A Dissertation

for the Degree of Doctor of Philosophy

Studies on factors affecting beef taste and the effects of dietary glycerin on growth performance and carcass characteristics in Korean cattle steers

한우 고기의 맛 조절 물질 탐색 및 사료 내 글리세린 첨가가 거세한우의 성장 및 도체특성에 미치는 영향 연구

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By

Min Yu Piao

Department of Agricultural Biotechnology

Graduate School

Seoul National University

농 학 박 사 학 위 논 문

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부위원장 _____ (인)

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Summary

Beef marbling score and quality grade positively affect meat sensory characteristics, including tenderness, juiciness, flavor, and overall palatability. Korean cattle beef is well known for good palatability because of the high quality grade and thus high marbling. Limited information is available and variability exists in data on the associations among marbling score/quality grade, meat characteristics, and sensory traits in Korean cattle beef. Korean consumers prefer Korean cattle beef to domestic Holstein and imported beef because they believe that the palatability of Korean cattle beef is superior to that of other breeds. Limited information is available on the factors that affect the preference of Korean consumers for Korean cattle beef over other breeds. Two studies (study 1 and 2) were conducted to elucidate 1) the associations among quality grade/marbling and various carcass characteristics and sensory traits in Korean cattle beef, and 2) the factors that contribute to differences in the sensory traits of *longissimus thoracis* of different breeds.

Glycerol, a by-product with biodiesel, also known as glycerin, serves as gluconeogenic substrate in the liver and kidney, and it has an energy value similar to corn on a pound-for-pound basis in dairy and feedlot cattle. Although many studies were conducted for the effect of glycerin on carcass composition and characteristics in various animal diets, the outcomes were not consistent. Also, limited information is available for effects of glycerin supplementation on meat characteristics in Korean cattle steers. Two studies

(study 3 and 4) were conducted to identify 3) the effects of dietary glycerin replacement on growth performance, carcass characteristics and sensory traits in Korean cattle steers fed diets based on the similar TDN level between experimental diets, and 4) the effects of crude glycerin supplementation on growth performance and carcass characteristics in Korean cattle steers fed diets based on the different TDN level between experimental diets.

1. Comparison of carcass and sensory traits, fatty acid profiles and volatile compounds among quality grades in *longissimus dorsi* and *semimembranosus muscles* of Korean cattle steer

This study was performed to compare carcass traits, sensory characteristics, physicochemical composition, nucleotides and collagen content, free amino acids, content and composition of fatty acids (FA), and volatile compounds among four quality grades (QG1++, 1+, 1, and 2), and to understand their association with carcass characteristics in *longissimus dorsi* (loin) and *semimembranosus* (rump) cuts of Korean cattle steers. This study confirms that marbling score (MS) and intramuscular fat (IMF) content are major positive determinants of QG in Korean cattle beef. Numeric values of tenderness, juiciness, and overall acceptability in loin tended to be highest in QG1++, and those of juiciness and overall acceptability tended to be lowest in QG 2. Juiciness and overall acceptability were strongly correlated with QG. Our results demonstrated that QGs are linked to sensory traits. However, the

nucleotide contents including inosine monophosphate (IMP) may not be major factors determining meat palatability of Korean cattle beef in this study. Glutamic acid and proline were significantly associated with tenderness, juiciness, and overall acceptability, although they did not differ significantly among QGs. In addition, beef QGs affected the compositions and contents of FAs and volatile compounds in loin and rump. Loin FA percentages, especially those of oleic acid (C18:1n9) and monounsaturated FA (MUFA), generally increased with increasing QGs. Some volatile compounds in loin and rump varied with QGs and were positively or negatively correlated with flavor.

2. Comparison of reducing sugar content, sensory traits, and fatty acids and volatile compound profiles of *longissimus thoracis* among Korean cattle, Holsteins, and Angus steers

This study was performed to compare intramuscular fat (IMF) and reducing sugar contents, sensory traits, and fatty acid (FA) and volatile compound profiles in *longissimus thoracis* (LT) among Korean cattle (KC), Holstein (HO), and Angus (AN) steers. The IMF, reducing sugar content, and sensory traits of the LT varied among KC, HO, and AN steers. The KC LT had the highest IMF and reducing sugar contents, and the best sensory traits (flavor, tenderness, juiciness, and overall acceptance). The IMF and reducing sugar contents were positively correlated with all of the sensory traits, suggesting

that these factors may positively affect beef flavor. Palmitic acid (C16:0), oleic acid (C18:1n9), and monounsaturated FA (MUFA) may positively affect sensory traits, whereas linoleic acid (C18:2), and polyunsaturated FA (PUFA) may negatively affect sensory traits. The percentages of different volatile compounds in the LT also varied among the three breeds. The KC had the highest percentage of volatile compounds, including acetaldehyde, 3-methyl butanal, and 3-hydroxy-2-butanone, and these compounds were positively correlated with flavor. Our results demonstrated that variations in IMF, reducing sugar content, and FA and volatile compound profiles may contribute to differences in the sensory characteristics of the LT among breeds. The results of this study enhance our understanding of the association of reducing sugar and volatile compound contents with the sensory traits of beef. This information may help in determining beef palatability.

3. Effects of dietary glycerin replacement on growth performance and rumen and carcass characteristics in Korean cattle steers

The study was performed to evaluate the effect of 3% dietary glycerin replacement on growth performance, blood metabolites, ruminal fermentation characteristics, carcass characteristics and sensory traits, hepatic gluconeogenic gene expression, and muscle glycogen contents in Korean cattle steers. Glycerin replacement in the finishing diet of Korean cattle steers had no impact on weight gain, average daily gain, and feed efficiency except

for increase in average daily concentrate intake. This increased intake may be attributed to the sweet taste of glycerin's property. Glycerin replacement did not affect carcass characteristics, chemical and physico-chemical composition, reducing sugar, glycogen, collagen, nucleotides, fatty acid, volatile compounds, and sensory traits in the *longissimus thoracis*. These results indicate that the glycerin inclusion level (3%) may be not enough to improve animal performance and carcass characteristics. In addition, feeding concentrate containing 3% of glycerin did not result in detrimental effects on growth performance, ruminal fermentation, animals' physical condition, and metabolism. This is important not only on animal performance and carcass characteristics but also for sustainable and economic aspects because glycerin is a biodiesel residue and it can potentially partially replace some expensive ingredients such as corn, molasses, distiller's dried grains with solubles as an energy source for beef cattle.

4. Effect of dietary crude glycerin supplementation on performance, blood metabolites, ruminal fermentation parameters, and carcass characteristics in Korean cattle steers

This study was performed to evaluate the effect of dietary glycerin supplementation on growth performance, blood metabolites, ruminal fermentation characteristics, carcass characteristics and sensory traits, glycogen content in liver and muscle, and hepatic gluconeogenesis gene

expression in Korean cattle steers finished in feedlot. This study confirms that dietary glycerin supplementation at 6.4% of DM did not lead to detrimental effect on feed intake in Korean cattle steers. Both glycerin and corn starch supplementation did not improve average daily gain and feed efficiency in Korean cattle steers. Glycerin supplementation also did not affect rumen fermentation characteristics, carcass characteristics, IMF content, reducing sugar content, glycogen content in both liver and muscle, and sensory traits of Korean cattle steers. Both glycerin and corn starch supplementation did not affect serum glucose concentration at initial and 8^{th} week, but glycerin supplementation slightly increased the average serum glucose concentration at 16^{th} week. Although glycerin supplementation had no impact on carcass and meat quality, glycerin could be potentially considered as a good energy source to maintain the animal's metabolism in finishing Korean cattle.

Key words: Korean cattle steers, quality grade, carcass traits, loin, rump, fatty acid, volatile compounds, intramuscular fat, sensory traits, reducing sugar, glycerin

Student number: 2013-31317

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List of Abbreviations

ADG: average daily gain

ADF: acid detergent fiber

AHSG: alpha 2-HS glycoprotein

AMP: Adenosine-5'-monophosphate

AN: Angus

BW: body weight

DDGS: dried distillers grains with solubles

DMI: dry matter intake

DNA: deoxyribonucleic acid

FA: fatty acid

FBP1: fructose-1,6-bisphosphatase

G6PC: glucose-6-phosphatase

GC: gas chromatograph

GHR: growth hormone receptor

GPD1: glycerol-3-phosphate dehydrogenase 1

GPD2: glycerol-3-phosphate dehydrogenase 2

GSTM1: glutathione S-transferase M1

GYK: glycerol kinase

HO: Holstein

IMF: intramuscular fat

IMP: inosine-5'-monophosphate

INHBA: inhibin beta A subunit

KC: Korean cattle

LM: longissimus muscle

LT: longissimus thoracis

MS: marbling score

MUFA: mono-unsaturated fatty acid

NDF: neutral detergent fiber

NEFA: non-esterified fatty acid

PC: pyruvate carboxylase

PCCA: propionyl-CoA carboxylase alpha-mitochondrial

PCCB: propionyl-CoA carboxylase beta-mitochondrial

PCDH19: protocadherin 19

PEPCK-C: phosphoenolpyruvate carboxykinase-cytosolic

PEPCK-M: phosphoenolpyruvate carboxykinase-mitochondrial

PUFA: poly-unsaturated fatty acid

QG: quality grade

RNA: ribonucleic acid

RT-PCR: real-time polymerase chain reaction

SCD: stearoyl-CoA

SFA: saturated fatty acid

TDN: total digestible nutrition

TMR: total mixed ration

USFA: unsaturated fatty acid

VFA: volatile fatty acid

WHC: water holding capacity

YG: yield grade

Units and Marks

℃: Celsius degree

dL; deciliter

Eq/L: equivalent per liter

g: gram

kg: kilogram

mg: milligram

mL: milliliter

mM: millimolar

㎜: millimeter

N: newton

ng: nanogram

CHAPTER ONE

General introduction

Quality grade (QG) in the Korean beef quality grading system is divided into five grades (QG1++, 1+, 1, 2, and 3), where QG1++ is the highest grade, and QG 3 is the lowest grade. The QG of beef in Korea is based on the marbling score (MS), fat color, meat color, texture and maturity of the *longissimus dorsi* muscle at the 13th rib interface (National Livestock Cooperatives Federation, 1998). A higher QG is achieved with a high MS, more red meat color, and more white fat color (Moon et al., 2003). It is generally agreed that intramuscular fat (IMF) and QG in beef are positive factors that affect meat sensory characteristics, such as tenderness, juiciness, flavor, and overall palatability (Emerson et al., 2013). A consumer survey revealed that tenderness is the most important factor in consumer eating satisfaction, followed by flavor and juiciness (Huffman et al., 1996). Increased cross-links in collagen fibrils (connective tissue) may contribute to increased toughness as the animal matures (Shorthose and Harris, 1990). In terms of meat flavor perception, 5'-ribonucleotides, adenosine monophosphate (AMP) and inosine monophosphate (IMP) can be considered as important factors because they cause umami taste characteristics (Durnford and Shahidi, 1998). In addition, fatty acid is also an important factor affecting beef palatability (Ba et al., 2013). Different types of fatty acid may affect the generation of volatile

compound types, and thus, flavor (Neethling et al., 2016). It has been thought that the palatability of Korean cattle beef is good because of the high QG and thus high MS. Limited information is available and variability exists in data on the associations among MS/QG, meat characteristics, and sensory traits in Korean cattle beef.

In Korea, domestic beef production is primarily based on Korean cattle, but Holstein steers are also produced. Since the self-sufficiency rate of beef consumption has decreased, Korea imports beef mainly from United States and Australia, from both of which predominantly imported Angus cattle (KREI, 2016). Korean consumers have historically preferred Korean cattle beef to domestic Holstein and imported beef because they believe that the palatability of Korean cattle beef is superior to that of Holstein and imported beef (Kim et al., 1993). In terms of factors affecting beef palatability, reducing sugars is also known to contribute to improved flavor of cooked meat by reacting with amino acids to produce many important volatile compounds via Maillard reactions (Aliani and Farmer, 2002). Currently, little information is available on the reducing sugar content of beef and its association with carcass and sensory traits. Also, limited information is available on the factors that affect the preference of Korean consumers for Korean cattle beef over other breeds.

Glycerol, also known as glycerin, is a colorless, odorless, sticky, sweet-tasting liquid. In the process of biodiesel production, it is produced via

transesterification of vegetable oil or animal fat with the use of methanol, leading to methyl-esters of glycerol and fatty acids (Ma and Hanna, 1999). Glycerin potentially serves as a gluconeogenic substrate for ruminants (Chung et al., 2007). Glycerol can be fermented to propionate in the rumen, which can also act as glucogenic precursor in ruminants. Since glycerol is one of precursors in gluconeogenesis, the effect of crude glycerin inclusion on carcass composition and characteristics in various animal diets as energy source has been evaluated by many studies. But the outcomes were not consistent, and limited information is available for the effect of glycerin on rumen fermentation characteristics, glucose/gluconeogenesis metabolism, carcass reducing sugar and glycogen contents in Korean cattle steers.

A series of studies were conducted to elucidate 1) the associations among quality grade/marbling and various carcass characteristics and sensory traits in Korean cattle beef, 2) the factors that contribute to differences in the sensory traits of *longissimus thoracis* of different breeds, 3) the effects of dietary glycerin replacement on growth performance, carcass characteristics and sensory traits in Korean cattle steers fed diets based on the similar TDN level between experimental diets, and 4) the effects of crude glycerin supplementation on the same parameters mentioned above in Korean cattle steers fed diets based on the different TDN level between experimental diets.

CHAPTER TWO

Literature Review

1. Overview of Korean cattle

1) History of Korean cattle

Korean native cattle, also called Hanwoo (*Bos taurus coreanae*), have been raised in the Korean Peninsula since 2,000 BC. In the ancient Agricultural Age, Korean cattle were raised primarily as an important draft tool or occasionally as an object of sacrificial rite. As the number of cattle in Korea was limited and religious and political issues existed in Korea, consumption of Korean cattle beef as an edible meat was very low. Along with the rapid growth of the economy in Korea since 1960s, the production of Korean cattle as meat-type cattle has developed. Therefore, the history of eating Korean cattle beef as a main meat food source is still very short (Jo et al., 2012). Due to the fact that Korean cattle have maintained stable traits by means of pure breeding, the present blood lineage is very valuable and is mainly widespread in the Korean Peninsula (Kim and Lee, 2000). In spite of high price, Korean cattle is very popular with both Koreans and foreigners, because Korean cattle beef is well known for its marbled fat, tenderness, flavor and juiciness (Lee et al., 2014). Currently, there are four breed types of Korean native cattle in Korea and each one has different coat color:

brown (Hanwoo), black (Jeju Black), black face (Heugu), and tiger color (Chikso), respectively. Among these four color variants brown coat color is the most common one **(Figure 1**).

Figure 1. Pictures of four types of Korean cattle. (Ⅰ) Brown Hanwoo, (Ⅱ) Jeju black, (Ⅲ) Heugu and (Ⅳ) Chikso. (source: Suh et al., 2014)

2) Korean cattle industry

Korean cattle are well known to possess relatively great fertility but their slow growth rate and low milking yield reduce total beef production. The Korean cattle industry has aimed to increase the cattle number to meet the growing demand of beef market in Korea (Kim and Lee, 2000). Carcass yield was

regarded as more important part compared to meat quality before the 1980s, because the overall beef supply had been insufficient to meet the demand in Korea. Along with the improvement of the economy in Korea, the consumers preferred more palatable meats with increased demand for meats. In Korea, per capita meat consumption has rapidly increased from 11.3 kg in 1980 to 51.3 kg in 2014 and beef consumption from 2.6 kg to 11.6 kg (KAPE, 2015). Korean cattle beef can be characterized by its highly marbled fat similar to Japanese Wagyu beef, thin muscle fibers, minimal connective tissue, and the characteristic flavor (Kim et al., 1994). Also, it has less subcutaneous fat depth with greater marbling scores and ossification scores compared to those of Australian Angus (Cho et al., 2005).

In Korea, approximately 3.1 million head of beef cattle were raised in 2016. The total number of slaughter cattle was 859,472, including 737,476 Hanwoo cattle, 57,642 Holstein cows, and 64,354 Holstein heifers and bulls (KAPE, 2016). The number of cattle farming households was 89,879, including 85,040 Hanwoo farmers (KAPE, 2016). During the last decade, the number of households raising Hanwoo cattle decreased from 186,000 households in 2006 to 85,040 in 2016 (KAPE, 2017). Korea has only a 37.7% self-sufficiency rate in beef market, and the rest imported mainly from Australia, USA, New Zealand, Mexico, as well as Canada (MAFRA, 2016). Despite Korean cattle beef's price being twice as expensive, Korean consumers prefer Korean cattle beef to

imported beef in Korean markets, as Korean cattle beef is considered to be fresher and to have better quality compared to imported beef (Kim et al., 2000). The average live and carcass weights of Korean cattle at slaughter (26~30 month of age) were 731 kg and 437 kg, respectively (KAPE, 2016).

3) Beef quality grading system in Korea

Beef quality grading system is that sorts out quality of livestock products according to certain criteria, and differentiates quality of beef. Korean consumers have a preference for high-marbled beef. Since Korea's carcass grading system was established and enforced from 1992, it gives a high priority to marbling. Grading system consists of two kinds of categories: 1) yield grade (**YG**), including YG A, B, and C, is used for evaluating meat amount; 2) quality grade (**QG**), including QG 1++, 1+, 1, 2, and 3, is used for evaluating meat quality (KAPE, 2017). Carcass of Korean cattle were evaluated by a meat grader using the Korean carcass-grading system of Korea Institute for Animal Products Quality Evaluation (KAPE, 2017) at 24h post-mortem. Carcass weight, longissimus muscle area, fat thickness, marbling score (MS), meat color, fat color, texture, and maturity are examined and reported by an official meat grader. Of these parameters, MS is mainly used for determining QG. Five grades (QG 1++, 1+, 1, 2, and 3) are assigned by meat graders. The MS of the Beef Marbling

Standard (BMS) ranges from 1 (devoid) to 9 (abundant); 8 or 9 is the MS for QG 1++, 6 or 7 is the MS for QG 1+, 4 or 5 is the MS for QG 1, 2 or 3 is the MS for QG 2, and 1 is the MS for QG 3 (**Figure 2**). In addition, meat color, fat color, texture, and maturity of the exposed longissimus muscle at the 13th rib interface are used for QG determination (NLCF, 1998).

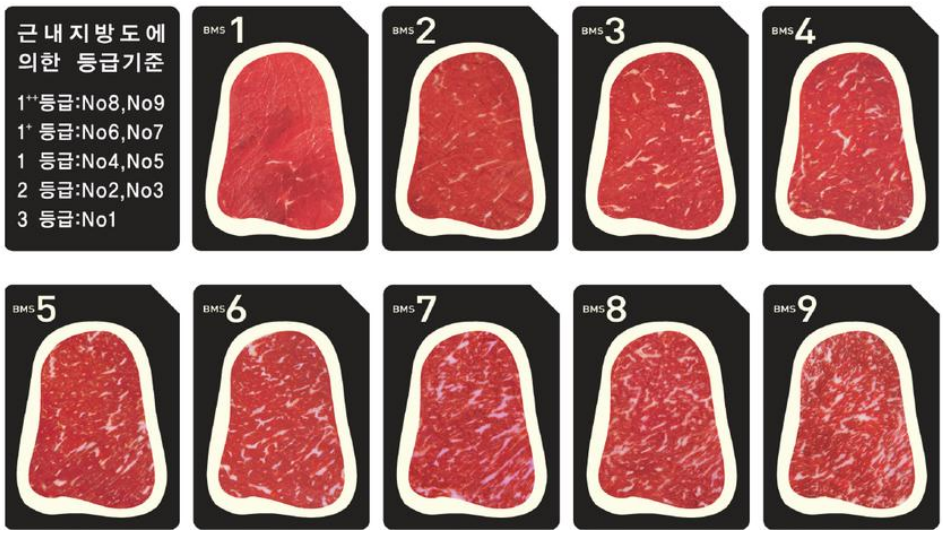

Figure 2. Beef marbling standard (BMS) used for estimating quality grade in Korean carcass grade system. (source: Korean Institute for Animal Products Quality Evaluation, 2017)

4) Characteristics of Korean cattle beef

Of Korean cattle slaughtered in 2016, QG 1++ accounts for 9.6%, QG 1+ 30.4%, QG 1 29.4%, QG 2 22.8%, and QG 3 7.6%, respectively (KAPE, 2016). In Korea, Korean cattle beef with QG 1++ or QG 1+ is evaluated a premium

class of beef. It was reported that the loin of Korean cattle with QG 1++ had the highest fat contents, whereas loin of cattle with QG 3 contained highest moisture content (Kim and Lee, 2003). Normally, it is accepted that intramuscular fat (IMF) content showed negative relation with moisture content. The loin of Korean cattle steers with QG 1++ had 23.8% IMF contents, followed by QG 1+ (17.3%), QG 1 (14.1%), and QG 2 (9.9%), respectively (Piao et al., 2015). Frank et al. (2016) reported that IMF content showed positive relations with sensory traits of beef loin including flavor, juiciness, and tenderness, whereas a low amount of fat induces a less positive response. Jo et al. (2012) reported that shear values of *longissimus dorsi* from Hanwoo beef with QG 1++ (3.5kg) were significantly lower than those of QG 2 (4.9kg), which indicates that shear force is negatively associated with IMF content in Korean cattle.

Korean cattle carcasses possessed less subcutaneous fat depth, whereas higher marbling scores and ossification scores compared with Australian Angus carcasses, when it was measured by USDA scoring systems. Also, the average IMF content of Korean cattle (11.3%) was evaluated higher value than that of Australian Angus (5.7%; Cho et al., 2005). Cho et al. (2011) reported that fat content of Korean cattle beef with QG 1+ and QG 1 were significantly higher than those of Australian Angus and crossbred in strip loin and loin, whereas protein content of these two QGs of Korean cattle were lower than those of Australian Angus and crossbred in these two cuts. In terms of mineral content

(Ca, Fe, Zn), there were no differences reported between Korean cattle and Australia Angus beef except that Zn content of Angus beef was higher than those of Korean cattle in top round. Free amino acids and peptides can influence flavor, taste, and aroma formation in meat during storage (Jo et al., 2012). Cho et al. (2008) reported that glutamic acid showed the highest amino acid content in ten different cuts (short plate, top sirloin, striploin, loin, chuck tender, eye of round, chuck roll, bottom round, top round, and brisket) of Korean cattle beef at 28-30 months of age. Of these different cuts, loin contained the least total amino acid content in Korean cattle beef. The particular amino acids, such as glutamic acid, may function as flavor enhancers to enhance the flavor contribution of other agents (Shahidi et al., 1986). Jo et al. (2012) reported similarly that glutamic acid can enhance an umami taste by reacting with inosine monophosphate (IMP). In addition, Sforza et al. (2001) reported that asparagine, serine, threonine, glycine, alanine, and glutamic acid were related to a sweet taste, whereas isoleucine, leucine, valine, methionine, phenylalanine, histidine, arginine, and proline were related to a bitter taste in meat.

Not only IMF, but also fatty acid is also known as an important factor affecting beef palatability (Ba et al., 2013a). Studies reported that oleic acid might positively relate to beef flavor, whereas polyunsaturated fatty acids (PUFA) and saturated fatty acid (SFA) might be negatively associated with beef flavor (Melton et al., 1982). Previous studies had showed that fatty acid composition

varies depending on breeds (Yang et al., 1999; Zembayashi and Nishimura, 1996) and that this has impact on palatability factors of beef, especially in Korean cattle (Cho et al., 2005) and Japanese Wagyu breeds (Xie et al., 1996). Cho et al., (2005) reported that Angus beef contained relatively higher n-3 PUFA, while Hanwoo beef had higher n-6 PUFA in three different muscles including *longissimus dorsi*, *triceps brachii*, and *semimembranosus*, which may be due to the different feeding conditions. In ruminant, although the fatty acid composition of meat is not a direct response to the dietary fatty acid profile because of biohydrogenation by rumen microbes, the difference in fatty acid profile of meat is probably caused by the effect of different diets, forage, and grain feeding (Enser et al., 1998). Thus, it can be easily assumed that Korean cattle beef contains a fatty acid profile characterized by high concentrate-fed animals.

Volatile compounds, including aldehydes, hydrocarbons, alcohols, ketones, furans, pyrazines, thiophenes, and thiazoles, etc. which are formed during cooking of meat, also affect palatability as well as flavor (Watkins et al., 2012). Korean cattle and imported Australian beef vary in volatile compounds. Cho et al. (2011) reported that the contents of heptanal, hexanal, octanal, E-2-decenal, E-2-octenal, nonenal, 2,4-decadienal, 2-undecenal, 2-butyl furan, and heptane of longissimus dorsi of Hanwoo beef were higher than those of imported beef. This is consistent with previous study which showed that beef of grain-fed cattle contained higher contents of 2-heptenal, hexanal, and 2,4-decadienal, which are

all formed by linoleic acid (Larick et al., 1990).

2. Factors affecting sensory traits

Overall beef palatability and eating satisfaction can be attributed to three factors: tenderness, flavor, and juiciness. In Korea, the overall acceptability of Korean cattle beef is decided by the consumers as follows: weights of tenderness 55%, flavor-likeness 27%, and juiciness 18% (Cho et al., 2010). Beef tenderness is regarded as one of the most important quality challenges facing the beef industry. Flavor after tenderness, a combination of taste and odor, is also one of the essential factors involved in a consumer's meat purchasing decision (Sitz et al., 2005). Juiciness is also a principal factor for sensory evaluation and it is attributed to the flow of juices from the actual meat and the moisture produced by saliva in the mouth during mastication (Winger and Hagyard, 1994).

1) Tenderness

Tenderness is a quality of meat gauging how easily it is chewed or cut. Since tenderness strongly affects consumer's perceptions of acceptability, tenderness is known as the most important eating quality traits. Meat tenderness is influenced by several factors, including the amount and solubility of connective tissue, the

composition and contractile state of muscle fibers, and the extent of proteolysis in rigor muscle (Joo et al., 2013). In addition, IMF content also indirectly influences tenderness. Tenderness is evaluated more important for red meat such as beef and lamb due to high composition of connective tissue and red muscle fibers than those of chicken and pork. The connective tissue content is associated with muscle fiber characteristics because of high proportion of muscle fibers (75-90%) in the muscle volume, and the morphology of the muscle fiber mainly acts as the determinant factor of mass (Lee et al., 2010). Although the relationship between meat tenderness and muscle fiber characteristics is still debatable, the heterogeneity of muscle fiber characteristics in different muscles has been considered to affect tenderness (Maltin et al., 2003). During the conversion of muscle to meat, muscles have different patterns of post-mortem change depending on diverse muscle fiber characteristics. In addition, sarcomere lengths in muscles are different due to the fact that each muscle fiber goes into rigor at different times in the whole of post-mortem period. As a result, meat tenderness varies depending on the rigor onset post-slaughter, and the rate and extent of glycolysis, which are all associated with muscle fiber characteristics as well as muscle temperature (Ali et al., 2008).

2) Flavor

Flavor is the sensory impression sensed by smell buds and taste, and it is a leading factor determining the meat quality and purchasing decision of the consumer (Khan et al., 2015). Flavor is also a principal factor for the eating quality of meat because consumer expect savory. Since meats are mainly composed of the lean portion and the fat portion, the meat flavor is primarily affected by the pool of flavor precursors in these two tissues (Joo et al., 2013). Meat flavor precursors are normally affected by several factors, including breed of animal, sex of animal, feed of animal, chiller aging, and meat cooking.

Breed of animal should be considered while evaluating consumer preferences of beef throughout aging (Monson et al., 2005). The animal breed mainly has impact on total fat, IMF, and fatty acid composition of meat. The primary variant for ruminant fatty acid composition is animal species; ruminant meat normally has more SFA content because of bio-hydrogenation process in the rumen compared with monogastric animals. Campo et al. (1999) reported that breed and slaughter weight of the animal influence the rate of sensory changes during aging. In that study, it was also reported that aging affects the tenderness and aging evolution of meat is attributed to breed type. Gorraiz et al. (2006) observed the difference in flavor and volatile compounds because of aging time in Friesian and Pirenaica heifers and bulls. Variation in some volatile compounds were found because the beef of Friesian breed contained a higher fatty flavor compared with Pirenaica after cooking. Ba et al. (2013b) summarized in their

study that breed of animal (Hanwoo vs Angus) plays a very important role in meat physico-chemical quality, sensory characteristics and volatile flavor compounds.

The sex of animal has significant impact on the flavor variations in meat. Gorraiz et al. (2006) observed difference in flavor, volatile compounds and odor because of aging time in Friesian and Pirenaica heifers and bulls. Bloody flavor which is associated with higher 2-propanone content and stronger liver-like flavor and odor were found in beef from bull, while beef from heifers contained higher characteristic flavor. Horcada et al. (1998) found the higher infiltration fat content in females. They reported that the females should have juicier meat compared with males. Forrest (1975) reported that the roast rib from steers were evaluated as juicier, tenderer, and more flavorful, and received higher scores in overall palatability compared with those of bulls. However, Franco et al. (2011) reported that there was no significant differences in meat quality between sexes and slaughter age.

Feed also has impact on the carcass conformation, physicochemical and organoleptic parameters of meat quality such as chemical composition, fatty acid composition, color and meat tenderness (Ramírez-Retamal and Morales, 2014). Lewis et al. (2002) reported that feeding system of the animals can affect the composition of carcass and the degree of fattening. The fatty acid profile of meat is mainly affected by the types of feed, and the meat of grass-fed animals is

recognized as healthier (Wood et al., 2008). Young et al. (1997) reported that terpenes and diterpenoids were attributed to feed as these volatile compounds (terpenes and diterpenoids) are normally found in cooked meat of pasture-fed sheep. Grain-based diets are known to have greater carbohydrate availability compared with pasture based (Young and Braggins, 1998).

In addition, meat cooking is also principal to achieve palatable and safe products (Tornberg, 2005). Meat cooking is well-known to lead to the development of meat aroma through lipoid oxidation, Maillard reaction, thermal degradation of thiamine, and interaction of these pathway products by utilizing non-volatile flavor precursors like reducing sugars, peptides, free amino acids, unsaturated fatty acids, vitamins, and nucleotides (Mottram, 1998). The cooking methods and cooking conditions (cooking temperature and time, and heating rate) can play an important role in the modification of chemical composition and subsequently the nutritional value of meat (Brugiapaglia and Destefanis, 2012). Of several cooking methods, the roasting of meat leads to increased oxidation because of the use of high temperature for a longer time period (Domínguez et al., 2014). Ames et al. (2001) concluded in their study that cooking temperature and volatile compounds produced with increased cooking temperature affect the flavor development through lipid oxidation and Maillard reaction.

3) Juiciness

Juiciness is defined as the amount of moisture squeezed out of a piece of meat by a few gentle chews (Ritchey and Hostetler, 1964). Juiciness is known to show positive relation with the IMF content and the water holding capacity (WHC) of meat. Hocquette et al. (2010) reported that the IMF content directly influences both juiciness and flavor. With the increased IMF content in meat, the human perception of juiciness can be increased (Jeremiah et al., 2003). For the consumers of pork, juiciness is evaluated as more important sensory trait than flavor or tenderness (Aaslyng et al., 2007), whereas meat tenderness is rated as the most important palatability trait for the consumers of beef (Cho et al., 2010). Luchak et al. (1998) reported that the IMF content influences juiciness through several pathways; by improving the WHC of meat, by increasing the tenderness, by lubricating the muscle fibers during cooking, or by stimulating salivary flow during mastication. Meat with lower IMF content is not easily deteriorated by severe short-heating under dry cooking conditions, whereas meat with high IMF content can improve the juiciness after long heating in a moist environment (Joo et al., 2013).

4) Precursors of flavor

There are some precursors affecting aroma flavor characteristics of cooked

meat, including the low molecular weight, water-soluble compounds and fats in meat constituents (Macey et al., 1970). These precursors of meat flavor such as free sugars, sugar phosphate, free amino acids, peptides, nucleotide-bound sugars, nucleotides, and vitamin, can be used either in the Maillard reaction or lipid oxidation/degradation and interaction upon heating to generate volatile flavor compounds, and then produce the final aroma flavor characteristics of meat (Ba et al., 2012). The adipose tissue and IMF not only play an important role in development of cooked meat flavor but also have a great influence on the characteristic-specific species flavors.

Reducing sugar in meat is well known as a precursor of Maillard reaction. Volatile compounds, such as aldehydes, hydrocarbons, ketones, alcohols, carboxylic acids, esters, lactones, furans, pyridines, pyrrans, pyrroles, pyrazines, thiophenes, thiazoles, thizolines, phenols, oxazoles, and other nitrogen or sulfuric compounds, can be produced via several pathways, including Maillard reaction of reducing sugars (e.g. glucose and ribose) with amino acids or peptides, thermal lipid degradation, and the interaction between Maillard reaction products and lipid oxidation products (Kosowska et al., 2017). In the Maillard reaction, glucose, ribose, glucose 6-phosphate and ribose 5-phosphate are well known as reducing sugars (Ba et al., 2012). It was reported that the flavor precursor components in meats can be affected by the type of diet. Koutsidis et al (2008) reported that diets had impact on the reducing sugars in *longissimus lumborum* of

cattle, and higher total reducing sugars were produced from beef of cattle fed concentrate-based diet compared to the grass-based diet, whereas beef from cattle fed with grass silage contained higher content of free amino acids.

Free amino acids can affect flavor, taste, and aroma formation in meat during storage (Jo et al., 2012). Among the amino acids contained in meat, cysteine and cystine are two sulfur-containing amino acids, which can react with other sugars to form many sulfur-containing flavor compounds (Elmore et al., 2002), while the reaction of other non-sulfur containing amino acids with sugars lead to formation of the nitrogen-containing products such as pyrazines (Ames et al., 1997).

In addition, thiamine is also known as an important precursor that provides wide range of sulfur compounds (Aliani and Farmer, 2005). Also, inosine-5'-monophosphate (IMP) is regarded as an important meat palatability factor as IMP enhances an umami taste (Aliani and Farmer, 2005). Hypoxanthine is also considered as an important component in the formation of meat flavor and it may impart a bitter flavor to the meat by conjugating with other free amino acids and some dipeptides (Tikk et al., 2006).

Some previous studies have been conducted to observe the effects of supplements or replacement of certain ingredient on the precursors of meat flavor mentioned above. Choi et al (2016) reported that supplementing flax seed to the

concentrates fed to finishing Hanwoo steers tended to improve flavor, umami, and overall palatability of *longissimus* muscle, resulting from increases in the concentration of free amino acids, glutamic acid, methionine, and α-aminoadipic acid, and peptides, anserine and carnosine, and their complex reactions.

3. Glycerin as feedstuff

The worldwide high demand for energy results in increasing production of biofuel, particularly liquid fuels for transportation, in order to replace fossil energy sources, to improve energy security and to respond to greenhouse gas emissions (Heinimo et al., 2009). This phenomenon resulted in an increasing competition of raw materials for food, feed, fuel usage, and the disposability of increasing by-products. For example, corn and vegetable oils are utilized for production of bioethanol and biodiesel, and one of the by-products is glycerin (Figure 3). Glycerol, also known as glycerin, is an essential structural component of triglycerides and phospholipids, and glucogenic properties of glycerol are well known (Cori and Shine, 1935). When animals are fasted off, they can use body fat reserves as energy source, and then free fatty acids and glycerol can be released into the bloodstream. In general, some portion of glycerol can enter the gluconeogenesis to be converted to glucose by the liver or kidney so that it can provide energy for cellular metabolism.

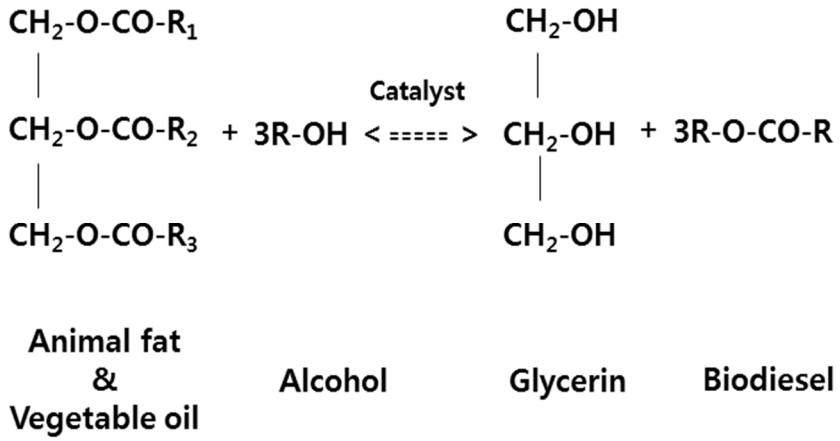

Figure 3. Glycerin and biodiesel production process.

1) Properties of glycerol

Glycerol is a colorless, odorless, sticky, and sweet-tasting liquid. As glycerol has a relatively high boiling point (290℃), it is difficult to separate from other non-volatile impurities. The three hydroxyl groups in glycerol chemical structure allow for hydrogen binding that makes glycerol viscous and gives strong water holding capacity (Myers, 2007). Glycerol is soluble in alcohols and water, and partially soluble in dioxane, ether and ethyl acetate. The water binding property makes glycerol to become a suitable moisturizing agent. At ambient temperature, refined glycerol is in the liquid state and its dry matter content is approximately 90% (Newman, 1968). This unique physical and chemical properties make the

glycerol to become an exceptional feed stuff in pelleting and increase flowability of liquid feeds.

2) Glycerol as energy source

The caloric value of glycerol was calculated to be equal to that of corn (NE_M=2.2 Mcal/kg; NE_G=1.5 Mcal/kg), but it doesn't contain protein, fat, or fiber (Preston, 2014). Glycerol is known as a feed additive of interest in the dairy industry for improving energy balance of cows. When fed a 500 mL oral bolus of glycerin to dairy cows 5 days postpartum, plasma non-esterified fatty acid (NEFA) was decreased, which suggested that the glucogenic property of glycerol enhanced energy balance (Ogborn, 2006). Carvalho et al. (2011) reported that dietary glycerol did not affect feed intake and milk production of cows fed 11.5% and 10.8% glycerol in prepartum and postpartum diets, respectively. It was reported that dry glycerin (dry powder; minimal 65% of food grade glycerol) is also known as an additive which has positive impact on the energy balance of dairy cows when top dressed during early lactation (Chung et al., 2007).

Glycerol is known to serve as an appetite stimulant due to the sweet taste of glycerin's property. Fisher et al. (1971) reported that when supplemented glycerin at 472g/d to the concentrate mix of a component based diet, the feed intake was increased. Fisher et al. (1973) also reported that when the experiment was

extended to 8 weeks treatment period using a larger number of cows, 174 or 347g/d of glycerol supplementation had no impact for improving feed intake relative to cows fed a conventional concentrate or propylene glycol. Parsons et al. (2009) reported that there is no change in dry matter intake (DMI) when glycerin was fed to crossbred heifer at either 0 or 2% of the diet, but reduction of DMI occurred when increasing glycerin to 4, 8, 12, and 16%. Pyatt et al. (2007) also reported that when glycerin was supplemented at 10% to a dry-rolled corn diet fed to steers, DMI was reduced by 10.1%. Trabue et al. (2007) reported that when lactate accumulation is increased, glycerol might be fermented slowly in the rumen, which can alter feed intake. Roger et al. (1992) stated that when the in vitro media was added glycerin at 5%, the growth and cellulolytic activity of rumen fungi and bacteria were inhibited, which indicated that small amount of glycerin inclusion might be favorable to animal growth, but concentrations more than 5% might lead to an unhealthy rumen, subsequently resulting in decreased feed intake. Average daily gain (ADG) was increased when glycerin was supplemented at 2, 4, 8% to diets fed to crossbred heifers, but reduction of ADG was occurred at 12 and 16% (Parsons et al., 2009). Mach et al. (2009) reported that glycerin supplementation did not affect ADG or feed efficiency, when glycerin was added at up to 12% to diets fed to finishing Holstein bulls. In contrast to these studies, Pyatt et al. (2007) reported that when corn was replaced by glycerin at 10% in a concentrate, glycerin improved ADG and feed conversion of Angus steers.

During the feedlot receiving phase, glycerol has been supplemented to the diet fed to feeder calves with the object of increasing energy intake and enhancing their immune function (Hales et al., 2013b). DMI was linearly decreased when glycerol was supplemented at levels up to 10% in receiving diets (control; 25% roughage) replacing grass hay. Hales et al. (2013b) explained that the reduction of intake may be attributed to an increase in energy density of the diet. In addition, although dietary glycerol was reported to have no impact on the health status of high risk calves suffering from bovine respiratory disease, mortality, or bovine rhinotracheitis, Hales et al. (2013b) reported that glycerin enhanced feed efficiency and acted as a viable feed ingredient when it was supplemented at 5% of receiving diets.

Glycerin was replaced for forage and grain in high-roughage diets. Glycerol was supplemented at 7.5% of growing steer diets (control; 40% roughage diet) substituting alfalfa or steam-flaked corn. However, there were no differences found in DMI and growth rate (Hales et al., 2013a). Ramos and Kerley (2012) also reported that when replacing grass hay with crude glycerin up to 20% of forage-based diets, there was no adverse effect on performance of heifers.

Glycerol was also included in high-concentrate bovine diets, which reported variable effects on cattle performance. Parsons et al. (2009) reported that when glycerol was supplemented at $\leq$ 2% levels to finishing diets fed to heifers, DMI was reduced. Anderson and Ilse (2008) reported that supplementing crude

glycerin up to 18% to finishing diets replacing dry-rolled corn and co-products linearly reduced DMI, but it had no effect on gains of heifers. Mach et al. (2009) reported that when supplement glycerin at up to 12% of DM to isocaloric and isonitrogenous high-concentrate diets fed to Holstein bulls, there were no detrimental effects on performance. Moreover, glycerol fed to steers elevated blood insulin and glucose levels after 24 and 48h of transportation, and potentially impeded breakdown of muscle proteins and preserved muscle quality (Parker et al., 2007).

