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Effects of dietary beet pulp on growth, rumen fermentation profiles, carcass traits, intramuscular adiposity, and lipogenic gene expression in Hanwoo steers

비육후기 한우 거세우 사료내 비트펄프 대체급여가 한우의 성장, 반추위 발효, 도체성적, 근내지방 축적 및 지방합성 유전자 발현에 미치는 영향에 대한 연구

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Abstract

In this study, we investigated the effects of partial replacement of cornflake in the late fattening stage diet with beet pulp pellet on the growth performance, ruminal volatile fatty acid profiles, blood characteristics, lipogenic gene expression, adipocyte cellularity, carcass traits, and economic efficiency of Korean cattle steers. Eighteen Korean cattle steers (body weight: 636 ± 10.9 kg; age: 25.9 ± 0.25 months) were divided into two groups: the corn flake (CF) group and the beet pulp (BP) group, considering body weight, age, and ultrasonically predicted intramuscular fat. Approximately 89% of the dry matter requirement was offered as a concentrate portion, while the remaining 11% was provided as tall fescue hay. The CF and BP groups received 78% and 72% of the pelleted basal concentrate, respectively, with the remaining 22% and 28% supplemented with corn flake or beet pulp, respectively. The dietary crude protein and energy levels were similar in both groups. The experiment was conducted for a duration of 25 weeks, including a 5−week adaptation period. The feeding of beet pulp did not affect average daily gain and feed efficiency ($P \geq 0.79$). However, the BP group exhibited a higher proportion of ruminal acetate ($P < 0.001$) and a lower proportion of ruminal propionate ($P < 0.001$) compared to the CF group. Additionally, the BP group tended to have higher beef yield grade ($P = 0.10$), quality grade ($P = 0.10$), and beef price per kg ($P < 0.001$) than the CF group. Furthermore, the intramuscular adipocyte size of longissimus thoracis (LT), determined through image analysis of histological sections, was larger ($P < 0.001$) in the BP group than in the CF group. The mRNA levels of fatty acid synthase, determined by qPCR, were higher ($P = 0.03$) in the BP

group compared to the CF group. Conversely, the CF group exhibited higher levels of lipolysis mRNA ($P < 0.08$) compared to the BP group. The increased expression of lipogenic genes and decreased expression of lipolytic genes may contribute, at least in part, to the observed increase in adipocyte size of the LT due to beet pulp feeding. In conclusion, beet pulp can serve as a lipogenic energy source to enhance beef quality grade without adversely affecting the growth performance of cattle.

Keywords: Beet pulp, Corn flake, rumen fermentation, Intramuscular fat, Adipocyte, Korean cattle steer

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List of abbreviations

ACC : Acetyl CoA carboxylase

ADF : Acid detergent fiber

ADG : Average daily gain

AOAC : Association of Official Agricultural Chemists

ATP : Adenosine triphosphate

BP : Beet pulp

BUN : Blood urea nitrogen

BW : Body weight

CD36 : Cluster of differentiation 36

CF : Corn flake

CF : Crude fiber

CIA : Computer image analysis

CP : Crude protein

DE : Digestible energy

DM : Dry matter

EDTA : Ethylenediaminetetraacetic acid

EE : Ether extract

FABP4 : Fatty acid binding protein 4

FAO : Food and agriculture organization of the united nations

FASN : Fatty acid synthase

GHG : Greenhouse gas

GOT : Glutamic-oxaloacetic transaminase

GPT : Glutamic-pyruvic transaminase

IGF-1 : Insulin like growth factor-1

IMF : Intramuscular fat

LPL : Lipoprotein lipase

LT : Longissimus thoracis

ME : Metabolizable energy

MP : Marbling particle

NDF : Neutral detergent fiber

NE : Net energy

NEFA : Non-esterified fatty acids

NFC : Non fiber carbohydrates

NFE : Nitrogen free extract

PCR : Polymerase chain reaction

PPARG : Peroxisome proliferator activated receptor gamma

SNU-IACUC : Seoul National University Institutional Animal Care and Use Committee

