]	1.45[a]	0.270	1.40[a]	1.60[a]	0.094	1.44[a]	1.23[a]	0.094
C18:2n6 (linoleic acid)	10.84[a]	8.72[b]	0.618	8.92[a]	5.92[b]	0.404	9.63[a]	5.69[b]	0.429
C18:3n6 (γ-linoleic acid)	0.11[a]	0.14[a]	0.027	0.03[a]	0.00[a]	0.011	0.01[a]	0.00[a]	0.006
C18:3n3 (α-linolenic acid)	1.91[a]	0.56[b]	0.192	1.36[a]	0.56[b]	0.116	1.45[a]	0.54[b]	0.109
C20:1n9 (eicosenoic acid)	0.39[a]	0.28[b]	0.024	0.28[a]	0.25[a]	0.033	0.34[a]	0.15[b]	0.019
C20:4n6 (arachidonic acid)	7.46[a]	6.02[b]	0.365	5.18[a]	4.01[b]	0.350	5.49[a]	4.06[b]	0.319
C20:5n3 (eicosapentaenoic acid)	0.46[a]	0.35[b]	0.036	0.26[a]	0.27[a]	0.030	0.34[a]	0.38[a]	0.058
C22:4n6 (adrenic acid)	2.64[a]	1.43[b]	0.242	2.07[a]	1.02[b]	0.197	1.91[a]	1.20[b]	0.147
C22:6n3 (docosahexaenoic acid)	0.32[a]	0.18[a]	0.052	0.27[a]	0.09[b]	0.029	0.26[a]	0.16[a]	0.036
SFA	40.39[a]	39.85[a]	1.006	41.98[a]	40.91[a]	1.027	38.72[b]	41.59[a]	0.803
UFA	59.61[a]	60.15[a]	1.006	58.02[a]	59.09[a]	1.027	61.28[a]	58.41[b]	0.803
MUFA	35.88[b]	42.77[a]	0.589	40.07[b]	47.20[a]	0.955	42.19[b]	46.47[a]	0.730
PUFA	23.73[a]	17.38[b]	1.320	17.95[a]	11.89[b]	1.009	19.09[a]	11.94[b]	0.929
MUFA/SFA	0.89[b]	1.08[a]	0.026	0.92[b]	1.16[a]	0.043	1.09[a]	1.12[a]	0.031
PUFA/SFA	0.59[a]	0.44[b]	0.048	0.46[a]	0.29[b]	0.031	0.50[a]	0.29[b]	0.031

657 [a-b] Means within a row with different superscript differ significantly at $p<0.05$.

KHA, KNBG fed high alfalfa (alfalfa: concentrate = 8:2); KLA, KNBG fed low alfalfa (alfalfa: concentrate = 2:8); SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Table 7. Effect of feeding alfalfa and concentrate on bioactive compounds of Korean native black goat loin during the cold storage

Bioactive compounds (mg/100 g)	Treatment	Storage (days)				SEM
		1	5	10	15	
CoQ_{10}	KHA	1.70^{Aa}	1.60^{Aa}	1.58^{Aa}	1.44^{Aa}	0.086
	KLA	1.43^{Ba}	1.30^{Ba}	1.34^{Aa}	1.31^{Aa}	0.066
	SEM	0.056	0.086	0.091	0.068	
L-carnitine (μmol/g)	KHA	2.80^{Bb}	3.42^{Ba}	3.56^{Ba}	3.73^{Aa}	0.103
	KLA	3.29^{Ab}	3.84^{Aa}	3.90^{Aa}	3.93^{Aa}	0.109
	SEM	0.105	0.117	0.069	0.124	
Creatinine	KHA	1.67^{Bc}	2.08^{Bb}	2.61^{Ba}	2.80^{Ba}	0.088
	KLA	2.71^{Ac}	2.97^{Ac}	3.46^{Ab}	3.96^{Aa}	0.079
	SEM	0.081	0.060	0.080	0.106	
Creatine	KHA	191.60^{Aab}	192.63^{Aa}	183.33^{Aab}	179.78^{Ab}	3.047
	KLA	184.35^{Aa}	179.76^{Ba}	178.43^{Aa}	171.39^{Bb}	1.727
	SEM	2.565	2.522	2.568	2.235	
Carnosine	KHA	46.90^{Ba}	46.09^{Ba}	46.51^{Ba}	39.67^{Aa}	2.057
	KLA	54.12^{Aa}	54.44^{Aa}	52.68^{Aa}	43.28^{Ab}	1.954
	SEM	1.695	1.741	1.571	2.779	
Anserine	KHA	52.98^{Aa}	51.66^{Aa}	50.96^{Aa}	46.52^{Aa}	3.526
	KLA	54.99^{Aa}	54.09^{Aa}	54.99^{Aa}	44.30^{Ab}	2.041
	SEM	3.070	3.146	2.568	2.698	

$^{A-B}$ Means within a column with different superscript differ significantly at $p<0.05$.

$^{a-c}$ Means within a row with different superscript differ significantly at $p<0.05$.