3) Glycerol fermentation in rumen

Three of the principle fates of dietary glycerol in ruminants have been estimated and include absorption through the rumen epithelium (45%), fermentation to volatile fatty acid (VFA, 25%), and escape through the omasal orifice (30%; Werner-Omazic et al., 2015). Glycerol absorption probably occurred primarily by passive diffusion not facilitated diffusion. Aquaporins are known as transport proteins which carry glycerol and water across cell membranes in various mammalian tissues such as rumen epithelium and gastrointestinal tract (Ishibashi et al., 2009). However, Werner-Omazic et al. (2015) reported that ruminal transport of glycerol was not inhibited by the presence of aquaporin inhibitor. Moreover, Werner-Omazic et al. (2015) showed

that transfer of glycerol across the rumen epithelium enhanced linearly with increased glycerol levels, suggesting reliance upon carriers for absorption was minimal.

The *Streptococcus bovis*, *Megasphaera elsdenii*, and *Selenomonas ruminantium* are primary bacterial species responsible for the anaerobic fermentation of glycerol. It was known that lactic acid derived from the fermentation of glycerol is converted to butyrate by *M. elsdenii* (Stewart et al., 1997). Wolin et al. (1997) reported that a major source of propionate in the rumen is produced from the decarboxylation of succinate by *S. ruminantium*. Propionate was identified as the primary product of glycerol fermentation by these *selenomonads* in sheep rumen fluid through *in vitro* techniques (Hobson and Mann, 1961). Boyd et al. (2013) reported that glycerin supplemented to ruminant diets is well known to cause a shift in VFA profiles, favoring propionate production at the expense of acetate. Rémond et al. (1993) reported that when glycerin (240g) was ruminally administered to cows fed maize silage, about 35-69% of carbons forming propionate are derived from glycerol. It was reported that total VFA concentration would be unchanged (Hales et al., 2013a) or increased because of accumulation of propionate in steers fed glycerol (Wang et al., 2009). Propionate is well known as a glucogenic compound which conserves carbons and acts as a hydrogen sink. Since glycerol can be converted extensively to propionate in the rumen, glycerin was supplemented with gradually increasing

level to forage-based diets, and CH_4 production was evaluated using *in vitro* techniques (Avila-Stagno et al., 2013). As a result, CH_4 emissions were linearly increased with increasing glycerol inclusion, which was attributed to the reduced state of glycerol compared with carbohydrates. Avila-Stagno et al. (2013) explained that since the conversion of glycerol to propionate lacks the incorporation of net electrons, the fermentation of glycerol to propionate in forage-based diets did not serve as a hydrogen sink.

Wang et al. (2009) stated that proteolytic activity was inhibited by glycerol inclusion in steers fed high-concentrate diets. Paggi et al. (1999) reported that when supplementing glycerol with increasing levels to bovine rumen fluid using *in vitro* techniques, proteolytic activity was reduced by 20%. In addition, previous studies found that ruminal lipolysis was inhibited by nearly 48-77% (Krueger et al., 2010) and 46-80% (Edwards et al., 2012) without affecting rumen DM digestion, when glycerol was included to rumen fluid using *in vitro* techniques. Although feed grade glycerol has been stated to reduce pH levels, it has no adverse effect on cellulolytic bacteria activity (Rémond et al., 1993). Other studies also reported that the reduction in pH without detrimentally affecting cellulose digestion was found (Schröder and Südekum, 1999; Wang et al., 2009). However, Roger et al. (1992) found a reduction in cellulose degradation by cellulolytic bacteria in *in vitro* media containing 5% glycerol.

4) Glycerol metabolism in ruminants

The density of glycerol is 1.261 g/cm^3, and this is similar to the optimal density of particles that pass from the rumen (Neel et al., 1995). Garton et al. (1961) reported that approximately 25% of glycerol was not detectable by 2h of incubation and over 90% of the glycerol disappeared after 8h of fermentation in *in vitro* test using sheep rumen contents. Bergner et al. (1995) found a glycerol disappearance rate of 90% within 2h in *in vitro* test. Trabue et al. (2007) performed similar *in vitro* test and estimated the ruminal metabolism of glycerol to be completed by 80% after 24h of incubation. Rémond et al. (1993) reported that glycerol administered (480g/d) in the rumen of cows fed maize silage was disappeared after 4h.

Boyd et al. (2013) reported no change in DMI or apparent digestibility when glycerol was supplemented up to 400g/d in diets fed to postpartum dairy cow. Winterholler et al. (2011) reported that when included 860g/d glycerol to beef cows for maintaining body condition score during late gestation, it was found no negative effects on total tract fiber digestibility. Hales et al. (2013) reported that when glycerol was used as a replacement for roughage in growing steer diets, it was observed a linear increase of apparent OM, and apparent and true starch digestibility.

Werner-Omazic et al. (2013) reported that glycerol can be rapidly absorbed

from the gastrointestinal tract of young calves. This glucogenic substrate can be phosphorylated to glycerol-3-phosphate by glycerol kinase (Montell et al., 2002). Then, glycerol-3-phosphate is oxidized to dihydroxyacetone phosphate (DHAP) by glycerol phosphate dehydrogenase, leading to generation of NADH and H^+. At this stage, DHAP enters either glucogenic or glycolytic pathway depending on the animal's energy status. As intake is increased over maintenance, the increased production of metabolites relative to demand leads to storage of energy.

The effects of glycerol on blood metabolites of bovines have been evaluated to further observe the impact of this glucogenic compound on metabolism. Carvalho et al. (2011) reported that blood glucose concentration was decreased, when supplemented glycerin in prepartum and postpartum diets at 11.5% and 10.8%, respectively. The author explained that hepatic gluconeogenesis is depressed because of increased propionate production. Liu et al. (2014) reported that when supplemented glycerol to diets fed to lactating dairy cows during heat stress period, the glucose concentration was increased while the NEFA concentration was decreased, which indicated that glycerol increased glucose utilization by peripheral tissues and diminished triglyceride mobilization. However, DeFrain et al. (2004) reported that the concentration of glucose and insulin were not changed when supplemented glycerol up to 7.2% to transition dairy cows.

5) Effects of glycerin on carcass characteristics and sensory traits

Crude glycerin may have impact on carcass characteristics and meat quality due to the fact that feeding glycerin may increase the availability of gluconeogenic compounds (Evans et al., 2008; Versemann et al., 2008). As mentioned previously, glycerol can be absorbed by the ruminal epithelium and then converted to glucose through gluconeogeneiss, while glycerol also can be converted to propionate in the rumen (Krehbiel, 2008). Krueger et al. (2010) suggested that glycerol would improve passage of unsaturated fatty acids (USFA) from the rumen and further enhance absorption by small intestine, which make monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) more easily available for incorporation into meat due to inhibited bacterial fat degradation by glycerol inclusion in ruminal contents. However, many previous studies reported that no differences were observed on dressing percentage and hot carcass weight when glycerin was supplemented to the diets (Barton et al., 2013; Lage et al., 2014). Mach et al. (2009) reported that adding glycerin at up to 12% to diets fed to Holstein bulls did not affect carcass weight, dressing percentage, backfat thickness, and LM area. Butterey et al. (2015) also reported that glycerin inclusion at up to 10% in diets fed to finishing crossbred steers did not affect carcass weight, dressing percentage, marbling score, as well as LM area. Thus, the outcomes were not consistent probably due to some factors including animal breed, sex, age or feed.

As glycerol and amino acids are well known as important substrates for gluconeogenesis in the liver and kidney of animals (Sunny and Bequette, 2011), glycerol supply can be expected to enhance glycogen reserves in the muscle through glycogenesis. Thus, it can be further expected that glycerin could potentially contribute to improvement of meat quality grades and sensory traits due to its gluconeogenic property. Since glycerin is one of precursors in gluconeogenesis, feeding glycerin may lead to a rise in blood insulin concentrations and lipogenesis. Thus, glucose derived from glycerin would increase lipid contents, which could improve the tenderness, flavor and juiciness of meat of cattle fed diet containing glycerin. Even though tenderness has been related to intramuscular fat content (Purchas et al., 2002), Eiras et al. (2014) reported that supplementing glycerin at up to 18% to diets fed to Purunã bulls did not affect tenderness, juiciness, and flavor. Egea et al. (2014) also reported that crude glycerin inclusion at up to 4% in Limousin bull diets did not affect aroma intensity, flavor intensity, hardness and chewiness, whereas it increased juiciness. However, van Cleef et al. (2017) reported that the increasing inclusion of crude glycerin at up to 30% in Nellore bull diets improved flavor intensity, juiciness, and greasy intensity, while the greatest tenderness of the beef was observed with the inclusion of 15% crude glycerin. The effects of crude glycerin on meat quality and sensory traits were also evaluated in pork. Schieck et al. (2010) reported that the inclusion of crude glycerin at 8% in crossbred pig diets did not affect juiciness, tenderness, pork flavor intensity, off-flavor intensity, and overall

desirability. Egea et al. (2016) also reported that the inclusion of crude glycerin at up to 10% in Iberian × Duroc crossbred pig diets did not affect aroma intensity, meat color, color intensity, flavor intensity, hardness, and juiciness.

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CHAPTER THREE

Comparison of carcass and sensory traits, fatty acid profiles and volatile compounds among quality grades in *longissimus dorsi* and *semimembranosus* muscles of Korean cattle steers

1. Abstract

This study was performed to compare carcass traits, sensory characteristics, physicochemical composition, nucleotides and collagen content, free amino acids, content and composition of fatty acids (FA), and volatile compounds among four quality grades (QG1++, 1+, 1, and 2), and to understand their association with carcass characteristics in *longissimus dorsi* (loin) and *semimembranosus* (rump) cuts of Korean cattle steers. Loin and rump samples were obtained from 48 Korean cattle steers with each of four quality grades (QG 1++, 1+, 1, and 2; average 32 months of age). Carcass weight and marbling score (MS) were highest in QG 1++, whereas texture score measured by a meat grader was highest in QG 2. A correlation analysis revealed that MS ($r = 0.98$; $P < 0.01$) and fat content ($r = 0.73$; $P < 0.01$)

had strong positive correlations with QG and that texture had a strong negative correlation (r = −0.78) with QG. Fat content in loin was highest but protein and moisture contents were lowest in QG 1++. Our results confirmed that a major determinant of QG is the MS; thus, intramuscular fat (IMF) content. The International Commission on Illumination L^*, a^*, and b^* values in loin were highest in QG 1++. Numeric values of shear force in loin were lowest in QG 1++, whereas those of tenderness, juiciness, and overall acceptability tended to be highest in QG 1++ without statistical significance. QG was strongly correlated with juiciness (r = 0.81; $P < 0.01$) and overall acceptability (r = 0.87; $P < 0.001$). All sensory characteristics were higher ($P < 0.05$) in loin than those in rump. Adenosine-5'-monophosphate (AMP) and inosine-5'-monophosphate (IMP) contents in both loin and rump did not differ among QGs. No nucleotide (AMP, IMP, inosine, hypoxanthine) was correlated with any of the sensory traits. Total, soluble, and insoluble collagen contents in loin were higher in QG 1++ than those in QG 1. All three collagens had lower content in loin than that in rump. All three collagens were positively correlated with tenderness, juiciness, and overall acceptability. Glutamic acid content did not significantly differ among the four QGs in either loin or rump. In addition, QG1++ and QG2 loins showed the highest ($P < 0.05$) and lowest percentages of oleic acid (C18:1n9) and monounsaturated FA, respectively. QG1++ loins had greater ($P < 0.05$) percentages of volatile hydrocarbons, including n-pentane, n-hexane, and 2-butene, and these compounds were positively correlated

(0.56 ≤ r ≤ 0.81; $P < 0.001$) with QG and crude fat content (g/100 g meat). Percentages of loin acetonitrile (r = 0.74, $P < 0.01$) and butanal (r = 0.71, $P < 0.01$) were positively correlated with flavor, whereas those of loin 2-methyl-2-propanol (r = -0.62, $P < 0.05$) and 3-methyl-2-butanone (r = −0.60, $P < 0.05$) were negatively correlated with flavor. In conclusion, it is confirmed that QG is associated with sensory traits but nucleotide contents in beef may not be a major factor determining meat palatability in the present study. Loin FA percentages, especially C18:1n9 and monounsaturated FA, tended to be higher with increasing QG. Loin volatile compounds, including n-pentane, n-hexane, and 2-butene, were also higher with increasing QG.

2. Introduction

Quality grade (QG) in the Korean beef quality grading system is divided into five grades (QG 1++, 1+, 1, 2, and 3), where QG 1++ is the highest grade, and QG 3 is the lowest grade. The QG of beef in Korea is based on the marbling score (MS), fat color, meat color, texture and maturity of the *longissimus dorsi* muscle at the 13th rib interface (National Livestock Cooperatives Federation, 1998). Of the 421,464 Korean cattle steer carcasses slaughtered in 2013, 17.1% were QG 1++, 32.7% were QG 1+, 33.9% were QG 1, 15.0% were QG 2, and 1.2% were QG 3 (KAPE, 2013). There are large differences in market price of beef among QGs in

Korean cattle. For example, the price of QG 1++ Korean cattle beef in 2013 was 2.1-fold higher than that of QG 3 (KAPE, 2013). Thus, Korean farmers have been made an effort to obtain good QGs (QG 1++ and QG 1+) to earn more income. As free-trade agreement negotiations have increased with other countries, Korean farmers have become more concerned about price competition between Korean cattle beef and imported beef.

Sensory characteristics, including tenderness, flavor, and juiciness, are important determinants of acceptability and palatability of beef (Savell et al., 1987). A consumer survey revealed that tenderness is the most important factor in consumer eating satisfaction, followed by flavor and juiciness (Huffman et al., 1996). In Korea, These three factors also account for 55%, 27%, and 18%, respectively (Cho et al., 2011). Increased cross-links in collagen fibrils (connective tissue) may contribute to increased toughness as the animal matures (Shorthose and Harris, 1990). In terms of meat flavor perception, 5'-ribonucleotides, adenosine monophosphate (AMP) and inosine monophosphate (IMP) can be considered as important factors because they cause umami taste characteristics (Durnford et al., 1998).

Several studies reported that fatty acid (FA) content, and not only IMF, is an important factor affecting beef palatability (Ba et al., 2013). For example, an increase in fat content up to 36% enhanced the umami and beef flavor intensity in the longissimus thoracis muscle of Japanese Black steers (Iida et al., 2015). Studies

reported that oleic acid might positively relate to beef flavor, whereas polyunsaturated fatty acids (PUFA) and saturated fatty acid (SFA) might be negatively associated with beef flavor (Melton et al., 1982). Since most studies have reported FA composition (%) from total FA, limited information is available regarding the correlation between FA content or amount with QG, flavor and palatability in Korean cattle beef. Content or amount (g FA/100 g meat) of FA per meat type, rather than the percentage of individual FA, may be more directly associated with QG, sensory quality and volatile compounds.

Several volatile compounds, including aldehydes, hydrocarbons, alcohols, ketones, furans, pyrazines, thiophenes, and thiazoles, are formed when meat is cooked, and these compounds affect palatability, as well as flavor (Watkins et al., 2012). There are several ways in which volatile compounds are formed, including thermal lipid degradation, the Maillard reaction of amino acids or peptides with reducing sugars (e.g., glucose or ribose), vitamin (e.g., thiamin) degradation during cooking, and the interaction between Maillard reaction products and lipid oxidation products (Ba et al., 2012a). The major volatile compounds are produced by thermal degradation of beef fat, and FA oxidation is primarily responsible for the development of flavor (Neethling et al., 2016). Therefore, the amount of fat and FA composition in beef can affect development of special characteristics of aroma and flavor upon thermal processing. For example, increased flavor desirability was associated with increased IMF (O'Quinn et al., 2012). Levels of 2-butanone, 2-

pentanone, and 3-hydroxy-2-butanone increased with increasing IMF content in beef (El-Magoli et al., 1996). Some volatile compounds, such as n-aldehydes (heptanal, octanal, nonanal), were negatively correlated with fat content (Legako et al., 2015). However, other studies reported that increased IMF content did not affect the generation of volatile flavor compounds (Mottram and Edwards, 1983). Therefore, clarification is needed regarding the association of fat content and volatile compounds.

Beef MS and QGs positively affect meat sensory characteristics, including tenderness, juiciness, flavor, and overall palatability (Savell et al., 1987). It has been thought that the palatability of Korean cattle beef is good because of the high QG and thus high MS. Limited information is available and variability exists in data on the associations among MS/QG, meat characteristics, and sensory traits in Korean cattle beef. In addition, different types of FA may affect the generation of volatile compound types, and thus, flavor (Neethling et al., 2016). In grilled beef from Aberdeen Angus and Holstein-Friesian steers, linoleic acid levels were higher in the muscle of concentrate-fed animals than that of silage-fed steers, and a greater amount of volatile compounds were derived from linoleic acid decomposition (Elmore et al., 2004). Currently, limited information is available regarding the association of FA content and volatile compound generation, which affects palatability of Korean cattle beef. In this study, we compared carcass traits, sensory characteristics, free amino acid content, FA and volatile compounds among QGs in

the loin and rump of Korean cattle steers. The associations among QGs and various carcass characteristics and sensory traits were also evaluated.

3. Materials and methods

1) Animals and sampling

Korean cattle steer were reared on a commercial farm (Yeongam-gun, Jeollanam-do, South Korea) according to the traditional Korean cattle feeding system. Briefly, Korean cattle bulls were weaned at 3 months of age and were then fed roughage (70%) and concentrate (30%) until 5 months of age. The bulls were castrated at 6 months of age, and steers were grown in pens (five steers/pen; pen size, 5 × 10 m) using group feeding. The steers were fed a total mixed ration (TMR) twice daily until slaughter at around 32 months of age. The TMRs were formulated to fit growing (7–12 months of age) and three fattening stages (early fattening, 13–18 months of age; middle fattening, 19–24 months of age; and late fattening, 25 months of age to slaughter; Table 1). The formula and chemical composition of the protein and energy concentrates used in the TMR are shown in Table 2. All steers had free access to water and mineral salt blocks.

The Korean cattle steers were transported to the Nong-Hyup Naju joint livestock products market (Naju, Korea), fasted for 24 h, but given free access to water. The animals were slaughtered the next day, and the carcasses were moved to

a cold room at 5°C. The carcasses were graded based on the Korean grading standard the following day. At this stage, carcass weight, *Longissimus* muscle (LM) area, fat thickness, marbling score, yield index, yield grade, meat color, fat color, texture, and maturity were examined and reported by an official meat grader.

Forty-eight loin and rump tissues (about 500 g) with 12 samples from each four QGs (QG 1++, 1+, 1, and 2) were collected from 48 Korean cattle steers, transported under ice (4°C) to a laboratory 24 h postmortem, and kept at −80°C for 1 week. All the samples were stored at −80°C until analysis due to different sample collection time and subsequent analysis. The samples were thawed at 4°C for 1 day before analysis. The beef samples were minced using a mini chopper (CH180, Kenwood, Shanghai, China) for 30 sec, and excess connective and fat tissues were removed before weighing. A part of the meat sample were aged further at 4°C for 7 days as part of the free amino acid analysis because fresh tissue has low free amino acid content. Minced beef samples were taken from various locations and pooled for analyses.

Table 1. Ingredient and chemical composition of the total mixed ration for Korean cattle steers

Item	Growing period	Fattening period (early)	Fattening period (middle)	Fattening period (late)
Ingredient, % (as-fed basis)				
Molasses	3.74	3.75	3.71	3.37
Corn, flaked	0.00	4.41	7.42	14.79
Cotton seed	0.00	2.65	2.97	3.63
Rice straw	0.00	6.62	7.42	9.60
Timothy, hay	5.98	0.00	0.00	0.00
Sugar beet pulp	4.48	4.41	4.95	2.59
Italian ryegrass silage	11.96	6.62	6.68	0.00
Alfalfa, hay	7.47	0.00	0.00	0.00
Liquid microbes	0.20	0.20	0.20	0.20
$NaHCO_3$ buffer	0.00	0.30	0.30	0.35
Ryegrass straw	4.48	6.62	3.71	0.00
Protein concentrate	24.36	20.96	18.55	12.97
Energy concentrate	0.00	4.41	8.16	15.57
Brewers grain, wet	8.07	8.16	7.42	7.26
Lupin hull	13.45	13.24	9.90	7.26
Oats, hay	4.48	0.00	0.00	0.00
Water	11.31	17.65	18.60	22.39
Chemical composition, % DM				
Dry matter (DM)	67.21	64.87	64.50	65.84
Crude protein	15.67	14.42	14.12	13.13
Crude fat	3.44	4.09	4.42	4.78
Crude fiber	22.86	20.54	18.45	15.26
Crude ash	6.88	6.85	6.56	5.89
NFE	51.14	54.11	56.45	60.95
NDF	54.51	51.47	47.15	39.26
ADF	33.15	31.92	29.33	24.35
TDN	70.78	74.01	75.32	78.22

Table 2. Ingredient and chemical composition of energy and protein concentrates

Item	Energy concentrate	Protein concentrate
Ingredient, % (as-fed basis)		
Corn	60.00	0.05
Wheat flour	3.00	5.00
Palm kernel meal	5.00	20.00
DDGS (Dried distillers grains with solubles)	–	4.85
Corn gluten feed	–	24.00
Rapeseed meal	–	6.10
Molasses	–	5.00
Rumen protected fat (Ca soup)	3.00	–
Condensed molassed soluble	–	2.00
Lupin, flake	19.00	–
Wheat bran	10.00	5.00
Coconut meal	–	20.00
Sesame meal	–	8.00
Chemical composition, % DM		
Dry matter (DM)	89.12	89.07
Crude protein	14.12	23.58
Crude fat	6.95	4.65
Carbohydrate	75.91	65.02
Crude ash	3.03	6.75
Crude fiber	7.19	12.17
Ca	0.65	0.30
P	0.35	0.74
NDF	17.98	41.46
ADF	9.48	20.16
TDN	90.71	80.84

2) Proximate composition, cooking loss, shear force, pH, and color

Moisture, crude protein, and crude fat contents were analyzed according to AOAC methods (Association of Official Analytical Chemists, 1996). Cooking loss was determined as the percentage weight loss of a meat sample (60 g) after cooking (Jung et al., 2013a). Three replicate samples from each animal were separately sealed in polyethylene bags, heated in a water bath at 75°C for 30 min, and cooled at room temperature for 30 min. Maximum shear force (kg) was measured according to the method described by Kim and Lee (2003) with some modifications. Each replicate cooking loss sample was cut into a 1.0 × 1.0 × 1.5-cm shape. Shear force was measured using a Warner–Bratzler shear attachment on a texture analyzer (CT3 10K, Brookfield Engineering Laboratories, Middleboro, MA, USA) with a 10-kg load cell and 2.0 mm/min cross-head speed. Each replicate was sheared once across the center of the sample perpendicular to the muscle fibers. The samples (10 g) were homogenized (T10 basic, Ika Works, Staufen, Germany) with distilled water (90 ml) at 1,130 × g for 1 min. pH of the loin meat was measured using a pH meter (SevenGo, Mettler-Toledo International, Inc., Schwerzenbach, Switzerland). Surface-color values (International Commission on Illumination (CIE) L^*, a^*, and b^* values representing lightness, redness, and yellowness, respectively) were measured using a colorimeter (CR-310, Minolta Co., Ltd., Osaka, Japan).

3) Nucleotide content

Nucleotide content was determined according to Jung et al. (2013b). Minced samples (5 g) were mixed with 20mL 0.7 M perchloric acid and homogenized to extract nucleic acids. The homogenate was centrifuged (Union 32R, Hanil Co., Ltd., Seoul, Korea) at 2,290 × g for 15 min at 4°C, filtered through filter paper (Whatman No. 4, Whatman PLC, Maidstone, UK), and the supernatant was adjusted to pH 5 (SevenGo, Mettler-Toledo international Inc.) with 7 N KOH. The supernatant was placed in a volumetric flask and adjusted to 50 mL with 0.7 M perchloric acid (pH 5, adjusted with 7 N KOH). After cooling for 30 min, the mixture was centrifuged at 3,000 rpm for 15 min (4°C), and the supernatant was analyzed by high-performance liquid chromatography (HPLC) (Ultimate 3000, Dionex, Sunnyvale, CA, USA). The analytical conditions for HPLC included a Synergi Hydro-RP 80 Å (250 × 4.6 mm, 4-μm particles; Phenomenex Inc., Seoul, Korea) column eluting 20 mM potassium phosphate monobasic (pH 5) at a 1.0 mL/min flow rate. Injection volume was 10 μL, and elution time was 25 min. Column temperature was maintained at 30°C, and detection was monitored at a wavelength of 254 nm. Nucleotide content was determined from a standard curve obtained using the standards AMP, IMP, inosine, and hypoxanthine (Sigma Aldrich, St. Louis, MO, USA) and calculated by area under each peak.

4) Total and insoluble collagen contents

Total and insoluble collagen contents were measured according to the modified methods of Jayasena et al. (2013). Sample contents were determined after

hydrolyzing 1 g of meat with 25 mL of 6 M HCl at 110°C for 16 h. The hydrolysates were neutralized to pH 2–6 with 6 M NaOH and diluted to 500 mL with distilled water. The hydrolysate (4 mL) and 2 mL of chloramine T solution (1.41-g chloramine T, 10mL distilled water, 10mL 1-propanol, and 80mL citric buffer; pH 6) were mixed in a test tube and left for 20 min at room temperature. Next, 2 mL of 4-dimethyl-aminobenzaldehyde (DABA) solution (10g DABA, 35mL 60% perchloric acid and 65mL 2-propanol) were added. The solutions were shaken and heated at 60°C for 60 min. The samples were cooled for 5 min in tap water and absorbance measured at 558 nm. Hydroxyproline content was determined from a standard curve, and collagen content was calculated from hydroxyproline content using the coefficient 8.

Samples (5 g) to determine insoluble collagen content were homogenized with 24mL Ringer's solution (1L distilled water, 8.6g sodium chloride, 0.3g potassium chloride, and 0.33g calcium chloride) and diluted 1:3 with distilled water. The homogenates were heated for 70 min at 77°C and centrifuged at 2,300 rpm for 30 min. The sediment was mixed with 24mL diluted Ringer's solution and centrifuged again. Next, the sediment was dried at 105°C, and the dried mass was hydrolyzed with 25 mL of 6 M HCl. Collagen content of the sediment was determined as described for total collagen.

5) Free amino acids

The free amino acid composition was determined by modifying the methods described by Jayasena et al. (2014). A defatted meat sample (5 g) was mixed with 20 mL of 2% TCA solution and homogenized at 13,500 rpm for 1 min. The homogenate was centrifuged for 15 min and filtered through 0.45 μm membrane filter. The filtrate was derivatized using the Waters AccQ-Tag method (Millipore Co., Milford, MA, USA), and 5 μL were injected into a reverse phase-HPLC (AccQ Tag column, 3.9 × 150 mm; Waters). The column temperature was 37°C, and a fluorescence detector (Waters 2475) was used with 250- and 395-nm excitation and emission wavelengths. Separation was performed using a gradient of buffers: A (Waters AccQ Tag eluent) and B (60%, v/v, acetonitrile).

6) Sensory evaluation

Beef samples were cut into sections (20 × 50 × 20 mm) and cooked on a preheated clam-type electric grill featuring double heating surfaces (1,400 W, Nova EMG-533, Evergreen Enterprise, Seoul, Korea). The internal temperature was monitored using a digital thermometer (YF-160A Type-K; YFE, Hsinchu City, Taiwan) placed in the center of the meat sample; the samples were removed from the grill after they reached an internal temperature of 72°C. Samples were assessed for their appearance, odor, taste, flavor, texture, juiciness, and overall acceptability. A 9-point hedonic scale, where 9 indicates "extremely like" and 1 indicates "extremely dislike", was employed for evaluating all the parameters. The samples were placed on transparent plastic white dishes and labeled randomly with a 3-digit

numerical code. All samples were provided to each of the panelists along with drinking water for rinsing their oral cavity following testing of each sample. This procedure of sensory evaluation was conducted in three independent experiments.

7) Fatty acid composition

Lipids in beef samples (20 g) were extracted with 200 mL of chloroform/methanol (2:1, v/v) according to the procedure of Folch et al. (1957). Extracted lipids were evaporated using N_2 gas (99.99%) and 1 g mixed with 2 mL of BF_3-methanol (14%, w/w) before being heated in a water bath (60°C) for 1 h. After cooling, hexane (2 mL) and deionized distilled water (5 mL) were added and centrifuged at 3,100 rpm for 10 min (HM-150IV, Hanil Co. Ltd., Korea). Next, the top hexane layer containing fatty acid methyl esters (FAME) was transferred to a vial, then the 1 μL of FAME in hexane was injected into a gas chromatograph (HP 7890, Agilent Technologies, Santa Clara, CA, USA). A split inlet (split ratio, 50:1) was used to inject the samples into a capillary column (SP^{TM} 2560 Capillary column; 100 m × 0.25 mm × 0.20 μm film thickness), and a ramped oven temperature was used (100°C for 5 min, increased to 240°C at 4°C/min and maintained for 20 min). The inlet temperature was 210°C. N_2 served as the carrier gas at a constant flow rate of 1 mL/min. Individual FAME were identified by comparison of the relative retention times of peaks from samples, with those of the standard mixture 37 component FAME Mix (Supelco, Bellefonte, PA). The FA composition of fat was

calculated based on the peak area. Relative quantities were expressed as weight percent of total FA.

Fatty acid contents (values per 100 g of beef) are also useful. When fatty acid contents are being calculated, the fact that the total fat in a food includes triglycerides (of which a proportion is glycerol, i.e. not fatty acid), phospholipids, and unsaponifiable components such as sterols should be considered. Thus, a conversion factor (0.953; 0.953 g total FA/g fat) was applied to obtain the individual FA content/100 g meat from FA percentage values and crude fat content, based on a previous report (Anderson et al., 1975). The conversion factor was also used for calculating FA content from FA percentage in other studies (Brugiapaglia et al., 2014; Horcada et al., 2016). Individual FA g/100 g meat was calculated as follows:

Individual FA content (g FA/100 g meat) = crude fat content (g fat/100 g meat) x individual FA percentage (%) x 0.953 (g FA/g fat)

8) Volatile compounds

To analyze the volatile compounds, samples were prepared according to the method described by Garcia-Esteban et al. (2004). Samples were thawed at 4℃, and 4 g of each sample was homogenized (IKA Works T10 basic homogenizer, Wilmington, NC, USA) with saturated 12 mL of NaCl solution at speed no. 6 for 1 min. In order to reduce the loss of flavor compounds during cooking, the 10 ml of homogenate was transferred to individually labeled 20 mL clear glass vial

(PerkinElmer® N9306078) and closed with a polytetrafluoroethylene septa and screw cap. Then, the samples were heated using a water bath (Thermo-minder Sm-05, Taitec, Tokyo, Japan) at 80°C for 30 min and then cooled in cold water. The vials were placed in the oven of the head-space sampler, and the extraction of the volatile compounds of the samples was performed using a headspace auto sampler. The transfer line from the headspace sampler was directly connected to the injector of the gas chromatograph (GC).

A PerkinElmer 680 GC (Perkin Elmer, Boston, Massachusetts, USA) equipped with a 600T MS detector was used. Volatiles were separated using a HP-PLOT Q column (30 m × 0.53 mm × 0.25 μm film thickness, Agilent, Wilmington, DE, USA). GC conditions were: initial oven temperature 35°C, held for 5 min, then programmed to 180°C at 7°C/min, then held for 0.0 min at 180°C, then increased 5°C/min to 250°C and held for 21.0 min. The transfer line temperature was maintained at 250°C. A mass spectrometer scanned from *m/z* 30 to *m/z* 250 at 0.2 s cycle time. The ion source was set at 250°C. Headspace was maintained at 85°C for 30 min and a ramped oven temperature was used (50°C for 3 min, increased to 240°C at 5°C/min and maintained for 9 min). The inlet temperature was 210°C. He served as the carrier gas at a constant flow rate of 20 mL/min. The resolved MS spectra obtained from the custom scripts were matched against reference mass spectra by using the National Institute of Standards and Technology (NIST) mass spectral search program for the NIST/EPA/NIH mass spectral library (version 2.0).

In the present study, internal standard for the volatile compounds was not used. GC chromatogram was used to quantify the volatile compounds, and the mass spectrometry was used to identify the volatile compounds. Results of volatile analyses were expressed as percentage of total chromatographic area.

9) Statistical analysis

The carcass characteristics, chemical compositions, physicochemical traits, sensory evaluation, nucleotide composition, collagen content, and free-amino acid data were analyzed by analysis of variance using SAS software (SAS Institute, Cary, NC, USA), using the General Linear Model Procedure (Proc GLM). Data of fatty acid and volatile compounds were analyzed by two-way analysis of variance to test the fixed effects of QG, cut type and their interaction by a generalized linear mixed model, using the Proc GLM procedure of SAS software (SAS Institute, Cary, NC, USA). Experimental unit was an individual animal for FA composition and volatile compound. Animal within QG or cut type was considered the random variable. The LSMEANS PDIFF option was used to compare differences among mean values at $P < 0.05$. Correlation coefficients were calculated using the SAS CORR procedure.

4. Result and discussion

1) Carcass characteristics of Korean cattle steers

The carcass weight of Korean cattle steers was highest ($P < 0.05$) in QG 1++ and lowest in QG 1+ (Table 3). Fat thickness and slaughtering age were lowest ($P < 0.05$) in QG 2, and no differences were observed among the other QGs (1++, 1+ and 1). *Longissimus* muscle (LM) area and MS were highest ($P < 0.05$) in QG 1++, and lowest in QG 2. Texture score, evaluated as 1 (fine) to 3 (coarse) by an official meat grade, was highest ($P < 0.05$) in QG 2, and no difference in the texture was observed among the other QGs (1++, 1+ and 1). Meat color, fat color, maturity, yield index, and yield grade did not differ ($P > 0.05$) among the four QGs. Our results indicate that MS were higher with better QG, whereas texture score was lower with better QG 2. These results agree with those of Cho et al. (2010), who reported that carcass weight was highest in QG 1++ and texture was highest in QG 2 in Korean cattle steers. Moon et al. (2003) also found that carcass weight and LM area increased with increasing QG; however, texture decreased with increasing QG. Overall, beef with the best QG revealed the highest MS but the lowest texture. MS was strongly correlated ($r = 0.98$) with QG, whereas texture was negatively correlated ($r = -0.78$) with QG. Similar strong MS with QG correlations have been reported previously (Dow et al., 2011). In Korean cattle, beef with higher marbling score revealed better quality grade (Lee et al., 2012).

Our results confirm that MS is a major determinant of QG.

Table 3. Means and standard errors of slaughter age and carcass characteristics of Korean cattle steers with different quality grades examined by an official meat grader

Item	Korean quality grade				SEM[7]	*P*-value
	1++	1+	1	2		
Slaughtering age (month)	32.4[a]	32.3[a]	33.5[a]	30.2[b]	0.36	0.01
Carcass weight (kg)	441[a]	397[b]	429[ab]	402[b]	6.45	0.05
LM area (㎠)	95.2[a]	83.3[bc]	88.3[b]	81.2[c]	1.38	0.01
Fat thickness (㎜)	14.7[a]	14.6[a]	15.6[a]	10.4[b]	0.79	0.03
Marbling score[1]	8.42[a]	6.17[b]	4.50[c]	2.58[d]	0.32	<0.01
Yield index	64.0	63.6	62.9	65.8	0.53	0.09
Yield grade[2]	19.2	18.3	15.8	21.7	1.06	0.06
Meat color[3]	4.50	4.33	4.42	4.67	0.07	0.50
Fat color[4]	3.00	3.00	3.00	3.00	0.00	-
Texture[5]	1.00[b]	1.00[b]	1.00[b]	2.00[a]	0.06	<0.01
Maturity[6]	2.00	2.08	2.25	2.00	0.04	0.06

n=12

[1] Marbling score: 1, devoid; 9, very abundant.

[2] Yield grade: A, 30; B, 20; C, 10.

[3] Meat color: 1, bright red; 7, dark red.

[4] Fat color: 1, white; 7 yellowish.

[5] Texture: 1, very fine; 3, very coarse.

[6] Maturity: 1, youthful; 9, mature.

[7] SEM is the standard error of the means.

[a-d] Means with different letter within a same row differ at P<0.05.

2) Chemical composition and physicochemical traits

Crude protein and moisture contents of loin were highest ($P < 0.05$) in QG 2, and lowest in QG 1++ (Table 4). However, crude fat content of loin was highest ($P < 0.05$) in QG 1++, and lowest in QG 2. Crude protein content in the rump was also highest ($P < 0.05$) in QG 2, and lowest in QG 1++. Crude fat content in the rump was highest ($P < 0.05$) in QG 1++. In a comparison by cut type, crude fat content was higher ($P < 0.01$) in loin than that in rump, but both crude protein and moisture contents were higher ($P < 0.05$) in rump than those in loin. Other studies have also reported an inverse relationship between moisture content and crude fat content in bovine muscle (Cho et al., 2010; Legako et al., 2014). Fat content was markedly higher in loin than that in rump, whereas protein content was lower in loin than that in rump. Cho et al. (2008) also showed that fat content was highest but protein content was lowest in loin among other cuts, including the short plate, top sirloin, striploin, chunk tender, eye of round, chunk roll, bottom round, rump, and brisket in Korean cattle steers. Our correlation analysis revealed that fat content in loin was strongly correlated ($r = 0.73$) with QG. Our results confirm that intramuscular fat (IMF) content is a major contributor to QG.

CIE L^* (lightness), a^* (redness), and b^* (yellowness) values of loin were highest ($P < 0.05$) in QG 1++, and lowest in QG 2. CIE a^* and b^* values in rump were highest ($P < 0.05$) in QG 1+, whereas the L^* value did not differ ($P > 0.05$)

among the four QGs. Meat color is important when selecting fresh meat at the market. The CIE L^*, a^*, and b^* values in loin were highest in QG 1++, which is consistent with a study reporting the highest L^*, a^*, and b^* values in QG 1++, among all QGs in Korean cattle steers (Lim et al., 2014). All CIE values were higher ($P < 0.01$) in loin than those in rump. Previous study also reported that the L* value is higher in loin than that in rump from Korean cattle (Cho et al., 2013). Our correlation analysis revealed that the L^*, a^*, and b^* values were correlated with QG, MS, and fat content in loin. Therefore, higher L^*, a^*, and b^* values in a good QG loin may reflect higher fat content. Sarriés and Beriain (2006) reported that IMF contributes the increase in b^*-value leading to increasing L^*-value in a positive relationship. A similar association between meat color values and IMF content has been reported (Jo et al., 2013).

Cooking loss of loin was highest ($P < 0.05$) in QG 2, and no difference in cooking loss was detected among the other QGs (1++, 1+ and 1). This result agrees with previous study showing greater cooking loss in lower QG loin from Korean cattle (Moon et al., 2006). Ozawa et al. (2000) reported that cooking loss of *longissimus thoracis* with a high MS was significantly lower than that with a low MS in Japanese black steers (Wagyu). Cooking loss of rump did not differ ($P > 0.05$) among the four QGs. Shear force of loin did not differ ($P > 0.05$) among the four QGs, but the numeric shear force value was lowest in QG 1++. Shear force of rump was lowest ($P < 0.05$) in QG 2, and no difference in shear force was observed

among the other QGs (1++, 1+ and 1). Both cooking loss and shear force were higher ($P < 0.01$) in rump than those in loin. Another study also reported that cooking loss is significantly lower in loin than that in several other cuts of Korean cattle (Cho et al., 2013). Our correlation analysis revealed that cooking loss was negatively correlated with loin fat content. Therefore, our results suggest that greater cooking loss with lower QG is due, in part, to lower fat content. Shear force values tended to be lowest in QG 1++, but the difference was not significant. Shear force was markedly lower in loin than that in rump. Several studies have reported that shear force in Korean cattle is lower in loin with a better QG (Moon et al., 2006; Lim et al., 2014).

Table 4. pH, chemical composition, cooking loss, meat color (CIE *L**, *a**, and *b**), and Warner-Bratzler shear force of loin and rump with different quality grades in Korean cattle steers

	Loin					Rump					*P*-value		
Item	1++	1+	1	2	SEM[1]	1++	1+	1	2	SEM	Grade	Cut	Grade*Cut
pH	5.47^{b}	5.48^{b}	5.51^{ab}	5.55^{a}	0.01	5.50	5.51	5.51	5.49	0.01	0.39	0.83	0.29
Protein (%)	17.9^{c}	19.7^{b}	20.8^{a}	21.3^{a}	0.25	21.4^{b}	21.5^{b}	22.9^{a}	23.0^{a}	0.16	<0.0001	<0.0001	0.007
Fat (%)	23.8^{a}	17.3^{b}	14.1^{bc}	9.90^{c}	1.00	6.20^{a}	4.09^{ab}	2.78^{b}	3.60^{b}	0.41	<0.0001	<0.0001	<0.0001
Moisture (%)	55.6^{c}	60.6^{b}	63.6^{b}	67.1^{a}	0.81	70.2	71.4	72.1	71.1	0.30	<0.0001	<0.0001	<0.0001
Cooking loss (%)	25.5^{b}	27.5^{b}	26.9^{b}	34.5^{a}	1.17	34.4	34.2	34.2	32.8	0.45	0.13	<0.0001	0.01
Shear force (kg)	3.83	4.79	5.39	5.23	0.26	13.7^{a}	14.0^{a}	15.1^{a}	9.30^{b}	0.65	0.01	<0.0001	0.0037
CIE *L**	43.6^{a}	41.4^{b}	40.4^{b}	40.1^{b}	0.32	39.6	39.6	38.9	39.3	0.15	<0.0001	<0.0001	0.003
CIE *a**	16.0^{a}	15.6^{a}	14.5^{ab}	13.8^{b}	0.31	11.6^{ab}	12.2^{a}	10.9^{b}	10.8^{b}	0.18	0.0012	<0.0001	0.53
CIE *b**	4.68^{a}	4.09^{a}	3.15^{b}	2.61^{b}	0.18	1.24^{a}	1.47^{a}	0.65^{b}	1.34^{a}	0.09	<0.0001	<0.0001	<0.0001

n=12

[1] SEM is the standard error of the means.
[a,b,c] Means with different letter within a same row in each cut differ at P<0.05.