TDN : Total digestible nutrient

TG : Triglyceride

VFA : Volatile fatty acid

WBSF : Warner-Bratzler shear force

WHC : Water holding capacity

Units and marks

% : Percent

$: dollar

d : Day

ADG : Average daily gain

dL : Deciliter

g : Gram

h : Hour

mm : Millimeter

mg : Milligram

ml : Milliliter

mM : Millimole

U : Unit

ng : Nanogram

μg : Microgram

μEq : Microequivalents

μm : Micrometer

N : Neuton

Ⅰ. Introduction

Under the current feed stuff market condition and livestock industry, the feed stuff that can reduce nutritional competition with human and enhance productivity is demanded. In Korean market, the intramuscular fat (IMF) deposition and marbling most critically affect flavor, palatability and consumer's demand of beef (Baik et al., 2023).

In general, feeding high level of grain-based diets is usual strategy to increase marbling content of beef (Pethick et al., 2004). However, consequences feeding high level of starch content diets such as corn grain have risk which cause decrease of ruminal pH and rumen acidosis (Enemark et al., 2002). Various of carbon precursors including acetate, glucose, lactate and etc. are converted into cytosolic acetyl-CoA to generate lipogenesis by adipose tissue (Vernon, 1981). Hocquette et al. (2010) reported that glucose is more preferred for IMF synthesis precursor compared with acetate. However, acetate can be converted into main substrate for lipogenesis in several adipose depots by acquired management (Nayananjalie et al., 2015). However, the evidence is insufficient to investigate preference between glucose and acetate for IMF lipogenesis.

Beet pulp is a byproduct obtained after extracting sugar from sugar beet, and used primarily as an animal feedstuff. The use of beet pulp as animal feed can avoid direct competition in nutritional system between human and livestock. Beet pulp highly contains digestible fiber sources including neutral detergent

fiber (NDF) and pectin, which are suitable to ruminants as it stabilizes rumen condition and stimulates acetate fermentation (Habeeb et al., 2017). In addition, beet pulp can be considered as alternative energy source with approximately 85% energy value of corn (Jeong et al., 2022). Furthermore, utilization of beet pulp in cattle diets decreases risk of rumen acidosis and digestive disorders derived from excessive starch intake and decline of ruminal pH (Flachowsky et al., 1993). Abo Zeid et al. (2017) verified that replacing corn grain with beet pulp up to 100% in growing buffalo calves' diet could reduce feed cost and obtain more economic benefits. Several studies were reported about feeding beet pulp as substitute of grain in ruminant diet. However, only few studies have been conducted to investigate effects of replacement of corn grain with beet pulp on economic efficiency and meat quality such as IMF deposition mechanisms and cellularity adipocyte growth in Korean cattle steer. Thus, the objectives of this study were to evaluate the effects of replacement corn flake with beet pulp pellet on growth performance, rumen fermentation, blood parameters, gene expression, cellularity adipocyte growth, marbling deposition, meat quality and economic efficiency in late fattening Korean cattle steers.

We hypothesized that partial replacement of corn flake with beet pulp as an energy source in fattening Korean cattle steer diets may enhance ruminal fermentation condition and acidosis stress. Moreover, it may increase ruminal acetate fermentation, lipogenic gene expression, IMF deposition and economic revenue without adverse effects of growth performance and productivity.

Ⅱ. Literature review

1. Beet pulp

Beet pulp as alternative feed stuff

Sugar beet is a plant which root contains sugar such as sucrose and scientific name is *Beta vulgaris*. It is grown generally for sugar production. In 2019 Russia, France, Germany, United states and Turkiye were the world's five largest sugar beet producing country by metric ton (FAO, 2019). Beet pulp is by-product after technological processing by sugar extraction from sugar beet. The root of sugar beet contains 75% water, 20% of sugar and 5% pulp. Sugar content of root has variation between 12 and 21% depending on the growing conditions and cultivar. One ton of sugar beet can produce about 0.15t sugar and 0.05t by-product. beet pulp is generally used as a final feedstuff of animal feed (Habeeb et al., 2017).