KHA, KNBG fed high alfalfa (alfalfa: concentrate = 8:2); KLA, KNBG fed low alfalfa (alfalfa: concentrate = 2:8)

668 **Table 8. Effect of feeding alfalfa and concentrate on antioxidant activities of Korean**

669 **native black goat loin during the cold storage**

Antioxidant activity (μmol TE/g dry matter)	Treatment	Storage (days)				SEM
		1	5	10	15	
DPPH	KHA	5.91^{Aa}	5.25^{Ba}	5.21^{Aa}	4.09^{Bb}	0.249
	KLA	6.74^{Aa}	6.81^{Aa}	5.82^{Aab}	4.99^{Ab}	0.321
	SEM	0.398	0.151	0.274	0.272	
ABTS	KHA	42.26^{Ba}	41.34^{Ba}	41.40^{Aa}	34.93^{Ab}	1.076
	KLA	46.93^{Aa}	47.04^{Aa}	41.06^{Ab}	37.79^{Ac}	0.729
	SEM	0.510	0.726	1.164	1.113	
FRAP	KHA	9.95^{Ba}	9.12^{Ba}	9.84^{Ba}	7.81^{Bb}	0.295
	KLA	14.17^{Aa}	13.32^{Aab}	13.35^{Aab}	12.45^{Ab}	0.269
	SEM	0.258	0.299	0.293	0.278	

670 $^{A-B}$ Means within a column with different superscript differ significantly at $p<0.05$.

671 $^{a-c}$ Means within a row with different superscript differ significantly at $p<0.05$.

672 KHA, KNBG fed high alfalfa (alfalfa: concentrate = 8:2); KLA, KNBG fed low alfalfa

673 (alfalfa: concentrate = 2:8)

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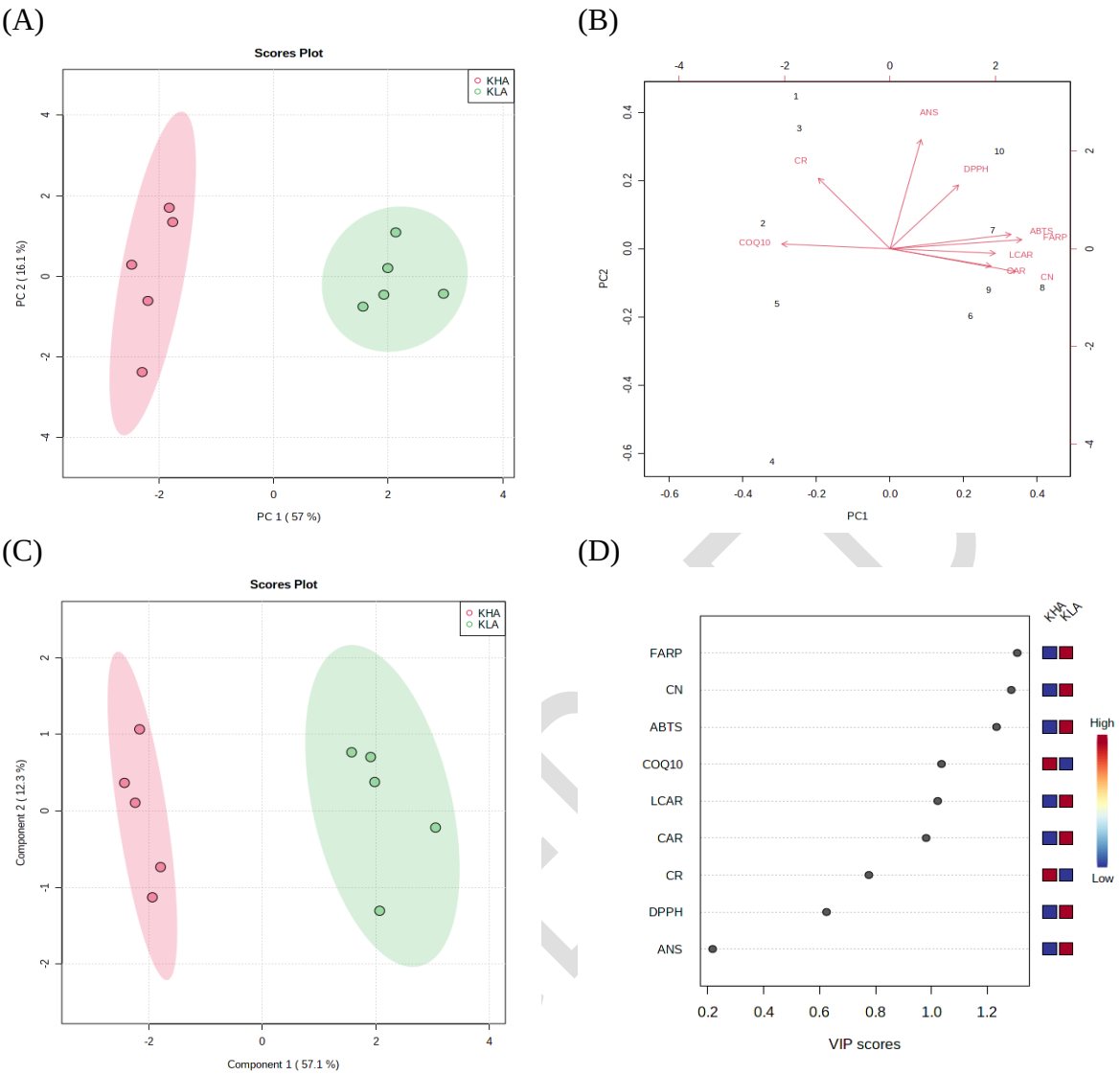

675 **Fig. 1. The principal component (PCA) and partial least squares-discriminant analysis**
676 **(PLS-DA) analysis for bioactive compounds and anti-oxidant activities of loin meat of**
677 **Korean native black goat feeding alfalfa and concentrate. (A) PCA score plot; (B) biplot**
678 **of PCA result; (C) PLS-DA score plot; (D) variable importance analysis.** KHA (no. 1-5),
679 KNBG fed high alfalfa (alfalfa: concentrate = 8:2); KLA (no. 6-10), KNBG fed low alfalfa
680 (alfalfa: concentrate = 2:8); CN, creatinine; COQ10, coenzyme Q_{10}; LCAR, L-carnitine; CAR,
681 carnosine; CR, creatine; ANS, anserine.
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