3) Sensory evaluation

The sensory characteristics, including flavor, tenderness, juiciness, and overall acceptability did not differ among the four QGs in loin and rump (Table 5). Numeric values of tenderness, juiciness, and overall acceptability in loin were highest in QG1++, and those of juiciness and overall acceptability were lowest in QG 2. All sensory characteristics were higher ($P < 0.05$) in loin than those in rump. Previous studies reported that flavor, tenderness, juiciness, and overall acceptability of loin were better in higher QGs (Jo et al., 2013; Lim et al., 2014). The juiciness and overall acceptability were positively correlated with QG, MS, and fat content in loin. All sensory characteristics were higher in loin than those in rump. Similar findings were observed by Legako (2014), who showed higher sensory characteristics in *longissimus lumborum* than those in *gluteus medius* muscle. Previous study has indicated that tenderness and juiciness are positively affected by IMF content (Renand et al., 2001). Therefore, the higher tenderness, juiciness, and overall acceptability values in QG 1++ and the higher sensory traits in loin than those in rump may be, in part, due to higher fat content. Overall, our results demonstrate that QG is tightly linked to sensory traits.

Table 5. Sensory characteristics of loin and rump with different quality grades in Korean cattle steers

	Loin					Rump					*P*-value		
Item[1]	1++	1+	1	2	SEM[2]	1++	1+	1	2	SEM	Grade	Cut	Grade*Cut
Appearance	6.45	6.45	6.38	6.37	0.07	6.17	5.70	4.85	5.00	0.26	0.20	0.0028	0.28
Odor	6.00	5.95	6.30	5.70	0.19	4.80	4.45	4.30	3.90	0.21	0.59	0.001	0.83
Taste	6.55	6.30	6.67	5.95	0.18	4.10	3.95	4.15	3.50	0.24	0.56	0.0001	0.99
Flavor	6.35	6.05	6.45	6.00	0.16	4.00	3.79	4.10	3.17	0.25	0.51	0.001	0.92
Tenderness	7.20	5.95	6.65	6.25	0.27	3.60	3.13	3.85	2.95	0.33	0.51	0.001	0.91
Juiciness	6.70	5.70	5.95	5.55	0.24	3.50	3.45	3.60	2.85	0.26	0.46	0.001	0.82
Overall acceptability	6.95	6.30	6.50	5.95	0.23	3.65	3.55	3.95	3.15	0.23	0.49	0.001	0.90

n=3

[1] The score was evaluated with 10 semi-trained panelists (1, extremely dislike; 5, neither dislike nor like; 9, extremely like).

[2] SEM is the standard error of the means.

4) Nucleotide content

Nucleotide content is often considered as an important meat palatability factor as IMP enhances an umami taste (Aliani and Farmer, 2005). AMP and IMP contents were not different among the four QGs in loin and rump (Table 6). Similarly, Lim et al. (2014) reported that AMP content in Korean cattle loin does not differ among QGs (1++, 1+, 1, and 2). However, in their study, IMP content increased with decreasing QG. Inosine contents of loin and rump were not different among the QGs. Hypoxanthine content of loin was highest ($P < 0.05$) in QG 1, and lowest in QG 1++. Similarly, hypoxanthine content of rump was highest ($P < 0.05$) in QG 2, but did not differ among the other three QGs (1++, 1+ and 1). IMP content was higher ($P < 0.01$) in rump than that in loin, whereas hypoxanthine content was higher ($P < 0.05$) in loin than that in rump. This is in agreement with other study showing lower IMP content in loin than that in rump (Cho et al., 2007). Hypoxanthine is an important component in the formation of meat flavor and may impart a bitter flavor to the meat by conjugating with other free amino acids and some dipeptides (Tikk et al., 2006). Correlation analyses revealed that none of the nucleotides (AMP, IMP, inosine, or hypoxanthine) were correlated with any of the sensory traits. Thus, nucleotide content in beef may not be a major factor determining meat palatability.

Table 6. Nucleotides contents (mg/100g) of loin and rump with different quality grades in Korean cattle steers

	Loin					Rump					*P*-value		
Nucleotide	1++	1+	1	2	SEM[2]	1++	1+	1	2	SEM	Grade	Cut	Grade*Cut
AMP[1]	2.86	2.68	3.05	3.05	0.08	2.37	2.35	2.05	2.75	0.11	0.16	0.0001	0.22
IMP[2]	120	135	116	104	6.50	196	184	189	173	5.16	0.27	0.0001	0.66
Inosine	65.7^{b}	77.0^{a}	80.2^{a}	78.3^{a}	1.18	66.8^{b}	74.5^{b}	67.6^{b}	88.9^{a}	1.92	0.0001	0.62	0.0001
Hypoxanthine	2.62^{c}	4.82^{bc}	8.48^{a}	8.10^{ab}	0.79	2.24^{b}	3.57^{b}	1.95^{b}	6.27^{a}	0.42	0.001	0.003	0.04

n=12

[1] SEM is the standard error of the means.

[a-d] Means with different letter within a same row in each cut differ at $P<0.05$.

5) Collagen contents

Total, soluble, and insoluble collagen contents of loin were highest ($p<0.05$) in QG 1++, and lowest in QG 1 (Table 7). All three types of collagen in rump were highest ($p<0.05$) in QG 1+, and lowest in QG 1. All three types of collagen were higher ($p<0.01$) in rump than those in loin. Muscle collagen content affects meat tenderness (Kim and Lee, 2003). Our correlation analyses revealed that content of all three collagens was correlated with sensory traits, including tenderness, juiciness, and overall acceptability. The relationship between collagen contents and tenderness is controversial; another study show no relationship between collagen contents and tenderness (Field et al., 1997), whereas others have shown lower collagen contents with better QG (Ryu et al., 1994).

Table 7. Collagen contents (mg/g) of loin and rump with different quality grades in Korean cattle steers

	Loin					Rump					*P*-value		
Collagen	1++	1+	1	2	SEM[1]	1++	1+	1	2	SEM	Grade	Cut	Grade*Cut
Total collagen	5.93^{a}	4.76^{bc}	3.90^{c}	5.04^{ab}	0.18	9.23^{a}	10.29^{a}	5.12^{c}	7.37^{b}	0.40	0.0001	0.0001	0.0001
Soluble collagen	2.45^{a}	2.06^{b}	1.64^{c}	2.13^{ab}	0.08	3.73^{b}	4.55^{a}	2.15^{d}	2.96^{c}	0.19	0.0001	0.0001	0.0001
Insoluble collagen	3.48^{a}	2.70^{bc}	2.26^{c}	2.91^{ab}	0.12	5.50^{a}	5.75^{a}	2.97^{c}	4.41^{b}	0.23	0.0001	0.0001	0.001

n=12

[1] SEM is the standard error of the means.

[a-d] Means with different letter within a same row in each cut differ at P<0.05.

6) Free amino acid contents

In the case of free amino acids, isoleucine, leucine, and tyrosine contents in loin were highest in QG 1++ and lowest in QG 2 (Table 8). In addition, glutamic acid content did not differ significantly among the four QGs in loin or rump. In addition, the glutamic acid, isoleucine, leucine, lysine, serine, tyrosine, and valine contents were higher in loin than those in rump, whereas arginine, cysteine, glycine, histidine, and threonine contents were higher in rump than those in loin. Alanine, arginine, cysteine, glycine, lysine, methionine, phenylalanine, serine, threonine, and valine contents did not differ among the four QGs. Lim et al. (2014) reported that threonine and alanine contents in Korean cattle loin were highest in QG 2 among QGs (1++, 1+, 1, and 2). Free amino acids can influence flavor, taste, and aroma formation in meat during storage (Jo et al., 2012). Our correlation analyses revealed that isoleucine, leucine, lysine, methionine, tyrosine, and valine contents in loin were correlated with flavor. In addition, some amino acids, such as glutamic acid and proline, were significantly associated with sensory traits, including tenderness, juiciness, and overall acceptability. Therefore, our results partially match with a previous report on the association between amino acid content and beef taste. In that study, asparagine, serine, threonine, glycine, alanine, and glutamic acid were related to a sweet taste, whereas isoleucine, leucine, valine, methionine, phenylalanine, histidine, arginine, and proline were related to a bitter taste in meat (Sforza et al., 2001). Shahidi et al. (1986) reported that the particular amino acids, such as

glutamic acid, may function as flavor enhancers, to enhance the flavor contribution of other agents. Jo et al. (2012) reported similarly that glutamic acid can enhance an umami taste by reacting with IMP. Further study is required to understand the functional significance of the relationships between individual amino acids and beef palatability.

Table 8. The concentration (mg/100g) of free amino acid of loin and rump with different quality grades in Korean cattle steers

	Loin					Rump					*P*-value		
Amino acid	1++	1+	1	2	SEM[1]	1++	1+	1	2	SEM	Grade	Cut	Grade*Cut
Alanine	14.7	14.4	14.1	15.9	0.36	15.6	15.4	16.1	14.7	0.32	0.94	0.16	0.14
Arginine	93.9	96.9	102	107	4.20	126	134	139	130	2.57	0.45	0.0001	0.72
Cysteine	3.04	2.46	2.50	2.75	0.10	3.15	2.96	2.80	2.96	0.09	0.09	0.04	0.76
Glutamic acid	7.20	6.47	6.91	7.10	0.26	5.11	5.49	5.70	6.13	0.15	0.48	0.0001	0.50
Glycine	4.83	4.73	5.03	5.32	0.08	5.13^{b}	5.34^{ab}	5.82^{a}	5.44^{ab}	0.09	0.009	0.0001	0.16
Histidine	18.4^{b}	18.5^{b}	19.9^{b}	31.8^{a}	1.42	26.4	27.8	31.6	24.9	0.92	0.02	0.0003	0.0001
Isoleucine	5.92^{a}	5.58^{ab}	5.70^{ab}	5.38^{b}	0.07	4.97^{c}	4.99^{c}	5.33^{b}	5.74^{a}	0.07	0.12	0.0001	0.0001
Leucine	9.43^{a}	8.48^{bc}	8.91^{ab}	8.02^{c}	0.14	7.17^{bc}	6.63^{c}	7.86^{ab}	8.71^{a}	0.22	0.03	0.0001	0.0001
Lysine	9.20	8.13	8.44	8.09	0.20	6.02	5.70	6.74	7.28	0.33	0.45	0.0001	0.15
Methionine	6.23	5.96	6.11	5.91	0.05	5.57^{b}	5.67^{b}	5.89^{b}	6.39^{a}	0.07	0.02	0.04	0.0001
Phenylalanine	7.67	6.97	6.91	6.84	0.17	6.39^{c}	6.42^{c}	6.89^{b}	7.42^{a}	0.10	0.37	0.09	0.0042
Proline	3.91^{a}	3.74^{ab}	3.67^{b}	3.87^{a}	0.03	3.69	3.65	3.82	3.80	0.03	0.08	0.14	0.02
Serine	7.27	6.95	7.13	7.15	0.12	5.79	6.31	6.80	6.97	0.18	0.22	0.0021	0.13
Threonine	21.2	20.8	23.1	19.3	1.07	25.7	25.3	25.9	30.1	1.08	0.85	0.0004	0.27
Tyrosine	8.24^{a}	7.70^{ab}	8.19^{a}	7.00^{b}	0.14	6.55^{b}	6.65^{b}	6.97^{b}	7.89^{a}	0.12	0.33	0.0001	0.0001
Valine	7.04	6.48	6.72	6.33	0.10	5.40^{b}	5.47^{b}	5.83^{b}	6.56^{a}	0.11	0.08	0.0001	0.0001

n=12

[1] SEM is the standard error of the means.

[a-c] Means with different letter within a same row in each cut differ at P<0.05.

7) Fatty acid compositions in loin and rump fat

In the current study, thirteen FA were detected and quantified in loin and rump fat muscles of Korean cattle steers (Table 9). Of these, the percentage of oleic acid (C18:1n9) was the highest in both loin and rump, ranging from 42% to 47%, followed by palmitic (C16:0) (25–27.4%) and stearic acid (C18:0) (9.3–10.4%). Our results are consistent with those of previous studies that reported C18:1n9 content was the highest among FA in beef from Korean cattle (Lee et al., 2010; Kim et al., 2009), American Angus (St. John et al., 1987), and Japanese Wagyu (Oka et al., 2002).

QG x cut type interactions were observed (P=0.01) for the percentage of C18:1n9 and monounsaturated fatty acid (MUFA). This indicates that C18:1n9 and MUFA percentages were differently associated with QG depending on cut type. The percentages of C18:1n9 and MUFA from loin fat were the highest ($P < 0.05$) in the QG1++ group and lowest ($P < 0.05$) in the QG2 group, although there were no statistical differences among the QG1++, QG1+, and QG1 groups. C18:1n9 and MUFA percentages from rump fat were not different between QG1++ and QG2, resulting in significant interaction between QG and cut type. Our results are in accordance with those of Hunt et al. (2016), who reported that QG differentially affects C18:1n9 percentage, depending on the muscle type. Lee et al. (2010) showed that numerical values of C18:1n9 were highest in QG1++ group among QGs for

loin of Korean cattle beef. Studies showed that C18:1n9 and MUFA percentages increased with increased fat or QG in the longissimus thoracis (Aldai et al., 2006). Similarly, heavy and medium carcass weight group had both higher IMF and C18:1n9 content (Jayasena et al., 2015). In our previous study using the same meat samples, we reported that the loin showed higher fat content and wider differences in fat contents among QGs than those observed in rump (Table 10; Piao et al., 2015). Thus, the significant interaction between QG and cut type for C18:1n9 and MUFA percentages observed in this study is most likely attributable to the differences in fat contents among QGs and between cut type.

A QG x cut type interaction was also observed ($P \leq 0.01$) for the percentages of pentadecanoic acid (C15:0), heptadecanoic acid (C17:0), linoleic acid (C18:2n6), cis-8,11,14-eicosatrienoic acid (C20:3n6), cis-5,8,11,14-eicosatetraenoic acid (C20:5), MUFA, PUFA, and PUFA/SFA, indicating that these FA percentages were also differently associated with QG, depending on the cut type. The percentages of fatty acids, 15:0, C17:0, C18:2n6, C20:3n6, C20:5, and sum of PUFA in loin fat were lowest ($P < 0.05$) in the QG1++ group and highest in the QG2, whereas the percentages of these FA in rump fat were lowest ($P < 0.05$) in the QG1 group. Our results were in accordance with a previous study that reported an increase in the percentage of MUFA, but a decrease in the percentage of PUFA, with increasing QG in loin (Legako et al., 2015). In addition, the accumulation of MUFA was higher

than that of PUFA as the crude fat increased, and thus, the percentage of MUFA increased (Raes et al., 2004a).

In this study, percentages of C16:0, C18:0, SFA, and USFA did not differ among QGs in either loin or rump fat. Lee et al. (2010) also reported no differences in these FA among the four QGs in the loin and rump. We observed that the C18:1n9 percentage in loin increased with increasing QG with no change in C18:0 percentage. Stearoyl-CoA desaturase (SCD) is the rate-limiting enzyme for MUFA (C16:1, C18:1n9) synthesis from SFA (C16:0, C18:0) (Ntambi, 1999), and it is important for oleic acid accumulation in both muscle and adipose tissues of ruminants (Wood et al., 2008). For example, the increase in oleic acid and MUFA was associated with higher SCD activity (Wang et al., 2005). Increased SCD1 expression in obese humans compared with lean subjects was also correlated with increased C18:1n9, and lower C16:0 and C18:0 (Hulver et al., 2005). However, FA uptake from dietary sources may also contribute to oleic acid accumulation, in addition to endogenous MUFA production by SCD from SFA, although the extent of MUFA accumulation from each of these two sources is not known. Our gene expression study also indicates that combined effects of de novo FA synthesis, FA uptake, and FA esterification contribute to IMF deposition in steers (Jeong et al., 2012, 2013). Archibeque et al. (2005) reported that differences in SFA seemed to be independent of SCD enzyme activity in both subcutaneous fat and the IMF tissues of beef steers. They suggested that duodenal concentrations of fatty acids were more

important in determining tissue fatty acid concentrations than endogenous desaturation by SCD. Thus, the increase in C18:1n9 percentage with increasing QG, with no change in C18:0 content in the loin, may be either because SCD activity was not enough for a change in C18:0 content or because the dietary origin of C18:1n9 contributed to increased C18:1n9 percentage.

In the comparison by cut type, the percentages C16:0, C18:0, C18:1n9, and MUFA were higher ($P < 0.05$), but those of C15:0, C17:0 and PUFA were lower ($P < 0.05$) in the loin fat than in the rump fat. Similar results regarding the content of these FA between cuts were reported by Schönfeldt et al. (2010). A recent study suggested that the differences in SFA and PUFA contents between cuts are correlated with the amounts of phospholipids and triacylglycerol in different locations (de Oliveira et al., 2015).

Table 9. Fatty acid composition (% of fat, fresh basis) of loin and rump with different quality grades (QG) for Korean cattle steers

	Loin					Rump					*P*-value		
Item	1++	1+	1	2	SEM	1++	1+	1	2	SEM	Grade	Cut	Grade*Cut
C14:0	3.53	3.57	3.83	3.39	0.10	2.71^{b}	2.54^{b}	3.66^{a}	2.60^{b}	0.14	0.01	0.001	0.29
C15:0	0.76^{c}	1.18^{b}	1.26^{b}	1.81^{a}	0.08	2.30^{a}	2.49^{a}	1.50^{b}	2.31^{a}	0.13	0.003	0.001	0.002
C16:0	27.41	27.03	26.88	25.79	0.28	24.59	25.41	26.57	25.76	0.35	0.48	0.01	0.11
C16:1	5.11	4.85	5.38	4.66	0.13	4.98	4.61	5.27	4.40	0.19	0.08	0.42	0.99
C17:0	0.76^{b}	0.66^{b}	0.98^{b}	1.37^{a}	0.07	1.62^{a}	1.76^{a}	1.01^{b}	1.45^{a}	0.08	0.03	0.001	0.001
C17:1	0.61	0.57	0.62	0.61	0.01	0.61	0.57	0.61	0.61	0.01	0.04	0.85	0.99
C18:0	10.44	10.26	9.74	10.34	0.18	9.39	9.26	9.69	9.97	0.17	0.59	0.02	0.41
C18:1n9t	0.84	0.76	0.80	0.88	0.03	0.64	0.60	0.75	0.79	0.03	0.051	0.002	0.57
C18:1n9	46.56^{a}	45.60^{a}	44.45^{ab}	43.14^{b}	0.38	42.41	41.50	44.06	42.16	0.36	0.04	0.001	0.01
C18:2n6	2.77^{c}	3.74^{b}	4.10^{b}	5.25^{a}	0.20	6.91^{a}	7.09^{a}	4.63^{b}	6.22^{ab}	0.30	0.02	0.001	0.001
C18:3n6	0.28^{a}	0.23^{ab}	0.20^{b}	0.22^{b}	0.01	0.23	0.19	0.19	0.24	0.01	0.02	0.11	0.22
C20:3n6	0.29^{c}	0.50^{b}	0.51^{b}	0.70^{a}	0.03	1.00^{a}	1.07^{a}	0.60^{b}	0.90^{a}	0.05	0.01	0.001	0.002
C20:5	0.63^{c}	1.05^{b}	1.23^{b}	1.84^{a}	0.09	2.61^{a}	2.92^{a}	1.46^{b}	2.60^{a}	0.14	0.001	0.001	0.001
SFA[1]	42.9	42.7	42.7	42.7	0.33	40.6	41.5	42.4	42.1	0.30	0.60	0.02	0.41
USFA[2]	57.1	57.3	57.3	57.3	0.33	59.4	58.5	57.6	57.9	0.30	0.60	0.02	0.41

MUFA[3]	53.1[a]	51.8[a]	51.3[ab]	49.3[b]	0.41	48.6[ab]	47.3[b]	50.7[a]	47.9[b]	0.41	0.01	0.001	0.01
PUFA[4]	3.97[c]	5.52[b]	6.04[b]	8.02[a]	0.30	10.8[a]	11.3[a]	6.88[b]	9.97[a]	0.49	0.002	0.001	0.001
MUFA/SFA	1.24	1.22	1.20	1.16	0.02	1.20	1.14	1.20	1.14	0.01	0.18	0.11	0.70
PUFA/SFA	0.09[c]	0.13[b]	0.14[b]	0.19[a]	0.01	0.27[a]	0.27[a]	0.16[b]	0.24[a]	0.01	0.01	0.001	0.001

n=12

[1] SFA (saturated fatty acids) = C14:0 + C15:0 + C16:0 + C17:0 + C18:0.

[2] USFA (unsaturated fatty acids) = C16:1 + C17:1 + C18:1n9t + C18:1n9 + C18:2n6 + C18:3n6 + C20:3n6 + C20:5.

[3] MUFA (monounsaturated fatty acids) = C16:1 + C17:1 + C18:1n9t + C18:1n9.

[4] PUFA (polyunsaturated fatty acids) = C18:2n6 + C18:3n6 + C20:3n6 + C20:5.

[a-c] Means with different letters within the same row in each cut differ ($P < 0.05$).

8) Fatty acid content of meat

Using the FA composition, the individual FA content (g FA/100 g loin or rump) was calculated based on a conversion factor (0.953, Anderson et al., 1975), as shown in Table 10. Among all FA contents, C18:1n9 content in loin meat was the highest, ranging from 4.13 to 10.6 g/100 g meat, followed by C16:0 (2.44–6.24 g/100 g meat) and C18:0 (0.94–2.38 g/100 g meat). Our results were in accordance with a previous study using sirloins of Angus beef (Prieto et al., 2010).

Interactions between QG and cut type were observed ($P < 0.01$) for contents of most FAs (Table 10). Contents (g/100 g meat) of palmitoleic acid (C16:1), C17:1, C18:0, C18:1n9, SFA, USFA, and MUFA were significantly influenced by QGs, but to different extents depending on the cut type, revealing a QG × cut type interaction. In loin meat, contents of C16:1, C17:1, C18:0, C18:1n9, SFA, USFA, and MUFA were highest ($P < 0.05$) in the QG1++ group and lowest in the QG2 group (Table 10). However, in rump meat, contents of these FAs were highest ($P < 0.01$) in the QG1++, but did not differ among other QGs (QG1+, QG1, QG2). In this study, C18:1n9 contents in loin muscles ranged widely between 10.57 and 4.13 g/100 g meat among the four QGs, whereas those in the rump muscles showed a narrower range (between 2.57 and 1.43 g/100 g meat). The differential degree of changes in FA contents among QGs depending on the cut type indicated significant interactions of these FAs between QGs and cut types.

Interactions between QGs and cut types were also observed (P = 0.001) for myristic acid (C14:0), C16:0, and C18:1 n9t contents (Table 10). Contents of C14:0, C16:0, and C18:1n9t in loin meat were highest ($P < 0.05$) in the QG1++ group and lowest in the QG2 group (Table 10), whereas contents of these FAs in rump meat did not differ among QGs.

In this study, we found significant interactions between QGs and cut types for many FAs. Loin fat contents decreased as the QG decreased from QG1++ to QG2, whereas rump fat contents were highest in QG1++, but similar among other QGs (QG1+, QG1, and QG2) (Table 10; Piao et al., 2015). Loin meat (16.3%: average of four QGs) had significantly higher fat content than did rump meat (4.2%: average of four QGs). Furthermore, loin meat (between 23.8% and 9.9%) had wider range of fat contents than did rump meat (between 6.2% and 2.78%) (Table 10; Piao et al., 2015). Thus, these differential extents of fat contents among QGs depending on cut type may cause significant interactions of FAs between QGs and cut types.

In this study, we found higher SFA and MUFA contents, but not PUFA contents, in QG1++ than in QG2 in both loin and rump meats. Total fat in a food includes triglycerides, phospholipids and other minor proportion of unsaponifiable components such as steroids. Triglycerides are mainly composed of SFA and MUFA, while phospholipids contain high amounts of PUFA (Raes et al., 2004b). Generally, the amount of triglycerides increased with an increase in total lipid content, while the amount of phospholipids was fairly constant (Wood et al., 2008). Thus, higher

SFA and MUFA, but not PUFA, found in the QG1++ meats are likely due to higher fat contents in this study.

In the comparison by cut type, all FA contents were higher ($P < 0.001$) in loin meat than in rump meat. These differences also might be caused by the higher fat content in the loin than in the rump (Piao et al., 2015). A previous study demonstrated that higher content of total C14:0, C16:0, C18:0, C18:1n9, SFA, MUFA, and PUFA in top sirloin than in top round (Pavan and Duckett, 2013).

Table 10. Total fatty acid content (g/100g meat, fresh basis) of loin and rump with different quality grades (QG) for Korean cattle steers [1]

	Loin					Rump					*P*-value		
Item	1++	1+	1	2	SEM	1++	1+	1	2	SEM	Grade	Cut	Grade*Cut
Crude fat[2], %	23.8^{a}	17.3^{b}	14.1^{bc}	9.90^{c}	1.00	6.20^{a}	4.09^{ab}	2.78^{b}	3.60^{b}	0.41	0.001	0.001	0.001
C14:0	0.82^{a}	0.59^{b}	0.52^{bc}	0.32^{c}	0.04	0.19	0.10	0.09	0.10	0.02	0.001	0.001	0.002
C15:0	0.16	0.19	0.16	0.16	0.01	0.10^{a}	0.09^{a}	0.04^{b}	0.08^{a}	0.01	0.03	0.001	0.29
C16:0	6.24^{a}	4.44^{b}	3.64^{b}	2.44^{c}	0.27	1.53	1.00	0.71	0.90	0.11	0.001	0.001	0.001
C16:1	1.18^{a}	0.80^{b}	0.74^{b}	0.46^{c}	0.06	0.33^{a}	0.18^{b}	0.14^{b}	0.16^{b}	0.03	0.001	0.001	0.004
C17:0	0.16	0.10	0.12	0.12	0.01	0.08^{a}	0.07^{ab}	0.03^{c}	0.05^{b}	0.00	0.01	0.001	0.10
C17:1	0.14^{a}	0.09^{b}	0.08^{bc}	0.06^{c}	0.01	0.04^{a}	0.02^{ab}	0.02^{b}	0.02^{b}	0.00	0.001	0.001	0.001
C18:0	2.38^{a}	1.70^{b}	1.29^{bc}	0.94^{c}	0.11	0.52^{a}	0.35^{b}	0.26^{b}	0.33^{b}	0.03	0.001	0.001	0.001
C18:1n9t	0.19^{a}	0.12^{b}	0.11^{b}	0.08^{b}	0.01	0.04	0.02	0.02	0.03	0.00	0.001	0.001	0.001
C18:1n9	10.57^{a}	7.54^{b}	5.98^{bc}	4.13^{c}	0.46	2.57^{a}	1.62^{ab}	1.18^{b}	1.43^{b}	0.18	0.001	0.001	0.001
C18:2n6	0.60	0.61	0.53	0.49	0.03	0.32^{a}	0.27^{ab}	0.12^{c}	0.21^{b}	0.02	0.002	0.001	0.26
C18:3n6	0.06^{a}	0.04^{b}	0.03^{bc}	0.02^{c}	0.00	0.01^{a}	0.01^{ab}	0.00^{b}	0.01^{a}	0.00	0.001	0.001	0.001
C20:3n6	0.06	0.08	0.07	0.06	0.00	0.05^{a}	0.04^{ab}	0.02^{c}	0.03^{b}	0.00	0.01	0.001	0.04
C20:5	0.14	0.17	0.16	0.17	0.01	0.12^{a}	0.11^{a}	0.04^{b}	0.09^{a}	0.01	0.02	0.001	0.01

SFA[3]	9.77[a]	7.03[b]	5.73[b]	3.98[c]	0.42	2.42[a]	1.62[ab]	1.13[b]	1.45[b]	0.16	0.001	0.001	0.001
USFA[4]	12.95[a]	9.47[b]	7.70[bc]	5.48[c]	0.54	3.48[a]	2.28[ab]	1.53[b]	1.98[b]	0.23	0.001	0.001	0.001
MUFA[5]	12.08[a]	8.56[b]	6.91[b]	4.73[c]	0.53	2.98[a]	1.85[ab]	1.35[b]	1.64[b]	0.21	0.001	0.001	0.001
PUFA[6]	0.86	0.91	0.78	0.75	0.04	0.50[a]	0.43[ab]	0.18[c]	0.34[b]	0.03	0.002	0.001	0.19
MUFA/SFA	1.24	1.22	1.20	1.16	0.02	1.20	1.14	1.20	1.14	0.01	0.18	0.11	0.70
PUFA/SFA	0.09[c]	0.13[b]	0.14[b]	0.19[a]	0.01	0.27[a]	0.27[a]	0.16[b]	0.24[a]	0.01	0.01	0.001	0.001

n=12

[1] Individual fatty acid content per meat (g/100g meat) was calculated as described in Materials and Methods.

[2] Crude fat (%) was published previously (Piao et al., 2015).

[3] SFA (saturated fatty acids) = C14:0 + C15:0 + C16:0 + C17:0 + C18:0.

[4] USFA (unsaturated fatty acids) = C16:1 + C17:1 + C18:1n9t + C18:1n9 + C18:2n6 + C18:3n6 + C20:3n6 + C20:5.

[5] MUFA (monounsaturated fatty acids) = C16:1 + C17:1 + C18:1n9t + C18:1n9.

[6] PUFA (polyunsaturated fatty acids) = C18:2n6 + C18:3n6 + C20:3n6 + C20:5.

[a-c] Means with different letters within the same row in each cut differ ($P < 0.05$).

9) Volatile compounds

Through GC/MS analysis, a total of 24 volatile compounds, including aldehydes (acetaldehydes, butanal, pentanal, hexanal, heptanal, benzaldehyde, octanal, and nonanal), hydrocarbons (methanethiol, carbon disulfide, n-pentane, n-hexane, and 2-butene), ketones (2-propanone, 2-butanone, 2,3-butanedione, and 3-methyl-2-butanone), alcohols (ethanol, 2-methyl-2-butanol, and 2-methyl-2-propanol), and others [acetonitrile, chloroform (data not shown), 2-ethoxy- 2-methylpropane (data not shown), and acetic acid (data not shown)] were detected in the loin and rump of Korean cattle steers (Table 11). The abundant volatile compounds, accounting for 17–29% of total volatile compounds, included acetaldehydes, 2-methyl-2-propanol, and 2-propanone. The moderate compounds, accounting for 1.0–6.5%, included butanal, hexanal, benzaldehyde, methanethiol, carbon disulfide, npentane, ethanol, chloroform, and 2-ethoxy-2-methylpropane. The minor compounds, accounting for less than 1%, included pentanal, heptanal, octanal, nonanal, n-hexane, n-butene, 2-butanone, 2,3-butanedione, 3-methyl-2-butanone, 2-methyl-2-butanol, acetonitrile, and acetic acid. The amount or variety of volatile compounds from cooked meat is affected by various factors, including diet, breed, postslaughtering ageing, cooking temperature and pH, irradiation, and techniques used for extraction and detection of volatile components (Ba et al., 2012b). Therefore, the major or minor component of volatile compounds detected from meat could vary with several factors, as described in this study.

In this study, most of the volatile compounds detected were aldehydes. Vasta and Priolo (2006) also reported that aldehydes were the major contributors to the volatile fraction in ruminant meat. Interaction between QG and cut type was observed ($P \leq 0.04$) for most of aldehyde percentages (butanal, pentanal, hexanal, heptanal, octanal, and nonanal), indicating that these were differently associated with QGs depending on the cut type (Table 11). In loin, pentanal, hexanal, and heptanal percentages were highest ($P < 0.05$) in QG1, and butanal and benzaldehyde percentages were highest ($P < 0.05$) in QG1+ and QG1, respectively. However, pentanal, hexanal, butanal, and benzaldehyde percentages in rump did not differ ($P > 0.05$) among the QGs. In loin, acetaldehyde, octanal, and nonanal percentages did not differ ($P > 0.05$) among the QGs. However, in rump, octanal and nonanal percentages were highest ($P < 0.05$) in QG2, and heptanal percentages were highest ($P < 0.05$) in QG1++ and QG2. Aldehydes arise from the thermal oxidation of USFAs such as C18:1n9, C18:2 n6, and C18:3 n6 (Cerny, 2007). Thus, interactions in these aldehydes might be related with significant interactions in the USFA contents (C18:1n9, C20:3n6) between QGs and cut types observed in this study.

Interactions between QGs and cut types were observed ($P = 0.001$) for contents of two hydrocarbons (n-pentane and 2-butene) (Table 11). In the loin, n-pentane and 2-butene percentages were highest ($P < 0.05$) in the QG1++ group and lowest in QG1 and QG2 groups. However, n-pentane and 2-butene percentages in rump meat

did not differ ($P > 0.05$) among the four QGs. The predominant hydrocarbon, n-pentane is mainly formed via the oxidation of linoleic acid (C18:2n6) (Seo, 1976). In this study, C18:2n6 was the major PUFA in loin and rump, and it was influenced by QGs and cut types (Table 9). Thus, significant interactions between QGs and cut types for n-pentane percentage may be due to differential C18:2n6 percentages among QGs and between cut types. In both loin and rump, n-hexane percentages were highest ($P < 0.05$) in the QG1++ group and lowest in QG1 and QG2 groups.

Hydrocarbons arise from the thermal oxidative decomposition of lipids, a reaction catalyzed by heme compounds, such as hemoglobin and myoglobin (Shahidi et al., 1986). Min et al. (1979) reported that nhexane and 2-butene were not significantly related to flavor because they possess relatively weak and non-beef-like odors. Our correlation data obtained from loin and rump muscle likewise indicated that these compounds did not have a significant relationship with flavor. A previous study found that straight chain hydrocarbons with less than 10 carbon atoms, such as hexane, do not contribute to the flavor of dry cured meat products because of their high threshold value (Ramirez and Cava, 2007). Contents of other hydrocarbons in the loin, including methanethiol and carbon disulfide, did not differ ($P > 0.05$) among the four QGs.

The ketone percentages varied among the QGs. Interaction between QG and cut type was observed ($P = 0.001$) for 2-butanone percentage (Table 11). The 2-butanone percentage in the loin was highest ($P < 0.05$) in the QG1+ group, whereas

it in rump was highest ($P < 0.05$) in the QG2 group. The autoxidation of C18 USFA (via hydroperoxides pathway) was proposed as the main mechanism for methyl-ketones formation (Larick and Turner, 1990). In our study, C18 contents (C18:1n-9, C18:2n-6, C18:3 n-6) were significantly affected by QG and cut type (Table 10). Thus, the interaction observed in 2-butanone percentage is probably related to differential C18 contents among QGs and between cut type. The 2-propanone percentage in the loin was lowest in the QG2 group, but it was similar among the other QG groups (QG1++, 1+, 1).

Table 11. Volatile compounds (%) of loin and rump with different quality grades (QG) in Korean cattle steers

	Loin					Rump					*P*-value		
Item	1++	1+	1	2	SEM	1++	1+	1	2	SEM	Grade	Cut	Grade*Cut
Aldehydes													
Acetaldehyde	27.1	30.8	30.2	27.8	0.77	25.4	24.1	24.1	24.2	0.74	0.74	0.001	0.34
Butanal	1.12^{b}	1.40^{a}	1.54^{a}	1.08^{b}	0.05	1.08	1.08	1.31	1.28	0.05	0.01	0.14	0.04
Pentanal	0.36^{b}	0.51^{b}	1.07^{a}	0.56^{b}	0.06	0.27	0.31	0.28	0.29	0.03	0.001	0.001	0.001
Hexanal	2.73^{b}	4.23^{b}	10.29^{a}	4.90^{b}	0.61	1.91	2.95	2.37	2.38	0.32	0.001	0.001	0.001
Heptanal	0.13^{b}	0.14^{b}	0.28^{a}	0.16^{b}	0.02	0.16^{ab}	0.11^{b}	0.10^{b}	0.21^{a}	0.01	0.07	0.17	0.001
Benzaldehyde	0.99^{b}	1.24^{ab}	1.42^{a}	1.15^{b}	0.05	1.08	1.19	1.37	1.30	0.05	0.004	0.62	0.65
Octanal	0.25	0.20	0.29	0.24	0.02	0.23^{b}	0.20^{b}	0.17^{b}	0.43^{a}	0.02	0.003	0.61	0.001
Nonanal	0.21	0.19	0.20	0.33	0.03	0.32^{b}	0.31^{b}	0.30^{b}	1.^{a}	0.08	0.001	0.001	0.01
Hydrocarbons													
Methanethiol	6.02	6.46	6.16	5.69	0.28	5.73^{b}	5.70^{b}	7.77^{a}	6.56^{ab}	0.29	0.22	0.36	0.14
Carbon disulfide	2.37	1.55	1.87	1.36	0.15	1.95	1.75	1.29	1.48	0.19	0.17	0.48	0.59
n-Pentane	3.35^{a}	1.81^{b}	1.04^{b}	1.12^{b}	0.20	0.94	1.04	0.65	1.26	0.08	0.001	0.001	0.001
n-Hexane	0.55^{a}	0.28^{b}	0.16^{c}	0.07^{c}	0.03	0.64^{a}	0.24^{b}	0.05^{b}	0.06^{b}	0.06	0.001	0.78	0.69
2-Butene	0.42^{a}	0.33^{a}	0.18^{b}	0.23^{b}	0.02	0.23	0.23	0.25	0.26	0.01	0.003	0.03	0.001
Ketones													

2-Propanone	17.1[ab]	18.8[a]	17.1[ab]	15.8[b]	0.34	15.9	16.5	18.4	17.5	0.62	0.44	0.91	0.14
2-Butanone	0.83[c]	1.00[a]	0.92[b]	0.82[c]	0.02	0.74[b]	0.81[b]	0.82[b]	0.94[a]	0.02	0.001	0.004	0.001
2,3-Butanedione	0.32	0.31	0.24	0.31	0.02	0.13[b]	0.14[b]	0.13[b]	0.30[a]	0.02	0.07	0.001	0.17
3-Methyl-2-Butanone	0.07	0.07	0.06	0.08	0.00	0.07[b]	0.09[b]	0.09[b]	0.12[a]	0.00	0.001	0.001	0.13
Alcohols													
Ethanol	7.10	2.72	3.34	6.43	0.77	6.57	6.25	5.12	4.53	0.66	0.24	0.47	0.23
2-Methyl-2-butanol	0.63	0.63	0.57	0.73	0.03	0.92	0.90	0.89	0.95	0.03	0.36	0.001	0.91
2-Methyl-2-propanol	22.1	23.7	20.0	27.7	1.21	29.1	28.9	28.1	29.6	1.14	0.28	0.001	0.57
Others													
Acetonitrile	0.72[b]	0.85[b]	1.09[a]	0.70[b]	0.04	0.88[a]	0.58[b]	0.42[b]	0.45[b]	0.04	0.01	0.001	0.001

n=12

[a-c] Means with different letters within a same row in each cut differ ($P < 0.05$).

10) Correlation analyses

Results of correlation analyses were presented in Tables 12-15. Briefly, MS was positively correlated with QG ($r = 0.98$; $P < 0.001$), crude fat content ($r = 0.76$; $P < 0.001$), juiciness ($r = 0.82$; $P < 0.01$), and overall acceptability ($r = 0.88$; $P < 0.001$); however, MS was negatively correlated with texture ($r = -0.75$; $P < 0.001$), moisture content ($r = -0.77$; $P < 0.001$), and crude protein content ($r = -0.80$; $P < 0.001$). QG was positively correlated with crude fat content ($r = 0.73$; $P < 0.001$), juiciness ($r = 0.81$; $P < 0.01$), and overall acceptability ($r = 0.87$; $P < 0.001$); however, it was negatively correlated with texture ($r = -0.78$; $P < 0.001$), moisture content ($r = -0.75$; $P < 0.001$), and crude protein content ($r = -0.75$; $P < 0.001$). Tenderness was positively correlated with juiciness ($r = 0.92$; $P < 0.001$), flavor ($r = 0.77$; $P < 0.01$), and overall acceptability ($r = 0.84$; $P < 0.01$). Tenderness was positively correlated with total collagen ($r = 0.75$; $P < 0.01$), soluble collagen ($r = 0.61$; $P < 0.05$), and insoluble collagen contents ($r = 0.72$; $P < 0.01$). Overall acceptability was also positively correlated with total collagen ($r = 0.70$; $P < 0.05$), soluble collagen ($r = 0.65$; $P < 0.05$), and insoluble collagen contents ($r = 0.63$; $P < 0.05$).

Tenderness was positively correlated with glutamic acid ($r = 0.72$; $P < 0.01$), leucine ($r = 0.77$; $P < 0.01$), lysine ($r = 0.75$; $P < 0.01$), proline ($r = 0.72$; $P < 0.01$), tyrosine ($r = 0.70$; $P < 0.05$), and valine contents ($r = 0.72$; $P < 0.01$). Juiciness was positively correlated with glutamic acid ($r = 0.68$; $P < 0.05$) and proline contents ($r = 0.82$; $P < 0.01$). Flavor was positively correlated with isoleucine ($r = 0.70$; $P <$

0.05), leucine (r = 0.75; P < 0.01), and lysine (r = 0.82; P < 0.01). Overall acceptability was positively correlated with glutamic acid (r = 0.60; P < 0.05) and proline contents (r = 0.78; P < 0.01).

Table 12. Pearson correlation coefficients between carcass traits and physico-chemical characteristics of loin in Korean cattle steers

Item	Carcass weight	LM area	Fat thickness	Marbling	Quality grade	Yield grade	Yield index	Meat color	Texture	Maturity
Slaughtering age	0.41**	0.36*	0.12	0.18	0.24	0.02	-0.10	-0.31*	-0.44**	0.11
Carcass weight	–	0.61***	0.34*	0.21	0.22	-0.27	-0.39**	-0.25	-0.20	0.14
LM area		–	0.16	0.46**	0.44**	0.17	0.01	0.03	-0.35*	0.13
Fat thickness			–	0.23	0.24	-0.80***	-0.96***	-0.11	-0.36*	-0.05
Marbling				–	0.98***	-0.07	-0.12	-0.09	-0.75***	-0.06
Quality grade					–	-0.08	-0.14	-0.13	-0.78***	-0.07
Yield grade						–	0.86***	0.17	0.23	-0.05
Yield index							–	0.18	0.27	0.05
Meat color								–	0.22	0.01
Texture									–	-0.17

* P<0.05; ** P<0.01; *** P<0.001.

