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Comparison of Fatty Acid Composition and Metabolite Compounds in the early stage of Hanwoo steers with different genetic potentials.

Abstract

The genetic sequencing of Hanwoo steers can be available for the selection of the best steers for high-quality meat production. This experiment aimed to examine differences in fatty acid profiles and metabolite compounds of Hanwoo steers at the late growth stage due to genetic selection for higher growth rate and better meat quality. Forty-eight Hanwoo steers were categorized based on age (10 months-10M, 13 months-13M) and further classified by genetic traits into growth (G) and quality (Q) subgroups. Muscle samples taken from live steers were examined using gas chromatography and nuclear magnetic resonance spectroscopy. Oleic acid, linoleic acid, Dihomo-γ-Linolenic, and eicosapentaenoic acid indicated significant differences between genetic trait groups regardless of the effect of age. The combined effect of age and genetic potential did not significantly alter the levels of most fatty acids, except elaidic acid, linolenic acid, and godonic acid. Simultaneously, significant differences were not observed between growth and quality traits within the same age group. All metabolite compounds categorized under genetic traits, without considering the age effect, were not significantly different between each group. However, metabolomic analysis revealed higher concentrations of protein synthesis-related amino acids and energy metabolism compounds in the 10M group. Simultaneously, PLS-DA data clearly distinguishes between the 10M and 13M groups. According to the Variable Importance in Projection score plot, this geographical variation is due to betaine, carnosine, creatine, isoleucine, anserine, inosine, and inosine monophosphate. Overall, genetic traits did not significantly impact fatty acid or metabolite profiles during the late growth stage, suggesting these effects may be more pronounced earlier in life. In contrast, age had a more notable influence, leading to distinct metabolic and fatty acid composition differences between 10M and 13M steers.

Keywords: Hanwoo, Growth stage, Metabolite compounds, Fatty acids, Genetic potential

Introduction

Since around 2000 B.C., Hanwoo has been raised on the Korean Peninsula through Korean agriculture. There are four types of Korean native cattle by their unit coat color, including Hanwoo (brown) [1]. They are highly valued for their superior meat quality, particularly their marbling, which is a significant factor in determining the flavor and texture of the beef [2]. According to statistics, since 2005, per capita beef consumption has been trending upward, while the pattern of beef imports has been declining [2]. These results indicate an increase in demand for Hanwoo beef in Korea. Due to the growing population and the growing demand for Hanwoo beef, Hanwoo's whole agricultural sector has been converted to a more industrialized and commercial industry, with a decrease in household farming. This forces scientists, as well as producers, to improve the meat quality and yield by engaging in several kinds of research to satisfy the consumers and cater to the increasing demand in a sustainable manner. The investigation of metabolomes and fatty acids provides information on how to achieve this target [3, 4].

Metabolites are identified as final or intermediate products of the regulatory processes of cells [5] that provide insight into the physiological state of the animal and the metabolic processes [6]. From a meat point of view, the profile of metabolites and fatty acids plays a decisive role in the development of taste and nutritional quality. Besides that, these profiles provide information to improve sustainable farming practices [7], act as biomarkers for diseases and monitor treatment responses [8], and predict or explain color, pH, marbling, and eating quality traits of meat before slaughtering the animal. Currently, there is a push to lower the slaughter age to reduce greenhouse gas emissions and resource scarcity [9]. The composition of fatty acids [10] and metabolite compounds [11] can be a good way to demonstrate the meat quality of animals of different ages, and to compare their meat quality.

Many factors affect the composition of metabolite compounds and fatty acids in animal bodies, such as age and developmental stages, like physiological factors, genetic factors, and nutritional factors. The age of cattle at slaughter is a critical factor that influences the physiological development of the animals, including fat deposition and metabolic processes. As the animals mature, their capability to accumulate fat, particularly intramuscular fat (IMF), improves significantly [12]. Adipose cells of muscle and subcutaneous fat tissue exhibit notable morphological, developmental, and metabolic differences with regard to fat deposition [13, 14]. Younger cattle generally exhibit higher feed conversion efficiency, which can be attributed to the developmental stages of muscle and fat tissues that differ with age, affecting how cattle metabolize energy and nutrients [15]. Similarly, Frampton et al. [16] showed, short-chain fatty acids produced in the rumen fermentation process show an effect on skeletal muscle metabolism.

According to [17], genes involved in lipid metabolism play a great role in fatty acid composition and intramuscular fat deposition. Growth rate and meat quality are two main dimensions that are considered by Hanwoo farmers [18], and both these factors directly correlate with the genetic background of the animal [19]. Genomic sequencing is the process of analyzing an animal's DNA to find and reveal information about its genetic heritage. This method evaluates the general genetic composition, and particular genes linked to characteristics like meat quality and growth rate can be identified [20]. Meat's metabolites and fatty acids can be used to predict Hanwoo's enhanced genetic potential as a consequence of selection and breeding. As a result of successful breeding, selection, and feeding practices, it would most likely be a good predictor of lowering the slaughter age of livestock species that resemble Hanwoo.

Although fatty acid and metabolite profiles are important, there was no detailed information on how the fatty acid and metabolite profiles of Hanwoo change with age, especially during the early stages of the life cycle. Therefore, the present study mainly deals with the differences in fatty acid and metabolite profiles in Hanwoo at two different ages: 10 months and 13 months, with the effect of the genetic potential for Growth and meat quality.

Materials and Methods

The current experiment was conducted on Forty-eight Hanwoo steers who were randomly assigned into two groups (10 mo, 13 mo) according to their age at the time of the muscle sample collection. To classify animals according to their genetic potential for meat quality (Q) and growth rate (G), genomic analysis was conducted using tail hair samples. Tail hair samples collection was conducted according to the protocol mentioned by Hao et al. [21]. The analyses were performed at the Animal Gene Testing Center (NongHyup Economic Holdings, Seosan, Chungnam, South Korea) employing the Illumina Infinium HTS assay with the BovineSNP50 v3 BeadChip. SNPs located within the AGPAT6, PPARGC1A, CMKLR1, SFT2D3, EMPP2, KDELC2, TMEM40, PHOX2A, IFFO2, MTIF3, and DMRT2 genes were considered for genetic potential group classification. Finally, all 48 steers resulted in 4 groups called 10Q, 10G, 13Q and 13G. All groups were reared under the same diet (Tables 1a and 1b present the feed ingredients and the chemical composition of the diet provided to all groups) and the same farming conditions at Sunchon National University (Sunchon, Jeollanam-do, South Korea).

The average weights of 10 mo and 13 mo old Hanwoo steers at the time of sample collection were 307.36 ±30.62 kg and 416.03±38.52 kg, respectively. The samples from the semitendinosus muscle of Hanwoo steers were collected under anesthetic conditions by a professional veterinarian. The protocol for minimal anesthesia induction was conducted in line with the procedure of [22]. Anesthesia was induced using 1 mL of xylazine hydrochloride and 3 mL of lidocaine hydrochloride administered through rapid injection into the cephalic vein. After collecting, collected samples were stored in sterilized tubes at 4°C and transported to the laboratory for further analysis. (The Institutional Animal Care and Use Committee of Sunchon National University (SCNU-IACUC) approved all animal procedures used in this study under permission number: SCNU IACUC-2023-11)

Analysis of fatty acid composition

The fatty acid composition of Hanwoo muscle samples was analyzed using a modified direct methylation method for fatty acid methyl ester (FAME) synthesis, originally described by O'Fallon et al. [23]. Briefly, 1 g of muscle tissue was mixed with 0.7 mL of 10 N potassium hydroxide (KOH) and 6.3 mL of methanol. The mixture was incubated in a water bath at 55°C for 1.5 h with vigorous agitation every 30 min. Following cooling in ice water for 2 min, 0.58 mL of 24 N sulfuric acid (H_2SO_4) was added, and the mixture was reheated under identical conditions. After completion, 3 mL of hexane was added, and the solution was transferred to vials using a Pasteur pipette. Samples were centrifuged at 1,100×g for 5 min (Combi-514R, Hanil Scientific, Incheon, Korea). FAMEs were analyzed using gas chromatography equipped with a flame ionization detector (GC-FID; Agilent 7890 series, Agilent Technologies, Wilmington, DE, USA). The injector operated in split mode (25:1) at 250°C. High-purity hydrogen, air, and helium served as carrier gases, with flow rates of 40 mL/min for hydrogen and 400 mL/min for air. Chromatographic separation was achieved using an HP-88 capillary column (60 m × 250 μm × 0.2 mm film thickness). Fatty acids were identified and quantified as relative percentages of the total detected fatty acids.