There are many countries that now in serious shortage of energy source for ruminant feed such as Egypt. Therefore, the alternative feed stuff that avoid direct competition of nutrition system between human and animal is demanded. The livestock feed industry largely depends on the grain like corn. Moreover, animal feed industry of many countries is depending on imported grain from some mass-producing countries. However, the use of cereal grains in ruminant diets is considered as direct interference for human nutritional systems (Abo

Zeid et al., 2017). On the other hands, Münnich et al. (2017) reported that feeding industrial by-product is a reasonable option to improve stability of livestock productivity by reducing the dietary proportion of products that human can consume. Thus, beet pulp can be considered for substitute of grain-based feedstuff.

Beet pulp contains about 25% of pectic materials and the chemical composition can vary depending on cultivation year and farming area (Taylor, 2012). Beet pulp usually processed form of pellet to transport and storage more easily. In addition, the molasses can be obtained by refining process of sugar beet. It can be added to improve palatability in diet. Furthermore, beet pulp is insoluble in water and rich for fibber contents such as cellulose, hemicellulose and pectin that can be used carbohydrate source for ruminants. Beet pulp fiber is readily digestible and palatable and pelleted beet pulp is less dusty than hay. For these reasons, beet pulp is considered popular feed for ruminants (Mustafa et al., 2009). However, when the beet pulp is used as final feed stuff, the price of beet pulp or other grains must be considered. Sugar beet pulp has a potential which improve the meat quality of ruminant such as beef cattle (Jeong et al., 2022).

On the other hands, depending on the country or region, the price of feed may be expensive or cheaper when beet pulp was supplemented in ruminant diet. Korea's beet pulp imports are 18.96 million dollars, ranking 10th in the world in 2021 and account for 2.49% of share in import value in world (WITS, 2021). The amount of beet pulp imports has been steadily declining since 2015.

Egypt, Ukraine, Canada, Turkiye and United states are five largest importing country and Egypt account for 68.6% of share in import value in Korea (WITS, 2021). Therefore, the feed price when supplement the beet pulp in ruminant diet fluctuates greatly with Egyptian export prices. The animal feed industry is largely variable depending on many of situation. Thus, if the beet pulp is used as feed stuff of ruminant, it is necessary to consider the break-even point carefully considering the fluctuating feed price and the increasing price of beef. Consequently, optimal beet pulp addition conditions and feeding strategies are required to utilize beet pulp as alternative feed stuff. When the break-even point is calculated, feed stuff price, animal' s feed intake, revenue from animal products should be considered.

Some of studies were conducted to evaluate the beet pulp as ruminant feed stuff in terms of growth performance. In general, the research suggests that beet pulp can be an effective feed ingredient for beef cattle, with little to no negative effects on growth performance, nutrient digestibility, or carcass characteristics. According to Abo Zeid et al. (2017), there were no any adverse effects when beet pulp was provided up to 100% in Egyptian buffalo claves diet. Similarly, the growth performance including body weight, average daily gain and dry matter intake did not different among Belgian Blue, Limousin and Aberdeen Angus when beet pulp was fed 50% in diet (Cuvelier et al., 2006). These studies suggest that beet pulp can be a viable feed stuff for beef cattle, especially when corn prices are high or when there is a surplus of beet pulp available. Furthermore, beet pulp has a high fiber content, which can improve rumen health

and promote better digestion and nutrient absorption. However, it is important to note that the optimal level of beet pulp in beef cattle diets may depend on factors such as animal age, weight, and breed, as well as the nutrient composition of the other feed ingredients in the diet.

In conclusion, the available research suggests that beet pulp can be an effective and beneficial feed option for beef cattle, as it can improve carcass weight and dressing percentage without compromising growth performance or rumen health. Further research is needed to determine the optimal inclusion rate of beet pulp in beef cattle diets and to investigate its effects on other production parameters, such as meat quality and shelf life.