Table 13. Pearson correlation coefficients among carcass traits and physico-chemical and sensory characteristics of loin in Korean cattle steers

Item	Moisture	Protein	Fat	Cooking loss	Shear force	pH	*L**	*a**	*b**	Tenderness	Juiciness	Flavor	Overall acceptability
Slaughtering age	-0.19	-0.15	0.14	-0.09	-0.06	0.04	0.14	0.03	0.14	0.36	0.45	0.43	0.52
Carcass weight	-0.14	-0.12	0.11	-0.18	-0.01	0.27	0.21	0.36*	0.33*	0.46	0.51	0.38	0.52
LM area	-0.43**	-0.42**	0.41**	-0.21	-0.20	-0.09	0.22	0.23	0.25	0.66*	0.75**	0.53	0.74**
Fat thickness	-0.07	-0.01	0.07	-0.13	0.14	0.19	0.14	0.26	0.35*	0.05	0.32	0.02	0.40
Marbling	-0.77***	-0.80***	0.76***	-0.32*	-0.33*	-0.37*	0.59***	0.46***	0.68***	0.55	0.82**	0.43	0.88***
Quality grade	-0.75***	-0.75***	0.73***	-0.37*	-0.30*	-0.39**	0.57***	0.41**	0.66***	0.51	0.81**	0.38	0.87***
Yield grade	-0.01	-0.07	-0.001	0.20	-0.14	-0.36*	-0.12	-0.22	-0.33*	0.26	0.10	0.37	0.10
Yield index	-0.04	-0.10	0.04	0.10	-0.19	-0.28	-0.12	-0.26	-0.33*	0.14	-0.09	0.13	-0.16
Meat color	0.21	0.03	-0.13	0.24	0.03	-0.04	-0.11	-0.27	-0.30*	-0.20	-0.11	-0.14	-0.06
Texture	0.55***	0.47**	-0.53***	0.42**	0.14	0.35*	-0.32*	-0.32*	-0.49***	-0.32	-0.56	-0.64*	-0.76**
Maturity	0.09	0.08	-0.06	0.07	0.01	0.09	-0.07	0.02	-0.04	-0.20	-0.15	0.09	-0.03
Moisture	–	0.91***	-0.98***	0.41**	0.29*	0.33*	-0.57***	-0.44**	-0.62***	-0.62*	-0.77**	-0.29	-0.71**
Protein		–	-0.93***	0.26	0.36*	0.20	-0.67***	-0.44**	-0.63***	-0.55	-0.77**	-0.32	-0.76**
Fat			–	-0.41**	-0.30*	-0.31*	0.59***	0.41**	0.60***	0.56	0.73**	0.30	0.70*
Cooking loss				–	0.13	0.15	-0.15	-0.06	-0.23	-0.52	-0.69*	-0.28	-0.67*
Shear force					–	-0.05	-0.35*	-0.25	-0.27	-0.32	-0.54	-0.19	-0.58
pH						–	0.06	0.02	-0.07	-0.29	-0.14	-0.42	-0.15
*L**							–	0.41**	0.67***	0.36	0.51	0.20	0.51

*a**	–	0.76***	0.28	0.51	0.16	0.54
*b**		–	0.04	0.30	-0.03	0.36
Tenderness			–	0.92***	0.77**	0.84**
Juiciness				–	0.68*	0.96***
Flavor					–	0.76**

* P<0.05; ** P<0.01; *** P<0.001.

Table 14. Pearson correlation coefficient between carcass traits and chemical composition, and sensory characteristics and collagen and nucleotide contents of loin in Korean cattle steers

Item	Total collagen	Soluble collagen	Insoluble collagen	AMP	IMP	Inosine	Hypoxanthine
Slaughtering age	0.03	0.03	0.03	0.19	-0.17	0.16	0.18
Carcass weight	0.08	0.07	0.08	0.13	-0.23	-0.26	0.21
LM area	0.09	0.09	0.08	-0.03	-0.08	-0.35*	0.001
Fat thickness	-0.03	0.01	-0.05	-0.04	-0.13	0.02	0.18
Marbling	0.30*	0.27	0.29*	-0.21	0.17	-0.58***	-0.43**
Quality grade	0.32*	0.29*	0.31*	-0.19	0.17	-0.57***	-0.42**
Yield grade	0.13	0.12	0.12	0	0.25	0.02	-0.29*
Yield index	0.03	0.003	0.04	-0.02	0.16	-0.06	-0.22
Meat color	-0.23	-0.15	-0.26	0.16	0.06	-0.06	0.03
Texture	0.06	0.07	0.05	0.15	-0.19	0.22	0.23
Maturity	-0.16	-0.12	-0.17	-0.10	-0.18	-0.02	0.29*
Moisture	-0.14	-0.13	-0.14	0.11	-0.06	0.51***	0.45**
Protein	-0.27	-0.24	-0.27	0.19	-0.003	0.63***	0.41**
Fat	0.14	0.10	0.15	-0.14	0.07	-0.54***	-0.45**
Cooking loss	-0.01	0.03	-0.03	0.14	-0.08	0.35*	0.19
Shear force	-0.23	-0.24	-0.20	0.20	0.22	0.26	-0.04
pH	0.05	0.01	0.06	0.01	-0.61***	0.09	0.68***
*L**	0.29*	0.17	0.35*	-0.17	-0.17	-0.43**	-0.15
*a**	0.09	0.08	0.09	-0.10	-0.09	-0.29*	-0.10
*b**	0.18	0.09	0.21	-0.20	-0.05	-0.41**	-0.22
Tenderness	0.75**	0.61*	0.72**	0.13	-0.32	-0.28	-0.31
Juiciness	0.79**	0.75**	0.69*	0.06	-0.55	-0.53	-0.12
Flavor	0.43	0.18	0.53	0.14	0.07	0.07	-0.18
Overall acceptability	0.70*	0.65*	0.63*	0.05	-0.49	-0.48	-0.02

* P<0.05; ** P<0.01; *** P<0.001.

Table 15. Pearson correlation coefficient between carcass traits and chemical composition, and sensory characteristics and amino acid contents of loin in Korean cattle steers

Item	Ala	Arg	Cys	Glu	Gly	His	iLe	Leu	Lys	Met	Phe	Pro	Ser	Thr	Tyr	Val
Slaughtering age	0.05	-0.15	0.13	0.05	0.05	-0.002	0.15	0.17	0.18	0.12	0.05	0.10	0.15	-0.08	0.14	0.14
Carcass weight	0.05	-0.10	0.16	0.23	-0.08	0.09	0.18	0.20	0.13	0.23	-0.01	0.25	0.17	-0.08	0.19	0.23
LM area	-0.11	-0.14	0.27	0.11	-0.25	-0.14	0.26	0.33*	0.12	0.29*	-0.06	0.11	0.11	0.15	0.28	0.26
Fat thickness	-0.11	-0.01	-0.05	-0.30*	-0.21	-0.16	-0.09	-0.08	-0.16	-0.13	0.11	-0.08	-0.14	-0.01	-0.01	-0.04
Marbling	-0.12	-0.15	0.10	0.05	-0.38**	-0.43**	0.36*	0.43**	0.26	0.22	0.28	0.13	0.03	0.04	0.34*	0.31*
Quality grade	-0.14	-0.18	0.14	-0.01	-0.37*	-0.48**	0.37**	0.44**	0.25	0.25	0.25	0.09	0.03	0.05	0.37**	0.31*
Yield grade	0.07	-0.04	0.22	0.18	0.04	0.07	0.09	0.10	0.15	0.13	-0.21	0.09	0.11	0.11	0.02	0.03
Yield index	0.05	-0.01	0.09	0.24	0.14	0.08	0.12	0.13	0.15	0.15	-0.11	0.04	0.12	0.08	0.05	0.06
Meat color	-0.03	-0.07	-0.16	-0.06	0.04	0.19	-0.20	-0.22	-0.23	-0.15	-0.23	0.07	-0.13	-0.09	-0.26	-0.23
Texture	0.25	0.15	0.05	0.06	0.37*	0.57***	-0.34*	-0.41**	-0.16	-0.22	-0.13	0.19	0.02	-0.14	-0.47**	-0.26
Maturity	0.05	0.11	-0.14	-0.01	0.07	-0.04	0.04	0.06	0.06	0.01	0.09	-0.18	-0.002	0.20	0.05	-0.002
Moisture	0.31*	0.04	-0.15	-0.10	0.47**	0.53***	-0.22	-0.30*	-0.14	-0.10	-0.26	-0.003	0.09	-0.13	-0.33*	-0.19
Protein	0.17	0.13	-0.14	-0.16	0.42**	0.30*	-0.17	-0.24	-0.15	-0.06	-0.22	-0.20	0.04	0.04	-0.16	-0.16
Fat	-0.33*	-0.06	0.15	0.06	-0.50***	-0.51***	0.17	0.25	0.10	0.06	0.24	0.02	-0.14	0.08	0.29*	0.14
Cooking loss	0.27	-0.01	0.10	0.10	0.39**	0.47	-0.23	-0.30*	-0.07	-0.31*	-0.19	0.19	0.13	-0.23	-0.43**	-0.24
Shear force	-0.04	0.07	-0.09	0.02	0.06	-0.01	-0.16	-0.18	-0.13	-0.17	-0.28	-0.25	-0.25	0.16	-0.17	-0.21
pH	0.38**	-0.10	-0.14	-0.03	0.22	0.64***	-0.15	-0.19	-0.06	-0.04	0.05	0.40**	0.20	-0.32*	-0.34*	0.002
*L**	0.08	-0.08	0.16	-0.06	-0.09	-0.06	0.23	0.25	0.24	0.21	0.29*	0.39**	0.23	-0.14	0.19	0.27
*a**	-0.13	-0.08	-0.07	0.31*	-0.26	-0.11	0.02	0.05	0.02	-0.01	0.08	0.15	0.01	-0.10	0.11	0.09

*b**	-0.13	-0.11	-0.12	0.19	-0.29*	-0.27	0.14	0.16	0.11	0.07	0.20	0.14	-0.01	-0.12	0.20	0.16
Tenderness	0.28	-0.14	0.60*	0.72**	-0.04	-0.65*	0.67*	0.77**	0.75**	0.63*	0.14	0.72**	0.58*	-0.02	0.70*	0.72**
Juiciness	0.13	-0.14	0.52	0.68*	-0.30	-0.66*	0.45	0.56	0.48	0.37	0.18	0.82**	0.39	-0.06	0.43	0.48
Flavor	0.52	-0.35	0.38	0.47	-0.18	-0.19	0.70*	0.75**	0.82**	0.62*	-0.18	0.48	0.52	-0.12	0.63*	0.63*
Overall acceptability	0.18	-0.23	0.43	0.60*	-0.43	-0.50	0.42	0.51	0.45	0.31	0.08	0.78**	0.32	-0.11	0.34	0.39

* P<0.05; ** P<0.01; *** P<0.001.

QG, MS, and fat content were positively correlated ($0.47 \leq r \leq 0.54$; $P < 0.01$) with the percentages of C18:1n9 and MUFA in loin fat (Supplementary Table 1). Indurain et al. (2006) also reported positive correlations between fat content and MUFA and between the percentage of C18:1n9 and IMF. In contrast, QG, MS, and fat content were negatively correlated ($-0.76 \leq r \leq -0.63$; $P < 0.001$) with the percentage of C18:2n6, C20:5, and PUFA in loin fat. A previous study found that high crude fat was related to high triacylglycerol that was rich in SFA and MUFA. However, PUFA content was relatively constant, and this may lead to the negative correlation between PUFA and fat content (Scollan et al., 2006). Conversely, meat texture score assigned by a meat grader was positively correlated with the percentage of PUFA ($r=0.60$, $P < 0.001$) in loin fat, whereas it showed a negative correlation with the percentages of C18:1n9 and MUFA (Supplementary Table 1). Beef texture mainly consists of several properties, including initial (first bite with incisors) and overall tenderness (after multiple chews), and more complex sensory attributes of chewing and mouthfeel with multiple descriptors (Juarez et al., 2011). According to the Korean beef grading system, texture scores ranged between 1 (very fine) and 3 (very coarse), where lower values indicate better texture. Our previous study, using the same set of samples, showed that the texture grade decreased with increasing QG and IMF (Piao et al., 2015), revealing that the beef texture improved with increasing QG and fat content. In this study, both QG and MS were positively correlated with the percentages of C18:1n9 and MUFA in loin fat, while they were negatively correlated with the percentage of PUFA

(Supplementary Table 1). Therefore, the positive or negative correlations between texture and FA percentages in this study are likely due to strong associations between texture and QG and MS, as reported in our previous study (Piao et al., 2015). Wood et al. (2003) also reported that texture is more likely to be affected by the total amount of FAs than by individual FA.

Correlation data between carcass traits and chemical and fatty acid composition in rump are presented in Supplementary Table 2. Briefly, crude fat content was negatively correlated ($-0.45 \leq r \leq -0.31$; $P < 0.05$) with percentages of C15:0, C18:0, and C18:2n6, whereas it was positively correlated ($0.32 \leq r \leq 0.34$; $P < 0.05$) with percentages of C16:0, C16:1, and MUFA in rump fat. Correlations between carcass traits and chemical compositions and fatty acid contents (g/100 g of meat) were also analyzed in both loin and rump. Briefly, QG, MS, and fat content had strong positive correlations ($0.63 \leq r \leq 0.99$; $P < 0.001$) with the contents of C16:0, C16:1, C18:0, C18:1n9, SFA, USFA, and MUFA in loin (Supplementary Table 3). QG and MS had positive correlations ($0.44 \leq r \leq 0.48$; $P < 0.01$) with C17:0 and C18:2n6 and PUFA contents in rump (Supplementary Table 4). Crude fat contents in rump had strong positive correlations ($0.61 \leq r \leq 0.99$; $P < 0.01$) with C14:0, C15:0, C16:0, C16:1, C17:0, C18:0, C18:1n9, C18:2n6, SFA, USFA, MUFA and PUFA.

Levels of hydrocarbons in loin, including n-pentane, n-hexane, and 2-butene, showed strong positive correlations ($0.56 \leq r \leq 0.81$; $P < 0.001$) with the content of

QG, MS, and crude fat (Table 16). We found the highest content of these compounds in the QG1++ group. These hydrocarbons are the main volatile compounds formed via lipid oxidation in beef (Hierro et al., 2004). Therefore, higher fat content in higher QG might contribute to the strong relationships between QG and volatile hydrocarbons. During lipid oxidation process, a labile hydrogen atom comes from a fatty acyl chain and a reactive free lipid radical is produced which further react with oxygen to produce a peroxyradical. Then, the peroxyradical abstracts a hydrogen from another hydrocarbon chain, resulting in generation of a hydroperoxide and a new free radical that can continue the chain reaction (Ladikos and Lougovois, 1990). These hydroperoxides decomposed to smaller volatile compounds such as aldehydes, ketones, alcohols, and hydrocarbons. Thermal oxidative decomposition of lipid also produces different saturated or unsaturated hydrocarbons. However, flavor did not show any significant correlations with the hydrocarbons (n- pentane, n-hexane, and 2-butene) in the present study. Therefore, our study convinced that these compounds might play a minor role in the generation of loin flavor due to high odor threshold value (Min et al., 1979), although these compounds proportionally increased with increases in QG because of the higher IMF.

The percentage of loin acetonitrile exhibited a strong positive correlation with flavor ($r = 0.74$, $P < 0.01$). Acetonitrile is known as a volatile compound with a sweet-burnt taste and ether odor (Gasparetto et al., 2012). Little information is available regarding the role of acetonitrile in beef flavor. The percentage (range

between 0.7% and 1.09%) of acetonitrile was relatively low in our study. Butanal and benzaldehyde also showed positive correlations with loin flavor, whereas 2-methyl-2-propanol and 3-methyl-2-butanone had negative correlations with loin flavor. Little information is available for the characteristics of butanal, benzaldehyde, 2-methyl-2-propanol, and 3-methyl-2-butanone.

Table 16. Pearson correlation coefficients between carcass traits, chemical composition, and sensory characteristics and volatile compound composition in loin of Korean cattle steers

Item	Methanethiol	2-Butene	Acetonitrile	n-Pentane	Butanal	2-Methyl-2-propanol	Chloroform	n-Hexane	3-Methyl-2-butanone-	Benzaldehyde
Slaughtering age	-0.07	0.01	0.28	0.09	0.15	-0.15	-0.11	0.09	-0.05	0.10
Carcass weight	0.11	0.02	0.05	0.06	0.09	-0.30*	0.06	0.16	-0.19	0.12
LM area	-0.13	0.29*	0.08	0.40**	0.04	-0.23	0.29*	0.41**	0.003	-0.08
Fat thickness	0.36*	-0.04	0.33*	-0.07	0.43**	0.37**	0.07	0.19	-0.24	0.44**
Marbling	0.03	0.56***	-0.07	0.63***	-0.05	-0.16	0.53***	0.79***	-0.13	-0.28
Quality grade	0.08	0.56***	-0.08	0.61***	-0.01	-0.18	0.54***	0.81***	-0.10	-0.22
Yield grade	-0.40**	0.12	-0.23	0.16	-0.29*	0.29*	0.04	-0.08	0.25	-0.31*
Yield index	-0.40**	0.13	-0.30*	0.18	-0.40**	0.35*	0.02	-0.08	0.27	-0.47**
Meat color	-0.27	0.07	-0.10	0.04	-0.15	0.21	0.21	-0.03	0.13	-0.20
Texture	-0.12	-0.25	-0.29*	-0.30*	-0.34*	0.30*	-0.21	-0.51***	0.27	-0.09
Maturity	-0.11	0.04	0.14	-0.02	0.03	-0.04	-0.11	-0.09	-0.08	0.03
Moisture	0.03	-0.59***	-0.08	-0.70***	0.01	0.16	-0.35*	-0.66***	0.20	0.16
Crude protein	0.09	-0.69***	0.001	-0.75***	0.13	0.04	-0.39**	-0.70***	0.01	0.32*
Crude fat	-0.05	0.63***	0.09	0.73***	-0.01	-0.14	0.40**	0.69***	-0.15	-0.18
Cooking loss	-0.20	-0.23	-0.08	-0.28	-0.23	0.23	-0.11	-0.29*	0.23	-0.06
Tenderness[1]	0.63*	0.12	0.33	0.36	0.46	-0.64*	-0.28	0.21	-0.69*	0.32
Juiciness[1]	0.65*	0.31	0.21	0.50	0.42	-0.58*	-0.11	0.46	-0.61*	0.22

Flavor[1]	0.16	-0.03	0.74**	0.13	0.71**	-0.62*	-0.25	0.09	-0.60*	0.68*
Overall acceptability[1]	0.50	0.32	0.34	0.47	0.52	-0.57	-0.05	0.49	-0.58*	0.33

n = 48 for all parameters except sensory characteristics. [1]n=12 for sensory characteristics (tenderness, juiciness, flavor, and overall acceptability).

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Correlation coefficients between carcass traits, chemical compositions, and sensory characteristics and content of volatile compound acetaldehyde, ethanol, carbon disulfide, 2-propanone, 2-butanone, 2,3-butanedione, propane-2-ethoxy-2-methyl, acetic acid, 2-butanol-2-methyl, pentanal, hexanal, heptanal, octanal, and nonanal were $r < 0.50$, and these values are not shown in this table. Correlation data of shear force, pH, and meat colors were also not shown in this table.

MS and QG had positive correlations ($0.50 \leq r \leq 0.65$; $P < 0.01$) with percentages of acetonitrile and n-hexane, whereas these had negative correlations ($-0.49 \leq r \leq -0.45$; $P < 0.01$) with percentages of 2-butanone, 3-methyl-2-butanone, and nonanal in rump (Table 17). Juiciness and overall acceptability had positive correlations ($0.63 \leq r \leq 0.85$; $P < 0.05$) with the percentages of acetonitrile in rump, whereas these had negative correlations ($-0.64 \leq r \leq 0.59$; $P < 0.05$) with the percentage of 2-butanone (Table 17). However, no correlation was observed between flavor and all volatile compounds in rump.

Percentages of both n-pentane and n-hexane in loin meat showed significant negative correlations ($-0.55 \leq r \leq -0.45$; $P < 0.01$) with percentages of C18:2n6 and PUFA in loin fat (Table 18). These did not exhibit significant correlations with the percentages of other major FA, including C16:0, C16:1, C18:0, and C18:1n9 in loin fat.

Percentage of methanethiol in rump showed negative correlations ($-0.37 \leq r \leq -0.32$; $P < 0.05$) with percentages of several FAs including C15:0, C17:0, C18:2n6, C20:3n6, C20:5, and PUFA in rump fat (Supplementary Table 5).

Percentages of hydrocarbon compounds, including 2-butene, npentane, and n-hexane exhibited strong positive correlations ($0.52 \leq r \leq 0.79$; $P < 0.001$) with contents (g/100 g of meat) of several FA, including C16:0, C16:1, C18:0, C18:1n9, SFA, MUFA, and USFA in loin (Table 19). We observed the highest percentage of hydrocarbons (npentane, n-hexane, and 2-butene) in the loin in the QG1++ group.

This might have been caused by the highest fat content and total FA content. As indicated earlier, hydrocarbons are produced from thermal oxidative degradation of lipid molecules. These three compounds had strong positive correlations with QG and fat content in loin. Taken together, the results of our study demonstrated that these hydrocarbon compounds are linked with beef QG even though they are not directly linked with beef flavor.

Percentage of acetonitrile showed positive correlations ($0.38 \leq r \leq 0.44$; $P < 0.01$) with contents of several FA, including C17:0, C18:0, C18:1n9, C18:2n6, C18:3n6, C20:3n6, C20:5, SFA, USFA, MUFA, and PUFA in rump (Supplementary Table 6). However, percentages of both methanethiol and benzaldehyde showed negative correlations ($-0.36 \leq r \leq -0.29$; $P < 0.05$) with contents of C17:0, C20:3n6, C20:5, and PUFA.

In this study, we presented both the percentage of individual FA (based on total FA) and the content (g/100 g of meat) of individual FA in loin and rump. From the results, content of FA per meat type, rather than the percentage of individual FA, is likely more directly associated with volatile compounds.

Overall, abundant volatile compounds (acetaldehyde, 2-propanone) and most of the moderate compounds (hexanal, methanethiol, carbon disulfide, n-pentane, and ethanol) in loin were not correlated with sensory characteristics, including flavor and overall acceptability. Minor compounds, including acetonitrile, butanal, and benzaldehyde in loin were positively correlated with flavor, whereas 2-methyl-2- propanol and 3-methyl-2-butanone were negatively correlated with flavor. A

recent study reported that acceptability and flavor were greater for longissimus lumborum steaks of USDA Prime and Choice than those of Standard grade, which is lower QG in the US (Legako et al., 2016). The authors also demonstrated that volatile compounds of grilled beef steaks varied with QG.

Table 17. Pearson correlation coefficients between carcass traits, chemical composition, and sensory characteristics and volatile compound composition in rump of Korean cattle steers

Item	Acetonitrile	2-Butanone	2,3-Butanedione	n-Hexane	2-Ethoxy-2-methylpropane	3-Methyl-2-Butanone	Octanal	Nonanal
Marbling	0.58**	-0.45**	-0.34*	0.54**	-0.30*	-0.49**	-0.40**	-0.49**
Quality grade	0.56**	-0.48**	-0.39**	0.50**	-0.34*	-0.48**	-0.39**	-0.45**
Texture	-0.26	0.45**	0.49**	-0.25	0.54**	0.50**	0.60**	0.59**
Crude protein	-0.45**	0.31*	0.20	-0.37**	0.07	0.21	0.14	0.20
Crude fat	0.39**	-0.17	0.03	0.18	0.002	-0.09	-0.12	-0.15
Tenderness[1]	0.55	-0.52	0.15	0.04	-0.46	-0.40	-0.17	-0.29
Juiciness[1]	0.63*	-0.64*	-0.09	0.29	-0.45	-0.50	-0.36	-0.47
Flavor[1]	0.43	-0.26	0.02	0.01	-0.51	-0.38	-0.43	-0.51
Overall acceptability[1]	0.63*	-0.59*	-0.21	0.36	-0.47	-0.54	-0.52	-0.61*

n = 48 for all parameters except sensory characteristics. [1]n=12 for sensory characteristics (tenderness, juiciness, flavor, and overall acceptability).

* $P < 0.05$; ** $P < 0.01$.

Table 18. Pearson correlation coefficients between fatty acid composition (% of fat, fresh basis) and volatile compound composition in loin of Korean cattle steers

Item	n-Pentane	n-Hexane
C14:0	0.002	-0.05
C15:0	-0.34*	-0.50***
C16:0	0.20	0.29*
C16:1	0.03	-0.04
C17:0	-0.31*	-0.39**
C17:1	-0.23	-0.15
C18:0	0.25	0.14
C18:1n9t	-0.03	-0.10
C18:1n9	0.23	0.38**
C18:2n6	-0.46**	-0.52***
C18:3n6	0.30*	0.28
C20:3n6	-0.43**	-0.54***
C20:5	-0.42**	-0.58***
SFA	0.15	0.09
USFA	-0.15	-0.09
MUFA	0.21	0.33*
PUFA	-0.45**	-0.55***
MUFA/SFA	0.03	0.11
PUFA/SFA	-0.45**	-0.54***

n = 48

* P < 0.05; ** P < 0.01; *** P < 0.001.

Correlation coefficients between fatty acid content (% of fat) and content of volatile compound acetaldehyde, methanethiol, 2-butene, acetonitrile, ethanol, carbon disulfide, 2-propanone, butanal, 2-propanol-2-methyl, chloroform, 2-butanone, 2,3-butanedione, propane-2-ethoxy-2-methyl, acetic acid, 2-butanol-2-methyl, 2-butanone-3-methyl, pentanal, hexanal, heptanal, benzaldehyde, octanal, and nonanal were r < 0.50, and these values are not shown in this table.

Table 19. Pearson correlation coefficients between fatty acid contents (g/100g meat) and volatile compound composition in loin of Korean cattle steers

Item	2-Butene	n-Pentane	Chloroform	2-Butanone	n-Hexane	2-Ethoxy-2-methylpropane
C14:0	0.55***	0.66***	0.30*	-0.02	0.53***	0.28
C15:0	0.19	0.16	-0.02	0.19	0.11	0.24
C16:0	0.63***	0.74***	0.41**	0.04	0.70***	0.35*
C16:1	0.52***	0.62***	0.33*	-0.04	0.52***	0.28
C17:0	0.10	0.15	0.18	-0.06	0.18	0.12
C17:1	0.52***	0.62***	0.34*	0.03	0.59***	0.23
C18:0	0.68***	0.79***	0.40**	0.06	0.70***	0.41**
C18:1n9t	0.52***	0.64***	0.33*	0.01	0.56***	0.28
C18:1n9	0.61***	0.71***	0.42**	0.07	0.69***	0.33*
C18:2n6	0.27	0.21	0.07	0.30*	0.30*	0.19
C18:3n6	0.57***	0.66***	0.28	0.03	0.60***	0.30*
C20:3n6	0.17	0.10	-0.09	0.40**	0.12	0.19
C20:5	0.05	-0.01	-0.09	0.22	-0.06	0.14
SFA	0.65***	0.76***	0.40**	0.04	0.69***	0.37**
USFA	0.61***	0.70***	0.40**	0.08	0.67***	0.33*
MUFA	0.61***	0.71***	0.41**	0.06	0.68***	0.32*
PUFA	0.27	0.22	0.04	0.30*	0.26	0.22
MUFA/SFA	0.02	0.03	0.1	0.15	0.11	-0.13
PUFA/SFA	-0.33*	-0.45**	-0.38**	-0.01	-0.54***	-0.06

n = 48

* P < 0.05; ** P < 0.01; *** P < 0.001.

Correlation coefficients between fatty acid content (g/100 g meat) and content of volatile compound acetaldehyde, methanethiol, acetonitrile, ethanol, carbon disulfide, 2-propanone, butanal, 2-propanol-2-methyl, 2,3-butanedione, acetic acid, 2-butanol-2-methyl, 2-butanone-3-methyl, pentanal, hexanal, heptanal, benzaldehyde, octanal, and nonanal were r < 0.50, and these values are not shown in this table.

Supplementary table 1. Pearson correlation coefficients between carcass traits and chemical compositions and fatty acid composition (% of fat, fresh basis) in loin of Korean cattle steers

Item	C18:1n9	C18:2n6	C20:5	MUFA	PUFA
Slaughtering age	0.06	-0.19	-0.21	-0.01	-0.20
Carcass weight	0.08	-0.04	-0.15	0.09	-0.08
LM area	0.19	-0.23	-0.31*	0.23	-0.25
Fat thickness	-0.02	-0.16	-0.27	0.03	-0.20
Marbling	0.48**	-0.68***	-0.76***	0.50***	-0.72***
Quality grade	0.49***	-0.64***	-0.73***	0.48**	-0.68***
Yield grade	0.02	0.001	0.08	-0.01	0.03
Yield index	0.06	0.08	0.19	0.03	0.12
Meat color	-0.13	0.10	0.20	-0.06	0.14
Texture	-0.40**	0.55***	0.65***	-0.43**	0.60***
Maturity	-0.05	-0.05	0.01	-0.01	-0.03
Moisture	-0.46**	0.62***	0.70***	-0.52***	0.65***
Crude protein	-0.43**	0.61***	0.65***	-0.46**	0.62***
Crude fat	0.47**	-0.63***	-0.69***	0.54***	-0.66***
Cooking loss	-0.46**	0.14	0.28	-0.49***	0.19
Shear force	-0.25	0.10	0.19	-0.19	0.13
pH	-0.12	0.31*	0.30*	-0.18	0.33*
L*	0.20	-0.47**	-0.47**	0.16	-0.48**
a*	0.22	-0.38**	-0.50***	0.25	-0.43**
b*	0.30*	-0.46**	-0.59***	0.31*	-0.51***

n = 48

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Correlation coefficients between carcass traits and chemical compositions and composition (% of fat) of fatty acid C14:0, C16:0, C16:1, C17:1, C18:0, C18:1n9t, SFA, USFA, and MUFA/SFA were $r < 0.50$, and these values are not shown in this table. Correlation data of having relatively low proportions (C15:0, C17:0, C18:3n6, C20:3n6) and PUFA/SFA ratio were deleted.

Supplementary table 2. Pearson correlation coefficients between carcass traits and chemical compositions and fatty acid composition (% of fat, fresh basis) in rump of Korean cattle steers

Item	C15:0	C16:0	C16:1	C17:0	C18:0	C18:1n9	C18:2n6	SFA	USFA	MUFA	PUFA
Marbling	0.11	-0.17	0.13	0.23	-0.20	-0.13	0.21	-0.21	0.21	-0.07	0.19
Quality grade	0.12	-0.22	0.09	0.26	-0.20	-0.08	0.24	-0.29*	0.29*	-0.05	0.22
Moisture	0.36*	-0.39**	-0.37*	0.29*	0.48**	-0.18	0.38**	-0.06	0.06	-0.35*	0.33*
Crude protein	0.16	-0.15	-0.18	0.05	0.34*	-0.07	0.06	0.09	-0.09	-0.15	0.07
Crude fat	-0.34*	0.32*	0.34*	-0.25	-0.45**	0.18	-0.31*	-0.01	0.01	0.33*	-0.28
pH	0.43**	-0.38**	-0.29*	0.32*	0.26	-0.24	0.45**	-0.14	0.14	-0.36*	0.39**

n = 48.

* $P < 0.05$; ** $P < 0.01$.

Correlation coefficients between carcass traits and chemical compositions and composition (% of fat, fresh basis) of fatty acid C14:0, C20:3n6, C20:5, MUFA/SFA and PUFA/SFA were $r<0.50$, and those values were not shown in this table. Correlation data of having relatively low proportions (C17:1, C18:1n9t, C18:3n6) were deleted.

Supplementary table 3. Pearson correlation coefficients between carcass traits and chemical composition and fatty acid content (g/100g meat, fresh basis) in loin of Korean cattle steers

Item	C16:0	C16:1	C18:0	C18:1n9	SFA	USFA	MUFA
Slaughtering age	0.15	0.07	0.19	0.14	0.16	0.13	0.13
Carcass weight	0.10	0.10	0.10	0.11	0.11	0.11	0.11
LM area	0.39**	0.41**	0.41**	0.41**	0.40**	0.41**	0.41**
Fat thickness	0.10	0.07	0.05	0.05	0.09	0.05	0.05
Marbling	0.76***	0.68***	0.73***	0.77***	0.75***	0.75***	0.76***
Quality grade	0.73***	0.63***	0.71***	0.74***	0.73***	0.72***	0.73***
Yield grade	-0.02	0.01	0.04	0.01	-0.01	0.001	0.01
Yield index	0.01	0.04	0.07	0.06	0.02	0.06	0.06
Meat color	-0.13	-0.04	-0.21	-0.13	-0.15	-0.12	-0.12
Texture	-0.54***	-0.49***	-0.50***	-0.54***	-0.53***	-0.53***	-0.54***
Maturity	-0.05	-0.02	-0.10	-0.07	-0.06	-0.07	-0.07
Moisture	-0.97***	-0.92***	-0.95***	-0.98***	-0.97***	-0.98***	-0.98***
Crude protein	-0.92***	-0.85***	-0.92***	-0.93***	-0.93***	-0.92***	-0.93***
Crude fat	0.99***	0.94***	0.96***	0.99***	0.99***	0.99***	0.99***
Cooking loss	-0.38**	-0.40**	-0.37*	-0.42**	-0.38**	-0.43**	-0.42**

n = 48

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Correlation coefficients between carcass traits and chemical compositions and contents (g/100g meat, fresh basis) of fatty acids C15:0, C17:0, C20:3n6, PUFA, and MUFA/SFA were $r < 0.50$, and these values are not shown in this table. Correlation data of having relatively low proportions (C14:0, C17:1, C18:1n9t, C18:2n6, C18:3n6, C20:5) and PUFA/SFA ratio were deleted.

Supplementary 4. Pearson correlation coefficients between carcass traits and chemical composition total fatty acid content (g/100g meat, fresh basis) in rump of Korean cattle steers

Item	C14:0	C15:0	C16:0	C16:1	C17:0	C18:0	C18:1n9	C18:2n6	SFA	USFA	MUFA	PUFA
Marbling	0.31*	0.36*	0.33*	0.34*	0.47**	0.37*	0.34*	0.46**	0.35*	0.36*	0.34*	0.44**
Quality grade	0.29*	0.36*	0.32*	0.33*	0.48**	0.37**	0.35*	0.48**	0.35*	0.37**	0.35*	0.45**
Moisture	-0.90**	-0.55**	-0.95**	-0.90**	-0.70**	-0.91**	-0.94**	-0.70**	-0.95**	-0.94**	-0.94**	-0.70**
Crude protein	-0.67**	-0.51**	-0.74**	-0.70**	-0.65**	-0.73**	-0.75**	-0.68**	-0.75**	-0.76**	-0.75**	-0.65**
Crude fat	0.94**	0.61**	0.99**	0.94**	0.77**	0.98**	0.99**	0.80**	0.99**	0.99**	0.99**	0.78**

n = 48

* $P < 0.05$; ** $P < 0.01$.

Correlation coefficients between carcass traits and chemical compositions and content (g/100g meat, fresh basis) of fatty acid C18:1n9t, C18:3n6, MUFA/SFA and PUFA/SFA were $r<0.50$, and those values were not shown in this table. Correlation data of having relatively low proportions (C17:1, C20:3n6, C20:5) were deleted.

Supplementary table 5. Pearson correlation coefficients between fatty acid composition (% of fat, fresh basis) and volatile compound composition in rump of Korean cattle steers

Item	Methanethiol	n-Pentane	n-Hexane
C14:0	0.20	0.08	-0.19
C15:0	-0.32*	0.01	0.11
C16:0	0.25	0.19	-0.13
C16:1	-0.04	0.12	-0.09
C17:0	-0.36*	0.03	0.12
C17:1	-0.07	-0.01	-0.08
C18:0	0.10	-0.11	-0.02
C18:1n9t	0.28	-0.04	-0.17
C18:1n9	0.30*	-0.26	0.01
C18:2n6	-0.34*	-0.03	0.16
C18:3n6	0.02	-0.12	0.08
C20:3n6	-0.33*	0.02	0.10
C20:5	-0.37**	0.14	0.13
SFA	0.22	0.21	-0.17
USFA	-0.22	-0.21	0.17
MUFA	0.26	-0.17	-0.05
PUFA	-0.35*	0.02	0.15
MUFA/SFA	0.05	-0.24	0.06
PUFA/SFA	-0.36*	-0.01	0.15

n = 48

* $P < 0.05$; ** $P < 0.01$.

Correlation coefficients between fatty acid composition (% of fat, fresh basis) and content of volatile compound acetaldehyde, 2-butene, acetonitrile, ethanol, carbon disulfide, 2-propanone, butanal, 2-methyl-2-propanol, chloroform, 2-butanone, 2,3-butanedione, 2-ethoxy-2-methylpropane, acetic acid, 2-methyl-2-butanol, 3-methyl-2-butanone, pentanal, hexanal, heptanal, benzaldehyde, octanal and nonanal were $r<0.50$, and those values were not shown in this table.

Supplementary table 6. Pearson correlation coefficients between fatty acid content (g/100g meat, fresh basis) and volatile compound composition in rump of Korean cattle steers

Item	Methanethiol	Acetonitrile	Carbon disulfide	n-Pentane	Benzaldehyde
C14:0	-0.10	0.30*	0.24	0.18	-0.30*
C15:0	-0.30*	0.34*	0.24	0.31*	-0.25
C16:0	-0.09	0.36*	0.28	0.16	-0.27
C16:1	-0.17	0.33*	0.24	0.19	-0.32*
C17:0	-0.33*	0.44**	0.28	0.31*	-0.36*
C17:1	-0.15	0.35*	0.26	0.14	-0.27
C18:0	-0.08	0.42**	0.28	0.14	-0.23
C18:1n9t	-0.03	0.30*	0.27	0.11	-0.22
C18:1n9	-0.08	0.39**	0.29*	0.10	-0.23
C18:2n6	-0.28	0.44**	0.29*	0.25	-0.26
C18:3n6	-0.02	0.40**	0.38**	0.13	-0.08
C20:3n6	-0.30*	0.40**	0.29*	0.29*	-0.32*
C20:5	-0.33*	0.39**	0.26	0.38**	-0.32*
SFA	-0.11	0.38**	0.29*	0.17	-0.28
USFA	-0.12	0.40**	0.30*	0.14	-0.25
MUFA	-0.09	0.39**	0.29*	0.12	-0.24
PUFA	-0.30*	0.43**	0.29*	0.29*	-0.29*
MUFA/SFA	0.05	0.14	0.03	-0.25	0.23
PUFA/SFA	-0.35*	0.18	0.10	-0.01	-0.09

n = 48

* $P < 0.05$; ** $P < 0.01$.

Correlation coefficients between fatty acid content (g/100g meat, fresh basis) and content of volatile compound acetaldehyde, 2-butene, ethanol, 2-propanone, butanal, 2-methyl-2-propanol, chloroform, 2-butanone, 2,3-butanedione, n-hexane, 2-ethoxy-2-methylpropane, acetic acid, 2-methyl-2-butanol, 3-methyl-2-butanone, pentanal, hexanal, heptanal, octanal and nonanal were $r<0.50$, and those values were not shown in this table.

5. Conclusion

Taken together, this study confirms that MS and IMF content are major positive determinants of QG in Korean cattle beef. Numeric values of tenderness, juiciness, and overall acceptability in loin tended to be highest in QG1++, and those of juiciness and overall acceptability tended to be lowest in QG 2. Juiciness and overall acceptability were strongly correlated with QG. Our results demonstrate that QGs are linked to sensory traits. However, the nucleotide contents including IMP may not be a major factors determining meat palatability of Korean cattle beef in this study. Glutamic acid and proline were significantly associated with tenderness, juiciness, and overall acceptability, although they did not differ significantly among QGs. In addition, beef QGs affected the compositions and contents of FAs and volatile compounds in loin and rump of Korean cattle steers. Loin FA percentages, especially those of C18:1n9 and MUFA, generally increased with increasing QGs. Some volatile compounds in loin and rump varied with QGs and were positively or negatively correlated with flavor.

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CHAPTER FOUR

Comparison of reducing sugar content, sensory traits, and fatty acids and volatile compound profiles of the *longissimus thoracis* among Korean cattle, Holsteins, and Angus steers

1. Abstract

Intramuscular fat (IMF) and reducing sugar contents, sensory traits, and fatty acid (FA) and volatile compound profiles in *longissimus thoracis* (LT) were compared among Korean cattle, Holstein, and Angus steers. The LT of Korean cattle had the highest ($P < 0.05$) IMF, the highest reducing sugar content, and the highest scores in the sensory evaluation (flavor, tenderness, juiciness, and overall acceptance). All of the sensory traits were positively correlated ($P < 0.001$) with IMF and reducing sugar content. Several FAs and volatile compound profiles varied among the breeds. Korean cattle LT had the highest ($P < 0.05$) concentrations of acetaldehyde, 3-methyl butanal, and 3-hydroxy-2-butanone, and these volatile compounds were positively correlated ($P < 0.05$) with all of the sensory traits. In conclusion,

variations in IMF and reducing sugar contents and FA and volatile compound profiles may contribute to differences in the sensory quality of LT among breeds.

2. Introduction

Per capita beef consumption in South Korea has increased nearly four-fold, from 2.6 kg in 1980 to 10.8 kg in 2014; this is likely a result of increasing levels of income and the westernization of Korean eating patterns (MAFRA 2015). Domestic beef production in South Korea is primarily based on Korean cattle (KC), but Holstein (HO) steers are also produced. The self-sufficiency rate of beef consumption in South Korea has decreased and reached as low as 37.7% in 2016 (KREI 2016). South Korea's main beef importers in 2016 were the United States and Australia, both of which predominantly imported Angus (AN) cattle (KREI 2016). Korean consumers have historically preferred KC beef to domestic HO and imported beef because they believe that the palatability (e.g., flavor, juiciness, and tenderness) of KC beef is superior to that of HO and imported beef (Kim *et al*. 1993). Limited information is available on the factors that affect the preference of Korean consumers for KC beef over other breeds.