Nutritional quality indices

Nutritional quality indices of Hanwoo muscle samples were assessed based on their fatty acid profiles. The atherogenicity index (AI) and thrombogenicity index (TI) were calculated according to the method described by Ulbricht et al. [24], while the hypocholesterolemic/hypercholesterolemic (HH) ratio was determined following the approach of Santos-Silva, Bessa [25]. The corresponding equations were applied to compute AI, TI, and HH values. Additional indices, including the ratio of polyunsaturated to saturated fatty acids (PUFA/SFA) and the ratio of n-6 to n-3 polyunsaturated fatty acids (n-6/n-3 PUFA), were also evaluated to further characterize the nutritional quality of the meat.

$$\mathrm{AI} = \frac{[\mathrm{C12{:}0} + 4 \times (\mathrm{C14{:}0}) + \mathrm{C16{:}0}]}{[\sum \mathrm{MUFA} + \sum \mathrm{PUFA}]}$$

$$\mathrm{TI} = \frac{[\mathrm{C14{:}0} + \mathrm{C16{:}0} + \mathrm{C18{:}0}]}{[0.5 \times (\sum \mathrm{MUFA} + \sum \mathrm{n6}) + 3 \times \sum \mathrm{n3} + \frac{\sum \mathrm{n3}}{\sum \mathrm{n6}}]}$$

$$HH = \frac{[C18{:}1cis9 + C18{:}2n6 + C20{:}4n6 + C18{:}3n3 + C20{:}5n3 + C22{:}5n3 + C22{:}6n3]}{[C14{:}0 + C16{:}0]}$$

Nuclear magnetic resonance spectroscopy (NMR)

Sample extraction and NMR analysis were conducted following the procedure described by [26]. The sample (5 g) was extracted using 20 mL of 0.6 M perchloric acid. The sample was homogenized, and the homogenate was centrifuged (Continent 512R, Hanil, Daejeon, Korea) at 3,500×g for 20 min. Then, the pH was adjusted to 7.0 using a KOH solution, and the supernatant was centrifuged once again under the same conditions. Each supernatant was filtered (Whatman No. 1) and lyophilized (Lyoph-Pride, LP03; Ilshin BioBase, Dongducheon, Korea).

Additionally, before NMR analysis, 20 mM phosphate buffer (pH 7.4) was used with D_2O containing 1 mM 3-(trimethylsilyl) propionic-2,2,3,3-d4 acid (TSP) to dilute the lyophilized sample. NMR analysis was performed using a JEOL 600 MHz NMR spectrometer. Next, spectral analysis was conducted using Chenomx NMR suit V8. 6.

Statistical analysis

Experimental data of each treatment were calculated with the Minitab 19 version (Minitab, LLC, State College, Pennsylvania, USA). The results presented “regardless of age” indicate the main effects averaged across both age groups, not interaction effects. A significance between mean values was performed using Tukey’s mean comparison test using one-way ANOVA and independent t-test with the confidence level of $p<0.05$. Partial least squares-discriminant analysis (PLS-DA) and Variable importance in projection (VIP) score were performed using MetaboAnalyst 6.0 (https://www.metaboanalyst.ca/).

Results and Discussion

Composition of fatty acids

Few studies have been published on the quality of Hanwoo beef meat of different age steers with different genetic potentials for higher growth and better meat quality. To the best of our knowledge, we are the first to investigate the composition of muscles, by collecting samples from live animals through surgery. In this study, the richest 14 fatty acids available in beef were identified and shown in Table 2. Oleic acid, Palmitic acid, stearic acid, and linoleic acid are the most abundant fatty acids with the availability of 30.38%-36.22%, 18.8%-21.76%, 10.37%-11.43%, and 8.87%-11.92% respectively. About 75% of fatty acids in these treatments are composed of the above-mentioned fatty acids. The remaining 25% is distributed among other fatty acids. But, as per the results presented by Abbas et al. and Hossain et al., more than 80% of the fatty acid profile comprised the above fatty acids, excluding linoleic acid, in Hanwoo aged between 24-28 months in their study [27, 28]. These changes may be due to the composition of animal feed and fatty acid metabolism at Hanwoo's young age. Conjugated linoleic acid (CLA) is produced mostly by rumen microorganisms using linoleic acid as a precursor. However, the Delta-9 desaturase enzyme can produce various isomers of CLA through linoleic acid [29].

Oleic acid is the most common monounsaturated fatty acid (MUFA) available in beef. According to Hwang and Joo [30], adult Hanwoo' s various primal cuts with marbling had oleic acids ranging from 41.89% to 48.38%. and a study conducted by [31], the oleic acid percentage was 53.27% in 28-month-old Hanwoo steers. However, in the present experiment, the oleic acid of the young Hanwoo muscle is lower than that of the adult Hanwoo, and it is obvious that an increase in oleic acid with the age of slaughter. According to the health perspective of oleic acid, high oleic acid consumption has been linked to improvements in cardiovascular risk factors such as high blood pressure [32]. But, Perdomo et al. showed that oleate has a protective effect against cardiovascular insulin resistance [33]. Palmitic acid (18.8-21.76%) is the second richest fatty acid in this study. Oleic and palmitic acids are abundant in meat because they are the primary products of lipid metabolism. Oleic acid is produced by desaturation of stearic acid via Stearoyl-CoA Desaturase (SCD) activity, whereas palmitic acid is created de novo by fatty acid synthase [34]. Their abundance reflects their critical roles in energy storage and membrane construction in muscle tissue.

Stearic acid (10.37-11.43%) and Linoleic acid (8.87-11.92%) ranked third and fourth. However, no differences (p>0.05) were observed in the most abounded fatty acids against their treatments. The literature shows palmitic acid 26.22±0.66%

[35], stearic acid 15.69% [30], linoleic acid 5.01% [36] in samples of 28-30 months old Hanwoo meat. Those values are comparatively higher than our readings. Aging affects the body's composition and energy metabolism. When animals reach their final body weight, metabolism tends to slow down, leading to the accumulation of fatty acids within the muscles. On the other hand, energy intake as feed accumulates as glycogen and fat intramuscularly and subcutaneously. Similarly, no marbling or fat distribution was observed in our muscle samples for this investigation. Linoleic acid plays a great role in growing animals. In their investigation, several scientists noted the significance of these particular fatty acids. Lack of a source of linoleic acid in the diet of young animals results in suboptimal growth rates [37]. In particular, it serves as an energy source, and structural component of membrane phospholipids [38].

According to the statistical analysis, only elaidic acid, linolenic acid, and gondoic acid show significant differences ($p<0.05$) between the tested age groups. But there are no significant differences observed between the genotype parameters within the same age group (table 2a). At the same time, the effect of genetic traits regardless of age (table 2b) has shown that oleic acid, linoleic acid, dihomo-γ-linolenic acid, and eicosapentaenoic acid are significantly different among each growth and quality group. The growth-related group had greater amounts of linoleic acid, dihomo-γ-linolenic acid, and eicosapentaenoic acid, whereas the quality-related group had higher levels of oleic acid. The composition of animal fatty acids depends slightly on the genotype and entirely on diet at the early stage of age, which mainly affects the composition of fatty acids [39]. The quality-related genetic trait (Q) has been selectively bred for better marbling and meat flavor. These animals tend to have higher expression of stearoyl-CoA desaturase (SCD1), an enzyme that converts stearic acid (C18:0) to oleic acid (C18:1) [34]. The growth-related line (G) prioritizes muscle growth and lean tissue accretion rather than intramuscular fat deposition. These animals have higher rates of phospholipid synthesis in lean muscle membranes [40], which are rich in polyunsaturated fatty acids (PUFAs) like linoleic acid. Dihomo-γ-linolenic acid is derived from linoleic acid through elongation and desaturation [41]. The higher linoleic acid content in G animals provides more substrate for dihomo-γ-linolenic acid synthesis. EPA is an omega-3 PUFA involved in energy metabolism and membrane function. Growth-related line animals, due to faster growth rates and higher oxidative muscle metabolism, often maintain more PUFAs like EPA to stabilize cell membranes and support higher mitochondrial activity [42]. Because linoleic, dihomo-γ-linolenic acid, and EPA are all PUFAs, their higher levels in the G group increase total PUFA content. Growth-oriented animals accumulate less intramuscular fat and more structural phospholipids, where PUFAs are concentrated. Conversely, quality-line animals have more adipocyte deposition dominated by MUFA (especially oleic acid). This may be the reason why only four fatty acids show significant differences between the genotype group and no significant differences between growth and quality traits within the same age group.