Beef flavor, juiciness, and tenderness are key factors in evaluation of meat palatability, and these three factors in combination are known to have great

influence on consumers' overall perceptions of the taste of beef (Neely *et al*. 1998). Intramuscular fat (IMF) is closely related to both beef flavor and tenderness (Joo *et al*. 2013). In addition to IMF contents, the composition and content of fatty acids (FAs) are also important factors in beef palatability (Ba *et al*. 2013). It has been suggested that oleic acid (C18:1n9) is positively associated with beef flavor, whereas polyunsaturated fatty acid (PUFA) may be negatively associated with beef flavor (Melton *et al*. 1982).

During the cooking process of meat, large quantities of volatile compounds are formed, such as aldehydes, ketones, hydrocarbons, furans, thiazoles, and pyrazines; these compounds directly affect flavor (Watkins *et al*. 2012). Volatile compounds can form via several pathways, including a Maillard reaction of amino acids or peptides with reducing sugars (e.g., glucose and ribose), thermal lipid degradation, and the interaction between Maillard reaction products and lipid oxidation products (Ba *et al*. 2012a). Of these pathways, thermal lipid oxidation is primarily involved in the formation of major volatile compounds, and FA oxidation is primarily responsible for the development of flavor (Neethling *et al*. 2016). Therefore, different types or amounts of FAs may influence the generation of volatile compounds, the development of aroma characteristics, and the intensity of flavor upon thermal processing.

Reducing sugars, such as glucose and ribose, have been reported to contribute

to improved flavor of cooked meat by reacting with amino acids to produce many important volatile compounds via Maillard reactions (Aliani and Farmer 2002). Currently, little information is available on the reducing sugar content of beef and its association with sensory traits. Furthermore, limited information is available regarding differences in reducing sugar content, sensory traits, and FA and volatile compound profiles among cattle breeds. This study was performed to compare the physico-chemical characteristics, reducing sugar content, sensory traits, and FA and volatile compound profiles of the *longissimus thoracis* (LT) among KC, HO, and imported AN breeds, and to identify correlations among these parameters.

3. Materials and methods

1) Beef sample preparation

The KC steers were from 12 female cows (implanted with the semen of seven different bulls), and were raised according to conventional Korean feeding methods. The HO steers were from 12 female cows (implanted with the semen of 11 different bulls), and were also raised according to conventional Korean feeding methods. Briefly, the KC male calves were weaned at 3 months of age and were then fed roughage (70%) and concentrate (30%) until 5 months of age. The calves were castrated at 6 months of age, and steers were grown in pens using group feeding. The steers were fed concentrate and roughage separately until slaughter at around

29 months of age. During growing stage (6 to 14 months of age), steers were fed a concentrate diet (1.6% of BW/steer) and a timothy hay (approximately 0.75% of BW/steer). During fattening stage (early fattening, 15 to 23 months of age; late fattening, 24 to 29 months of age), steers were fed a concentrate diet (approximately 1.5% of BW/steer) and rice straw (1kg/steer). The HO male calves were weaned at two months of age, then fed starter diet (30%) and hay (70%) until four months of age, and were castrated at 5 months of age. The steers were fed concentrate and roughage separately until slaughter at around 20 months of age. During growing stage (5 to 8 months of age), steers were fed a concentrate diet (approximately 2.4% of BW/steer) and a timothy hay (approximately 0.75% of BW/steer). During fattening stage (early fattening, 9 to 13 months of age; late fattening, 14 to 20 months of age), steers were fed a concentrate diet (approximately 1.8% of BW/steer) and rice straw (1kg/steer). Cattle were slaughtered in a local municipal slaughterhouse (Bucheon, Republic of Korea). Carcass traits were evaluated by a meat grader using the Korean carcass-grading system of the Korean Institute for Animal Products Quality Evaluation (KAPE, 2013) at 24 h post mortem. The LT samples (about 500 g each) of KC with an average age of 31 ± 0.42 months, an average carcass weight of 431 ± 12.5 kg, and a quality grade (QG) of 1+ were obtained from the joint livestock products market (Bucheon, Republic of Korea) immediately after grading. The LT samples of HO cattle with an average age of 24 ± 0.54 months, an average carcass weight of 402 ± 7.81 kg, and a QG of 2 were also

obtained from the same market. Samples were then vacuum-packaged, transported under ice to a laboratory, and stored at 4°C for 42 days until the analysis. We selected a QG of 1+ for KC beef because the largest proportion (44.4%) of KC steer beef was of this grade (KAPE 2016). Similarly, a QG of 2 for HO beef was selected because the largest proportion (48.6%) of HO steer beef was of this grade. The LT samples of the AN steers (choice grade) had an average age of about 20 months; they were slaughtered on the same day as the KC and HO cattle in the United States, shipped at 4°C, imported by a beef delivery company (A-Meat Corp., Seoul, Republic of Korea), and stored in vacuum-packaging at 4°C for 42 days until analysis. The choice grade of the 12 AN LT samples was randomly purchased from approximately 100 choice beef options. We selected a choice grade because it is the most popular and abundant grade in the United States. The AN cattle were raised using a conventional beef production system in the United States. After 42 days of storage, the packages containing the LT samples of the different breeds were opened and the external fat was trimmed away. The LT samples were minced using a mini chopper (CH180; Kenwood, Shanghai, China) for 30 s. Minced LT samples from various locations were pooled and stored at –70°C for analysis of the chemical composition, and collagen, reducing sugar, FA, and volatile compound contents. Samples assessed for shear force and sensory traits were collected but not minced. Some samples were used immediately for shear force evaluation, while others were stored at –70°C for the sensory evaluation.

2) Proximate composition, color, pH, shear force, and collagen content

Moisture, crude protein, and crude fat contents were analyzed according to the methods of the Association of Official Analytical Chemists (AOAC) (AOAC 1996). Surface-color values (International Commission on Illumination [CIE]; L^*, a^*, and b^* values represent lightness, redness, and yellowness, respectively) were measured using a colorimeter (CR-310; Minolta Co., Ltd., Osaka, Japan). The samples (1 g) used for pH measurement were homogenized with distilled water (9 mL) at Lv.6 (30,000 rpm) for 30 sec (T10 Basic; Ika Works, Staufen, Germany) and centrifuged at 2,265 ×g for 10 min (Continent 512R; Hanil Co., Ltd., Incheon, Korea). The sample pH was measured with a pH meter (SevenGo; Mettler-Toledo, Inc., Schwerzenbach, Switzerland). The shear force (N) was measured as described previously (Piao *et al*. 2015) using a Warner-Bratzler shear attached to a texture analyzer (CT3 10K; Brookfield Engineering Laboratories, Middleboro, MA, USA), based on slight modifications of the method developed by Kim and Lee (2003).

The total insoluble collagen content was measured using a spectrophotometer, as described previously (Piao *et al.* 2015), according to a modified method of Jayasena *et al*. (2013).

3) Reducing sugars

Sugars were extracted from LT sample (1 g) by homogenization (T10 Basic; Ika Works) with 5 mL of 80% ethanol (50°C) and were centrifuged at 2,265 ×g for

10 min (Continent 512; Hanil Co., Ltd.). The extracts were then centrifuged (Continent 512R; Hanil Co., Ltd.) at 2,265 ×g for 10 min. The resulting supernatants were filtered (filter paper No.1; Whatman International Ltd., Springfield Mill, Kent, England) separately into 15-mL tubes and evaporated using N_2 gas (99.999%). Next, distilled water (2 mL) was added to each tube and vortexed to dissolve the sugars. The mixture was transferred to a 2-mL microtube and centrifuged at 18,500 ×g for 10 min (HM-150IV; Hanil Co., Ltd.). The reducing sugar content was measured using a dinitrosalicylic (DNS) acid method, as described by the Korean Society of Food Science and Nutrition (2000). In brief, 1 mL of each extract was mixed with 2 mL of the DNS solution (0.5 g of DNS acid, 8.0 g of sodium hydroxide, and 150 g of Rochelle salt in 500 mL of distilled water) in a 15-mL test tube and heated in a water bath (90°C) for 10 min. The mixture was then cooled under running water for 5 min and the absorbance was measured at 550 nm using a spectrophotometer (X-ma 3100; Human Co., Ltd., Seoul, Korea). The reducing sugar content was calculated using a standard curve developed with glucose (Sigma Corp., St. Louis, MO, USA).

4) Sensory evaluation

LT samples were cut into sections (15 × 40 × 15 mm^3) and cooked. The internal temperature was monitored using a digital thermometer (YF-160A Type-K; YFE, Hsinchu City, Taiwan) that was placed in the center of the LT samples; the samples

were removed from the grill after they reached an internal temperature of 72°C. Ten untrained panelists evaluated the cooked samples for appearance, odor, taste, flavor, tenderness, juiciness, and overall acceptability. A 9-point hedonic scale, where 9 indicated *extremely liked* and 1 indicated *extremely disliked*, was employed to evaluate all of the parameters. For the evaluation, samples were placed into randomly coded white dishes and served together with drinking water to rinse the panelists' oral cavities, following a test of each sample. The sensory characteristics were evaluated in three independent experiments.

5) Fatty acid composition

The FA composition was measured as described previously (Piao *et al*. 2017). Briefly, lipids in beef samples (5 g) were extracted with chloroform/methanol (100 mL) according to the procedure of Folch *et al*. (1957). Extracted lipids were methylated with BF_3-methanol (14%, w/w), and FA methyl esters (FAMEs) were injected into a gas chromatograph (HP 7890; Agilent Technologies, Santa Clara, CA, USA). A split inlet (split ratio, 50:1) was used to inject the samples into a capillary column (SP^{TM} 2560 Capillary column; 100 m × 0.25 mm × 0.20 μm film thickness). The inlet temperature was 210°C. N_2 served as the carrier gas, at a constant flow rate of 1 ml/min. The column oven temperatures were as follows: 100°C for 5 min, increased to 240°C at 4°C/min, then held at 240°C for 20 min. Individual FAMEs were identified by comparison of the relative retention times of the peaks from the

samples with those of the standard mixture (37-component FAME Mix; Supelco, Bellefonte, PA, USA). The FA composition of the fat was calculated based on the peak area. Relative quantities were expressed as weight percent of total FAs.

FA content (values per 100 g of beef) is also important to measure, and was obtained by using FA percentage in LT fat and fat content in LT as previously described (Piao *et al*. 2017). Individual FA content (g/100 g meat) was calculated as follows:

Individual FA content (g FA/100 g meat) = crude fat content (g fat/100 g meat) × individual FA percentage (%) × 0.953 (g FA/g fat)

6) Volatile compounds

To analyze the volatile compounds, samples were minced and grilled on a hot plate (PC-420D; Corning, Corning, NY, USA) until they reached an internal temperature of 72℃. Then, the sample (10 g) was immediately transferred to individually labeled 20 ml clear glass vials (N9306078; PerkinElmer, Boston, MA, USA) and closed with a polytetrafluoroethylene septa and screw cap. One minute later, the vials were placed in the oven of a headspace sampler, and extraction of the volatile compounds of the samples was performed using a headspace autosampler. The transfer line from the headspace sampler was directly connected to the injector for the gas chromatography (GC).

A PerkinElmer 680 GC equipped with a 600T mass spectrometry (MS) detector was used to analyze the volatile compounds. The compounds were separated using a HP-PLOT Q column (Agilent, Wilmington, DE, USA; 30 m × 0.53 mm × 0.25-μm film thickness). The GC conditions were as follows: initial oven temperature, 35°C; held for 5 min, increased by 7°C/min to 180°C, held for 0.0 min at 180°C, and then increased by 5°C/min to 250°C and held for 21 min. The transfer line temperature was maintained at 250°C. A mass spectrometer scanned from *m/z* 30 to *m/z* 250 with a 0.2 s cycle time. The ion source was set at 250°C. Headspace was maintained at 85°C for 30 min and a ramped oven temperature was used (50°C for 3 min, increased by at 5°C/min to 240°C, and maintained for 9 min). The inlet temperature was 210°C. Helium served as the carrier gas, delivered at a constant flow rate of 20 mL/min. The resolved MS spectra obtained from the custom scripts were matched against reference mass spectra by using the National Institute of Standards and Technology (NIST) mass spectral search program for application to the NIST/US Environmental Protection Agency (EPA)/National Institutes of Health (NIH) mass spectral library (ver. 2.0). A GC chromatogram was used to quantify the volatile compounds, and MS was used to identify the volatile compounds. Results of the analyses are expressed as percentages of the total chromatographic area.

7) Statistical analysis

Data (12 animals/group) were analyzed by analysis of variance using the general linear model procedure (Proc GLM) of SAS software (SAS Institute, Cary, NC, USA). The LSMEANS PDIFF option was used to compare differences among mean values at $P < 0.05$. The CORR procedure of SAS was used to calculate Pearson's correlation coefficients.

4. Result and discussion

1) Chemical composition, physico-chemical parameters, collagen and reducing sugar contents, and sensory traits

The chemical compositions, physico-chemical parameters, collagen and reducing sugar contents, and sensory traits of the LT of KC, HO, and AN breeds are shown in Table 20. The KC had the highest ($P < 0.05$) IMF content (21.1%), followed by HO (7.08%) and AN (3.14%), whereas KC had the lowest ($P < 0.05$) moisture and crude protein contents. This is consistent with previous studies that reported higher ($P < 0.05$) crude fat content in the loin of KC versus that of imported beef (Kim *et al*. 2000; Cho *et al*. 2011).

The LT of both KC and AN had similar CIE a^* (redness) values, but the LT of HO had a lower ($P < 0.05$) value than that of the two other breeds (Table 20). CIE L^*- (lightness) and CIE b^*-value (yellowness) did not differ ($P > 0.05$) among the

three breeds. Cho et al. (2011) also reported that the CIE L^* value of the loin did not differ among KC with a QG of 1+, KC with a QG of 1, Australian AN, and crossbred breeds (AN and Hereford mix). Imported AN had the highest ($P < 0.05$) pH, followed by KC and HO.

The shear force of the LT was the lowest ($P < 0.05$) in KC, followed by HO and AN (Table 20). Shear force is known to be an indicator of meat tenderness. A previous study reported that tenderness increased with a decrease in shear force (Destefanis *et al*. 2008), and that shear force in turn decreased with an increase in QG (Obuz *et al*. 2004). In our study, the lowest shear force was seen in KC; this may be attributable to that meat having the highest IMF content. Total collagen content in the LT did not differ ($P > 0.05$) among the three breeds (Table 20). Soluble collagen content was highest ($P < 0.05$) in AN, but did not differ between KC and HO. Insoluble collagen content was highest ($P < 0.05$) in HO, and again did not differ between KC and AN.

The reducing sugar content of the LT was highest ($P < 0.05$) in KC (0.42%), and the content did not differ between HO (0.18%) and AN (0.19%) (Table 20). Our study is the first to show that KC had the highest reducing sugar content among several breeds. Volatile flavor compounds mainly produce meat aromas and flavors during cooking. Maillard reactions between amino acids or peptides with reducing sugar are key processes in generating volatile compounds (Mottram and Nobrega

2002). The reducing sugar for this reaction included ribose, glucose, glucose 6-phosphate, and ribose 5-phosphate (Ba *et al*. 2012b). Meinert *et al*. (2009) reported that reducing sugar may act as important precursors of flavor in chicken meat. Thus, the two-fold higher reducing sugar content in the LT from KC may influence the production of volatile compounds during cooking.

All sensory parameters, including flavor, tenderness, juiciness, and overall acceptance, were highest ($P < 0.05$) in KC, and no differences in flavor or overall acceptance were observed between HO and AN (Table 20). The IMF content positively affected the sensory quality (Joo *et al*. 2013; Piao *et al*. 2015). In our study, the LT of KC had a significantly higher IMF content than that of the other two breeds, and the shear force of LT from KC was the lowest among the three breeds. Collectively, a higher IMF content and lower shear force may have contributed to the higher sensory quality of the LT of KC compared with the other two breeds. Additionally, the reducing sugar content was highest in KC, which may also in part contribute to the superior sensory traits of this breed, as discussed above.

Table 20. Chemical composition, physico-chemical parameter, collagen and reducing sugar contents, and sensory traits in the *longissimus thoracis* from Korean cattle (KC), Holstein (HO), and Angus (AN) steers

Item	KC	HO	AN	s.e.m.	*P*-value
Chemical composition					
Moisture, %	57.6[c]	67.4[b]	73.7[a]	0.99	0.001
Crude protein, %	18.2[b]	22.1[a]	21.5[a]	0.28	0.001
Crude fat, %	21.1[a]	7.08[b]	3.14[c]	1.14	0.001
Physico-chemical parameter					
CIE *L**	45.8	45.3	45.4	0.27	0.82
CIE *a**	17.0[a]	16.1[b]	17.5[a]	0.18	0.01
CIE *b**	3.72	3.00	3.35	0.16	0.27
pH	5.43[b]	5.19[c]	5.71[a]	0.04	0.001
Shear force, N	24.3[c]	32.0[b]	39.6[a]	1.73	0.001
Collagen content					
Total, mg/g	4.64	5.18	5.03	0.15	0.19
Soluble, mg/g	1.92[b]	1.91[b]	2.46[a]	0.11	0.03
Insoluble, mg/g	2.72[b]	3.27[a]	2.57[b]	0.11	0.01
Reducing sugar, %	0.42[a]	0.18[b]	0.19[b]	0.02	0.001
Sensory traits [A]					
Appearance	5.90[a]	5.70[a]	5.33[b]	0.07	0.004
Odor	6.23[a]	4.70[b]	5.23[b]	0.14	0.001
Taste	6.80[a]	3.90[b]	4.47[b]	0.25	0.001
Flavor	6.77[a]	4.03[b]	4.60[b]	0.23	0.001
Tenderness	6.73[a]	5.23[b]	3.90[c]	0.22	0.001
Juiciness	6.03[a]	4.70[b]	3.97[c]	0.18	0.001
Overall acceptance	6.83[a]	4.23[b]	4.73[b]	0.23	0.001

[A] The score was evaluated with 10 semi-trained panelists (1, extremely dislike; 5, neither dislike nor like; 9, extremely like). Values are means (n=12).

[a-c] Means with different letters within the same row differ ($P < 0.05$).

2) Percentages and contents of FA

Of the 18 FAs identified in this study, the percentage of C18:1n9 was the highest, ranging from 39% to 44%, followed by palmitic acid (C16:0) (26.5%–30%) and stearic acid (C18:0) (11.4–15.9%) (Table 21). The percentages of C16:0, palmitoleic acid (C16:1), and C18:1n9 were lowest ($P < 0.01$) in AN, and did not differ between KC and HO. Cho et al. (2011) also reported higher proportions of C16:1 and C18:1n9 in the loin fat of KC versus AN. The percentage of monounsaturated fatty acids (MUFAs) in the LT of KC and HO was higher ($P < 0.05$) than that in AN beef. This may be because the proportion of C18:1n9 was higher in KC and HO than in AN. The MUFA deposition in the triacylglycerol fraction increased with increasing IMF content, whereas PUFA was preferentially incorporated into the phospholipid fraction in the cell membranes (Raes *et al*. 2003). Thus, the higher IMF of KC compared with AN may have contributed directly to the higher MUFA content of KC. The percentages of C18:0, linoleic acid (C18:2), and arachidonic acid (C20:4) were lowest ($P < 0.01$) in KC. The PUFA percentage of LT fat was lowest ($P < 0.01$) in KC and highest in AN. Cho *et al*. (2011) also reported lower C18:2 and PUFA percentages for the *longissimus* muscle fat of KC versus AN. Mandell *et al*. (1998) reported that C18:1n9 was positively associated with beef flavor, whereas PUFAs were negatively associated with beef flavor. Taken together, variation in the proportions of these FAs may in part have contributed to differences in the sensory traits of the LT of different breeds.

Using the FA percentage and fat content of the LT, individual FA contents (g FA/100 g LT) were calculated based on a conversion factor (0.953; Anderson *et al*. 1975). Among all FAs, the C18:1n9 content of the LT was the highest, ranging from 1.9 to 8.33 g/100 g meat, followed by C16:0 (1.27–5.62 g/100 g meat) and C18:0 (0.76–2.11 g/100 g meat) (Supplementary Table 1). The content of most FAs in the LT, including C16:0, C16:1, C18:0, and C18:1n9, were highest ($P < 0.001$) in KC and lowest ($P < 0.001$) in AN. These results are likely attributable to the IMF content in the LT being highest in KC and lowest in AN.

Table 21. Fatty acid composition (% of fat) in the *longissimus thoracis* from Korean cattle (KC), Holstein (HO), and Angus (AN) steers

Fatty acid	KC	HO	AN	s.e.m.	*P*-value
C12:0	0.11[a]	0.11[a]	0.00[b]	0.01	0.001
C13:0	0.04	0.06	0.10	0.01	0.14
C14:0	4.09[a]	3.90[a]	3.02[b]	0.14	0.004
C14:1	1.39[a]	1.36[a]	0.68[b]	0.09	0.001
C15:0	0.23[c]	0.33[b]	0.50[a]	0.02	0.001
C16:0	30.0[a]	29.3[a]	26.5[b]	0.36	0.001
C16:1	5.12[a]	5.01[a]	3.39[b]	0.19	0.001
C17:0	0.36[c]	0.92[b]	1.94[a]	0.15	0.001
C17:1	0.50[c]	0.66[b]	1.12[a]	0.05	0.001
C18:0	11.4[b]	12.4[b]	15.9[a]	0.42	0.001
C18:1t	0.22[b]	0.22[b]	0.42[a]	0.03	0.002
C18:1n9	44.0[a]	41.6[a]	39.0[b]	0.54	0.001
C18:2	1.60[b]	2.55[b]	5.20[a]	0.31	0.001
C18:3	0.04[b]	0.09[b]	0.19[a]	0.01	0.001
$C18:2_{9c11t}$ + $C18:2_{10t12c}$	0.36	0.31	0.37	0.02	0.54
C20:1	0.27[a]	0.18[b]	0.22[ab]	0.01	0.02
C20:3	0.14[b]	0.30[a]	0.36[a]	0.03	0.001
C20:4	0.17[c]	0.72[b]	1.04[a]	0.08	0.001
SFA[A]	46.2	47.0	48.0	0.40	0.24
USFA[B]	53.8	53.0	52.0	0.40	0.25
MUFA[C]	51.5[a]	49.1[a]	44.9[b]	0.64	0.001
PUFA[D]	2.31[c]	3.97[b]	7.16[a]	0.42	0.001

[A] SFA (saturated fatty acids) = C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0; [B] USFA (unsaturated fatty acids) = C14:1 + C16:1 + C17:1 + C18:1t + C18:1c + C20:1; [C] MUFA (monounsaturated fatty acids) = C14:1 + C16:1 + C17:1 + C18:1t + C18:1c + C20:1; [D] PUFA (polyunsaturated fatty acids) = C18:2 + C18:3 + C20:3 + C20:4 + $C18:2_{9c11t}$ + $C18:2_{10t12c}$. Values are means (n=12).

[a-c] Means with different letters within the same row differ ($P < 0.05$).

3) Volatile compounds

A total of 31 volatile compounds, including aldehydes, hydrocarbons, ketones, pyrazines, alcohols, and terpenes, were detected in the LT of the three cattle breeds (Table 22). The abundant volatile compounds, accounting for 7.28–60% of the total volatile compounds, included acetaldehyde and 2-propanone. The moderate compounds, accounting for 1.26–25.9% of the total volatile compounds, included 2-methyl butanal, hexanal, and methanethiol. The minor compounds, accounting for 0.01–8.5% of the total volatile compounds, included propanal, butanal, 3-methyl butanal, pentanal, heptanal, benzaldehyde, octanal, nonanal, butane, carbon disulfide, 2-butene, n-pentane, hexane, heptane, octane, ethylacetate, 2-butanone, 2,3-butanedione, 3-hydroxy-2-butanone, 2-heptanone, 2,3-octanedione, dimethyl sulfide, ethanol, isopropyl alcohol, 1-pentanol, and 1,3,5-cycloheptatriene.

The percentages of produced aldehydes varied among the three breeds. Acetaldehyde and 3-methyl butanal contents were highest ($P < 0.05$) in KC, followed by HO and AN (Table 22). The 2-methyl butanal content was similar between KC and HO, and was lowest in AN. In contrast, propanal, pentanal, hexanal, heptanal, and octanal contents were highest ($P < 0.05$) in AN, and similar between KC and HO. During the formation of volatile compounds, Strecker degradation is one of the most important reactions related to the generation of the precursors of thermal formation aromas. The Strecker aldehydes acetaldehyde

(sharp, penetrating, fruity), 2-methyl propanal (penetrating, green), 3-methyl butanal (malty, green), and 2-methyl butanal (ethereal, bitter, almond, green), which are well-known volatile components of cooked beef, may be derived from alanine, valine, leucine, and isoleucine, respectively (Huang and Ho 2012). Of these aldehydes, acetaldehyde is associated with many reactions that may explain the formation of meaty aromas: 3,5-dimethyl-1,2,4-trithiolane, trithioacetaldehyde, thialdine, 2,4-dimethylthiazole, 2,4-dimethyl-5-ethylthiazole, and 2,4,5-trimethyl-3-thiazoline together appear to impart a complex meat flavor during cooking. Acetaldehyde provides the carbon skeleton on their structures (Huang and Ho 2012). In addition, cysteine is also a well-known precursor of meat flavor. Some compounds, including mercaptoacetaldehyde, hydrogen sulfide, and acetaldehyde, were identified as common products when cysteine was boiled with various carbonyl compounds. Of these compounds, acetaldehyde is produced by Strecker degradation or cysteine hydrolysis (Fujimaki *et al*. 1969). Acetaldehyde was the most abundant volatile compound detected in this study. Among the three cattle breeds, the acetaldehyde content was significantly higher in KC than in HO and AN, and there was a positive correlation between acetaldehyde content and sensory scores. The reducing sugar content was also significantly higher in KC, which may in part be due to the relatively high content of sugars in this type of beef; reducing sugar is a substrate in Maillard reactions or complex Maillard and Strecker reactions. Thus, higher percentages of acetaldehydes may in part have contributed to the

higher sensory scores of KC beef.

Hydrocarbon percentages varied among the three breeds. The methanethiol percentage in the LT was highest ($P < 0.01$) in HO, and lowest in AN. Butane, hexane, and ethylacetate were present in similar proportions in HO and AN, while KC had a lower butane percentage ($P < 0.05$) than that of the other two breeds. The percentages of n-pentane and heptane were highest ($P < 0.01$) in AN, while the KC and HO breeds had similar percentages of these compounds. Carbon disulfide and octane percentages did not differ ($P > 0.05$) among the three breeds. Hydrocarbons are derived from lipid thermal oxidation, which is a reaction catalyzed by heme compounds such as myoglobin and hemoglobin (Shahidi *et al*. 1986). Although hydrocarbon components make up a certain proportion of volatile compounds, Min *et al*. (1979) reported that both saturated and unsaturated hydrocarbons play a minor role in the flavor of roast beef, as they contain relatively weak, non-beef-like odors. In this study, the correlations between flavor and the percentages of most hydrocarbons, including butane, carbon disulfide, 2-butene, n-pentane, hexane, heptane, octane, and ethylacetate were not significant (data not shown). Collectively, hydrocarbon variation among the three breeds did not seem to significantly affect the flavor of LT.

Among the ketones, 2-propanone percentages were similar in both KC and HO LT, and lowest ($P < 0.01$) in AN LT. The percentage of 2-butanone was highest (*P*

<0.01) in HO LT, and lowest in AN LT. Both 2,3-butanedione and 3-hydroxy-2-butanone percentages were highest ($P < 0.01$) in KC, while HO and AN had similar percentages of these compounds. Both 2-heptanone and 2,3-octanedione percentages were highest ($P < 0.01$) in AN, while KC and HO had similar contents of these compounds. Previous reports have shown that 2,3-butanedione and 3-hydroxy-2-butanone contents in AN were higher in prime grades compared with choice or standard grades (Legako *et al*. 2015). Ketones are also related to fat content, and ketone content increases with increasing fat content (El-Magoli *et al*. 1996).

Table 22. Volatile compounds (%) in the *longissimus thoracis* from Korean cattle (KC), Holstein (HO), and Angus (AN) steers

Volatile compound	KC	HO	AN	s.e.m.	*P*-value
Aldehyde					
Acetaldehyde	60.0[a]	49.1[b]	31.5[c]	2.43	0.001
Propanal	0.29[b]	0.53[b]	2.48[a]	0.19	0.001
Butanal	0.06[b]	1.17[a]	0.33[ab]	0.22	0.1
2-Methyl butanal	2.79[a]	2.76[a]	1.26[b]	0.13	0.001
3-Methyl butanal	1.25[a]	0.98[b]	0.54[c]	0.06	0.001
Pentanal	0.81[b]	1.56[b]	8.50[a]	0.66	0.001
Hexanal	1.76[b]	3.81[b]	25.9[a]	2.07	0.001
Heptanal	0.07[b]	0.14[b]	0.92[a]	0.08	0.001
Benzaldehyde	0.24[a]	0.29[a]	0.17[b]	0.01	0.004
Octanal	0.05[b]	0.09[b]	0.58[a]	0.04	0.001
Nonanal	0.02	0.01	0.01	0.00	0.79
Hydrocarbon					
Methanethiol	8.89[b]	12.8[a]	3.98[c]	0.75	0.001
Butane	0.94[b]	1.08[a]	1.12[a]	0.03	0.02
Carbon disulfide	1.77	2.01	0.96	0.20	0.13
2-Butene	0.29[b]	0.36[a]	0.30[b]	0.01	0.02
n-Pentane	0.73[b]	1.70[b]	6.47[a]	0.49	0.001
Hexane	0.41[b]	0.83[a]	0.65[ab]	0.06	0.01
Heptane	0.05[b]	0.06[b]	0.53[a]	0.04	0.001
Octane	0.45	1.56	1.30	0.48	0.63
Ethylacetate	0.32[b]	1.27[a]	0.62[ab]	0.17	0.1
Ketone					
2-Propanone	14.8[a]	13.9[a]	7.28[b]	0.68	0.001
2-Butanone	0.96[b]	1.49[a]	0.38[c]	0.11	0.001
2,3-Butanedione	0.08[a]	0.04[b]	0.03[b]	0.00	0.001

3-Hydroxy-2-butanone	0.89[a]	0.14[b]	0.17[b]	0.08	0.001
2-Heptanone	0.04[b]	0.05[b]	0.24[a]	0.02	0.001
2,3-Octanedione	0.05[b]	0.14[b]	1.01[a]	0.09	0.001
Pyrazine					
Dimethyl sulfide	0.48	0.53	0.65	0.05	0.29
Alcohol					
Ethanol	1.07	0.88	0.45	0.18	0.35
Isopropyl alcohol	0.26[b]	0.49[a]	0.24[b]	0.03	0.001
1-Pentanol	0.06[b]	0.08[b]	0.72[a]	0.06	0.001
Terpene					
1,3,5-Cycloheptatriene	0.18[b]	0.22[b]	0.66[a]	0.05	0.001

Values are means (n=12).

[a-c] Means with different letters within the same row differ ($P < 0.05$).

4) Correlation

We analyzed Pearson correlation coefficients for the chemical composition, physical-chemical parameters, and sensory traits of pooled LTs from KC, HO, and AN (Supplementary Table 2). Fat content was one of the factors most strongly associated with sensory traits (flavor, tenderness, juiciness, and overall acceptance); these results were consistent with our previous study that showed positive correlations between fat content and sensory traits (juiciness and overall acceptance) (Piao *et al*. 2015).

Little information about the correlation between beef carcass characteristics and reducing sugar content is available. In this study, we analyzed Pearson correlation coefficients for chemical composition and sensory traits with the reducing sugar content of pooled LTs from the three breeds. The reducing sugar content in the LT was positively correlated ($0.63 \leq r \leq 0.71$; $P < 0.001$) with fat content and sensory traits (flavor and overall acceptance) (Table 23). We also found that KC LT had the highest reducing sugar content and the best sensory traits among the three breeds. As mentioned above, reducing sugar, especially glucose, can contribute to an improved meat flavor by reacting with amino acids to produce important volatile compounds via Maillard reactions. Our study thus implies that higher reducing sugar content may positively affect flavor and overall acceptance.

Crude fat content was positively correlated with C14:0, C16:0 ($0.48 \leq r \leq 0.50$,

$P < 0.01$), C16:1, C18:1n9, and MUFA percentages ($0.53 \leq r \leq 0.73$, $P < 0.001$), and negatively correlated with C18:0, C18:2, and PUFA percentages ($-0.75 \leq r \leq -0.71$, $P < 0.001$), in the LT fat (Supplementary Table 3). This is generally consistent with our previous study (Piao *et al*. 2017) that showed positive correlations of C18:1n9 and MUFA percentages, and negative correlations of C18:2 and PUFA percentages, with LT fat content.

Table 23. Pearson correlation coefficients of chemical composition and sensory traits with reducing sugar contents of pooled *longissimus thoracis* from Korean cattle (KC), Holstein (HO), and Angus (AN) steers

Item	Reducing sugar content
Chemical composition	
Moisture	-0.70***
Crude protein	-0.72***
Crude fat	0.71***
Sensory traits	
Flavor	0.63***
Tenderness	0.59***
Juiciness	0.55***
Overall acceptance	0.63***

n=36. *** $P < 0.001$.

Sensory traits, including flavor, tenderness, and juiciness, were positively correlated ($0.35 \leq r \leq 0.61$, $P < 0.05$) with C16:0, C18:1n9, and MUFA percentages in LT fat, and negatively correlated ($-0.70 \leq r \leq -0.42$, $P < 0.05$) with C18:2 and PUFA percentages (Table 24). Mandell *et al*. (1998) reported that C18:1n9 might be positively associated with beef flavor, whereas PUFA might be negatively associated with beef flavor. Other studies have also shown that C18:1n9 is positively associated with beef flavor, whereas C18:2 is negatively associated with beef flavor (Garmyn *et al*. 2011). O'Quinn (2012) reported that MUFAs, such as lauric acid (C12:1), myristic acid (C14:1), C16:1, and C18:1n9, were all positively correlated with overall flavor desirability (beefy, browned/grilled, brothy, buttery, and sweet flavors), and negatively correlated with bloody/metallic, grassy, gamey, livery, fishy, and sour flavor intensities, which can impart bad flavor to the beef. Collectively, C18:1n9, and MUFA percentages may positively affect sensory traits, whereas C18:2 and PUFA percentages may negatively affect sensory traits. In this study, we found that C18:1n9 and MUFA percentages were highest, and C18:2 and PUFA percentages were lowest, in KC LT fat among the three breeds. Taken together, the high percentages of C18:1n9 and MUFA, and low percentages of C18:2 and n-6 PUFA, in KC LT fat may contribute to the sensory traits of KC beef being rated as the best for Korean consumers.

Table 24. Pearson correlation coefficients of sensory traits with fatty acid composition (% of fat) of pooled *longissimus thoracis* from Korean cattle (KC), Holstein (HO), and Angus (AN) steers

Item	C14:0	C16:0	C16:1	C18:0	C18:1n9	C18:2	SFA	USFA	MUFA	PUFA
Flavor	0.24	0.35*	0.21	-0.30	0.35*	-0.42*	-0.07	0.07	0.35*	-0.46**
Tenderness	0.43**	0.61***	0.49**	-0.58***	0.53***	-0.70***	-0.18	0.18	0.57***	-0.70***
Juiciness	0.32	0.50**	0.37*	-0.50**	0.51**	-0.59***	-0.21	0.20	0.52***	-0.60***
Overall acceptance	0.26	0.37*	0.24	-0.30	0.32	-0.43**	-0.03	0.03	0.33	-0.47**

n=36. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

We analyzed the correlation coefficients for chemical composition, physico-chemical parameters, reducing sugar content, and sensory traits with the volatile compound content of pooled LTs from KC, HO, and AN. Both the crude fat ($0.63 \leq r \leq 0.70$, $P < 0.001$) and reducing sugar contents ($0.39 \leq r \leq 0.57$, $P < 0.05$) were positively correlated with several volatile compounds, including acetaldehyde, 3-methyl butanal, 2,3-butanedione, and 3-hydroxy-2-butanone (Table 25). The percentages of acetaldehyde, 3-methyl butanal, 2,3-butanedione, and 3-hydroxy-2-butanone were also positively correlated ($0.39 \leq r \leq 0.66$, $P < 0.05$) with flavor and/or overall acceptance. Our results are consistent with previous studies that reported positive correlations between the 3-methyl butanal contents of loins from AN, HO, and American Wagyu with buttery/beef fat flavor (O'Quinn *et al*. 2016). Both 2-methyl butanal and 3-methyl butanal have been associated with nutty and buttery odors, which positively affect flavor (Giri *et al*. 2010). Other studies have also reported that concentrations of 2,3-butanedione and 3-hydroxy-2-butanone had positive correlations with beef flavor (Legako *et al*. 2015; O'Quinn *et al*. 2016). Both 2,3-butanedione and 3-hydroxy-2-butanone are widely used to produce an artificial buttery flavor, and they are also known to impart a buttery flavor to beef (Peterson *et al*. 1975; Machiels *et al*. 2004). We also found that crude fat and reducing sugar contents as well as the percentages of 3-methyl butanal, 2,3-butanedione, and 3-hydroxy-2-butanone, were highest in KC LT among the three breeds, which may in part contribute to high levels of volatile compounds in KC

beef. This in turn may play a positive role in the superior flavor of KC beef versus the other two breeds for Korean consumers. The impact of 2,3-butanedione and 3-hydroxy-2-butanone on beef flavor in this study may have been minor because these compounds were present in relatively low amounts (less than 1.0% of all volatile compounds). Further study is warranted to elucidate the role of reducing sugar in the formation of volatile compounds, and their contribution to beef flavor.

In contrast, both crude fat ($-0.71 \leq r \leq -0.60$, $P < 0.001$) and reducing sugar contents ($-0.49 \leq r \leq -0.39$, $P < 0.05$) were negatively correlated with several volatile compounds, including propanol, pentanal, hexanal, heptanal, octanal, and n-pentane (Table 25). None of these volatile compounds showed significant correlations with flavor and overall acceptance, although they were negatively correlated with tenderness and juiciness.

Table 25. Pearson correlation coefficients of chemical composition, physico-chemical parameter, reducing sugar, and sensory traits with volatile compounds of pooled *longissimus thoracis* from Korean cattle (KC), Holstein (HO), and Angus (AN) steers

Item	Acetaldehyde	Propanal	2-Methyl butanal	3-Methyl butanal	Pentanal	Hexanal	Heptanal	Octanal	Methanethiol	n-Pentane	2-Propanone	2-Butanone	2,3-Butanedione	3-Hydroxy-2-butanone
Chemical composition														
Moisture	-0.73***	0.68***	-0.68***	-0.73***	0.72***	0.72***	0.66***	0.74***	-0.38*	0.75***	-0.72***	-0.26	-0.69***	-0.67***
Crude protein	-0.50**	0.39*	-0.35*	-0.43**	0.42*	0.39*	0.37*	0.40*	-0.04	0.46**	-0.44**	0.08	-0.56***	-0.59***
Crude fat	0.70***	-0.62***	0.65***	0.69***	-0.67***	-0.67***	-0.60***	-0.68***	0.35*	-0.71***	0.68***	0.20	0.67***	0.63***
Physico-chemical parameters														
pH	-0.47**	0.63***	-0.77***	-0.50**	0.67***	0.69***	0.64***	0.71***	-0.84***	0.63***	-0.56***	-0.69***	-0.22	0.05
Shear force	-0.43**	0.52***	-0.43**	-0.60***	0.46**	0.42*	0.47**	0.49**	-0.21	0.43**	-0.37*	-0.23	-0.26	-0.36*
Reducing sugar	0.52**	-0.40*	0.36*	0.56***	-0.44**	-0.42*	-0.39*	-0.40*	-0.01	**-0.49****	0.40*	-0.04	**0.39***	**0.57*****
Sensory traits														
Flavor	0.40*	-0.20	0.21	0.52***	-0.23	-0.26	-0.17	-0.27	-0.12	-0.29	0.28	-0.07	**0.54*****	**0.66*****
Tenderness	0.67***	-0.61***	0.64***	0.79***	-0.64***	-0.67***	-0.57***	-0.68***	0.34*	-0.66***	0.64***	0.31	0.56***	0.58***
Juiciness	0.58***	-0.54***	0.55***	0.72***	-0.57***	-0.60***	-0.49**	-0.57***	0.28	-0.59***	0.57***	0.29	0.62***	0.62***
Overall acceptance	0.39*	-0.22	0.20	0.52***	-0.25	-0.28	-0.19	-0.28	-0.13	-0.31	0.31	-0.03	0.52***	0.66***

n=36. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Correlation coefficient values of several volatile compounds (butanal, butane, carbon disulfide, octane, ethylacetate, benzaldehyde, nonanal, 2-butene, hexane, heptane, 2-heptanone, 2,3-octanedione, dimethyl sulfide, ethanol, isopropyl alcohol, 1-pentanol, 1,3,5-cycloheptatriene) were $r < 0.50$ or $P > 0.05$, and these data are not shown in this table.

We analyzed the correlation coefficients for FA percentages (in LT fat) with volatile compounds. The percentages of C16:0, C16:1, C18:1n9, and MUFA of LT fat were positively correlated ($0.41 \leq r \leq 0.78$, $P < 0.05$) with acetaldehyde, 2-methyl butanal, 3-methyl butanal, and 2-propanone percentages (Table 26). The percentages of C18:1n9 and MUFA of LT fat were also positively correlated ($0.49 \leq r \leq 0.61$, $P < 0.01$) with 2,3-butanedione and 3-hydroxy-2-butanone, which impart a buttery scent to beef. Both C18:1n9, and MUFA are known to be positively associated with beef flavor (Mandell *et al*. 1998). Currently, limited information is available on the generation of volatile compounds from specific FAs. In contrast, the C16:0, C16:1, C18:1n9, and MUFA percentages of LT fat were negatively correlated ($-0.81 \leq r \leq -0.50$, $P < 0.01$) with several volatile compounds, including propanol, pentanal, hexanal, and n-pentane (Table 26). Pentanal and hexanal can impart pungent, fishy, and grassy odors, which are negatively associated with flavor (Kaseleht *et al*. 2010). Our study showed that these compounds were negatively correlated with tenderness and juiciness, and not correlated with flavor or overall acceptance (Table 25).

The percentages of C18:0, C18:2, and PUFA of LT fat were negatively correlated ($-0.74 \leq r \leq -0.38$, $P < 0.05$) with acetaldehyde, 2-methyl butanal, 3-methyl butanal, 2-propanone, 2,3-butanedione, and 3-hydroxy-2-butanone percentages, whereas all of these FAs were positively correlated ($0.60 \leq r \leq 0.83$, $P < 0.001$) with propanal, pentanal, hexanal, butane, and n-pentane (Table 26). C18:0

is negatively correlated with beef fat aroma and flavor (Melton *et al*. 1982). Also, PUFAs, including C18:2, are known to be oxidized easily, so they can easily exert a negative effect on beef flavor (Wood *et al*. 2003).

Overall, our results indicate that specific FAs can be positively or negatively correlated with volatile compounds. Our data imply that FA composition may in part affect the sensory traits of beef, including flavor, through the generation of volatile compounds.

In our study, both KC and HO were raised in Korea with a Korean cattle feed regimen, while AN were raised in the United States with a typical US cattle feed regimen. These dietary and environmental differences may affect beef characteristics. Therefore, our study has limitation for interpretation of results.

Table 26. Pearson correlation coefficients between fatty acid composition (% of fat) and volatile compounds of pooled *longissimus thoracis* from Korean cattle (KC), Holstein (HO), and Angus (AN) steers

Item	Acetaldehyde	Propanal	2-Methyl butanal	3-Methyl butanal	Pentanal	Hexanal	Methanethiol	Butane	n-Pentane	2-Propanone	2,3-Butanedione	3-Hydroxy-2-butanone
C14:0	0.39*	-0.43**	0.50**	0.47**	-0.46**	-0.47**	0.43**	-0.19	-0.49**	0.46**	0.21	0.15
C16:0	0.41*	-0.52***	0.59***	0.71***	-0.50**	-0.50**	0.43**	-0.17	-0.51**	0.45**	0.28	0.24
C16:1	0.58***	-0.60***	0.65***	0.48**	-0.66***	-0.66***	0.56***	-0.35*	-0.68***	0.65***	0.29	0.21
C18:0	-0.68***	0.69***	-0.74***	-0.57***	0.74***	0.74***	-0.60***	0.37*	0.76***	-0.71***	-0.52***	-0.38*
C18:1n9	0.73***	-0.59***	0.58***	0.42**	-0.69***	-0.70***	0.33*	-0.52***	-0.71***	0.64***	0.61***	0.50**
C18:2	-0.72***	0.63***	-0.72***	-0.68***	0.77***	0.79***	-0.48**	0.62***	0.83***	-0.74***	-0.52***	-0.43**
SFA	-0.48**	0.45**	-0.36*	-0.06	0.47**	0.45**	-0.29	0.24	0.44**	-0.44**	-0.42*	-0.30
USFA	0.48**	-0.44**	0.36*	0.06	-0.46**	-0.45**	0.29	-0.23	-0.43**	0.44**	0.41*	0.30
MUFA	0.78***	-0.67***	0.68***	0.47**	-0.78***	-0.78***	0.46**	-0.56***	-0.81***	0.75***	0.61***	0.49**
PUFA	-0.73***	0.60***	-0.70***	-0.67***	0.75***	0.77***	-0.43**	0.63***	0.82***	-0.72***	-0.54***	-0.46**

n=36. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Correlation coefficient values of several volatile compounds (butanal, carbon disulfide, octane, ethylacetate, 2-butanone, heptanal, benzaldehyde, octanal, nonanal, 2-butene, hexane, heptane, 2-heptanone, 2,3-octanedione, dimethyl sulfide, ethanol, isopropyl alcohol, 1-pentanol, 1,3,5-cycloheptatriene) were $r < 0.50$ or $P > 0.05$, and these data are not shown in this table.

Supplementary Table 1. Fatty acid contents (g/100g meat)[A] in the *longissimus thoracis* from Korean cattle (KC), Holstein (HO), and Angus (AN) steers

Fatty acid	KC	HO	AN	s.e.m.	*P*-value
C12:0	0.02[a]	0.01[b]	0.00[c]	0.00	0.001
C13:0	0.01	0.01	0.01	0.00	0.61
C14:0	0.77[a]	0.34[b]	0.15[c]	0.05	0.001
C14:1	0.27[a]	0.12[b]	0.03[c]	0.02	0.001
C15:0	0.04[a]	0.03[b]	0.02[b]	0.00	0.001
C16:0	5.62[a]	2.51[b]	1.27[c]	0.33	0.001
C16:1	0.97[a]	0.43[b]	0.17[c]	0.06	0.001
C17:0	0.06	0.08	0.10	0.01	0.31
C17:1	0.09[a]	0.06[b]	0.06[b]	0.00	0.001
C18:0	2.11[a]	1.05[b]	0.76[c]	0.11	0.001
C18:1t	0.04[a]	0.02[b]	0.02[b]	0.00	0.005
C18:1n9	8.33[a]	3.55[b]	1.90[c]	0.51	0.001
C18:2	0.30[a]	0.22[b]	0.23[b]	0.01	0.003
C18:3	0.01	0.01	0.01	0.00	0.35
$C18:2_{9c11t}$ + $C18:2_{10t12c}$	0.07[a]	0.03[b]	0.02[b]	0.01	0.001
C20:1	0.05[a]	0.02[b]	0.01[b]	0.00	0.001
C20:3	0.03[a]	0.03[a]	0.02[b]	0.00	0.01
C20:4	0.03[c]	0.06[a]	0.04[b]	0.00	0.001
SFA[B]	8.63[a]	4.02[b]	2.30[c]	0.48	0.001
USFA[C]	10.2[a]	4.53[b]	2.50[c]	0.61	0.001
MUFA[D]	9.75[a]	4.19[b]	2.18[c]	0.59	0.001
PUFA[E]	0.43[a]	0.34[b]	0.31[b]	0.02	0.002

[A] Individual fatty acid content per meat (g/100g meat) was calculated as described in Materials and Methods. [B] SFA (saturated fatty acids) = C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0. [C] USFA (unsaturated fatty acids) = C14:1 + C16:1 + C17:1 + C18:1t + C18:1c + C20:1. [D] MUFA (monounsaturated fatty acids) = C14:1 + C16:1 + C17:1 + C18:1t + C18:1c + C20:1. [E] PUFA (polyunsaturated fatty acids) = C18:2 + C18:3 + C20:3 + C20:4 + $C18:2_{9c11t}$ + $C18:2_{10t12c}$. Values are means (n=12).

[a-c] Means with different letters within the same row differ ($P < 0.05$).

Supplementary Table 2. Pearson correlation coefficients among chemical composition, physico-chemical parameters, and sensory traits of pooled *longissimus thoracis* from Korean cattle (KC), Holstein (HO), and Angus (AN) steers

Item	Crude protein	Crude fat	CIE L*	CIE a*	CIE b*	pH	Shear force	Flavor	Tenderness	Juiciness	Overall acceptance
Chemical composition											
Moisture	0.82***	-0.98***	-0.09	0.10	-0.24	0.08	0.53***	-0.71***	-0.82***	-0.79***	-0.68***
Crude protein	–	-0.88***	-0.04	-0.05	-0.14	-0.14	0.32	-0.71***	-0.54***	-0.55***	-0.67***
Crude fat		–	0.05	-0.05	0.23	-0.08	-0.48**	0.71***	0.76***	0.73***	0.67***
Physico-chemical parameters											
CIE *L**			–	0.25	0.49**	0.26	-0.03	0.14	0.13	0.08	0.14
CIE *a**				–	0.75***	0.30	0.07	0.30	-0.01	0.06	0.29
CIE *b**					–	0.16	-0.03	0.41*	0.34*	0.38*	0.40*
pH						–	0.14	0.23	0.03	0.12	0.26
Shear force							–	-0.27	-0.45**	-0.39*	-0.30
Sensory traits											
Flavor								–	0.80***	0.84***	0.99***
Tenderness									–	0.96***	0.81***
Juiciness										–	0.85***

n=36. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Supplementary Table 3. Pearson correlation coefficients of chemical composition and physico-chemical parameters with fatty acid composition (% of fat) of pooled *longissimus thoracis* from Korean cattle (KC), Holstein (HO), and Angus (AN) steers

Item	C14:0	C16:0	C16:1	C18:0	C18:1n9	C18:2	SFA	USFA	MUFA	PUFA
Chemical composition										
Moisture	-0.49**	-0.55***	-0.56***	0.73***	-0.70***	0.77***	0.39*	-0.39*	-0.76***	0.79***
Crude protein	-0.32	-0.28	-0.35*	0.50**	-0.56***	0.51***	0.36*	-0.35*	-0.59***	0.56***
Crude fat	0.48**	0.50**	0.53***	-0.71***	0.67***	-0.72***	-0.39*	0.38*	0.73***	-0.75***
Physico-chemical parameters										
pH	-0.52***	-0.56***	-0.61***	0.66***	-0.28	0.56***	0.16	-0.16	-0.43**	0.51**
Shear force	-0.49**	-0.63***	-0.47**	0.45**	-0.12	0.47**	-0.10	0.11	-0.23	0.46**

n=36. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

5. Conclusion

In conclusion, the IMF, reducing sugar content, and sensory traits of the LT varied among KC, HO, and AN steers. The KC LT had the highest IMF and reducing sugar contents, and the best sensory traits (flavor, tenderness, juiciness, and overall acceptance). The IMF and reducing sugar contents were positively correlated with all of the sensory traits, suggesting that these factors may positively affect beef flavor. C16:0, C18:1n9, and MUFA may positively affect sensory traits, whereas C18:2 and PUFA may negatively affect sensory traits. The percentages of different volatile compounds in the LT also varied among the three breeds. The KC had the highest percentage of volatile compounds, including acetaldehyde, 3-methyl butanal, and 3-hydroxy-2-butanone, and these compounds were positively correlated with flavor. Our results demonstrated that variations in IMF, reducing sugar content, and FA and volatile compound profiles may contribute to differences in the sensory characteristics of the LT among breeds. The results of this study enhance our understanding of the association of reducing sugar and volatile compound contents with the sensory traits of beef. This information may help in determining beef palatability. This study has limitations of interpretation, considering that beef samples were collected in cattle with different conditions including feeding method, diet type, slaughter weight, and slaughter age.

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CHAPTER FIVE

Effects of dietary glycerin replacement on growth performance and rumen and carcass characteristics in Korean cattle steers

1. Abstract

This objective of this study was to evaluate the effects of dietary glycerin replacement on growth performance, blood metabolites, rumen characteristics, and carcass and sensory characteristics in Korean cattle steers. Steers (n=20; average 27±0.2 months old; 647±10.5kg of body weight) were assigned into a control (n=10; conventional feeding) and a glycerin (n=10; 3% glycerin replacement) group, respectively. A concentrate were individually fed with the amount of 1.5% of body weight using an automatic feeding station, and 1kg of rice straw were also individually fed twice daily (0.5kg/time) for 5 months. Steers were weighed at 4 weeks interval, and blood samples and rumen fluid were also collected at 4 weeks interval. Steers were slaughtered at average 31.8±0.2 months old and *longissimus thoracis* (LT) samples were collected for carcass measurements. Crude glycerin replacement increased ($P < 0.05$) concentrate intake. Average daily gain (ADG) and

feed efficiency (FE) were not changed ($P > 0.05$) by glycerin replacement. Glycerin replacement decreased blood glucose concentrations at 4 weeks but tended to increase ($P = 0.10$) those at 20 weeks. Glycerin replacement did not affect ($P > 0.05$) acetate, propionate, butyrate, and total VFA concentrations, but tended ($P = 0.06$) to decrease acetate/propionate ration at 16 weeks but not at other sampling times. Glycerin replacement did not affect ($P > 0.05$) carcass characteristics including LM area, marbling score, quality grade, and yield grade. Glycerin replacement did not affect ($P > 0.05$) chemical and physicochemical compositions, reducing sugar and glycogen contents, sensory traits (taste, flavor, tenderness, overall acceptance), and fatty acid contents in the LT. These results indicate that dietary glycerin replacement increased feed intake and resulted in minor changes of blood glucose concentrations and acetate/propionate ratio in the rumen, but it did not change growth performance, carcass and sensory traits of Korean cattle steers.

2. Introduction

Energy sources of feedstuff, such as corn and molasses, are essential feed ingredients for beef cattle diets. However, because of constantly rising cost of these energy sources, alternative feed ingredients needs to be developed for the cost-effective and sustainable livestock industry. Biodiesel industry worldwide has developed rapidly with its high production (Benedeti et al., 2016). The biodiesel is

commonly yielded via catalyzed reactions between alcohol and triacylglycerides in animal fats and vegetable oils (Van Gerpen, 2005). Of these oil or fat used to produce biodiesel, approximately ten percent of glycerin are generated (Parsons et al., 2009).

Glycerin potentially serves as a gluconeogenic substrate for ruminants (Chung et al., 2007). In the rumen, glycerol can be fermented to propionate that can act as glucogenic precursor in ruminants. The glycerol that escapes ruminal fermentation can be converted to glycerol-3-phosphate via phosphorylation first, which is catalyzed by glycerol kinase, and then enters gluconeogenesis in the liver through glyceraldehyde-3-phosphate and dihydroxyacetone phosphate (Krehbiel, 2008). Glucose is main energy source, which can be stored as glycogen in the muscle, and is also carbon source used for fatty acid synthesis, which is important for improving marbling of meat (Benedeti et al., 2015).

Previous studies have been conducted for the effect of glycerin on carcass composition and characteristics in various animal diets, including poultry (Cerrate et al., 2006), swine (Lammers et al., 2007), and cattle (Egea et al., 2014; Mach et al., 2009). Especially, the effect of crude glycerin inclusion in diets fed to beef cattle was evaluated by many studies. But the outcomes were not consistent, since some studies show no differences in animal performance and carcass characteristics (Mach et al., 2009; Ramos and Kerley, 2012; van Cleef et al., 2014), while other studies showed increase in oleic acid or monounsaturated fatty acid concentration of

longissimus muscle (Carvalho et al., 2014; Lage et al., 2014), improvement in overall acceptability of the meat (Prado et al., 2015), and improvement in weight gain and feed efficiency (San vito et al., 2016). Little information is available for the effect of glycerin on rumen fermentation characteristics, glucose/gluconeogenesis metabolism, carcass reducing sugar and glycogen contents and volatile compounds in beef cattle. Moreover, no glycerin inclusion study has been conducted in Korean cattle steers.

Flavor is known as one of the most important factors for determining meat quality. During the cooking process of meat, large quantity of volatile compounds such as aldehydes, hydrocarbons, ketones, are formed and these compounds directly relate to meat flavor (Watkins et al., 2012). Reducing sugars such as glucose and ribose, have been reported to contribute to the improved flavor of cooked meat by reacting with amino acids to produce many important volatile compounds via Maillard reaction during cooking (Aliani and Farmer, 2002). Limited information is available for dietary glycerin effect on generation of glucose and its effect on beef sensory traits including beef flavor.

We have hypothesized that glycerin included in the concentrate diet of finishing cattle may be used as energy source after conversion to either propionate or glucose. The generated glucose by glycerol replacement may affect muscle glycogen deposition, and the resulting glycogen may affect beef characteristics including flavor generation. The objective of this study was to evaluate the effect of

dietary glycerin replacement on growth performance, blood metabolites, ruminal fermentation characteristics, carcass characteristics and sensory traits, hepatic gluconeogenic gene expression, and muscle glycogen contents in Korean cattle steers.

3. Materials and methods

Experimental procedures were approved by the Seoul National University Institutional Animal Care and Use Committee.

1) Animals, housing, and diets

Twenty Korean cattle steers were reared at the University Animal Farm of the College of Agriculture and Life Sciences, Pyeongchang Campus of Seoul National University in South Korea and they were used in a complete randomized design. In order to assign the cattle uniformly into two groups, measurements of the longissimus muscle area (LM area), backfat thickness, and marbling score (MS) were obtained by ultrasound. From one week before experiment, all animals were fed an experimental control concentrate diet (approximately 1.5% of body weight [BW] per animal) and a rice straw (1kg/day per animal). After an adaptation period of 7 days with these animals consuming control concentrate without glycerin, animals were weighed and started the study with an average initial BW of 647 ±

10.5kg and age of 27.0 ± 0.2 mo. According to individual BW, age and estimated MS of ultrasound, steers were randomly assigned to 1 of 2 groups containing: control (n=10, conventional fattening diet) or 3% glycerin group (n=10, 3% glycerin replacement) on a DM basis. Crude glycerin inclusion level was chosen after a preliminary manufacture of concentrate was conducted to assess pellet quality based on physical property and energy value of crude glycerin. An attempt was made to include more than 5% glycerin, but pellet quality was moderately and severely reduced at 5% inclusion levels due to its viscosity and decomposition. Therefore, the glycerin inclusion level in concentrate of glycerin group was determined at 3%. The glycerol-added diet was made by adding 99.7% purified glycerol (Palm Oleo SDN. BHD., Selangor, Malaysia) adsorbed ground wheat to give final 3% glycerol during pelleting process of a concentrate diet. Molasses and DDGs (distiller's dried grain with solubles) were partially replaced with 3% glycerin to give iso-energy content between control and glycerol diet. Ingredient and chemical composition of two experimental diets are presented in Table 27. All steers were individually allowed to receive the commercial fattening concentrate diets with the daily amount of 1.5% of individual BW using an automatic feeding station (Dawoon system, Incheon, Korea), and they were individually fed total 1.0kg/d of rice straw with twice daily at 0800 am and 1700 pm. The animals had free access to water and mineral block. Experimental feeding periods were 20 weeks in duration (July 24th to December 15th). Rice straw and concentrate consumptions were recorded daily.

Daily feed intake of a concentrate diet was recorded automatically online using a computer with the Dawoon system. Steers BW were measured at 0900 am before the feeding at start date and at an interval of 4 weeks throughout the feeding trial. Samples of concentrate and rice straw were collected at an interval of two weeks, and stored at -20℃ until further analysis. The animals were observed once daily throughout the feeding trial to record animals' physical condition.

Table 27. Ingredients and chemical composition of the experimental diets

Item	Control	Glycerin
Ingredients, % of DM		
Ground wheat	20.36	22.54
Steamed flaked corn	18.29	18.41
Ground corn	9.40	8.65
Distiller's dried grains with solubles	9.97	6.02
Wheat bran	6.41	6.11
Palm kernel meal	6.76	6.81
Copra meal	4.88	4.91
Molasses	3.62	0.91
Whole cottonseed	3.97	4.00
Corn gluten feed	4.65	5.07
Rice bran	2.00	4.04
Soyhull	2.05	2.87
Palm oil	1.48	0.87
Alfalfa pellet	0.97	0.98
Cottonseed hull	0.96	0.96
Purified glycerin	0.40	3.57
Condensed molasses solubles	0.22	0.00
Sodium bentonite	0.29	0.30
Magnesium oxide	0.36	0.36
Sodium bicarbonate	0.91	0.89
Salt	0.25	0.25
Urea	0.00	0.21
Limestone	1.57	1.06
Mineral-vitamin premix[1]	0.22	0.23
Chemical composition, % of DM		
DM	89.6	89.7
Crude protein	14.5	14.5
Crude fat	4.99	4.70
Ash	6.77	6.53
NDF	31.5	31.4
ADF	14.2	13.6
Calcium	0.84	0.72
TDN	85.4	85.2
ME, Mcal/kg	3.30	3.29

[1]Provided following nutrients per kg of additive (Grobic-DC, Bayer Health Care, Leverkusen, Germany): Vit. A, 2,650,000 IU; Vit. D3, 530,000 IU; Vit. E, 1,050 IU; Niacin, 10,000 mg; Mn, 4,400 mg; Zn, 4,400 mg; Fe, 13,200 mg; Cu, 2,200 mg; I, 440 mg; Co, 440 mg.

2) Blood and rumen fluid collections and measurements

Blood was collected at 0800 am after 8 h fast, and also collected at 1300 pm 3 h post-feeding. Blood was collected at 4 weeks interval by jugular venipuncture with both a non-heparinized vacutainer (20mL; Becton-Dickinson, Franklin Lakes, NJ, USA) and ethylenediaminetetraacetic acid-treated vacutainer (20ml). Both serum and plasma were separated by centrifugation at 1,500 x g at 4℃ for 15 min, and stored at -80℃ until analysis. The blood serum was used for metabolite analysis. Reagent to analyze glucose was purchased from JW Medical (Seoul, Korea). The analytical reagent for non-esterified fatty acid (NEFA) was obtained from WAKO (Osaka, Japan). Both of items were analyzed using an automated chemistry analyzer (Hitachi 7180; Hitachi, Tokyo, Japan). Plasma ghrelin was analyzed using a bovine ghrelin ELISA kit ((Mybiosource, Cat.No: MBS013058). All of the analysis methods were verified in our laboratory, as previously reported (Kang et al., 2016). Rumen fluid sample was harvested 3 hours post-feeding using oral-stomach tube method as described by Shen et al. (2012). Rumen fluid pH was measured immediately with pH meter (Ohaus Corp., 7 Campus Drive, Suite 310, Parsippany, NJ07054, USA). For VFA analyses, 1mL of rumen fluid was mixed with 0.2mL of 25% meta-phosphoric acid, and stored at -20℃ until analysis, while 50mL of rumen fluid was collected for ruminal NH_3-N analyses, and stored at -20℃

until analysis. The VFA and NH_3-N concentrations were analyzed as described previously (Piao et al., 2012). The NH_3-N concentration was determined using a modified colorimetric method (Chaney and Marbach, 1962), and VFA concentrations were determined by gas chromatography using a Agilent Tech 7890A (Hewlett Packard Strasse 876337, Waldbronn, Germany) for which a Supelco (30 m×0.25 mm×0.25 μm, fused silica capillary column) column was used. Fecal samples were collected at 4 weeks interval, and both of diet and fecal samples were ground and stored at -20℃ until further analysis. Nutrient digestibility was estimated using indirect digestibility method by the lignin ratio technique as described by Wallace and Van dyne (1970). Feces and feed samples were used for the analysis of DM, OM and crude fat content according to AOAC (1996), and NDF and lignin were determined according to the method of Van Soest et al. (1991).

3) Slaughter procedures and tissue sample collections

Steers were transported to a local municipal slaughterhouse (Bucheon, Korea) after 144 d of study with final BW of 734 ± 12.2kg and age of 31.8 ± 0.2 month. At slaughter, longissimus hot-carcass was taken within around 60 min post-mortem after the carcass had been split and transferred to a chiller. The *longissimus thoracis* (LT) sample was obtained from the 12^{th} vertebrae for glycogen content analysis, and liver sample was also collected for gene expression analyses. Immediately visible fat was trimmed away from LT sample at the slaughterhouse, and the LT and liver

samples were frozen in liquid nitrogen and later stored at -80℃ for glycogen content analysis and gene expression analyses, respectively. Carcass of Korean cattle steers were evaluated by a meat grader using the Korean carcass-grading system of Korea Institute for Animal Products Quality Evaluation (KAPE, 2013) at 24 h post-mortem. Carcass weight, longissimus muscle area, fat thickness, MS, meat color, fat color, texture, and maturity were examined and reported by an official meat grader. Among these parameters, MS was mainly used for determining quality grade (QG). Five quality grades (QG 1++, 1+, 1, 2, and 3) are assigned by meat graders. The MS of the Beef Marbling Standard (BMS) ranges from 1 (devoid) to 9 (abundant); 8 or 9 was the MS for QG 1++, 6 or 7 was the MS for QG 1+, 4 or 5 was the MS for QG 1, 2 or 3 was the MS for QG 2, and 1 was the MS for QG 3. In addition, meat color, fat color, texture and maturity of the exposed longissimus muscle at the 13^{th} rib interface were used for QG determination (NLCF, 1998). After slaughter and a 24-h chill, LT cold carcass from the 12^{th} vertebrae was also obtained. *Longissimus thoracis* samples (about 500g) were vacuum-packaged, transported under ice (4℃) to a laboratory within 30 h post-mortem. After transportation to laboratory, the packages containing the LT samples of 20 steers were opened and the external fat was trimmed away. The LT samples were minced using a mini chopper (CH180, Kenwood, Shanghai, China) for 30s. Minced LT samples from various locations were pooled, and some samples were used immediately for the evaluation

of pH and chemical composition, while others were stored at -70℃ for the analysis of collagen content, reducing sugar, nucleotide content, fatty acid content, and volatile compounds. Samples assessed for shear force, meat color (CIE value) and sensory traits were collected but not minced, and some samples were used immediately for the analysis of shear force and meat color, while some samples were stored at -70℃ for the sensory evaluation.

4) mRNA levels in the liver

Total RNA from liver was isolated using TRIzol reagent (Molecular Research Center, Inc. 5645 Montgomery Rd Cincinnati, OH 45212 USA) as described in Kang et al. (2015). Total RNA was quantified using the absorbance at 260 nm, and the integrity of the total RNA was checked by agarose gel electrophoresis and ethidium bromide staining of the 28S and 18S bands. Total RNA (2 μg) was reverse-transcribed into cDNA using an iScript cDNA Synthesis kit (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. Real-time polymerase chain reaction (PCR) was performed using QuantiTect SYMBR Green RT-PCR master mix (QIAGEN, Venlo, Netherlands). Briefly, the PCR was conducted in a 25 μL reaction volume containing 200 ng cDNA, 12.5 μL SYBR Green RT-PCR master mix, and 1.25 μL of 10 μM primers. The thermal cycling parameters were as follows: 95°C for 15 min, followed by 40 cycles at 94°C for 15 s, 60°C for 30 s and 72°C for 30 s. The primers of interest were designed using integrated DNA

technology based on published sequences at the National Center for Biotechnology Information (Supplementary table 1). The ΔΔCT method was used to determine the relative fold changes (Livak and Schmittgen, 2001), and all data were normalized with the housekeeping RPS9 gene.

5) Chemical and physicochemical compositions, color, pH, and shear force

Chemical and physicochemical compositions, color, pH, and shear force of LT samples were analyzed using the same methods, as described in Chapter four.

6) Reducing sugar content

The reducing sugar contents of LT samples were measured using the same method, as described in Chapter four.

7) Sensory evaluation

The sensory characteristics were evaluated using the same method, as described in Chapter four.

8) Glycogen concentration

To analyze the glycogen in LT, grounded meat (2 g) was suspended with 8.5% perchloric acid (10 ml) and homogenized at Lv. 6 for 30 sec (T10, Ika Works, Staufen, Germany). The homogenate was centrifuged (Combi 514R, Hanil Co., Ltd., Incheon, Korea) and filtered through glass wool. The pellet was re-extracted with

8.5% perchloric acid (5 ml) and supernatants were obtained in the same manner. Iodine color reagent [1.3 ml of a solution containing 0.26 g of iodine and 2.6 g of potassium iodide (in 10 ml distilled water) with 100 ml calcium chloride] (2.6 ml) was added to glycogen extracts (0.4 ml) and measured the absorbance at 460 nm (X-ma 3100, Human Co., Ltd., Seoul, Korea) after 30 min. The amount of glycogen was calculated using a standard curve developed with glycogen (Sigma-Aldrich, St. Louis, MO, USA).

9) Fatty acid profile

Fatty acid (FA) content was measured as described previously with a few modification (Piao et al., 2017). Briefly, lipids in beef samples were extracted according to the procedure of Folch et al. (1957). Extracted lipids were evaporated using N_2 gas (99.99%) and 0.5 g was weighed into a 15 ml test tube with 1 ml of internal standard (1 mg of triundecanoate in 1 ml of iso-octane) and 1.5 ml of 0.5 N sodium hydroxide. The samples were heated at 85℃ for 10 min and for methylation, 2 ml of 14% BF_3-methanol was added after cooling and heated at 85℃ for 10 min. After cooling, 2 ml of iso-octane and 1 ml of saturated sodium chloride were added and centrifuged at 1,573 ×g for 3 min (Continent 512R, Hanil Co., Ltd., Incheon, Korea). Upper layer containing fatty acid methyl ester (FAME) was dehydrated with anhydrous sodium sulfate and transferred to a vial and analyzed using a gas chromatograph (HP 7890, Agilent Technologies, Santa Clara, CA, USA)

with a split ratio (200:1). A capillary column (SP^{TM}-2560, 100 m × 0.25 mm × 0.20 μm, Supelco, Bellefonte, PA, USA) was used. The injector and detector temperatures were maintained at 240°C and 260°C, respectively. The column oven temperature were as follows: 100°C for 5 min, increased to 240°C at 3°C/min, then held at 240°C for 25 min. Nitrogen was used as a carrier gas at linear flow of 0.7 ml/min. Individual FAME was identified by comparison of the relative retention times of peaks from samples, with those of the external standards (37 FAME mix and CLA mix, Supelco, Bellefonte, PA, USA) calculated based on the Korean Food Standards Codex (MFDS, 2017).

10) Volatile compound

The volatile compounds contents of LT samples were measured using the same methods, as described in Chapter four.

11) Statistical analyses

Data (10 animals/group) except blood metabolites were analyzed by analysis of variance using the General Linear Model Procedure (Proc GLM) in SAS software (SAS Institute, Cary, NC, USA). The data for blood metabolites, including glucose, NEFA and ghrelin, were analyzed by two-way analysis of variance to test the fixed effects of diet, time and their interaction by a generalized linear mixed model, using the Proc GLM procedure of SAS software. Experimental unit was an individual

animal for blood metabolites. Animal within diet or time type was considered the random variable. The LSMEANS PDIFF option was used to compare differences among mean values at $P < 0.05$.

4. Result and discussion

1) Intake, animal performance, and digestibility

Feed intake and animal performance results over the feeding trial are presented in Table 28. By design, there were no differences ($P > 0.05$) in initial age and initial BW, and final BW and average daily gain were numerically greater in glycerin replacement group. Average daily concentrate intake in glycerin replacement group was higher ($P < 0.01$) than that in control group, and rice straw intake (0.9kg/d of DM) did not differ ($P > 0.05$) between two groups, thus total DMI was higher ($P < 0.01$) in glycerin replacement group. However, no significant differences was also observed in feed efficiency ($P > 0.05$). This result was somewhat similar to Ogborn (2006) who reported the increased DMI at 5% glycerol inclusion in prepartum dairy cattle, whereas DMI tended to be reduced at 3.3% glycerol in the total ration after calving. However, Egea et al. (2014) reported that crude glycerin inclusion in the diet fed to Limousin bulls during the finishing period did not affect average daily feed intake when supplementing glycerin to diet at inclusion rates similar to this study. For feed efficiency, the result was in agreement with Mach et al. (2009) who

reported increasing glycerin to 4%, 8%, and 12% DM did not affect feed efficiency, final BW, as well as average daily gain in Holstein bulls. In addition, glycerin replacement did not affect ($P > 0.05$) apparent digestibilities of total dry matter, organic matter, fat and NDF in this study. Previous study with castrated crossbred cattle fed diets containing 60% of concentrate and 40% of roughage have also reported that glycerin inclusion in the diets up to 20% of DM had no effects on digestibilities of dry matter, organic matter and ether extract (Fávaro et al., 2015).

Table 28. Intake, performance, and nutrient digestibility of Korean cattle steers fed control and glycerin replacement diet

Item	Control	Glycerin	SEM	*P*-value
Initial age, month	27.1	26.8	0.15	0.37
Final age, month	31.9	31.6	0.15	0.30
Initial BW, kg	647	648	10.49	0.97
Final BW, kg	731	737	12.19	0.58
Average daily gain, kg/d	0.57	0.61	0.03	0.55
Concentrate intake, kg of DM/d	6.2	7.1	0.18	0.001
Rice straw intake, kg of DM/d	0.9	0.9	0.00	—
Total feed intake, kg of DM/d	7.1	8.0	0.16	0.001
Feed efficiency, kg gain/kg intake	0.08	0.076	0.003	0.59
Digestibility, %				
Dry matter	56.6	58.1	1.14	0.47
Organic matter	61.2	62.7	0.98	0.42
Fat	84.0	81.8	1.46	0.43
NDF	29.7	30.7	1.72	0.74

n=10 per group

2) Blood parameters

Blood metabolites pattern are shown in Table 29. Chung et al. (2007) reported that glycerin supplementation to Holstein dairy cows improved energy availability (higher blood glucose, lower blood BHBA, and lower urine ketones) during lactation. Glycerin fed to ruminants was well known to be converted to propionic acid in the rumen, which was in turn absorbed into the rumen wall (Kijora et al., 1998). After absorption into the rumen, the propionic acid and some glycerin are moved to liver and kidney through bloodstream, in which the propionic acid and glycerin component can be converted to glucose through gluconeogenesis and provides energy for cellular metabolism (Krebs et al., 1966). In this study, we also focused on the glucose concentration out of blood metabolites. The average glucose concentration of serum was lower (P = 0.045) in glycerin replacement group than that in control group at 4th week. However, the average glucose level was higher (P = 0.047) in glycerin replacement group at 16th week. Thus, glycerin supplemented to concentrate diet may affect gluconeogenesis pattern in Korean cattle steers at 16th week. In addition, serum concentration of NEFA almost did not differ (P > 0.05) between two groups throughout the feeding trial.

Ghrelin hormone secreted from the digestive tract is also an important metabolite in this study. Ghrelin can act as a signal of hunger and thus it may stimulate feed intake (Wertz et al., 2004). In this study, the average ghrelin concentration of plasma was lower (P = 0.04) in glycerin replacement group than

that in control group at 16th week. Ghrelin is also well known as an appetite-stimulating hormone, which is mainly secreted from the stomach (Salfen et al., 2004). Thus, ghrelin is an important variable in meal initiation in mice (Asakawa et al., 2001), rats (Nakazato et al., 2001), and humans (Cummings et al., 2001). In Japanese black cattle, the plasma ghrelin levels at 4 h post-feeding were intermediate between plasma ghrelin level pre-feeding and 1 h post-feeding (Wertz et al., 2004). In this study, average daily concentrate intake was higher in glycerin replacement group, but the average plasma ghrelin concentration was lower in glycerin group, which is not consistent with the ghrelin's mechanism in the body mentioned above. Similar result was reported that ghrelin barely influenced feed intake and energy metabolites of lambs administered daily over a period of 4 days (Krueger and Melendez, 2012). The lower average plasma ghrelin concentration relative to higher concentrate intake warrants further investigation to determine the impact of plasma ghrelin concentrations on the feed intake in Korean cattle steers.

Table 29. Serum metabolites and plasma ghrelin concentrations of Korean cattle steers fed control and glycerin replacement diet

	8 hours post-fasting		3 hours post-feeding			*P* - value		
Item	Control	Glycerin	Control	Glycerin	SEM	Diet	Time	Diet x Time
Glucose, mg/dL								
4 week	55.2^{ab}	50.4^{b}	59^{a}	55.7^{ab}	1.06	0.045	0.03	0.70
8 week	65.8^{a}	66^{a}	61.2^{ab}	59.9^{b}	0.79	0.69	<0.001	0.59
12 week	69.2^{a}	69.6^{a}	62.2^{b}	62.7^{b}	0.74	0.66	<0.001	0.96
16 week	70.2	71.7	69.9	72.2	0.47	0.047	0.91	0.67
20 week	66.9^{b}	67.8^{b}	71.7^{ab}	75.3^{a}	0.83	0.10	<0.001	0.32
NEFA, Eq/L								
4 week	358	322	324	308	10.1	0.21	0.25	0.62
8 week	410^{a}	386^{ab}	320^{bc}	296^{c}	13.3	0.30	<0.001	0.99
12 week	405	366	308	325	17.1	0.75	0.04	0.41
16 week	319	271	287	256	13.3	0.15	0.38	0.75
20 week	360^{a}	340^{ab}	291^{b}	386^{a}	13.8	0.16	0.68	0.04
Ghrelin, ng/ml								
16 week	316^{a}	302^{ab}	298^{b}	290^{b}	2.86	0.04	0.01	0.63
20 week	283	294	295	300	3.78	0.31	0.22	0.69

n=10 per group

$^{a-c}$ Means with different letter within a same row differ ($P < 0.05$).

3) Ruminal characteristics

Ruminal fermentation pattern are shown in Table 30. Average rumen pH, total rumen VFA concentration and the ratio of acetate to propionate did not differ ($P > 0.05$) between two groups throughout the whole feeding trial except that the ratio of acetate/propionate in glycerin group tended to be lower ($P = 0.06$) than that in control group at 16^{th} week. Rumen individual VFA and NH_3-N content also did not differ ($P > 0.05$) between two groups. In disagreement to these results, DeFrain et al. (2004) reported that glycerin-supplementation in diets at minimum 430g/d fed to Holstein cows increased ruminal propionate and total VFA concentration, and decreased acetate/propionate ratio, but did not affect acetate and butyrate. Also, Linke et al. (2004) reported that providing the Holstein cows with 1kg/d of glycerin by drenching or tubing increased ruminal propionate concentration. In this study, the actual average glycerin intake in glycerin replacement group was average 213g/d. Thus, it may be assumed that glycerin replacement in diet at 3% was too insufficient to affect the ruminal fermentation.

Table 30. Ruminal VFA and NH_3-N concentrations of Korean cattle steers fed control and glycerin replacement diet

Item	Control	Glycerin	SEM	*P*-value
pH				
4th week	6.26	6.20	0.07	0.64
8th week	6.41	6.43	0.07	0.88
12th week	6.41	6.52	0.08	0.50
16th week	6.74	6.73	0.09	0.94
20th week	6.67	6.83	0.08	0.37
Acetate, mM				
4th week	60.6	56.6	1.84	0.30
8th week	57.0	55.8	2.32	0.84
12th week	67.8	61.3	3.12	0.19
16th week	57.4	49.9	2.96	0.30
20th week	61.9	51.4	3.47	0.17
Propionate, mM				
4th week	18.7	25.3	2.69	0.26
8th week	20.7	21.6	1.79	0.84
12th week	22.7	23.1	1.84	0.94
16th week	16.6	21.7	1.86	0.16
20th week	18.5	14.9	1.21	0.20
Butyrate, mM				
4th week	17.7	16.9	1.21	0.72
8th week	15.5	15.0	0.99	0.83
12th week	18.6	16.8	1.18	0.22
16th week	16.0	17.2	1.56	0.74
20th week	16.3	15.1	1.21	0.61
Total VFA, mM				
4th week	101.8	103.9	4.16	0.84
8th week	98.1	97.6	4.51	0.96
12th week	114.9	106.8	5.24	0.44

16th week	94.7	93.5	5.52	0.92
20th week	101.2	85.9	5.93	0.24
Acetate:propionate ratio				
4th week	3.29	2.77	0.19	0.16
8th week	3.07	2.71	0.18	0.37
12th week	3.14	3.11	0.23	0.96
16th week	3.58	2.74	0.21	0.06
20th week	3.39	3.56	0.09	0.39
NH_3-N, mg/100ml				
4th week	11.5	8.22	1.72	0.31
8th week	7.33	6.63	1.23	0.80
12th week	10.5	8.37	1.48	0.46
16th week	7.95	6.63	1.08	0.62
20th week	9.39	7.95	1.22	0.53

n=10 per group

4) Carcass characteristics

Carcass characteristics are presented in Table 31. Glycerin replacement did not affect ($P > 0.05$) carcass weight, LM area, backfat thickness, MS, meat color, meat quality, and yield grade. This result was in agreement with Mach et al. (2009), who reported that dietary glycerin replacing other ingredients up to 12% did not affect carcass and meat quality in Holstein bulls, including carcass weight, backfat thickness, and LM area. San Vito et al. (2015) also reported that the supplementation of animals with glycerin up to 28% DM did not affect carcass characteristics of Nellore young bulls. In this study, some ingredients (such as molasses, DDGS) were replaced by glycerin at 3%, and the TDN level of glycerin replacement group was same as that of control group. The glycerin replacement levels (3%) may not be enough to affect carcass characteristics in this study.

Chemical composition and physico-chemical composition are presented in Table 32. Content of chemical composition, including crude fat, crude protein, and moisture, did not differ ($P > 0.05$) between two groups in this study. This result was consistent with Egea et al. (2014) who reported crude glycerin used at 2 and 4% did not affect the chemical composition of longissimus muscle in Limousin bulls. Volpi-Lagreca and Duckett (2016) also reported that feeding 4.3% crude glycerin via drinking water to finishing cattle did not affect the contents of moisture, total lipids, crude protein, as well as ash of longissimus muscle.

CIE L^* (brightness), a^* (redness), and b^* (yellowness) are known to be indicators of meat color. In this study, the CIE L^*, a^*, and b^* value did not differ ($P > 0.05$) between two groups. This result is consistent with previous studies where San Vito et al. (2015) reported that the inclusion of crude glycerin up to the level of 28% of dry matter in the supplement did not affect CIE values of longissimus muscle in Nellore bulls. Similarly, Egea et al. (2014) also found no differences on the color parameters (L^*, a^* and b^*) between control and crude glycerin (2 and 4%) groups in Limousin bull meat.

pH of LT in glycerin replacement group (pH=5.56) was lower ($P < 0.05$) than that in control group (pH=5.64). There are several factors affecting the rate of pH decline, including stress (Apple et al., 1995), electrical stimulation (Chrystall et al., 1984), chilling temperature (Bowling et al., 1978), glycogen, ATP, creatine phosphate reserves, lactic acid (de Lima Júnior et al., 2016), animal sex (Brown et al., 1990), species (Varnham and Sutherland, 1995), breed (Sanz et al., 1996), season (Brown et al., 1993), and possibly animal age (Varnham and Sutherland, 1995). Of these factors, ATP, creatine phosphate, and lactic acid would be associated with the difference of pH of muscle between control and glycerin group in this study, because other factors were similar between two groups. It is not clear for the reason of decline of pH of LT in glycerin replacement group due to limited information. Even though the pH of glycerin group was lower than that of control group, the pH of LT from both two groups were lower than 5.7, which are in the pH

range (5.4-5.7) of normal meat of an unstressed animal. Thus, glycerin replacement can be evaluated not to affect the pH of LT, as well as meat quality in this study.

Table 31. Carcass characteristics of LT from Korean cattle steers fed control and glycerin replacement diet

Item	Control	Glycerin	SEM	*P*-value
Slaughtering age, month	31.9	31.6	0.15	0.37
Carcass weight, kg	433	436	7.73	0.87
LM area, ㎠	96.4	97	2.29	0.92
Backfat thickness, ㎜	12.4	11.8	0.50	0.47
Marbling score[1]	5.70	5.50	0.34	0.82
Yield index	65.8	66.2	0.36	0.59
Meat color[2]	4.60	4.80	0.11	0.34
Fat color[3]	3.00	3.00	0	—
Texture[4]	1.10	1.10	0.07	1.00
Maturity[5]	2.30	2.50	0.11	0.34
Yield grade[6]	2.10	1.90	0.10	0.34
Quality grade[7]	2.40	2.50	0.17	0.82

n=10 per group

[1] Marbling score: 1, devoid; 9, very abundant.