Table 2b. show the effect of genetic trait and effect of age on fatty acid composition separately. Elaidic acid was found in higher concentrations in 10 mo group compared to 13 mo group. However, in comparison with other studies conducted with older Hanwoo steers, our values are lower than theirs [28]. Elaidic acid is a monounsaturated trans fatty acid and it is identified as a trans isomer of oleic acid. Elaidic acid can increase the bad cholesterol in blood serum compared to its cis-isomer, oleic acid [43]. Linolenic acid (18:3) is lowest in 10 mo group (p<0.05), whereas the highest is in 13 mo groups (p<0.05). Gondoic acid shows significantly lower values in 10 mo, while it shows the highest values in 13 mo group. During the rapid growth stage of young animals, changes in rumen microflora and energy metabolism are somewhat complex [44]. Gondoic acid-like fatty acids can be presented to energy metabolism when needed. This will be the reason that gondoic acid expresses a lower concentration in the 10 mo group.

The fatty acid compositions of these four treatments were compared using a partial least squares discriminant analysis (PLS-DA) to check if there were any overall differences (Accuracy = 0.393, R^2 = 0.588, Q^2 = 0.406). The differences between the four treatments of Hanwoo steers with Component 1 account for 92.2% of the total variance and primarily separated animals by age with 10 mo groups positioned on the negative side and 13-month groups toward the positive side of the axis (Fig. 1a). Furthermore, the variable importance injection score showed MUFA, n-6, oleic, PUFA, palmitic, arachidonic, linoleic, n-6/n-3 and SFA reflecting the importance of the variables in PLS-DA for treatment discrimination (Fig. 1b).

Fig. 2a-d shows nutritional quality indices of identified fatty acids. The lowest n-6/n-3 (11.98) was found in 13Q and the highest (14.27) in 10G (p<0.05) (fig.2a). Recommended ratio for n-6/n-3 should be less than 4, as this is related with an increased risk of heart attack and thrombosis [45]. However, in this study, higher values were observed for n-6/n-3. 13Q showed the lowest n-6 (15.28) while showing the highest value (21.95) in 10G (p>0.05) (fig.2b). n-3 (fig.2c) and P/S (fig.2d) both indicate the highest value (1.46,0.74) in 10G and the lowest (0.74,0.48) in 10MQ (p>0.05). n-3 fatty acids are beneficial for human health because of their benefits related to coronary heart diseases, brain development, cancers, and other diseases such as rheumatoid arthritis and inflammatory bowel disease [46]. However, many studies mentioned the importance of maintaining the ratio of n-6/n-3.

Fig.3-5 shows atherogenicity (AI) indices, thrombogenicity (TI), and hypocholesterolemic/hypercholesterolemic (HH) index accordingly. Within the four treatments of Hanwoo steers, no significant differences (p>0.05) were observed in AI and HH, while results varied between 0.45 to 1.50 and 2.21 to 2.60, respectively. The ranges of TI were from 0.14 to 1.06. Observed TI values were higher (p<0.05) in the 13G and 13Q. Ruminant animals become more capable of biohydrogenation as they develop. Production of saturated fatty acids like myristic acid, palmitic acid, and stearic acid at elevated levels in the process of biohydrogenation may be the reason for the higher TI values in the 13 mo group. AI and TI indicate the stimulus potential of platelet aggregation. It provides ideas about the nutritional and health values of some fatty acid classes concerning CVD [47]. The lower the AI and TI, the greater the amount of antiatherogenic fatty acid in meat. Therefore, the higher platelet aggregation leads to the prevention of coronary heart disease [48].

Table 3a-b shows the findings of the NMR analysis, which was conducted for four treatments of Hanwoo muscles. Twenty-one metabolite compounds were quantified under five main categories (amino acids, bioactive compounds, energy metabolism-related compounds, nucleotide-related compounds, and other compounds). Alanine, glutamate, isoleucine, leucine, phenylalanine, valine, N, N-dimethyl glycine, and carnitine were significantly higher (p<0.05) in the 10 mo group. On the other hand, all explored free amino acids except betaine, glycine, methionine, and tyrosine have been shown to have higher levels in the 10 mo group (p<0.05). Free amino acids are essential for protein synthesis. Beyond their structural role, free amino acids are essential for several metabolic processes in animal bodies as well [49]. This might be the reason for elevated levels of free amino acids in 10 mo group.

The score plot visually represents how well the PLS-DA model (accuracy=0.705, R^2=0.762, Q^2 = 0.6) distinguishes between the four groups (Fig. 6a). The 10 and 13 mo Hanwoo steers are distinctly separated along Component 1, which explains 54.5% of the variation. The 13-month animals (13G and 13Q) were tightly clustered on the positive side of the axis, whereas the 10-month animals (10G and 10Q) were positioned on the negative side. The VIP (Variable Importance in Projection) score plot identifies the most important metabolites for distinguishing between the two age groups based on their VIP scores (Fig. 6b). Betaine, carnosine, creatine, isoleucine, anserine, inosine, and IMP have the highest VIP scores, indicating they are the most significant in differentiating between 10 and 13 mo Hanwoo steer.

Glutamate is one of the amino acids that acts as a main precursor for the umami taste in meat. However, within animal bodies, it provides energy by involving the tricarboxylic acid and purine nucleotide cycles [50]. It participates in many physiological activities, such as protein synthesis [51]. Transamination of branched-chain amino acids, protein decomposition, and intake are the main paths that provide glutamate to skeletal muscles [50]. Similarly, Isoleucine increases muscle mass through fat accumulation inside myocellular and myogenesis. [52] as well as it is also compulsory for blood sugar and energy regulation and hemoglobin formation [53]. Leucine and valine are identified as important for tissue regeneration. Isoleucine, leucine, and valine, which were relatively abundant in younger muscles, provide carbon skeletons for acetyl-CoA and succinyl-CoA production, feeding into both the TCA and fatty acid synthesis pathways [54].

Phenylalanine is an essential amino acid in the fragrance of cattle and performs many functions in the body. In the kidneys and liver, it can be transformed into tyrosine [55]. The synthesis of thyroxine and triiodothyronine, which control metabolism, growth, and energy levels, needs tyrosine. According to the bioactive compounds we identified, the 10 mo group had greater levels of carnosine and anserine. Carnosine levels, however, are not significant ($p>0.05$). As antioxidants, anserine and carnosine help to lower the oxidative damage brought on by increased metabolic activity. The 10 mo group had significantly greater carnosine and anserine concentrations, indicating antioxidant protection against reactive oxygen species produced by high metabolic and oxidative activity [56], particularly in PUFA-rich tissues prone to lipid peroxidation.

Carnitine is also a compound that is involved in energy metabolism. One example is beta-oxidation, in which long-chain fatty acids are oxidized in mitochondria [57]. It was found in higher ($p<0.05$) concentrations in the 10 mo group. In this study, creatine, lactate, and succinate were quantified as directly energy metabolism-related compounds; among them, creatine and succinate only showed significant differences. The highest creatine content ($p<0.05$) and succinate ($p>0.05$) were observed in 10Q. In this study, the Q group had higher amounts of oleic acid (C18:1) and monounsaturated fatty acid (MUFA), as well as creatine and succinate. These molecules are strongly linked to energy buffering and mitochondrial activity [58], implying that Q-line animals may have a more efficient energy cycle in muscle tissues, which promotes lipid deposition and marbling. Hypoxanthine ($p<0.05$) and IMP ($p>0.05$) were lower in 10 mo group. But not significantly different from G and Q. Hypoxanthine is one of the most important metabolites in the nucleotide salvage and purine metabolism pathways. It can be created when ATP is broken down when there is a strong need for energy [59].

These findings indicate a metabolic connection between fatty acid content and muscle metabolites. The Q trait exhibits metabolic adaptation that favors lipid storage and oleic acid synthesis, enhancing marbling and flavor potential, while the G trait's metabolism focuses on oxidative energy production, β-oxidation, and PUFA maintenance. The distinct physiological priorities of each genetic line are highlighted by these coordinated alterations in fatty acid and metabolite profiles: growth performance and metabolic activity in G, and energy efficiency and flavor quality in the Q group.

Conclusion

The presented results of fatty acid profile and metabolic compounds of 10 and 13-mo-old Hanwoo steers separated by genetic potential (growth and quality), the results of fatty acid showed significant differences in elaidic, linolenic, and gondoic acids. However, no significant differences were observed between the growth and quality traits within each age group. The majority of the metabolomic substances that were investigated showed distinct age-group separation. Differences were caused by betaine, carnosine, creatine, isoleucine, anserine, inosine, and IMP according to PLS-DA. Both fatty acid and metabolites data collected during this study showed that the free amino acids related to protein synthesis and compounds related to energy metabolism were observed in higher concentrations in the 10-month-old group. However, a significantly clear demarcation was not observed between the genetic traits of each age group. Only four fatty acids showed significant differences between the genetic trait groups regardless of age. This may be because animals need more time to express their genetic traits related to growth and meat. Finally, it can be concluded that the concentration of water-soluble metabolites and the composition of fatty acids in young Hanwoo steers in the late growth stage are more influenced by the age of the steers than by their genetic characteristics. After slaughtering these Hanwoo cattle, additional data will be collected and compared with the current study for a better understanding of the influence of genetic traits on fatty acids and metabolite compounds in Hanwoo cattle.

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Tables and Figures

Table 1a. **Ingredients of the ration that all groups were fed.**

Ingredients	As Fed (%)	% of DM
Corn	11.00	12.45
Corn flakes	29.00	32.83
Wheat	10.15	11.49
Corn gluten feed, imported	20.00	22.64
Wheat bran, domestic	5.00	5.66
Rice bran	6.00	6.79
Molasses	4.50	5.09
Limestone, powdered	2.50	2.83
Salt	0.55	0.62
Palm kernel meal	10.00	11.32
Mineral premix	0.10	0.06
Vitamin premix	0.10	0.11
Feed additives	1.00	1.13

Table 1b. **Chemical composition of the ration**

Chemical composition, DM basis	Forage (%)	Concentrates (%)
Dry matter	93.30	88.33
Crude protein	4.35	16.76
Crude fat	1.46	4.23
Crude fiber	41.71	8.15
Crude ash	5.53	6.86
Ca	0.20	1.14
P	0.08	0.52
ADF	44.82	13.21
NDF	74.92	29.71

Table 2a. **Composition of fatty acid profile of Hanwoo steers at the late-growth phase with genetic effect.**

Fatty Acid		**Age × Genetic trait**				
		10G	**10Q**	**13G**	**13Q**	***P*-Value**
Capric	10.0	0.00	0.01	0.00	0.00	0.526
Lauric	12.0	0.01	0.03	0.02	0.01	0.434
Myristic	14.0	1.76	2.00	1.52	1.78	0.576
Palmitic	16.0	18.80	20.66	20.86	21.76	0.080
Palmitoleic	16.1	2.81	3.07	3.13	3.71	0.215
Stearic	18.0	10.71	11.43	10.95	10.37	0.172
Elaidic	18.1T	0.34[a]	0.29[ab]	0.22[bc]	0.19[c]	**0.000**
Oleic	18.1	30.38	34.97	34.07	36.22	0.081
Linoleic	18.2	11.92	9.09	10.33	8.87	0.100
Linolenic	18.3	0.36[b]	0.45[ab]	0.48[a]	0.48[a]	**0.014**
Godonic	20:1	0.06[bc]	0.02[c]	0.22[ab]	0.27[a]	**0.001**
Dihomo-γ-Linolenic	20:3	1.53	1.07	1.37	1.17	0.111
Arachidonic	20:4	8.13	5.56	5.93	5.34	0.104
Eicosapentaenoic	20:5	1.10	0.81	0.94	0.79	0.143
SFA		31.28	34.13	33.35	33.94	0.133
UFA		56.64	55.32	56.69	56.94	0.142
MUFA		34.01	39.14	38.36	40.91	0.099
PUFA		22.01	16.18	18.34	16.03	0.103

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[a-c] Different letters within the same row differ significantly (p < 0.05).

10G,10 months old group having growth related genetic trait; 10Q, 10 months old group having quality related genetic trait; 13G, 13 months old group having growth related genetic trait; 13Q, 13 months old group having quality related genetic trait; G, growth trait regardless of age; Q, quality trait regardless of age

SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, mono-unsaturated fatty acids; PU FA, poly-unsaturated fatty acid

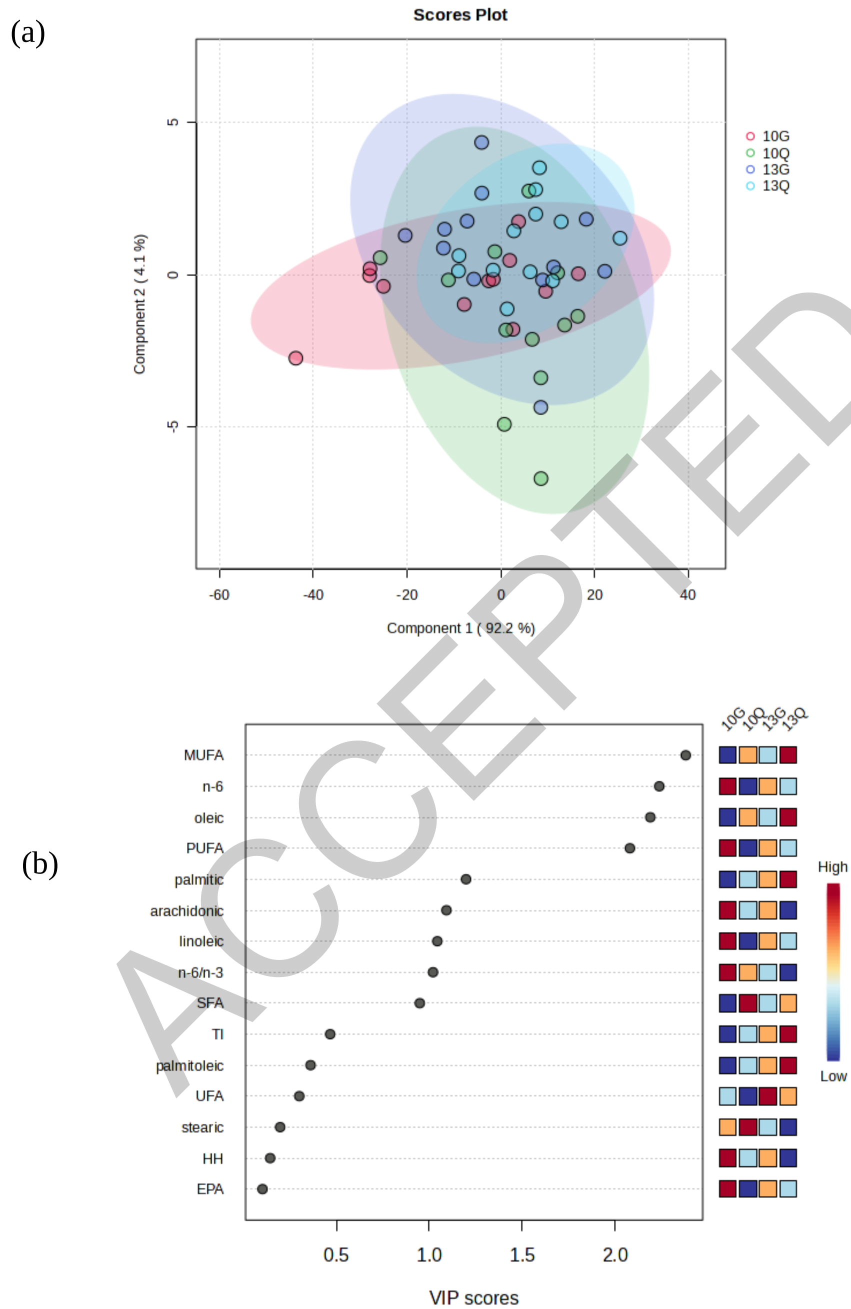
Table 2b. **Effect of genetic trait and effect of age at late-growth phase on fatty acid composition of Hanwoo.**