[2] Meat color: 1, bright red; 7, dark red.

[3] Fat color: 1, white; 7, yellowish.

[4] Texture: 1, very fine; 3, very coarse.

[5] Maturity: 1, youthful; 9, mature.

[6] Yield grade: A=1; B=2; C=3.

[7] Quality grade: 1^{++}=1, 1^{+}=2, 1=3, 2=4, 3=5.

5) Reducing sugar and glycogen contents in the LT

Reducing sugar contents of LT did not differ ($P > 0.05$) between two groups (Table 32). Volatile compounds, such as aldehydes, hydrocarbons, ketones, furans, and pyrazines, are formed during the cooking process of meat (Watkins et al., 2012). Volatile compounds can form via several pathways, including a Maillard reaction of amino acids or peptides with reducing sugars (e.g. glucose and ribose), thermal lipid degradation, and the interaction between Maillard reaction products and lipid oxidation products (Ba et al., 2012a). Of these pathways, Maillard reactions are key processes in generating volatile compounds (Mottram and Nobrega, 2002). It is well known that ribose, glucose, glucose 6-phosphate, and ribose 5-phosphate are the main reducing sugar for this reaction (Ba et al., 2012b). In addition, glycerol can be converted into glucose in the liver of cattle. In this study, the glycerin replacement did not affect the reducing sugar content, which may be due to the limited inclusion level (3%) of glycerin in diet.

Glycogen content in LT did not differ ($P > 0.05$) between two groups (Table 32). Glycerol, also known as glycerin, is utilized as an important substrate for gluconeogenesis, mainly in the liver and kidneys (Hagopian et al., 2008). The glycerol absorbed can be transformed to glucose through phosphorylation to glycerol 3-phosphate, which is catalyzed by glycerol kinase, and then stored as glycogen in the liver and muscle (Mourot et al., 1994). Thus, with expansion of the biodiesel industry the glycerin is widely utilized as an alternative energy source for

livestock (Mach et al., 2009; Parsons et al., 2009; Benedeti et al., 2016). In this study, glycerin replacement did not affect the glycogen content in LT, which may be also due to the limited inclusion level (3%) of glycerin in diet.

Table 32. Chemical and physico-chemical compositions, reducing sugar and glycogen contents, and sensory traits of LT from Korean cattle steers fed control and glycerin replacement diet

Item	Control	Glycerin	SEM	*P*-value
Chemical composition				
Moisture, %	61.9	60.0	0.99	0.46
Crude protein, %	19.0	19.1	0.27	0.90
Crude fat, %	16.1	17.9	1.10	0.51
Physico-chemical composition				
CIE *L**	43.5	45.9	0.76	0.18
CIE *a**	30.7	30.3	0.42	0.67
CIE *b**	25.9	26.5	0.2	0.13
pH	5.64	5.56	0.01	0.001
Shear force, kg	7.05	7.11	0.37	0.94
Reducing sugar				
Reducing sugar content, %	0.34	0.34	0.01	0.89
Glycogen				
Glycogen content, mg/g	5.12	4.08	0.48	0.37
Sensory traits[1]				
Appearance	5.88	5.88	0.04	1.00
Odor	5.97	6.09	0.15	0.76
Taste	6.06	6.27	0.24	0.71
Flavor	6.12	6.18	0.21	0.92
Tenderness	5.18	6.03	0.32	0.19
Juiciness	5.76	5.85	0.27	0.89
Overall acceptance	6.09	6.06	0.20	0.95

n=10 per group

[1] The score was evaluated with 10 semi-trained panelists (1, extremely dislike; 5, neither dislike nor like; 9, extremely like).

6) Fatty acid and volatile compound profiles

In the current study, sixteen FA were detected and quantified in LT of Korean cattle steers (Table 33). Of these FA, the oleic acid (C18:1n9c) content was the highest in LT, ranging from 36.7 to 36.9 g/100g meat, followed by palmitic acid (C16:0) (21.6-22.0 g/100g meat) and stearic acid (C18:0) (8.59-8.82 g/100g meat). Our results are consistent with previous studies that reported C18:1n9c was the highest among FA in beef from Korean cattle (Kim et al., 2009; Piao et al., 2017), Aberdeen Angus and Limousin (Prieto et al., 2010), and Aberdeen Angus and Holstein-Friesian (Warren et al., 2008). In this study, FA content of LT did not differ ($P > 0.05$) between two groups. This result was consistent with previous studies where San Vito et al. (2015) reported that the inclusion of crude glycerin up to level of 28% of dry matter in the supplement did not affect the carcass characteristics or the FA profile of longissimus muscle in Nellore bulls, while Terré et al. (2011) also reported that the glycerin inclusion up to 100g/kg of dry matter did not affect growth performance, blood metabolites, and FA composition of lamb meat. This result may be related to the similar crude fat content, MS, or backfat thickness of animals between two groups in this study.

A total of 31 volatile compounds, including aldehydes, hydrocarbon, ketone, alcohol, and others, were detected in the LT of Korean cattle steers (Table 34). The abundant volatile compounds, accounting for 12.8-44.8% of the total volatile compounds, included 2-propanone and hexanal. The moderate compounds,

accounting for 1.04-9.62% of the total volatile compounds, included propanal, pentanal, heptanal, 2-methyl propanal, methanethiol, butane, n-pentane, 2-butanone, 3-hydroxy-2-butanone, and ethyl-acetate. The minor compounds, accounting for 0.01-1.09% of the total volatile compounds, included butanal, octanal, nonanal, 3-methyl butanal, benzaldehyde, 2-butene, carbon disulfide, methyl acetate, hexane, heptane, octane, 2-pentanone, 2-heptanone, 2,3-butanedione, 2,3-octanedione, isopropyl alcohol, 1-pentanol, dimethyl sulfide, and 1,3,5-cycloheptatriene.

In this study, the most various compound detected during cooking is aldehyde. This is consistent with Legako et al. (2015) who reported the aldehyde compounds were the most various volatile compounds in *Longissimus lumborum*, *Psoas major*, *Gluteus medius*, and *Semimembranosus* of beef. However, all the contents of aldehydes compound, including 3-methyl butanal (buttery, beefy), pentanal (rancid), hexanal (grassy, fatty), octanal (gamey, fishy), benzaldehyde (off-flavor, fishy), and so on, did not differ ($P > 0.05$) between two groups. During cooking, the main reactions that produce aroma volatile compounds are the Maillard reaction between reducing sugars and amino acids, and the thermal degradation of lipid (Mottram, 1998). Cerny (2007) reported that during cooking, aldehydes are mainly formed by oxidation of FAs, such as oleic acid, linoleic acid, and linolenic acid. There were no differences on aldehyde compounds contents in this study. This result may be associated with the similar content of reducing sugar and FA, as well as crude fat content of LT of animals between two groups.

Hydrocarbon is known to arise from the lipid oxidation catalyzed by heme compound such as hemoglobin and myoglobin (Shahidi et al., 1986). Min et al. (1979) reported that the presence of saturated and unsaturated straight chain hydrocarbon seems to have a limited influence on meat flavor due to their high threshold values. In this study, the hydrocarbon compounds contents also did not differ ($P > 0.05$) between two groups.

Most of ketone compounds contents did not differ ($P > 0.05$) between two groups except 2,3-butanedione and 2,3-octanedione. Ketones compound, especially 2-ketone, can highly contribute to aroma of meat and meat products, which impart some special aroma such as buttery, spicy, and blue cheese, when these compounds production is high (Novelli et al. 1995). In this study, 2,3-butanedione (buttery) content was higher ($P < 0.05$) in glycerin replacement group, whereas 2,3-octanedione (oxidized fat flavor) content was lower ($P < 0.05$) in glycerin group than that in control group.

Table 33. Fatty acid contents (g/100g meat) of LT from Korean cattle steers fed control and glycerin replacement diet

Fatty acid	Control	Glycerin	SEM	*P*-value
C12:0	0.05	0.05	0.01	0.72
C14:0	2.48	2.50	0.11	0.94
C14:1	0.82	0.73	0.03	0.13
C15:0	0.17	0.18	0.01	0.36
C16:0	22.0	21.6	0.51	0.69
C16:1	3.80	3.84	0.11	0.88
C17:0	0.50	0.55	0.02	0.10
C18:0	8.82	8.59	0.23	0.63
C18:1(n-9)t	0.13	0.09	0.01	0.10
C18:1(n-9)c	36.7	36.9	0.70	0.91
C18:2(n-6)c	1.64	1.62	0.06	0.87
C20:1	0.21	0.22	0.01	0.55
C18:3(n-3)	0.06	0.06	0.00	0.91
CLA[1]	0.33	0.34	0.01	0.82
C20:3(n-6)	0.10	0.08	0.01	0.34
C20:4	0.13	0.11	0.01	0.34
SFA[2]	34.1	33.5	0.77	0.75
USFA[3]	44.0	44.0	0.71	0.97
MUFA[4]	41.7	41.8	0.73	0.95
PUFA[5]	2.27	2.22	0.07	0.76
MUFA/SFA	1.23	1.26	0.03	0.72
PUFA/SFA	0.05	0.05	0.00	0.76

n=10 per group

[1] CLA, conjugated linoleic acid ($C18:2_{9c11t}$ + $C18:2_{10t12c}$).

[2] SFA (saturated fatty acid) = C12:0 + C14:0 + C15:0 + C16:0 + C16:0 + C18:0

[3] USFA (unsaturated fatty acid) = C14:1 + C16:1 + C18:1(n-9)t + C18:1(n-9)c + C18:2(n-6)c + C20:1 + C18:3(n-3) + CLA + C20:3(n-6) + C20:4

[4] MUFA (monounsaturated fatty acid) = C14:1 + C16:1 + C18:1(n-9)t + C18:1(n-9)c + C20:1

[5] PUFA (polyunsaturated fatty acid) = C18:2(n-6)c + C18:3(n-3) + CLA + C20:3(n-6) + C20:4

Table 34. Volatile compounds (%) of LT from Korean cattle steers fed control and glycerin replacement diet

Volatile compound	Control	Glycerin	SEM	*P*-value
Aldehyde				
Propanal	1.64	1.33	0.27	0.41
Pentanal	5.77	5.93	1.17	0.90
Butanal	0.26	1.08	0.38	0.32
Hexanal	13.7	12.8	2.35	0.70
Heptanal	1.04	1.32	0.28	0.48
Octanal	0.42	0.56	0.11	0.40
Nonanal	0.43	0.40	0.06	0.70
2-Methyl propanal	4.19	4.58	0.33	0.33
3-Methyl butanal	1.09	1.00	0.07	0.15
Benzaldehyde	0.78	0.92	0.07	0.18
Hydrocarbon				
Methanethiol	8.07	9.62	1.10	0.33
2-Butene	0.47	0.58	0.05	0.33
Carbon disulfide	0.84	1.09	0.10	0.18
Methyl acetate	0.04	0.01	0.02	0.37
Butane	1.53	1.63	0.11	0.72
n-Pentane	2.30	1.75	0.27	0.24
Hexane	0.60	0.49	0.07	0.30
Heptane	0.22	0.18	0.02	0.39
Octane	0.37	0.25	0.04	0.10
Ketone				
2-Propanone	44.8	38.9	3.18	0.12
2-Pentanone	0.18	0.13	0.02	0.07
2-Heptanone	0.22	0.20	0.02	0.47
2-Butanone	2.05	1.87	0.13	0.34
2,3-Butanedione	0.38	0.61	0.06	0.04
3-Hydroxy-2-butanone	3.89	7.08	1.16	0.19
2,3-Octanedione	0.67	0.45	0.09	0.04
Alcohol				
Isopropyl alcohol	0.66	0.52	0.07	0.30
1-Pentanol	0.01	0.00	0.00	0.34
Other				
Dimethyl sulfide	0.96	0.98	0.08	0.93
1,3,5-Cycloheptatriene	0.47	0.56	0.04	0.13
Ethyl-acetate	1.86	3.13	0.45	0.18

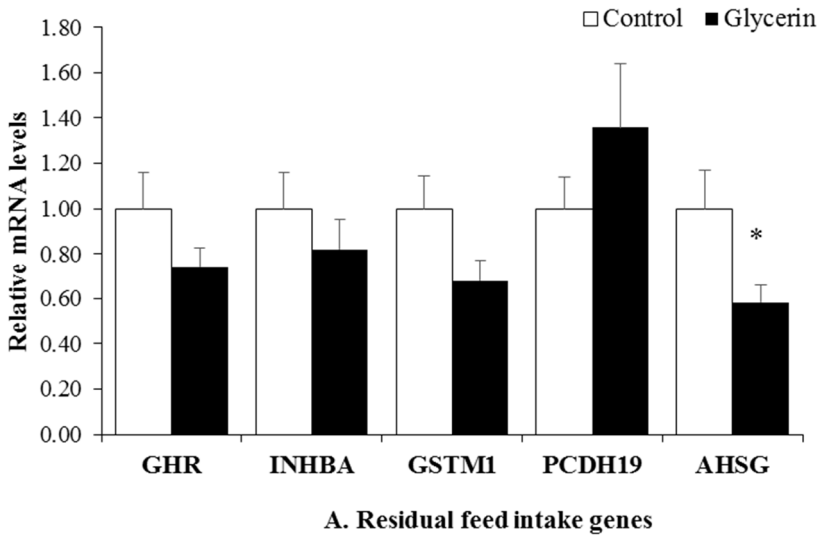
n=10 per group

7) Gene expression related to gluconeogenesis

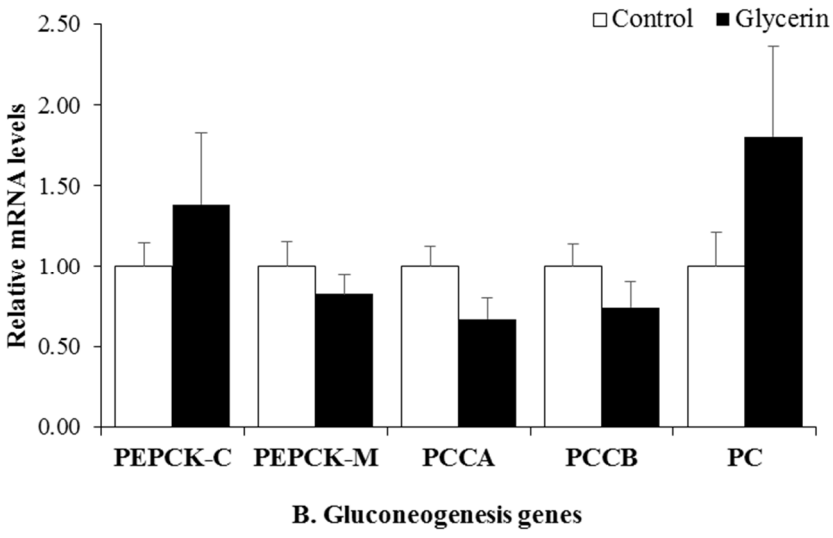
The relative mRNA expression of fifteen genes are shown in Figure 4. The relative mRNA expression of growth hormone receptor (GHR), inhibin beta A subunit (INHBA), glutathione S-transferase M1 (GSTM1), and protocadherin 19 (PCDH19) did not differ ($P > 0.05$) between control and glycerin replacement group. However, alpha 2-HS glycoprotein (AHSG) mRNA level was lower ($P < 0.05$) in glycerin replacement group than that in control group. Al-Husseini et al. (2014) reported that these five genes (GHR, INHBA, GSTM1, PCDH19, and AHSG) are highly associated with residual feed intake and could potentially be used to predict residual feed intake, and AHSG showed negative correlation with residual feed intake. Residual feed intake is known as the difference between the amount of feed an animal is expected to eat based on its size and growth over a specified period and the animal's actual eats (Arthur and Herd, 2008). Therefore, the result of Al-Husseini et al. (2014) indicated that the expression level of AHSG would showed positive correlation with actual feed intake. In this study, average daily concentrate intake was higher ($P < 0.05$) in glycerin replacement group than that in control group, whereas the relative mRNA expression of AHSG was lower in glycerin group, indicating that the mRNA expression of AHSG was negatively associated with feed intake in this study. Thus, this result is not consistent with Al-Husseini et al. (2014). The lower expression level of AHSG relative to higher concentrate intake in glycerin group warrants further investigation to determine the relationship of feed

intake with expression level of AHSG. In addition, Stefan et al. (2006) revealed that AHSG is a plasma protein, which is primarily produced by the liver, and its circulating concentration is positively related to liver fat accumulation and insulin resistance in human.

In addition, the relative mRNA expression of phosphoenolpyruvate carboxykinase-cytosolic (PEPCK-C), phosphoenolpyruvate carboxykinase-mitochondrial (PEPCK-M), propionyl-CoA carboxylase alpha-mitochondrial (PCCA), propionyl-CoA carboxylase beta-mitochondrial (PCCB), pyruvate carboxylase (PC), fructose-1,6-bisphosphatase (FBP1), glycerol kinase (GYK), glycerol-3-phosphate dehydrogenase 1 (GPD1), glycerol-3-phosphate dehydrogenase 2 (GPD2), and glucose-6-phosphatase (G6PC) did not differ ($P > 0.05$) between two groups.



A. Residual feed intake genes



B. Gluconeogenesis genes

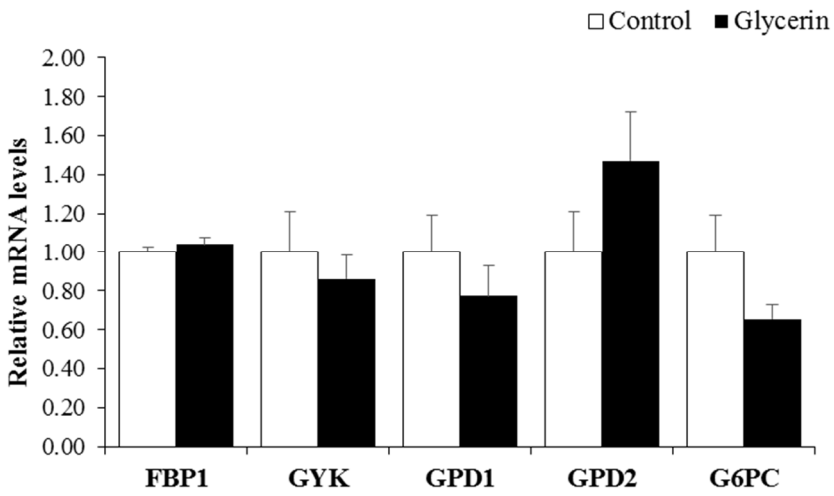


Figure 4. Comparison of the hepatic mRNA levels of residual feed intake, gluconeogenesis, and glucogenic substrate incorporation genes between the control and glycerin replacement groups in Korean cattle steers. The mRNA levels were determined by real-time PCR, and the results were normalized with the RPS9 gene. The mRNA levels of the control group were normalized to 1.0. Values are the means±SEM (n=10). GHR, growth hormone receptor; INHBA, inhibin beta A subunit; GSTM1, glutathione S-transferase M1; PCDH19, protocadherin 19; AHSG, alpha 2-HS glycoprotein; PEPCK-C, phosphoenolpyruvate carboxykinase, cytosolic; PEPCK-M, phosphoenolpyruvate carboxykinase, mitochondrial; PCCA, propionyl-CoA carboxylase alpha, mitochondrial; PCCB, propionyl-CoA carboxylase beta, mitochondrial; PC, pyruvate carboxylase; FBP1, fructose-1, 6-bisphosphatase; GYK, glycerol kinase; GPD1, glycerol-3-phosphate dehydrogenase 1; GPD2, glycerol-3-phosphate dehydrogenase 2; G6PC, glucose-6-phosphatase; PCR, polymerase chain reaction.

Supplementary table 1. Primer sequences (5' to 3') for quantitative real-time PCR

Gene name	GenBank ID	Primer	Sequence	Length (bp)
Growth hormone receptor (*GHR*)	NM_176608	Forward	CACACCAGCTTTCCTTGTCA	102
		Reverse	GAACGGCACTTGGTGAATTT	
Inhibin beta A subunit (*INHBA*)	NM_174363.2	Forward	GAACTTATGGAGCAGACCTC	132
		Reverse	GAAGAGCCAGATTTCTGCAC	
Glutathione S-transferase M1 (*GSTM1*)	NM_175825.3	Forward	ACATCGCTCGCAAGCACAAC	90
		Reverse	AGCGGACATCCATAACCTGG	
Protocadherin 19 (*PCDH19*)	XM_003588123.4	Forward	AACTACGTGAACAGCCGAGC	145
		Reverse	TGTCACAATACAGGCTCCGC	
Alpha 2-HS glycoprotein (*AHSG*)	NM_173984.3	Forward	GATACCCTGGAAACCACCTG	139
		Reverse	AACAGCACGGAAAACTGGCC	
Phosphoenolpyruvate carboxykinase, cytosolic (*PEPCK-C*)	AY145503	Forward	GGTGTGATCAAGAGGCTGAAG	125
		Reverse	ATGGGCACCGTATCTCTTTG	
Phosphoenolpyruvate carboxykinase, mitochondrial (*PEPCK-M*)	NM_001205594	Forward	TGGATGAGGTTTGACAGTGATG	127
		Reverse	TGGTGTTACTCTGGATTGTGG	
Propionyl-CoA carboxylase alpha, mitochondrial (*PCCA*)	NM_001083509	Forward	AAATGAACACGAGACTCCAG	135
		Reverse	AGCCGTTGATGGGAATATCG	

Propionyl-CoA carboxylase beta, mitochondrial (*PCCB*)	NM_001038548	Forward	CACATGCCCAAAAGATCTGC	115
		Reverse	GCCAAAGATTCCACTCCCTC	
Pyruvate carboxylase (*PC*)	NM_177946	Forward	TCCCCAACATCCCATTCCAG	116
		Reverse	TGTCCATGCCATTCTCCTTG	
Fructose-1, 6-bisphosphatase (*FBP1*)	NM_001034447.2	Forward	TCACCGAGTATGTCCAGAGG	146
		Reverse	GGGGCTTTTCTTGTTAGCTG	
Glycerol kinase (*GYK*)	NM_001075236.1	Forward	GCTTCGTTGGCTCCTTGACA	131
		Reverse	TACAATGGACCCCTCCACTG	
Glycerol-3-phosphate dehydrogenase 1 (*GPD1*)	NM_001035354.1	Forward	CACCCAATTTCCGCATCACG	126
		Reverse	CCTTGGTGTTGTCGCCAAAG	
Glycerol-3-phosphate dehydrogenase 2 (*GPD2*)	NM_001100296.1	Forward	TTCCTCGTGCGAGAGGATCA	106
		Reverse	AGGCCTAGAACAGTGGCAAG	
Glucose-6-phosphatase (*G6PC*)	NM_001076124.2	Forward	AGCTGTGGGCATCAAACTCC	116
		Reverse	AGTAGTCGGTATCCAAAACC	
Ribosomal protein S9 (*RPS9*)	NM_001101152	Forward	CCTCGACCAAGAGCTGAAGCCTC	64
		Reverse	CAGACCTCACGTTTGTTC	

PCR, polymerase chain reaction.

5. Conclusion

In general, glycerin replacement in the finishing diet of Korean cattle steers had no impact on weight gain, average daily gain, and feed efficiency except for increase in average daily concentrate intake. The increased intake may be attributed to the sweet taste of glycerin's property. Glycerin replacement did not affect carcass characteristics, chemical and physico-chemical composition, reducing sugar, glycogen, collagen, nucleotides, fatty acid, volatile compounds, and sensory traits in the LT. These results indicate that the glycerin inclusion level (3%) may be not enough to improve animal performance and carcass characteristics. In addition, feeding concentrate containing 3% of glycerin did not result in detrimental effects on growth performance, ruminal fermentation, animal health, and metabolism. This is important not only on animal performance and carcass characteristics but also for sustainable and economic aspects because glycerin is a biodiesel residue and it can potentially partially replace some expensive ingredients such as corn, molasses, DDGS as an energy source for beef cattle.

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CHAPTER SIX

Effects of dietary crude glycerin supplementation on growth performance, blood metabolites, ruminal fermentation parameters, and carcass characteristics in Korean cattle steers

1. Abstract

This study investigated the effects of dietary glycerin supplementation on performance, carcass characteristics, blood metabolites, and ruminal fermentation in Korean cattle steers. Steers (n=15; average 30.5±0.2 months old; 607±15.2kg of initial body weight [BW]) were assigned into three supplement groups: 1) control (n=5; no supplement), 2) glycerin (n=5), or 3) corn starch (n=5) group, respectively. Steers were individually fed diets twice daily (0800 am and 1600 pm), and allowed to free access to water and mineral block. Steers were weighed at 4 weeks interval, and blood sample and rumen fluid were collected at 8 weeks interval throughout the whole feeding trial period. Steers were slaughtered at average 34.3±0.2 months old and *longissimus thoracis* (LT) samples were collected for evaluation of carcass characteristics. Animal performance, such as weight gain, average daily gain, feed

efficiency did not differ ($P > 0.05$) among groups. Average daily total intake and concentrate intake did not differ ($P > 0.05$) among three groups. Carcass weight, longissimus muscle (LM) area, backfat thickness, and marbling score did not differ ($P > 0.05$) among groups. Glycerin supplementation did not alter ($P > 0.05$) chemical composition, meat color parameters (CIE a^* and b^* except for L^*), and glycogen content in both liver and LT, reducing sugar content, and sensory traits. Biochemical parameters of blood did not differ ($P > 0.05$) among groups except that glucose concentration was different ($P = 0.03$) among three groups at 16th week of feeding trial. Glycerin supplementation also did not affect ($P > 0.05$) ruminal fermentation patterns except that the total volatile fatty acid (VFA) concentration were higher ($P < 0.05$) in control and glycerin group compared with corn starch group at 16th week. These results indicate that crude glycerin supplementation may not affect growth performance, carcass characteristics and ruminal fermentation of Korean cattle finishing steers.

2. Introduction

Biodiesel industry worldwide has developed fast with its high production. It is produced via transesterification of vegetable oil or animal fat with the use of methanol, bring about methyl-esters of glycerol and fatty acids (Ma and Hanna, 1999). Glycerol, a by-product with biodiesel, also known as glycerin, is a colorless, odorless, sticky, sweet-tasting liquid. The amount of crude glycerol produced

accounts for about 10 percentage of the weight of the oil or fat used in the biodiesel production process (Ilham and Saka, 2016). Feed-grade glycerol is refined partially to remove residual methanol, because methanol is toxic to cattle.

Glycerol has an energy value similar to corn on a pound-for-pound basis in dairy and feedlot cattle (Lardy, 2008). Mach et al. (2009) reported that the metabolizable energy content of crude glycerin was estimated as 3.47 Mcal/kg of dry matter in high concentrate diets fed to Holstein bulls, which enabled the glycerin to replace some ingredients, such as corn in the feed. Glycerol, the 3 carbon backbone in triglyceride, is also known as substrate for gluconeogenesis in the liver and kidney, which can provide energy for cellular metabolism. It is phosphorylated by glycerol kinase to glycerol 3-phosphate, which is a more physiologically important form of glycerol, and then to dihydroxyacetone phosphate, thus, facilitating its entry into the gluconeogenic pathway at the level of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate, and then glucose is produced through gluconeogenesis and can be stored as glycogen in the liver and muscle (Krehbiel, 2008; Hagopian et al., 2008). Glycerol is also a carbon source used for fatty acid synthesis, which is important for improvement of marbling of meat (Benedeti et al., 2015). Therefore, crude glycerin has the potential as an alternative energy source for animal's diet in beef cattle industry.

In terms of the fate of glycerol entering the rumen, the glycerol consumed may be 1) fermented to produce volatile fatty acids in the rumen, 2) absorbed across the

rumen epithelium, or 3) escape the rumen by outflow via the omasal orifice (Werner Omazic et al., 2015).

Crude glycerin has been extensively evaluated as an ingredient in diets for several species of animal: swine (Schieck et al., 2010), poultry (Jung and Batal, 2011), dairy cattle (Chung et al., 2007), beef cattle (Mach et al., 2009), and sheep (Gunn et al., 2010a). Especially, the effect of crude glycerin inclusion in diets fed to cattle on meat quality has been tested by many previous studies (Mach et al., 2009; Ramos and Kerley, 2012; Egea et al., 2014; Benedeti et al., 2016). However, the outcomes were not consistent, since there is no significant difference in animal performance and carcass characteristics in some studies, while other studies reported the changes in fat content and oleic acid concentration (Carvalho et al., 2014), sensory trait, for example overall acceptability (Prado et al., 2015), and gain and feed efficiency (San vito et al., 2016). Furthermore, Edwards et al. (2012) reported that the dietary supplementation of glycerin decreased in ruminal lipolysis without adverse effect in rumen digestion, indicating that glycerin may contribute to increasing the fat content or marbling score (MS).

The objective of this study was to evaluate the effect of dietary glycerin supplementation on growth performance, blood metabolites, ruminal fermentation characteristics, carcass characteristics and sensory traits, glycogen content, and hepatic gluconeogenesis gene expression in finishing Korean cattle steers.

3. Materials and methods

Experimental procedure were approved by the Seoul National University Institutional Animal Care and Use Committee.

1) Animals, housing, and diets

Fifteen Korean cattle steers at the University Animal Farm of the College of Agriculture and Life Sciences, Pyeongchang Campus of Seoul National University in South Korea were used in this experiment. From ten days before experiment, all animals were fed an experimental concentrate diet (approximately 1.35% of body weight [BW] per animal) and an oat straw (1kg/day per animal). After an adaptation period of ten days with these animals consuming experimental concentrate without supplements, animals (initial BW of 607±15.2kg and age of 30.5±0.2 mo.) were randomly assigned to 1 of 3 treatment groups: **control** (no supplement), 6.4% glycerin supplementation (**Glycerin**), or 8.7% corn starch supplementation (**Corn starch**; Table 35) according to individual BW, age and average daily gain (ADG). Glycerin is a colorless, odorless, viscous, and sweet-tasting liquid. In order to apply the glycerin to diet fed to Korean cattle conveniently and effectively, glycerin supplement was made by adsorbing glycerin to the wheat bran at the ratio of 58% of glycerin to 42% of wheat bran on dry matter (DM) basis. Cattles in the glycerin group received glycerin at 6.4% of concentrate DM supply daily, whereas those in the corn starch group received corn starch at 8.7% of concentrate DM supply daily.

The supplements were poured on top of the feed trough for individual cattle. The cattle in the glycerin and corn starch group were supplied supplements to make doses correspond to equal TDN intake. The intended doses of glycerin and corn starch used in this study were calculated based on amount of TDN intake per day, where the glycerin amount of 6.4% of concentrate corresponded to the corn starch amount of 8.7% of concentrate DM supply daily. Ingredient and chemical composition of experimental diets are presented in Table 35. All steers were individually allowed to receive the commercial fattening concentrate diet with the daily amount of 1.35% of individual BW, and the concentrate was individually manually supplied two times daily with 1.0kg/d of oat straw per animal at 0800 am and 1600 pm. We recorded the amount of remaining diet after the animals consumed diet in each feeding time. The animals had free access to water and mineral block. Experimental feeding periods were 16 weeks in duration (February 24^{th} to June 16^{th}). Oat straw and concentrate consumptions were recorded daily. Body weight was measured at 0900 am before the feeding at start date and at an interval of 8 weeks throughout the feeding trial. Samples of concentrate and oat straw were collected at an interval of two weeks, and stored at -20℃ until further analysis. The animals were observed once daily throughout the feeding trial to record animals' physical condition.

Table 35. Ingredients and nutrient composition of the experimental concentrate

Item	Concentrate		
Ingredient, % of DM			
Steamed flaked corn	25.42		
Ground wheat	23.84		
Corn gluten feed	16.73		
Palm kernel meal	9.66		
Ground corn	3.53		
Soyhulls	5.52		
Wheat flour	2.98		
Molasses	3.17		
Condensed molasses solubles	2.10		
Whole cottonseed	1.49		
Distiller's dried grains with solubles	1.45		
Palm oil	0.18		
Ammonium chloride	0.14		
Sodium bicarbonate	0.82		
Salt	0.18		
Limestone	2.23		
Porphyry	0.40		
Mineral-vitamin premix [1]	0.19		
Chemical composition, % of DM			
DM	91.5		
Crude protein	14.15		
Crude fat	5.08		
Ash	6.51		
NDF	28.56		
ADF	10.55		
Calcium	1.21		
Phosphorus	0.48		
TDN	80.66		
ME, Mcal/kg	2.85		
Supplements, % of concentrate	Control	Glycerin	Corn starch
Wheat bran	4.7	4.7	4.7
Glycerin	—	6.4	—
Corn starch	—	—	8.7

[1] Provided following nutrients per kg of additive (Grobic-DC, Bayer Health Care, Leverkusen, Germany): Vit. A, 2,650,000 IU; Vit. D3, 530,000 IU; Vit. E, 1,050 IU; Niacin, 10,000 mg; Mn, 4,400 mg; Zn, 4,400 mg; Fe, 13,200 mg; Cu, 2,200 mg; I, 440 mg; Co, 440 mg.

2) Blood and rumen fluid collections and measurements

Blood was collected at 0800 am after 12 h fast, and also collected at 1300 pm 3h post-feeding. Blood was collected at an interval of 4 weeks by jugular venipuncture with both a non-heparinized vacutainer (20mL; Becton-Dickinson, Franklin Lakes, NJ, USA) and ethylenediaminetetraacetic acid- treated vacutainer (20ml). Both serum and plasma were separated by centrifugation at 1,500 × g at 4℃ for 15min, and stored at -80℃ until analysis. The blood serum was used for metabolite analysis. Reagents to analyze glucose and triglyceride (TG) were purchased from JW Medical (Seoul, Korea). The analytical reagent for non-esterified fatty acid (NEFA) was obtained from WAKO (Osaka, Japan). All of these items analyzed using an automated chemistry analyzer (Hitachi 7180; Hitachi, Tokyo, Japan). All of the analysis methods were verified in our laboratory, as previously reported (Kang et al., 2016). Rumen fluid sample was harvested 3 hours post-feeding using oral-stomach tube method as described by Shen et al. (2012). Rumen fluid pH was measured immediately with pH meter (Ohaus Corp., 7 Campus Drive, Suite 310, Parsippany, NJ07054, USA). For VFA analyses, 1 mL of rumen fluid was mixed with 0.2mL of 25% meta-phosphoric acid, and stored at -20℃ until analysis, while 50 mL of rumen fluid was collected for ruminal NH_3-N analyses, and stored at -20℃ until analysis. The VFA and NH_3-N concentrations

were analyzed as described previously (Piao et al., 2012). The NH_3-N concentration was determined using a modified colorimetric method (Chaney and Marbach, 1962), and VFA concentrations were determined by gas chromatography using a Agilent Tech 7890A (Hewlett Packard Strasse 876337, Waldbronn, Germany) for which a Supelco (30m×0.25mm×0.25μm, fused silica capillary column) column was used. Fecal samples were collected at an interval of 4 weeks, and both of diet and fecal samples were ground and stored at -20℃ until further analysis. Nutrient digestibility was estimated using indirect digestibility method by the lignin ratio technique as described by Wallace and Van dyne (1970). Feces and diet samples were used for the analysis of DM, OM and crude fat content according to AOAC (1996), and lignin was determined according to the method of Van Soest et al. (1991).

3) Slaughter procedures and tissue sample collections

Steers were transported to a local municipal slaughterhouse (Bucheon, Korea) after 112 d of study with final BW of 666±18.2kg and age of 34.3±0.2 mo. At slaughter, longissimus hot-carcass was taken within around 60 min post-mortem after the carcass had been split and transferred to a chiller. The *longissimus thoracis* (LT) sample was obtained from the 12^{th} vertebrae for glycogen content analysis, and liver sample was also collected for glycogen content analysis and gene expression analyses. Immediately visible fat was trimmed away from LT sample at the

slaughterhouse, and the LT and liver samples were frozen in liquid nitrogen and later stored at -80℃ for glycogen content analysis and gene expression analyses, respectively. Carcass of Korean cattle steers were evaluated by a meat grader using the Korean carcass-grading system of Korea Institute for Animal Products Quality Evaluation (KAPE, 2017) at 24h post-mortem. Carcass weight, longissimus muscle area, fat thickness, MS, meat color, fat color, texture, and maturity were examined and reported by an official meat grader. Among these parameters, MS was mainly used for determining quality grade (QG). Five grades (QG 1++, 1+, 1, 2, and 3) are assigned by meat graders. The MS of the Beef Marbling Standard (BMS) ranges from 1 (devoid) to 9 (abundant); 8 or 9 was the MS for QG 1++, 6 or 7 was the MS for QG 1+, 4 or 5 was the MS for QG1, 2 or 3 was the MS for QG 2, and 1 was the MS for QG 3. In addition, meat color, fat color, texture and maturity of the exposed longissimus muscle at the 13^{th} rib interface were used for QG determination (NLCF, 1998). After slaughter and 24-h chill, LT cold carcass from the 12^{th} vertebrae was also obtained. *Longissimus thoracis* samples were vaccum-packaged, transported under ice (4℃) to a laboratory within 30h post-mortem, and were subsequently stored at 4℃ for 6 days. After total 7 days of storage post-mortem, the packages containing the LT samples of 15 steers were opened and the external fat was trimmed away. The LT samples were minced using a mini chopper (CH180, Kenwood, Shanghai, China) for 30s. Minced LT samples from various locations

were pooled, and some samples were used immediately for the analysis of pH, water holding capacity, and chemical composition, while others were stored at -70℃ for the analysis of reducing sugar content. Samples assessed for shear force, meat color (CIE value) and sensory traits were collected but not minced, and the LT samples were used immediately for the analysis of shear force, meat color, and sensory evaluation.

4) mRNA levels in the liver

The mRNA levels in the liver were measured using the same method, as described in Chapter five. The primers of interest were designed using integrated DNA technology based on published sequences at the National Center for Biotechnology Information (Supplementary table 1).

5) Chemical and physicochemical compositions, color, pH, shear force, and water holding capacity

Chemical and physico chemical compositions, color, pH, and shear force of LT samples were analyzed using the same methods, as described in Chapter four.

Meat sample (5g) was placed into a centrifugation tube with a filter paper (Whatman No. 4, Whatman PLC., Kent, UK), and centrifuged at 2,265 × g for 10 min. After centrifugation, water holding capacity was calculated as the remaining moisture in the meat sample on the basis of the moisture content of the original

meat sample.

6) Reducing sugar content

The reducing sugar contents of LT samples were measured using the same method, as described in Chapter four.

7) Sensory evaluation

The sensory characteristics of LT samples were evaluated using the same method, as described in Chapter four.

8) Glycogen concentration

The glycogen concentration of LT samples was measured using the same method, as described in Chapter five.

9) Statistical analysis

Data (5 animals/group) except blood metabolites were analyzed by analysis of variance using the General Linear Model Procedure (Proc GLM) in SAS software (SAS Institute, Cary, NC, USA). The data for blood metabolites, including triglyceride, glucose and NEFA, were analyzed by two-way analysis of variance to test the fixed effects of supplement, time, and their interaction by a generalized linear mixed model, using the Proc GLM procedure of SAS software. Experimental unit was an individual animal for blood metabolites. Animal within supplement or

time type was considered the random variable. The LSMEANS PDIFF option was used to compare differences among mean values at $P < 0.05$.

4. Result and discussion

1) Intake, animal performance, and digestibility

Concentrate intake in both glycerin and corn starch supplementation groups were lower ($P = 0.0349$) than that in control group at 16th week (Table 36). However, both glycerin and corn starch supplementation did not affect ($P > 0.05$) average daily concentrate intake (5.3 ± 0.25 kg/d of DM), total straw intake (0.95 ± 0.0 kg/d of DM), and total DMI (6.9 ± 0.24 kg/d of DM) during the whole feeding trial period. Parsons et al. (2009) reported that 2% glycerin in the total ration did not affect DMI in crossbred finishing heifers, but increasing glycerin to 4, 8, 12, or 16% gradually reduced DMI. However, Buttrey et al. (2015) reported that glycerin inclusion in the diet up to 10% of DM did not affect DMI in crossbred finishing steers. In present study, the glycerin was supplemented at 6.4% of DM. Supporting our results, some previous studies conducted with beef cattle have showed no negative effects on DMI when supplementing glycerin to diets at inclusion rates similar with the present study (Mach et al., 2009; Bartoň et al., 2013). However, feed intake particularly decreased when glycerin was fed to lactating Holstein cows in high amounts up to 30% (Ezequiel et al., 2015). Indeed, supplementing high

levels of glycerin to diets was reported to have a negative impact on rumen fermentation through decreased fiber digestion, bacterial populations, and acetate production (Abo El-nor et al., 2010). In our previous study, 3% glycerin inclusion in diet fed to Korean cattle steers increased average daily concentrate intake. However, 6.4% glycerin supplementation did not affect concentrate intake in current study. As mentioned in our previous study, the increased feed intake may be attributed to the sweet taste of glycerin's property. Also, the glycerin was included in the concentrate pellet in the previous study, which may induce the steers to eat more concentrate. But in the current study, the glycerin was separately supplied as supplement, and the supplementation level was increased to 6.4% that is higher than that of previous study, and thus more energy supply was derived from glycerin. In addition, the glycerin supplement was fed with straw by steers first before feeding concentrate at each feeding time. After feeding glycerin and straw, the concentrate was subsequently supplied. For these reason, it can be assumed that the glycerin may not affect the concentrate intake in this study.