Fatty Acid		**Effect of genetic traits, regardless of age**			**Effect of age, regardless of genetic trait**		
		G	**Q**	***P*-Value**	**10 mo**	**13 mo**	***P*-Value**
Capric	10.0	0.00	0.01	0.297	0.00	0.00	0.909
Lauric	12.0	0.02	0.02	0.451	0.02	0.02	0.826
Myristic	14.0	1.64	1.89	0.294	1.88	1.65	0.343
Palmitic	16.0	19.83	21.21	0.101	19.73	21.31	0.058
Palmitoleic	16.1	2.97	3.39	0.178	2.94	3.42	0.124
Stearic	18.0	10.83	10.90	0.837	11.07	10.66	0.235
Elaidic	18.1T	0.28	0.24	0.252	0.32[x]	0.20[y]	**0.000**
Oleic	18.1	32.22[b]	35.59[a]	**0.046**	32.67	35.14	0.146
Linoleic	18.2	11.13[a]	8.98[b]	**0.027**	10.51	9.60	0.361
Linolenic	18.3	0.42	0.47	0.179	0.41[y]	0.48[x]	**0.011**
Godonic	20:1	0.14	0.15	0.932	0.04[y]	0.24[x]	**0.000**
Dihomo-γ-Linolenic	20:3	1.46[a]	1.12[b]	**0.023**	1.30	1.27	0.852
Arachidonic	20:4	7.03	5.40	0.077	6.84	5.58	0.174
Eicosapentaenoic	20:5	1.02[a]	0.80[b]	**0.037**	0.95	0.87	0.421
SFA		32.31	34.04	0.074	32.70	33.65	0.334
UFA		56.66	56.13	0.330	55.98	56.82	0.124
MUFA		36.49	40.02	0.055	36.88	39.63	0.137
PUFA		20.17[a]	16.10[b]	**0.037**	19.10	17.18	0.344

[a-b] Different letters within the same row differ significantly ($p < 0.05$).
[x-y] Different letters within the same row differ significantly ($p < 0.05$).

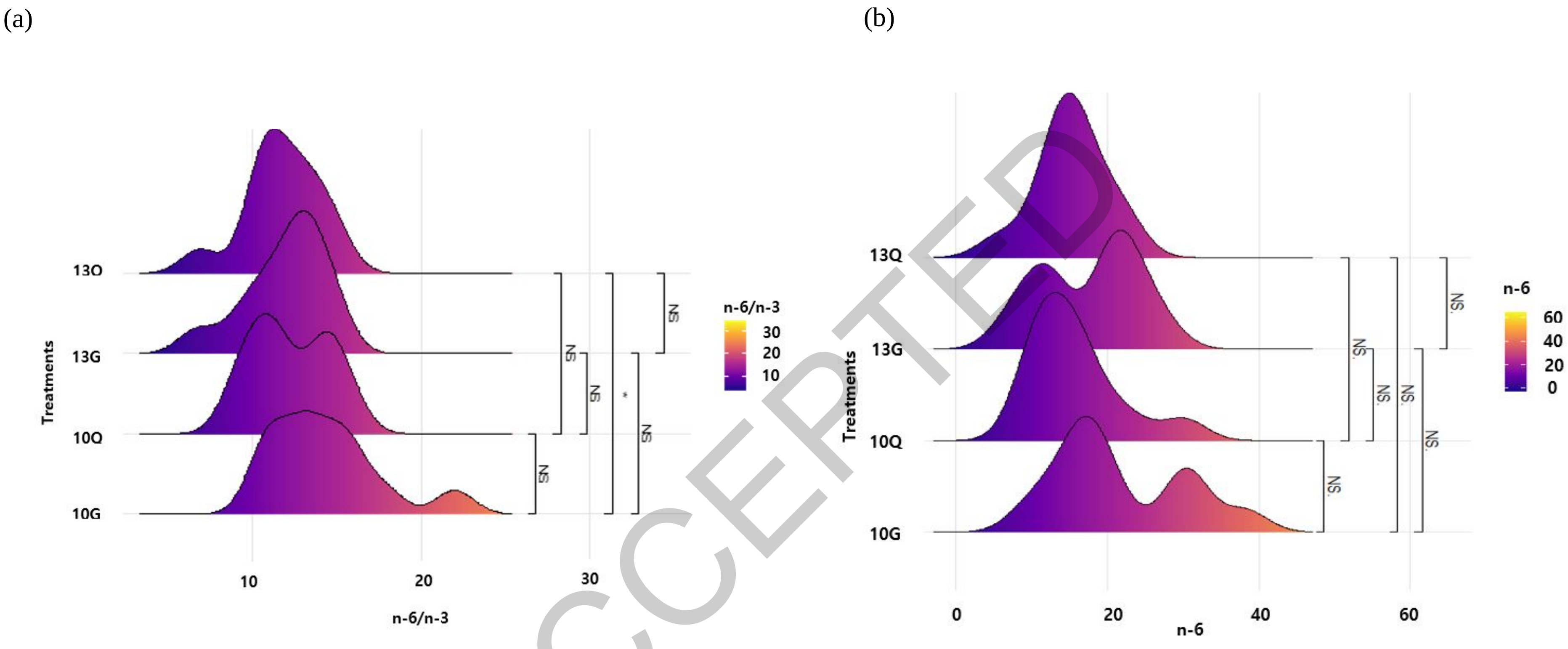
G, growth trait related genetic factor; Q, quality trait related genetic factor.

Fig. 1 **Results of (a) PLS-DA and (b) VIP score of fatty acids composition of 10-month and 13-month-old**

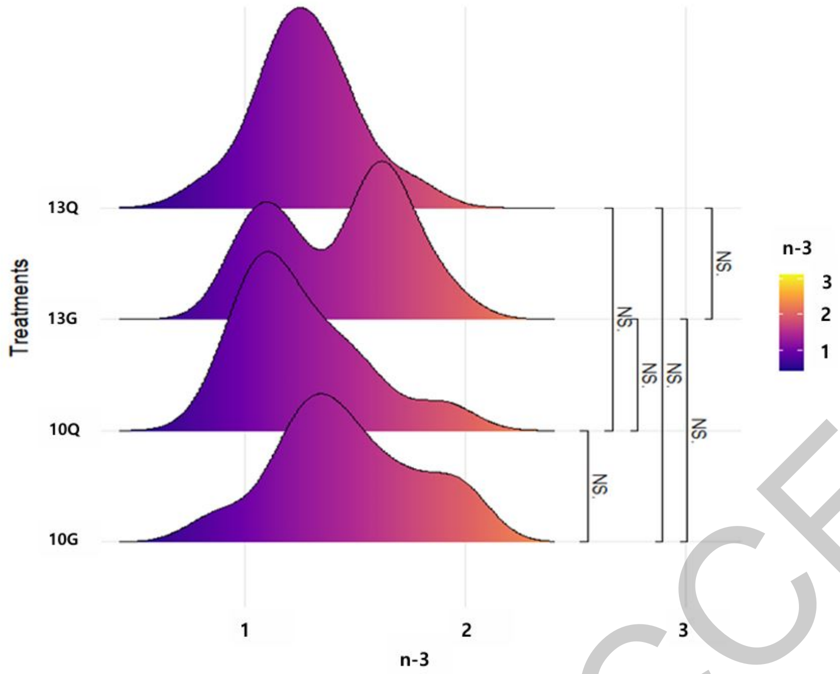


10G,10 months old group having growth-related genetic trait; 10Q, 10 months old group having quality-related genetic trait; 13G, 13 months old group having growth-related genetic trait; 13Q, 13 months old group having quality-related genetic trait.