Average daily gain (ADG) in glycerin supplementation group was higher (P = 0.0487) than that in control group at 4th week, and feed efficiency in both glycerin and corn starch supplementation groups were higher (P = 0.0079) than that in control group at 4th week. However, both glycerin and corn starch supplementation by top-dressing method did not affect ($P > 0.05$) ADG and feed efficiency during the whole feeding trial period. Supporting our results, Mach et al. (2009) reported

that glycerin replacing cereals in the diet as an energy ingredient did not have negative impact on ADG and feed efficiency in Holstein finishing bulls. Also, the addition of glycerin at levels up to 10% of DM to the diet fed to crossbred steers (Buttrey et al., 2015), as well as at levels up to 21% of DM fed to crossbred finishing steers (Chanjula et al., 2016) had no impact on growth performance. However, Parsons et al. (2009) reported positive effect on ADG and feed efficiency when glycerin was added to crossbred finishing heifer diets at less than 10% of DM. In contrast, negative effects on ADG and feed efficiency were also observed when glycerin was included to finishing heifer diets at 16% of DM (Parsons et al., 2009), and to wether lamb diets at 45% of DM (Gunn et al., 2010b), respectively. In addition, dietary glycerin supplementation did not affect ($P > 0.05$) apparent digestibility of total dry matter, organic matter, and fat in this study.

Table 36. Effects of glycerin or corn starch supplementation on growth performance, feed intake (% of DM), feed efficiency, and nutrient digestibility in Korean cattle steers

Item	Supplement			SEM	*P*-value
	Control	Glycerin	Corn starch		
Age, month					
Initial	30.7	30.5	30.4	0.24	0.91
4 weeks	31.6	31.4	31.3	0.25	0.91
8 weeks	32.6	32.3	32.3	0.25	0.89
12 weeks	33.5	33.3	33.2	0.24	0.91
Final	34.4	34.2	34.1	0.25	0.91
Body weight, kg					
Initial	607	604	610	15.2	0.95
4 weeks	620	629	631	17.0	0.81
8 weeks	633	645	648	17.7	0.66
12 weeks	652	661	659	18.2	0.86
Final	666	667	666	18.2	0.99
Average daily gain, kg/d					
4 weeks	0.44[b]	0.88[a]	0.76[ab]	0.11	0.0487
8 weeks	0.49	0.56	0.61	0.08	0.84
12 weeks	0.67	0.59	0.39	0.06	0.23
16 weeks	0.49	0.21	0.26	0.07	0.33
Total period	0.52	0.56	0.50	0.04	0.86
Total intake, kg/d (DM basis)					
4 weeks	6.6	6.8	7.0	0.27	0.86
8 weeks	6.9	6.8	7.1	0.27	0.82
12 weeks	7.0	6.9	6.9	0.25	0.99
16 weeks	7.5	7.0	6.8	0.25	0.31
Total period	7.0	6.9	6.9	0.24	0.97
Concentrate intake, kg/d (DM basis)					
4 weeks	5.3	5.1	5.1	0.26	0.96
8 weeks	5.6	5.1	5.4	0.27	0.66
12 weeks	5.7	5.1	5.0	0.26	0.29
16 weeks	6.1[a]	5.2[b]	4.9[b]	0.27	0.0349
Total period	5.7	5.1	5.1	0.25	0.37
Oat straw intake, kg/d (DM basis)					
4 weeks	1.0	1.0	1.0	0.001	0.30
8 weeks	0.9	0.9	0.9	0.001	0.45
12 weeks	0.9	0.9	0.9	0.001	0.45
16 weeks	0.9	0.9	0.9	0.001	0.20
Total period	0.95	0.95	0.95	0	—
Supplement intake, kg DM/d					
Wheat bran intake					
4 weeks	0.35	0.28	0.28	0.01	0.12
8 weeks	0.36[a]	0.33[a]	0.28[b]	0.01	0.008
12 weeks	0.36	0.35	0.37	0.004	0.49

16 weeks	0.38	0.37	0.38	0.003	0.27
Total period	0.36	0.33	0.33	0.006	0.067
Glycerin intake					
4 weeks	—	0.392	—	—	—
8 weeks	—	0.451	—	—	—
12 weeks	—	0.490	—	—	—
16 weeks	—	0.506	—	—	—
Total period	—	0.456	—	—	—
Corn starch intake					
4 weeks	—	—	0.522	—	—
8 weeks	—	—	0.545	—	—
12 weeks	—	—	0.615	—	—
16 weeks	—	—	0.593	—	—
Total period	—	—	0.564	—	—
Feed efficiency, kg gain/kg intake					
4 weeks	0.063[b]	0.124[a]	0.106[a]	0.01	0.0079
8 weeks	0.067	0.083	0.084	0.01	0.81
12 weeks	0.097	0.083	0.055	0.01	0.15
16 weeks	0.065	0.030	0.038	0.01	0.34
Total period	0.073	0.080	0.071	0.004	0.70
Digestibility, %					
DM	49.1	50.2	57.5	1.87	0.21
OM	55.3	55.0	61.6	1.66	0.29
Fat	80.3	81.3	86.5	1.61	0.31

n=5 per group

2) Ruminal fermentation

Ruminal fermentation pattern are shown in Table 37. Both glyceirn and corn starch supplementation did not affect ($P > 0.05$) average rumen pH throughout the feeding trial. The ruminal NH_3-N content in glycerin supplementation group was lower ($P = 0.03$) than that in control group at 8th week. This result was consistent with that reported by Paiva et al. (2016), where crude glycerin inclusion at levels up to 21% in diet fed to Holstein cows decreased the ruminal NH_3-N content. Paiva et al. (2016) also suggestd that ruminal degradation of glycerin would be faster compared to those of corn and soybean meal. Many previous studies (Mach et al., 2009; Khattab, 2015; Benedeti et al., 2016) reported that glycerin could be effectively used as an alternative energy source to substitute for cereals in the diet. In this study, the decreased ruminal NH_3-N content in glycerin group may be due to the fact that the readily degradable crude glycerin may be utilized with ruminal NH_3-N to synthesize microbial protein by synchronization, which may decrease ruminal NH_3-N content. Although previous studies reported that microbial protein synthesis was not affected when crude glycerin was included up to 15% of the diet, indicating no influence of glycerin on ruminal nitrogen supply (Donkin et al., 2009; Shin et al., 2012), the current result warrants further investigation to determine the level of microbial protein synthesis in glycerin replacement group.

The acetate concentration in corn starch supplementation group was lower ($P = 0.04$) than that in control group at 16th week, but did not differ between glycerin

supplementation and control group. The total VFA concentration in corn starch supplementation group was lower ($P = 0.02$) than that in glycerin supplementation or control group at 16th week. The ratio of acetate to propionate in glycerin supplementation group tended to ($P = 0.07$) be lowest at 8th week, followed by that in corn starch group and control group. However, there was no impact observed on growth performance and rumen fermentation characteristics in the steers of control or glycerin group. In addition, no differences were observed in rumen concentrations of propionate and butyrate throughout the whole feeding trial. In disagreement to our results, some previous studies reported that animals fed glycerin had higher total rumen VFA concentration, higher ruminal propionate concentration, and lower ratio of acetate to propionate than animals without feeding glycerin (van Cleef et al., 2015; San Vito et al., 2016). Linke et al. (2004) also reported that the administration of 1kg of dietary glycerol supplement by oral drench via rumen tube that corresponded to the amount in our study increased ruminal propionate concentration.

Table 37. Effect of glycerin or corn starch supplementation on rumen metabolites of Korean cattle steers

Item	Supplement			SEM	*P*-value
	Control	Glycerin	Corn starch		
pH					
0 week	6.26	6.55	6.70	0.11	0.27
8 weeks	6.36	6.31	6.37	0.13	0.99
16 weeks	5.89	6.02	6.19	0.09	0.38
NH_3-N, mg/100ml					
0 week	16.2	17.7	10.5	1.60	0.13
8 weeks	17.6[a]	8.6[b]	11.4[ab]	1.56	0.03
16 weeks	12.7	14.9	12.8	1.89	0.91
Acetate, mM					
0 week	60.0	58.9	49.6	2.84	0.31
8 weeks	61.5	46.8	53.8	3.53	0.30
16 weeks	66.0[a]	59.4[ab]	47.1[b]	3.14	0.04
Propionate, mM					
0 week	20.5	17.6	16.9	1.72	0.71
8 weeks	15.2	29.0	25.4	4.17	0.47
16 weeks	34.2	23.1	16.9	3.59	0.17
Butyrate, mM					
0 week	15.9	15.8	11.7	0.89	0.07
8 weeks	16.9	12.0	12.0	1.16	0.21
16 weeks	16.1	17.8	12.2	1.31	0.26
Total VFA, mM					
0 week	100.8	96.9	82.0	5.28	0.35
8 weeks	97.8	92.3	95.3	7.49	0.97
16 weeks	121[a]	105[a]	79.5[b]	6.40	0.02
A/P ratio					
0 week	3.09	3.58	3.17	0.19	0.61
8 weeks	4.08	2.25	2.80	0.32	0.07
16 weeks	2.54	2.85	3.50	0.36	0.64

n=5 per group

3) Blood metabolites

The effects of dietary glycerin supplementation on blood metabolite pattern are shown in Table 38. Serum glucose concentration was not affected ($P > 0.05$) by glycerin supplementation at initial and 8th week of feeding trial. However, the average serum glucose concentration was increased ($P = 0.03$) in glycerin group at 16th week. Previous study reported that feeding glycerin to Holstein dairy cows enhanced energy availability (higher blood glucose, lower urine ketones, and lower blood BHBA) during lactation (Chung et al., 2007). In ruminants, glycerin was well known to be converted to propionic acid in the rumen, which can be absorbed into rumen wall (Kijora et al., 1998). Consequently, both of propionic acid and some glycerin absorbed by rumen wall can be moved to liver and kidney through bloodstream, where the propionic acid and glycerin component can be converted to glucose via gluconeogenesis, and then provides energy for cellular metabolism (Krebs et al., 1966). In this study, although the serum glucose concentration was not influenced by glycerin supplementation at initial and 8th week, the glucose level was slightly increased at 16th week. The result was to some extent consistent with previous study mentioned above. The reason for little effects of glycerin on blood metabolites may be due to the fact that glycerin supplement was taken up over a longer period of time compared to oral drenching (Bartoň et al., 2013).

In addition, glycerin supplementation did not affect ($P > 0.05$) concentrations of serum TG and NEFA. However, the aveage serum TG concentrations for 12

hours post-fasting were higher ($P < 0.05$) than those for 3 hours post-feeding at initial and 16th week. Also, the average serum NEFA concentrations for 12 hours post-fasting were higher ($P < 0.05$) than those for 3 hours post-feeding throughout the whole feeding trial. The higher average concentrations of serum TG and NEFA for 12 hours post-fasting may reflect body fat mobilization in response to negative energy balance during fasting period.

Table 38. Effect of glycerin or corn starch supplementation on serum metabolites in Korean cattle steers

Item	12 hours post-fasting			3 hours post-feeding			SEM	*P* -value		
	Control	Glycerin	Corn starch	Control	Glycerin	Corn starch		Supplement	Time	Supplement x Time
TG, mg/dL										
0 week	23.2^{a}	22.8^{a}	24^{a}	15^{b}	15.8^{b}	18.6^{ab}	1.11	0.60	0.0017	0.84
8 weeks	25	24.8	30.2	22.8	22.2	25.8	1.19	0.25	0.21	0.92
16 weeks	31.4^{ab}	29.2^{abc}	37.4^{a}	28.4^{bc}	22.2^{c}	25.6^{bc}	1.34	0.11	0.0034	0.29
Glucose, mg/dL										
0 week	69.4	83.4	81.8	82	78.6	84.2	2.42	0.47	0.49	0.36
8 weeks	80.4	75.4	73	78.2	79.2	71.4	1.75	0.27	1.00	0.76
16 weeks	84^{bc}	97.4^{a}	85.6^{bc}	90.2^{ab}	87.8^{ab}	75^{c}	2.05	0.03	0.20	0.12
NEFA, Eq/L										
0 week	199^{a}	180^{ab}	191^{a}	91.5^{c}	99.4^{c}	115^{bc}	12.7	0.87	0.0003	0.80
8 weeks	210^{a}	129^{abc}	172^{ab}	120^{bc}	82.9^{c}	128^{abc}	13.4	0.14	0.02	0.69
16 weeks	218^{a}	215^{a}	211^{a}	116^{b}	103^{b}	113^{b}	11.6	0.90	<0.0001	0.91

n=5 per group

[a-c] Means with different letter within a same row differ ($P < 0.05$).

4) Carcass and meat quality characteristics

Carcass and meat quality characteristics are shown in Table 39. Both glycerin and corn starch supplementation did not affect ($P > 0.05$) carcass weight (378±10.6 kg), LM area (85±1.3㎠), and backfat thickness (8.8±0.9㎜). Previous studies reported that the addition of glycerin at levels up to 10% of DM to the diet fed to finishing cattle did not affect carcass weight, backfat thickness, and LM area, the values of which are similar to those of the current study (Buttrey et al., 2015; Egea et al., 2014). In addition, both glycerin and corn starch supplementation did not affect ($P > 0.05$) the marbling score, texture, and QG. In this study, it was hypothesized that glycerin supplementation would increase rumen propionate concentration. In addition, the increased propionate and certain proportion of glycerin, which are glucose precursors, can be converted to glucose in the liver of cattle. Therefore, it was expected that glucose production would increase in steers of glycerin group, leading to an increase in blood insulin concentration and lipogenesis and subsequent MS. Thus, our results were not consistent with the hypothesis. It is not clear for the reason due to limited information.

The effects of glycerin supplementation on meat quality parameters are shown in Table 40. Both glycerin and corn starch supplementation did not affect ($P > 0.05$) the content of moisture, crude protein and crude fat. These results were agreed with previous studies which reported that the inclusion of glycerin at levels up to 18% of

DM to the diet fed to finishing cattle did not affect the content of moisture, crude protein, and total lipids (Egea et al., 2014; Eiras et al., 2014; Prado et al., 2015). Volpi-Lagreca and Duckett (2016) also reported that feeding 4.3% crude glycerin via drinking water to finishing cattle did not affect the contents of moisture, total lipids, crude protein, as well as ash of longissimus muscle.

Glycerin supplementation had no effect ($P > 0.05$) on pH and color parameters (a^*, b^*, c^* and H^*) except that CIE L^* value was higher ($P = 0.0075$) in glycerin supplementation group than those of control and corn starch supplementation group. These results were consistent with Egea et al. (2014) who reported that the inclusion of glycerin at levels up to 4% of DM to the diet fed to finishing Limousin bulls did not affect the pH of beef 7 days after slaughter and the color parameters (a^*, b^*, c^* and H^*). Similar results were also reported by other previous studies which showed no effect of glycerin inclusion on pH and color parameters between control and glycerin (5 and 12%) group in Nellore bull meat (Françozo et al., 2013), nor between control and glycerin (16.1% of DM) group in crossbred finishing bulls meat (Prado et al., 2015). In this study, 6.4% glycerin supplementation increased CIE L^* value of LT in Korean cattle steers. However, many previous studies reported that glycerin inclusion at up to 28% of DM to diet did not affect CIE L^* of longissimus muscle of finishing cattle (San Vito et al., 2015; Chanjula et al., 2016; Favaro et al., 2016). It is not clear for the reason of increase of CIE L^* of LT in glycerin supplementation group due to limited information.

In addition, glycerin supplementation had no effect ($P > 0.05$) on water holding capacity (WHC) and shear force. Supporting our result, Mach et al. (2009) reported that the inclusion of glycerin to the diet fed to finishing Holstein bulls had no effect on Warner-Bratzler shear force between control and glycerin (4, 8, 12% of DM) groups. Previous study has showed the relationship between tenderness and intramuscular fat content, as well as with shear force (Purchas et al., 2002). Although the glycerin supplementation used in our study had no impact on shear force of LT, shear force results (<4.0kg) ensure a tenderness that bring about high consumer acceptance (Miller et al., 2001). Also, previous studies reported that the inclusion of glycerin had no effect on WHC between control and glycerin (2 and 4%) group in Limousin bull meat (Egea et al, 2014), nor between control and glycerin (7, 14 and 21%) group in crossbred steer meat (Chanjula et al., 2016). In contrast, different results for WHC were observed in the non-ruminant. Mourot et al. (1994) reported that the inclusion of glycerin at 5% of DM to the diet fed to pigs reduced water losses and cooking losses in pork due to the increased cell osmotic pressure, leading to increased intracellular water content, which would improve the WHC. This difference between species may be due to the fact that the glycerin can be absorbed without transformation in the non-ruminant stomach, but in ruminant 80% of glycerin would be transformed into volatile fatty acids or other metabolites (Mach et al., 2009), leading to a low absorption rate of untransformed glycerin molecule. Collectively, glycerin supplementation may not have impact on WHC in the present study.

Table 39. Effects of glycerin or corn starch supplementation on carcass characteristics of *longissimus thoracis* in Korean cattle steers

Item	Supplement		SEM	*P*-value
	Control	Glycerin	Corn starch		
Carcass weight, kg	382	377	376	10.6	0.86
LM area, ㎠	87.4	81.8	85.8	1.25	0.11
Backfat thickness, ㎜	8.4	11.0	7.0	0.87	0.13
Marbling score [1]	5.4	6.0	5.2	0.38	0.74
Yield index	68.4	66.1	69.2	0.66	0.08
Meat color [2]	4.8	4.4	4.6	0.13	0.60
Fat color [3]	3.0	3.0	3.0	0	—
Texture [4]	1.2	1.0	1.2	0.09	0.66
Maturity [5]	2.2	2.0	2.2	0.09	0.66
Yield grade [6]	1.4	1.8	1.2	0.17	0.18
(A:B:C)	(3:2:0)	(2:2:1)	(4:1:0)		
Quality grade [7]	2.6	2.2	2.8	0.19	0.51
(1+:1:2)	(3:1:1)	(4:1:0)	(2:2:1)		

n=5 per group

[1] Marling score: 1, devoid; 9, very abundant

[2] Meat color: 1, bright red; 7, dark red

[3] Fat color: 1, white; 7, yellowish

[4] Texture: 1, very fine; 3, very coarse

[5] Maturity: 1, youthful; 9, mature

[6] Yield grade: A=1, B=2, C=3

[7] Quality grade: 1++=1, 1+=2, 1=3, 2=4, 3=5

Table 40. Effect of glycerin or corn starch supplementation on chemical and physico-chemical composition, glycogen, reducing sugar and sensory traits of *longissimus thoracis* in Korean cattle steers

Item	Supplement			SEM	*P*-value
	Control	Glycerin	Corn starch		
Chemical composition					
Moisture, %	64.5	62.1	63.8	0.73	0.53
Crude protein, %	20.2	19.6	20.0	0.24	0.70
Crude fat, %	13.1	17.0	14.6	0.93	0.32
Physico-chemical composition					
pH	5.54	5.56	5.62	0.02	0.31
WHC, %	71.1	76.4	74.6	1.22	0.29
Shear force, N	23.4	19.6	22.6	2.35	0.71
CIE *L** [1]	35.5^{b}	39.6^{a}	35.6^{b}	0.72	0.0075
CIE *a**	14.7	14.9	15.4	0.38	0.71
CIE *b**	11.5	12.0	11.4	0.31	0.52
*C**	18.7	19.2	19.2	0.46	0.85
*H**	37.8	38.9	36.6	0.51	0.09
Glycogen					
Liver, mg/g	26.3	32.0	42.8	3.08	0.11
Muscle, mg/g	1.05	0.68	0.91	0.19	0.67
Reducing sugar					
Muscle, mM	15.6	11.7	14.8	0.77	0.10
Sensory traits [2]					
Appearance	5.88	6.04	5.88	0.13	0.92
Odor	5.67	5.67	5.58	0.09	0.96
Taste	6.38	6.38	6.33	0.05	0.97
Flavor	6.17	6.46	6.33	0.09	0.64
Tenderness	5.96	6.13	6.46	0.17	0.67
Juiciness	6.21	6.42	6.46	0.13	0.84
Overall acceptance	6.33	6.33	6.29	0.11	0.99

n=5 per group

[1] *L**: lightness; *a**: redness; *b**: yellowness; *C**: chroma; *H**: hue.

[2] The score was evaluated with 12 semi-trained panelists (1, extremely dislike; 5, neither dislike nor like; 9, extremely like).

5) Reducing sugar and glycogen contents in the LT

Reducing sugar content in LT, and glycogen contents in LT and liver are also shown in Table 40. Glycerin supplementation had no effect ($P > 0.05$) on the reducing sugar content in LT. A big amount of volatile compounds, such as aldehydes, hydrocarbons, ketones, furans, pyrazines, thiophenes, sulfuric compounds, and so on, can be produced during cooking process of meat, and play an important role in flavor. These volatile compounds can be generated via several pathways, including a Maillard reaction of amino aicds or peptides with reducing sugars (e.g. glucose and ribose), thermal lipid degradation, and the interaction between Maillard reaction products and lipid oxidation products (Kosowska et al., 2017). Of these pathways, Maillard reactions are key processes in volatile compoudns generation (Mottram and Nobrega, 2002), and glucose, ribose, glucose 6-phosphate and ribose 5-phosphate are well known as main reducing sugar for this reaction (Ba et al., 2012). In addition, glycerin can be converted to glucose through gluconeogenesis in the liver of cattle. In this study, 6.4% glycerin supplementation did not affect reducing sugar content. It is not clear for the reason of no difference on reducing sugar content in LT of Korean cattle steers due to limited information. This warrants further investigation to determine the impact of glycerin supplementatin on reducing sugar content in Korean cattle steers.

Also, glycerin supplementation had no effect ($P > 0.05$) on the glycogen content in both LT and liver. Little difference on glycogen content could be

indicative of similar glycolytic activity post-mortem and/or similar glycogen levels before slaughter. Daly et al. (1999) reported that muscle glycogen concentrations tend to be increased under grain-based diets system, independent of the higher calorific intake normally related to those diets, possibly in response to changes in rumen fatty acid production. In the current study, the average daily concentrate intake (5.3±0.25 kg) did not differ among three groups. For these reasons, there was probably no difference on the content of reducing sugar in LT, and glycogen in both LT and liver.

6) Gene expression related to gluconeogenesis

The relative mRNA expression of five genes are shown in Figure 5. Glycerin supplementation had no effect ($P > 0.05$) on hepatic mRNA levels of genes for glucogenic substrate incorporation in gluconeogenesis pathway in Korean cattle. Glycerol is known as one of the major substrates for gluconeogenesis. Under fasting conditions, several glycogenic precursors, such as glycogenic amino acids, lactate, and glycerol, can be utilized for gluconeogenesis to maintain blood glucose concentrations within a normal range, and then provide energy for glucose-dependent tissues. In one of gluconeogenesis pathways, glycerol can be phosphorylated by glycerol kinase (GYK), and converted to glycerol-3-phosphate. Then, glycerol-3-phosphate can be oxidized to dihydroxyacetone phosphate and used for gluconeogenesis, which is catalyzed by glycerol-3-phosphate dehydrogenase (GPD) including GPD1 and GPD2 (Sato et al., 2016). Glucose-6-

phosphatase (G6PC) is also known as an important enzyme that mediates gluconeogenesis from glycerol and its gene expression is inhibited by insulin (Lee et al., 2015). Also, fructose-1,6-bisphosphatase (FBPase) is known as a regulatory enzyme in the process of gluconeogenesis (Visinoni et al., 2012). In the current study, the relative mRNA expression of GYK, GPD1, GPD2, G6PC, and FBP1 did not differ among three groups, which indicates that glycerin supplementation has no impact on these gene expression, and furthermore on the gluconeogenesis in Korean cattle steers.

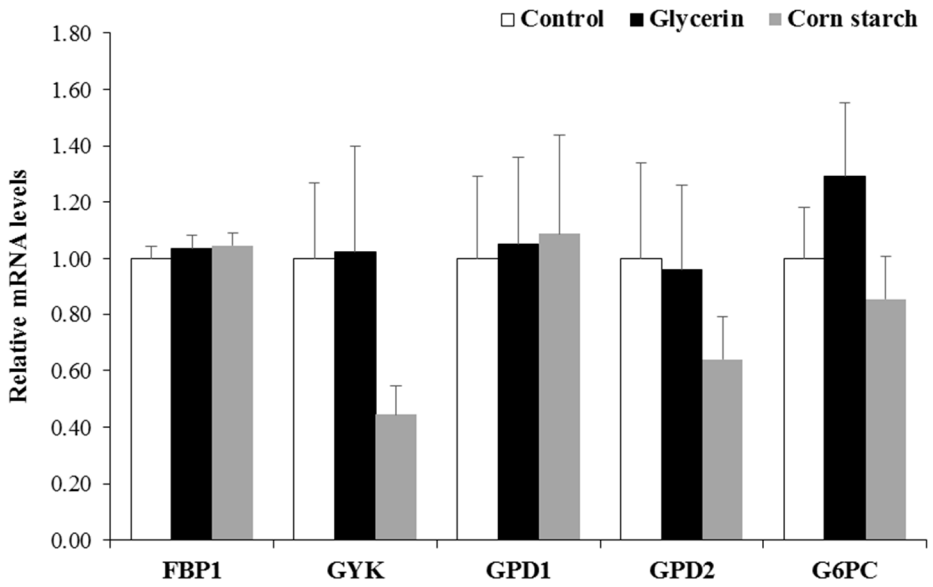

Figure 5. Hepatic mRNA levels of genes for glucogenic substrate incorporation in gluconeogenesis pathway in Korean cattle. The mRNA levels were determined by real-time PCR and normalized with the RPS9 gene. The mRNA levels of the control

group were normalized to 1.0. Values are the means±SEM (n=5). FBP1, fructose-1, 6-bisphosphatase; GYK, glycerol kinase; GPD1, glycerol-3-phosphate dehydrogenase 1; GPD2, glycerol-3-phosphate dehydrogenase 2; G6PC, glucose-6-phosphatase.

Supplementary table 1. Primer sequences (5' to 3') for quantitative real-time PCR

Gene name	GenBank ID	Primer	Sequence	Length (bp)
Fructose-1, 6-bisphosphatase (*FBP1*)	NM_001034447.2	Forward	TCACCGAGTATGTCCAGAGG	146
		Reverse	GGGGCTTTTCTTGTTAGCTG	
Glycerol kinase (*GYK*)	NM_001075236.1	Forward	GCTTCGTTGGCTCCTTGACA	131
		Reverse	TACAATGGACCCCTCCACTG	
Glycerol-3-phosphate dehydrogenase 1 (*GPD1*)	NM_001035354.1	Forward	CACCCAATTTCCGCATCACG	126
		Reverse	CCTTGGTGTTGTCGCCAAAG	
Glycerol-3-phosphate dehydrogenase 2 (*GPD2*)	NM_001100296.1	Forward	TTCCTCGTGCGAGAGGATCA	106
		Reverse	AGGCCTAGAACAGTGGCAAG	
Glucose-6-phosphatase (*G6PC*)	NM_001076124.2	Forward	AGCTGTGGGCATCAAACTCC	116
		Reverse	AGTAGTCGGTATCCAAAACC	
Ribosomal protein S9 (*RPS9*)	NM_001101152	Forward	CCTCGACCAAGAGCTGAAGCCTC	64
		Reverse	CAGACCTCACGTTTGTTC	

PCR, polymerase chain reaction.

5. Conclusion

In general, dietary glycerin supplementation at 6.4% of DM did not lead to detrimental effect on feed intake in Korean cattle steers. Both glycerin and corn starch supplementation did not improve average daily gain and feed efficiency in Korean cattle steers. Glycerin supplementation also did not affect rumen fermentation characteristics, carcass characteristics, IMF content, reducing sugar content, glycogen content in both liver and muscle, and sensory traits of Korean cattle steers. Both glycerin and corn starch supplementation did not affect serum glucose concentration at initial and 8^{th} week, but glycerin supplementation slightly increased the average serum glucose concentration at 16^{th} week. Although glycerin supplementation had no impact on carcass and meat quality, glycerin could be potentially considered as a good energy source to maintain the animal's metabolism in finishing Korean cattle.

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CHAPTER SIX

General conclusion

It is well known that beef marbling score and quality grade play an important role in meat sensory characteristics, including tenderness, juiciness, flavor, and overall palatability. It has been thought that the palatability of Korean cattle beef is good due to the high QG and thus high MS. But limited information is available and variability exists in data on the associations among MS/QG, meat characteristics, and sensory traits in Korean cattle beef. In study 1, we compared carcass traits, sensory characteristics, and free amino acid content, fatty acid profile and volatile compounds among QGs in loin and rump of Korean cattle steer. We also evaluated the associations among QGs and various carcass characteristics and sensory traits. This study confirms that marbling score (MS) and intramuscular fat (IMF) content are major positive determinants of QG in Korean cattle beef. Numeric values of tenderness, juiciness, and overall acceptability in loin tended to be highest in QG1++, and those of juiciness and overall acceptability tended to be lowest in QG 2. Juiciness and overall acceptability were strongly correlated with QG. Our results demonstrated that QGs are linked to sensory traits. However, the nucleotide contents including inosine monophosphate (IMP) may not be major factors determining meat palatability of Korean cattle beef in this study. Glutamic acid and proline were significantly associated with tenderness, juiciness, and overall

acceptability, although they did not differ significantly among QGs. In addition, beef QGs affected the compositions and contents of FAs and volatile compounds in loin and rump. Loin FA percentages, especially those of oleic acid (C18:1n9) and monounsaturated FA (MUFA), generally increased with increasing QGs. Some volatile compounds in loin and rump varied with QGs and were positively or negatively correlated with flavor.

Korean consumers have historically preferred Korean cattle beef to domestic Holstein and imported Angus beef because they believe that the palatability of Korean cattle beef is superior to those of other breeds. But limited information is available on the factors that affect the preference of Korean consumers for Korean cattle beef over other breeds. In study 2, we compared the physico-chemical characteristics, reducing sugar content, sensory traits, and fatty acid and volatile compound profiles of the *longissimus thoracis* among Korean cattle, Holstein, and imported Angus breeds, and also identified correlations among these parameters. This study confirms that Korean cattle *longissimus thoracis* contained higher IMF and reducing sugar contents, and better sensory traits compared to *longissimus thoracis* of Holstein and Angus. The IMF and reducing sugar contents showed positive correlation with sensory traits, suggesting that these factors may positively affect beef flavor. Palmitic acid (C16:0), oleic acid (C18:1n9), and monounsaturated FA (MUFA) may positively affect sensory traits, whereas linoleic acid (C18:2), and polyunsaturated FA (PUFA) may negatively affect sensory traits. The percentages

of different volatile compounds in the *longissimus thoracis* also varied among the three breeds. Korean cattle *longissimus thoracis* had the highest percentage of acetaldehyde, 3-methyl butanal, and 3-hydroxy-2-butanone, and these compounds showed positive correlation with flavor. Variations in IMF, reducing sugar content, and FA and volatile compound profiles may contribute to differences in the sensory characteristics of the *longissimus thoracis* among breeds. The results of this study enhance our understanding of the association of reducing sugar and volatile compound contents with the sensory traits of beef. This information may help in determining beef palatability.

Glycerol, a by-product with biodiesel, also known as glycerin, serves as gluconeogenic substrate in the liver and kidney. It is well known that glycerol can be absorbed by the ruminal epithelium and then converted to glucose or converted to propionate in the rumen. Also, glycerol has an energy value similar to corn on a pound-for-pound basis in dairy and feedlot cattle. Although many studies were conducted for the effect of glycerin on carcass composition and characteristics in various animal diets, the outcomes were not consistent. Also, limited information is available for effects of glycerin inclusion in diets on meat characteristics in Korean cattle. In study 3, we evaluated the effect of dietary glycerin replacement on growth performance, blood metabolites, ruminal fermentation characteristics, carcass characteristics and sensory traits, hepatic gluconeogenic gene expression, and muscle glycogen contents in Korean cattle steers. This study confirms that glycerin

replacement in the finishing diet of Korean cattle steer had no impact on weight gain, average daily gain, and feed efficiency except for increase in average daily concentrate intake. The increased intake may be attributed to the sweet taste of glycerin's property. Glycerin replacement did not affect carcass characteristics, chemical and physico-chemical composition, reducing sugar, glycogen, collagen, nucleotides, fatty acid, volatile compounds, and sensory traits in the *longissimus thoracis*. These results indicate that the glycerin inclusion level (3%) may be not enough to improve animal performance and carcass characteristics. In addition, feeding concentrate containing 3% of glycerin did not result in detrimental effects on growth performance, ruminal fermentation, animals' physical condition, and metabolism. This is important not only on animal performance and carcass characteristics but also for sustainable and economic aspects because glycerin is a biodiesel residue and it can potentially partially replace some expensive ingredients such as corn, molasses, distiller's dried grains with solubles as an energy source for beef cattle. Finally, in study 4, we evaluated the effect of dietary glycerin supplementation on growth performance, blood metabolites, ruminal fermentation characteristics, carcass characteristics and sensory traits, glycogen content in the liver and muscle, and hepatic gluconeogenesis gene expression in Korean cattle steers finished in feedlot. We confirms that dietary glycerin supplementation at 6.4% of DM did not lead to detrimental effect on feed intake in Korean cattle steers. Both glycerin and corn starch supplementation did not improve average daily gain and feed efficiency in Korean cattle steers. Glycerin supplementation also did not affect

rumen fermentation characteristics, carcass characteristics, IMF content, reducing sugar content, glycogen content in both liver and muscle, and sensory traits of Korean cattle steers. Both glycerin and corn starch supplementation did not affect serum glucose concentration at initial and 8th week, but glycerin supplementation slightly increased the average serum glucose concentration at 16th week. Although glycerin supplementation had no impact on carcass and meat quality, glycerin could be potentially considered as a good energy source to maintain the animal's metabolism in finishing Korean cattle.

Summary in Korean

소고기 마블링 점수와 육질 등급은 연도, 다즙성, 풍미와 종합적 기호도 등의 관능 특성에 긍정적인 영향을 주는 것으로 알려져 있다. 한우육은 높은 마블링 함량과 육질 등급을 가지며, 기호성이 좋은 고기로 잘 알려져 있다. 하지만 한우육의 마블링 점수/육질 등급, 도체 특성 및 관능 특성 사이의 상관관계에 대한 자료가 미비하고, 일부 진행된 연구 결과에서는 결과값의 변이가 컸다. 국내 소비자를 대상으로 소고기 선호도를 조사한 결과 국내산 홀스타인육과 수입산 소고기에 비해 한우를 더 선호한다는 결과가 나타났다. 그 이유는 한우육의 기호성이 기타 품종 소고기에 비해 훨씬 뛰어나다는 것이었다. 하지만 한우육을 선호하는 현상에 영향을 주는 요인에 대한 규명은 비교적 제한된 상황이다. 따라서 연구 1에서는 한우육의 마블링 점수/육질 등급, 도체 특성 및 관능 특성 간의 상관관계를 알아보았고, 연구 2에서는 품종별 소고기 등심의 관능 특성의 차이에 영향을 주는 요인에 대해 알아보았다.

바이오디젤유의 생산과정에서 나오는 글리세린은 간과 신장에서 포도당 신생합성의 기질로써 작용하며, 옥수수와 비슷한 에너지 값을 가지고 있는 것으로 알려져 있다. 글리세린이 도체 특성에 주는 영향에 관한 연구들이 이미 다양한 동물에서 진행되어 왔지만 그 결과들이 서로 일치하지

않는 보고들이 있다. 또한 글리세린 첨가가 거세한우의 도체 특성에 미치는 영향에 관한 연구가 비교적 제한되어 있다. 따라서 연구 3에서는 글리세린의 급여 효과를 알아보고자 비육말기 한우거세우의 마무리 사료 내 일부 원료를 글리세린으로 대체하고 그룹간 사료의 TDN 함량을 비슷하게 맞추었을 때, 거세한우의 생산성, 도체 특성 및 관능 특성의 변화를 관찰하였고, 연구 4에서는 비육말기 한우거세우의 마무리 사료에 글리세린을 추가로 첨가하고 그룹간 사료의 TDN 함량을 달리 하였을 때 거세한우의 생산성, 도체 특성의 변화를 관찰하였다.

1. 한우 고기 등급별 및 부위별 도체 및 관능 특성, 지방산과 휘발성 물질 비교 분석

본 연구는 한우 거세우 고기의 등급별 및 부위별 도체 특성, 관능 특성, 이화학적 특성, 핵산 관련 물질, 콜라겐 함량, 아미노산 함량, 지방산 함량 및 휘발성 물질 함량을 비교, 분석하고 그들 간의 상관관계를 알아보기 위하여 수행하였다. 실험 결과 마블링 점수와 근내지방 함량은 한우 육질 등급의 결정요인으로 확인되었다. 등심 내 맛의 관능 검사는 1++등급에서 연도, 다즙성 및 종합적 기호도가 가장 높은 경향을 보였고 2등급에서 다즙성과 종합적 기호도가 가장 낮은 경향을 보였다. 다즙성과 종합적 기호도는 육질 등급과 정의 상관을 보였다. 따라서 육질 등급이 관능

특성과 밀접한 관계가 있는 것으로 확인되었다. 본 실험에서 이노신산(IMP)을 비롯한 핵산 관련 물질들이 소고기의 기호성에 영향을 주지 않는 것으로 확인되었다. 글루타민산과 프롤린 함량은 등급 간에 차이가 없지만 연도, 다즙성 및 종합적 기호도와 밀접한 관계가 있는 것으로 확인되었다. 지방산 함량과 휘발성 물질 함량은 육질 등급에 따라 달랐고, 올레인산(C18:1n9)과 단일불포화지방산(MUFA) 함량은 육질등급이 높아짐에 따라 증가하였다. 일부 휘발성 물질 함량은 등급과 부위에 따라 달랐고, 풍미와 정 또는 부의 상관을 보였다.

2. 소 품종별 등심 환원당 함량, 관능 특성, 지방산 및 휘발성 물질 함량 비교 분석

본 연구는 한우고기와 홀스타인 육우 및 앵거스 수입육간의 근내지방 함량, 환원당 함량, 관능 특성, 지방산 함량 및 휘발성 물질 함량을 비교, 분석하고 한우고기의 우수한 맛에 영향을 미치는 요인을 발굴하기 위하여 수행하였다. 실험 결과 근내지방 함량, 환원당 함량 및 관능 특성(풍미, 연도, 다즙성, 종합적기호도)은 한우에서 가장 높았다. 근내지방 함량과 환원당 함량은 관능 특성과 정의 상관을 보였다. 따라서 이러한 물질들이 소고기 풍미에 긍정적인 영향을 주는 요인으로 평가되었다. 팔미트산(C16:0), 올레인산(C18:1n9) 및 단일불포화지방산(MUFA) 함량은 관능 특

성과 정의 상관을 보였다. 반면 리놀레인산(C18:2)과 다가불포화지방산(PUFA) 함량은 관능 특성과 부의 상관을 보였다. 소고기 품종에 따라 휘발성 물질 함량도 달랐다. Acetaldehyde, 3-methyl butanal 및 3-hydroxy-2-butanone 함량은 모두 한우에서 가장 높았고, 이런 물질들은 소고기 풍미와 정의 상관을 보였다. 종합적으로, 높은 근내지방 함량, 환원당 함량, 지방산 함량(C16:0, C18:1n9, MUFA), 및 휘발성 물질 함량(acetaldehyde, 3-methyl butanal, 3-hydroxy-2-butanone)이 한우의 풍미를 다른 품종들과 차별화하는 요인들로 평가되었다. 이와 같이 환원당 함량, 휘발성 물질 함량 및 관능 특성 간의 상관관계는 본 연구에서 처음으로 확인되었고, 여기서 밝혀낸 결과들은 한우고기의 우수성을 과학적으로 입증할 수 있는 중요한 자료로 활용할 수 있을 것으로 판단된다.

3. 사료 내 글리세린 대체 첨가가 거세한우의 생산성, 반추위 발효 특성 및 도체 특성에 미치는 영향

본 연구는 비육말기 한우 마무리사료 내 일부 원료(당밀, DDGS 등)를 3% 글리세린으로 대체하였을 때, 한우의 생산성, 혈액 성상, 반추위 발효 특성, 도체 및 관능 특성, 간 조직 내 포도당 신생합성 관련 유전자 발현량 및 근육 내 글리코겐 함량의 변화를 조사하였다. 글리세린 대체 사료는

사료섭취량을 증가시켰지만 일당 증체량과 사료효율에는 영향을 주지 않았다. 사료섭취량의 증가 원인은 글리세린의 단맛을 내는 특성에 기인한 것으로 보인다. 또한 글리세린 처리 사료는 도체 특성, 일반 성분 및 이화학적 물질 함량, 환원당 함량, 글리코겐 함량, 콜라겐 함량, 핵산 관련 물질, 관능특성, 지방산 및 휘발성 물질 함량에 영향을 주지 않았다. 이러한 결과를 통하여, 본 연구에서 사용한 글리세린의 대체비율이 동물의 생산성과 도체 특성을 변화시킬 수 있는 요건을 충족시키기에 충분하지 않은 것으로 추정된다. 한편 사료 내 글리세린 3% 대체 첨가가 거세한우의 생산성, 반추위 내 발효, 동물의 건강 상태 및 대사에 해로운 영향을 주지 않았다. 또한 사료 원료 사용상의 지속가능성과 경제적인 측면에서 볼 때 비육말기 한우사료 내 글리세린 부산물의 이용은 생산비를 줄이는 데 도움이 될 것으로 사료된다.

4. 사료 내 글리세린 첨가가 거세한우의 생산성, 혈액 성상, 반추위 발효 특성 및 도체 특성에 미치는 영향

본 연구에서는 비육말기 거세한우 마무리사료에 글리세린을 첨가 급여하였을 때 한우의 생산성, 혈액 성상, 반추위 내 발효 특성, 도체 및 관능특성, 간과 등심 내 글리코겐 함량 및 간 조직 내 포도당 신생합성 관련

유전자 발현량에 미치는 영향을 조사하였다. 하루 농후사료급여량의 6.4%에 해당하는 글리세린 첨가제는 사료섭취량에 해로운 영향을 주지 않았다. 또한 글리세린 및 옥분 첨가 (글리세린과 동량의 에너지 첨가)는 일당 증체량, 사료효율, 반추위 내 발효 특성, 도체 및 관능 특성, 근내지방 함량, 환원당 함량 및 간과 등심 내 글리코겐 함량에 영향을 주지 않았다. 글리세린 및 옥분 첨가는 실험 초기와 실험 8주에 혈청 glucose 농도에 영향을 주지 않았지만, 실험 16주에는 글리세린 첨가구에서 상승하였다. 글리세린 첨가는 거세한우의 도체 특성과 육질 향상에 영향을 주지 않았지만 사료 자원의 측면에서 보았을 때 지속 가능한 부산물로 이용할 수 있을 것으로 기대된다.