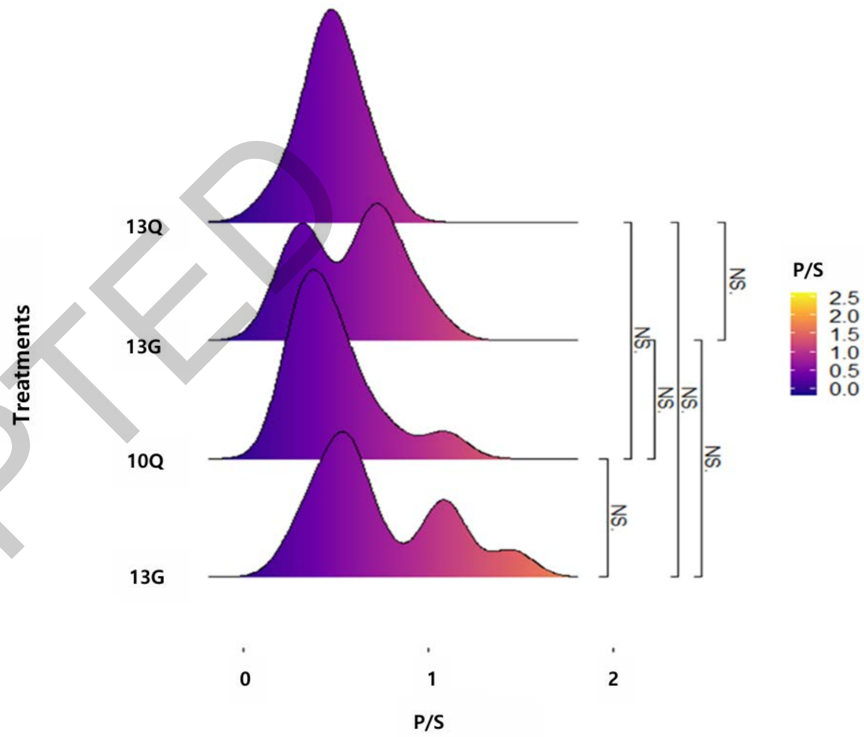
Fig. 2 **Ridgeline plots showing the results of nutritional quality indices of Hanwoo muscle.**



(c)



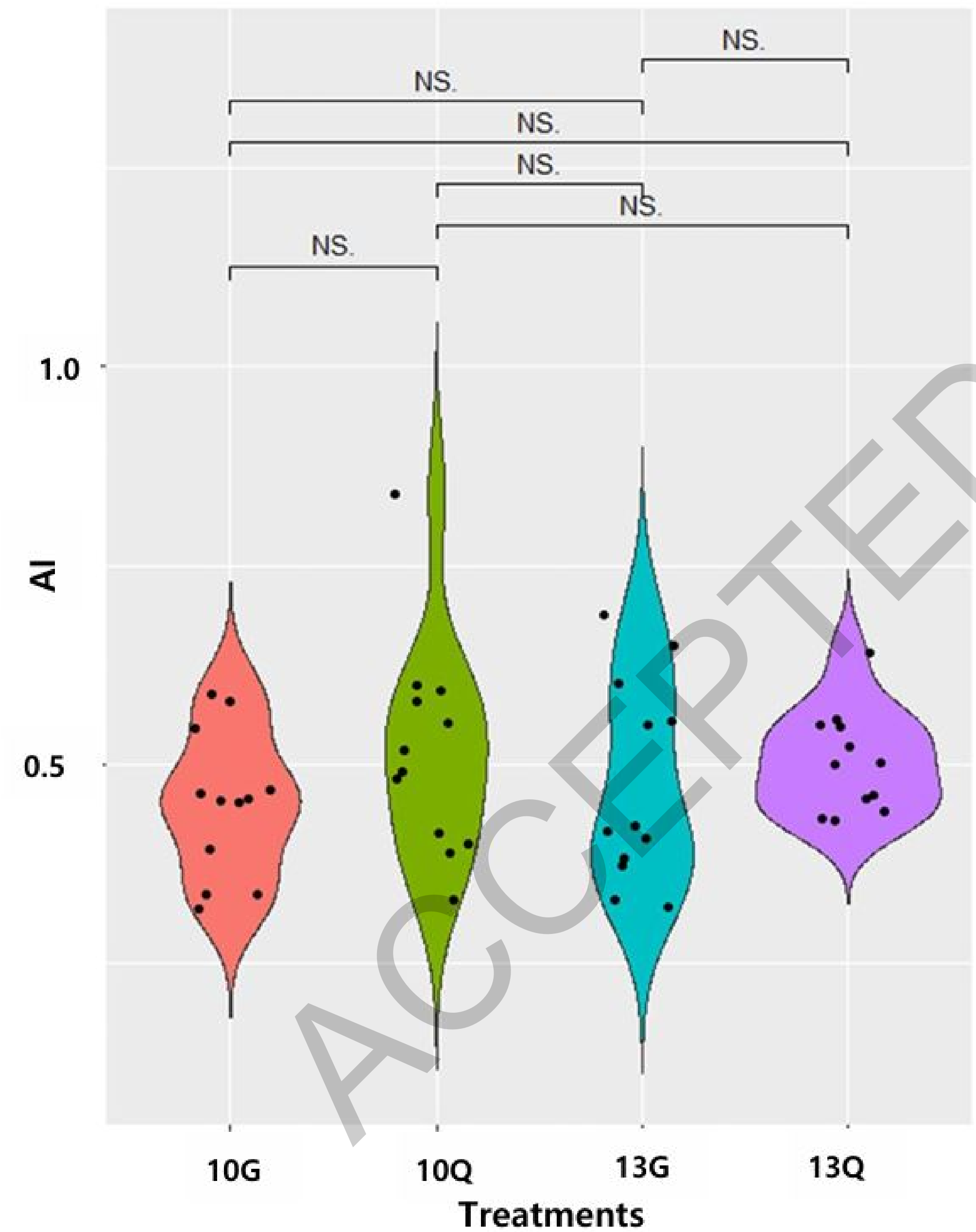
(d)



(a) n-6 to n-3 fatty acid ratio, (b) n-6, (c) n-3, (d) polyunsaturated to saturated fatty acid ratio

NS, significantly not different ($p > 0.05$); ***, significantly different ($p < 0.05$); 10G,10 months old group having growth-related genetic trait; 10Q, 10 months old group having quality-related genetic trait; 13G, 13 months old group having growth-related genetic trait; 13Q, 13 months old group having quality-related genetic trait.

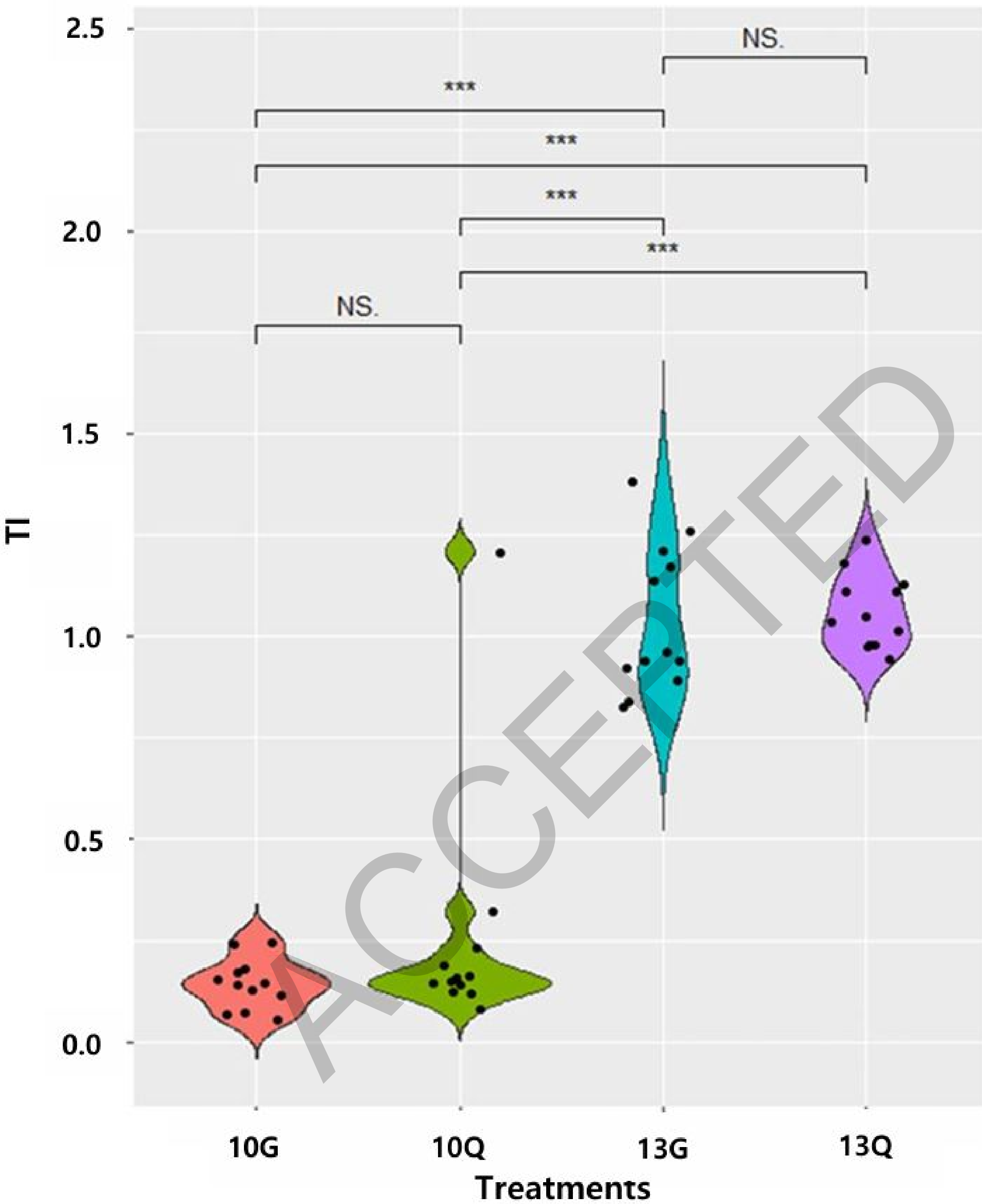
Fig. 3 **Atherogenicity (AI) indices of each treatment**



NS, significantly not different (p > 0.05)

10G,10 months old group having growth-related genetic trait; 10Q, 10 months old group having quality-related genetic trait; 13G, 13 months old group having growth-related genetic trait; 13Q, 13 months old group having quality-related genetic trait.

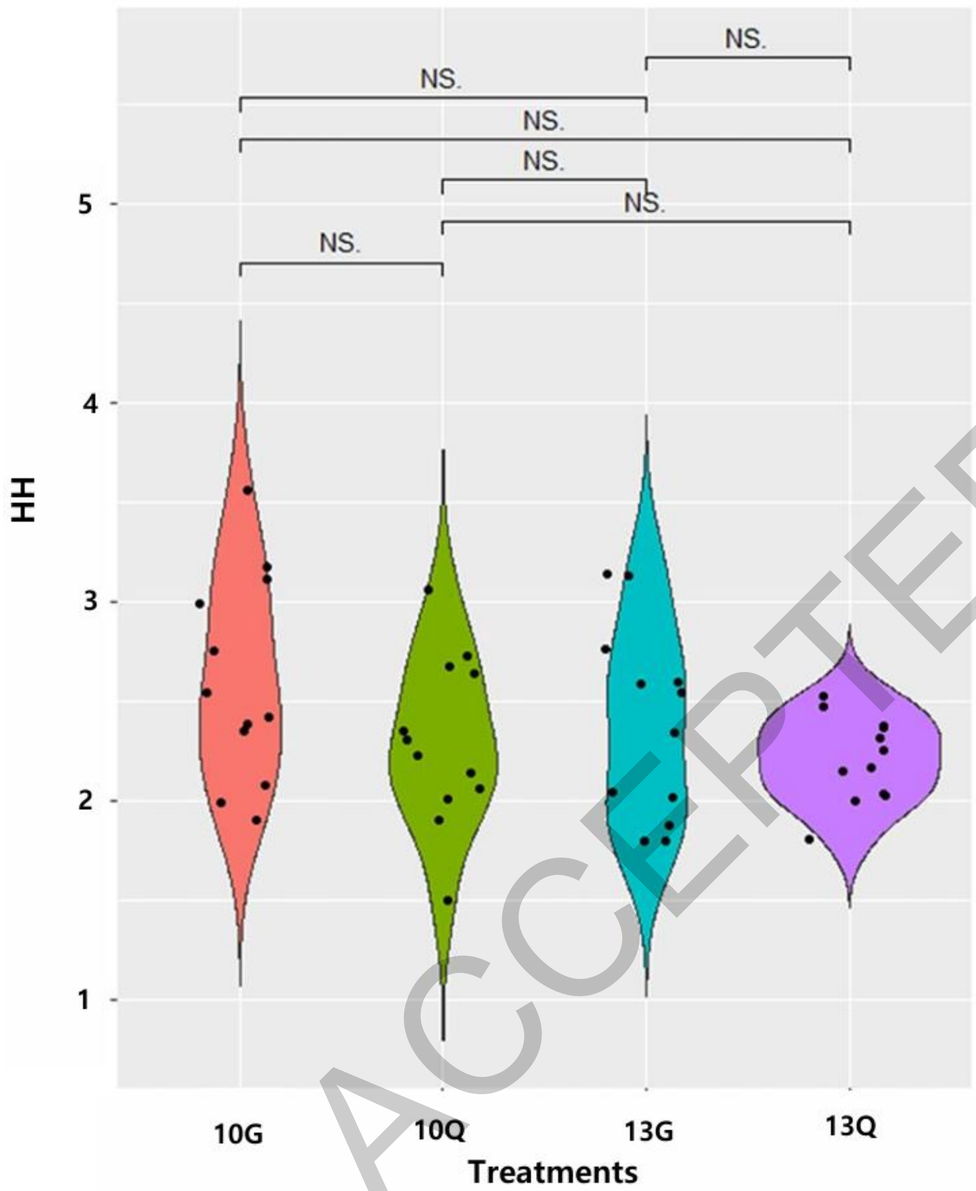
Fig. 4 **Thrombogenicity (TI) indices of each treatment**



NS, significantly not different (p > 0.05); ***, significantly different (p < 0.05)

10G,10 months old group having growth-related genetic trait; 10Q, 10 months old group having quality-related genetic trait; 13G, 13 months old group having growth-related genetic trait; 13Q, 13 months old group having quality-related genetic trait.

Fig. 5. **Hypocholesterolemic/hypercholesterolemic (HH) indices of each treatment**



NS, significantly not different (p > 0.05)

10G,10 months old group having growth-related genetic trait; 10Q, 10 months old group having quality-related genetic trait; 13G, 13 months old group having growth-related genetic trait; 13Q, 13 months old group having quality-related genetic trait.

Table 3a. Composition of metabolite compounds of Hanwoo steers at the late-growth phase with genetic effect**.**

	Age × Genetic trait				
Amino acids	**10G**	**10Q**	**13G**	**13Q**	***P*-Value**
Alanine	0.41^{a}	0.47^{a}	0.35^{b}	0.34^{b}	**0.023**
Betaine	5.61^{b}	5.57^{b}	12.79^{a}	13.59^{a}	**0.000**
Glutamate	2.98^{a}	2.88^{a}	1.44^{b}	1.43^{b}	**0.000**
Glycine	0.24	0.36	0.31	0.32	0.168
Isoleucine	3.88^{a}	4.26^{a}	0.03^{b}	0.03^{b}	**0.000**
Leucine	0.14^{a}	0.16^{a}	0.04^{b}	0.04^{b}	**0.000**
Methionine	0.34^{ab}	0.44^{a}	0.20^{b}	0.22^{ab}	**0.021**
Phenylalanine	0.10^{a}	0.12^{a}	0.03^{b}	0.04^{b}	**0.000**
Tyrosine	18.59	24.18	20.47	20.73	0.076
Valine	1.18^{a}	1.44^{a}	0.03^{b}	0.03^{b}	**0.000**
N, N-Dimethylglycine	0.42^{a}	0.58^{a}	0.02^{b}	0.02^{b}	**0.000**
Bioactive compounds					
Anserine	36.67	34.25	32.43	32.53	0.610
Carnitine	3.26^{a}	3.74^{a}	1.97^{b}	2.08^{b}	**0.000**
Carnosine	30.67^{ab}	32.15^{a}	22.13^{b}	24.81^{ab}	**0.008**
Energy metabolism-related compounds					
Creatine	15.77^{b}	22.03^{a}	11.23^{b}	11.69^{b}	**0.000**
Lactate	17.14	18.81	15.92	18.50	0.247
Succinate	0.10^{ab}	0.12^{a}	0.09^{b}	0.08^{b}	**0.009**
Nucleotide-related compounds					
Hypoxanthine	0.38^{b}	0.42^{b}	1.15^{a}	1.21^{a}	**0.000**
Inosine mono phosphate (IMP)	4.52^{b}	4.39^{b}	6.42^{ab}	8.31^{a}	**0.000**
Inosine	27.88	34.11	26.02	25.84	0.054
Other					
Acetate	0.13^{ab}	0.17^{a}	0.11^{b}	0.12^{ab}	**0.020**

[a-b] Means with a column with different letters are significantly different ($p < 0.05$).

10G,10 months old group having growth related genetic trait; 10Q, 10 months old group having quality related genetic trait; 13G, 13 months old group having growth related genetic trait; 13Q, 13 months old group having quality related genetic trait.

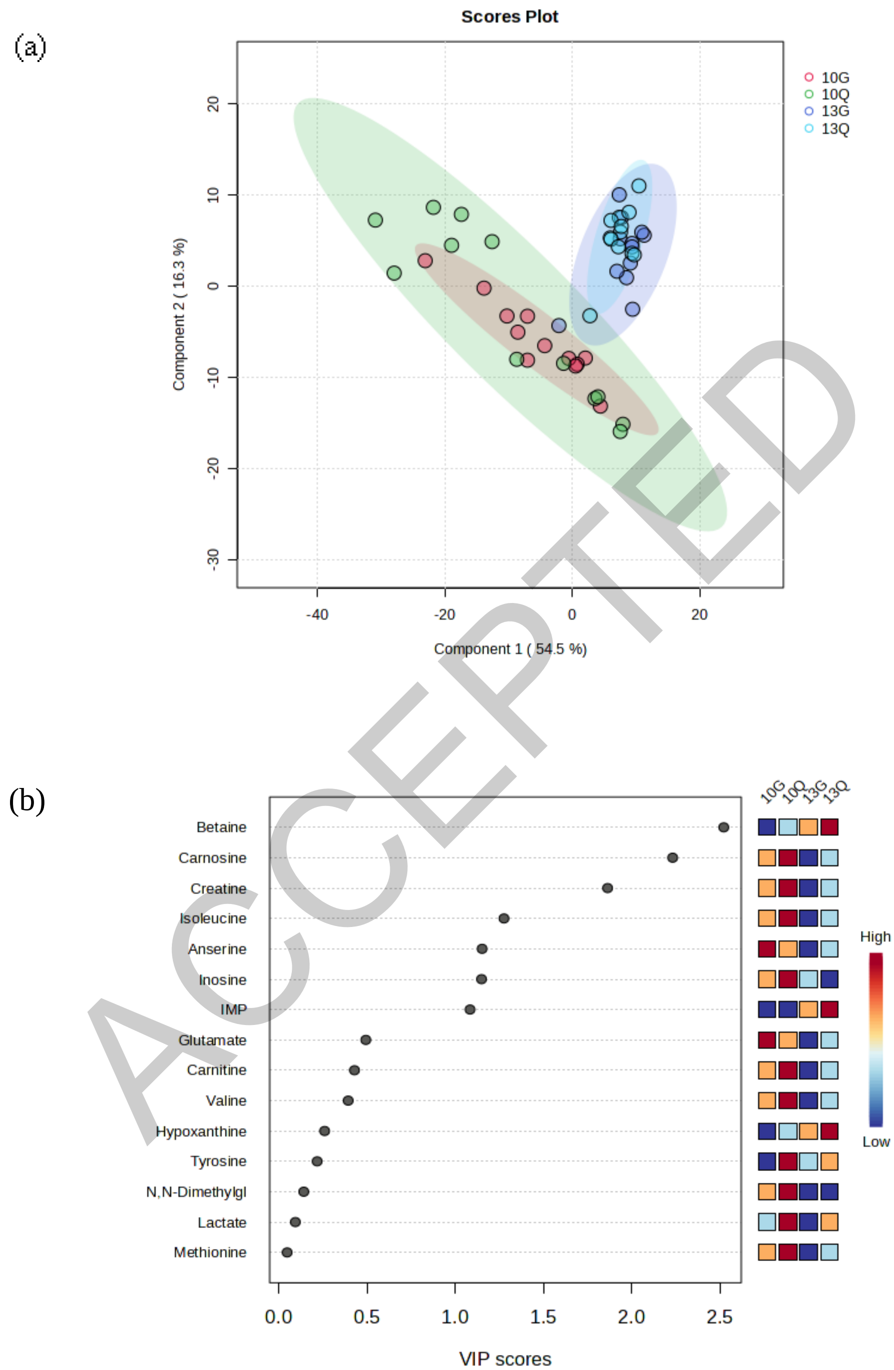
Table 3b. Effect of genetic trait and effect of age at late-growth phase on metabolite compound composition of Hanwoo.

	Effect of genetic traits regardless of age			Effect of age regardless of genetic trait		
Amino acids	**G**	**Q**	***P*-Value**	**10 mo**	**13 mo**	***P*-Value**
Alanine	0.37	0.40	0.312	0.43^{x}	0.35^{y}	**0.011**
Betaine	9.20	9.58	0.772	5.59^{y}	13.19^{x}	**0.000**
Glutamate	2.21	2.16	0.872	2.93^{x}	1.43^{y}	**0.000**
Glycine	0.27	0.33	0.098	0.30	0.31	0.651
Isoleucine	1.96	2.15	0.769	4.07^{x}	0.03^{y}	**0.000**
Leucine	0.09	0.10	0.658	0.15^{x}	0.04^{y}	**0.000**
Methionine	0.27	0.33	0.366	0.39^{x}	0.21^{y}	**0.004**
Phenylalanine	0.06	0.08	0.395	0.11^{x}	0.03^{y}	**0.000**
Tyrosine	19.53	22.45	0.061	21.39	20.60	0.617
Valine	0.60	0.74	0.535	0.31^{x}	0.03^{y}	**0.000**
N, N-Dimethylglycine	0.22	0.30	0.345	0.50^{x}	0.02^{y}	**0.000**
Bioactive compounds						
Anserine	34.55	33.40	0.648	35.46	32.48	0.238
Carnitine	2.62	2.91	0.336	3.50^{x}	2.03^{y}	**0.000**
Carnosine	26.40	28.48	0.410	31.41^{x}	23.47^{y}	**0.001**
Energy metabolism-related compounds						
Creatine	13.50	16.86	0.083	18.90^{x}	11.46^{y}	**0.000**
Lactate	16.53	18.65	0.059	17.98	17.21	0.500
Succinate	0.09	0.10	0.392	0.11^{x}	0.08^{y}	**0.002**
Nucleotide-related compounds						
Hypoxanthine	0.77	0.81	0.722	0.40^{y}	1.18^{x}	**0.000**
Inosine mono phosphate (IMP)	5.47	6.35	0.250	4.45^{y}	7.36^{x}	**0.000**
Inosine	26.95	30.00	0.225	31.00^{x}	25.93^{y}	**0.038**
Other						
Acetate	0.12	0.15	0.066	0.15^{x}	0.12^{y}	**0.019**

a-b Different letters within the same row differ significantly ($p < 0.05$).
x-y Different letters within the same row differ significantly ($p < 0.05$).

G, growth trait related genetic factor; Q, quality trait related genetic factor.

Fig. 6 **Results of (a) PLS-DA and (b) VIP score of metabolite compounds between 10 and 13 months of Hanwoo steers**



10G,10 months old group having growth-related genetic trait; 10Q, 10 months old group having quality-related genetic trait; 13G, 13 months old group having growth-related genetic trait; 13Q, 13 months old group having quality-related genetic trait.