Beet pulp as source for improving beef quality

As described above, sugar beet pulp can be used as alternative feed stuff that provide carbohydrate precursors and contains considerable amount of pectic materials. The chemical composition of beet pulp can be changed by growing conditions, cultivar and farming area (Taylor, 2012). In general, beet pulp contains rich fiber contents such as digestible NDF, pectin etc. according to Mustafa et al. (2009), sugar beet pulp contains 9.1% of CP, 31% of ADF, 0.72% of calcium, and of 0.20% phosphorus. In addition, Abo et al. (2017) reported beet pulp contains rich digestible NDF about 40% and it mainly consists of pectic substances that readily fermentable in rumen. The dried sugar beet pulp contains approximately 10% of moisture and it has sufficient energy in a

palatable form. Furthermore, beet pulp can be considered as alternative energy source with approximately 85% energy value of corn (Jeong et al., 2022). The nutritional information of sugar beet pulp and other feed stuffs summarized in Table 1 (Habeeb et al., 2017). According to the table, beet pulp has approximately 87.8% of energy value of corn. And also, beet pulp has low starch and fat contents compared with corn. On the other hands, protein and fiber contents in beet pulp are higher than corn. Furthermore, beet pulp contains relatively high level of calcium content.

Table 1 Nutritional analysis of whole sugar beets, sugar beet pulp, corn silage, corn and cob meal and shelled corn on dry matter basis %.

Items	DM	CP	TDN	Ca	P
Whole Sugar Beets	20	6.8	81	0.24	0.24
Sugar Beet Pulp	89	9.1	69	0.85	0.07
Corn grain	86	8.4	85	0.02	0.23
Corn and Cob Meal	87	9.0	82	0.10	0.24
Corn Silage	35	8.0	69	0.30	0.20
Shelled Corn	87	9.5	88	0.01	0.30

Cited by Habeeb et al., 2017 and Jeong et al., 2022

When sugar beet pulp is used as feed stuff in ruminant feed, ruminating and chewing were stimulated and ruminal fermentation is

promoted (Mojtahedi and Mesgaran, 2011). Beet pulp also has great digestibility in ruminant. In Egyptian buffalo calves, replacing of dietary maize grains with increasing levels of sugar beet pulp (0, 33.3, 66.7 and 100%) increased ruminal apparent digestibility (Abo et al., 2017). The increased digestibility from feeding increasing levels of sugar beet pulp can be explained by microbial growth and cellulolytic and hemicellulolytic bacteria and fungi activity (Mehrez et al., 2008). Moreover, as mentioned above, since beet pulp contains digestible NDF, pectin, utilization of beet pulp in cattle diets decreases risk of rumen acidosis and digestive disorders derived from excessive starch intake and decline of ruminal pH (Flachowsky et al., 1993). Likewise, replacing beet pulp up to 24% did not affect ruminal pH in dairy cow (Petri et al., 2019). These factors of beet pulp can change rumen fermentation condition.

Moreover, beet pulp makes ruminal liquid dilution lower, and liquid escaped slower than diet contained more corn (Voelker and Allen 2003). As a result, the produced VFA was better absorbed into the rumen wall. It can improve digestibility of beet pulp and cause enhanced feed efficiency. Feeding beet pulp can affect comprehensive ruminal VFA fermentation. It is still unclear which kind of VFA affects which specific location of fat depot depending on preference for hyperplasia or hypertrophy. Thus, beet pulp has a potential to verify preference of VFAs for intramuscular fat in Korean cattle steer. Since rich fibber contents of beet pulp, acetate is more fermented than propionate. In

addition, total VFA fermentation can increase by feeding beet pulp because of high digestibility (Münnich et al., 2018). Therefore, replacement of beet pulp can affect significantly on C2:C3 ratio. In agreement with these, feeding up to 100% of beet pulp stimulated1 acetate fermentation while propionate fermentation was inhibited in Egyptian buffalo calves (Abo Zeid et al., 2017). In addition, Voelker and Allen (2003) reported that substitution of pelleted beet pulp up to 24% was increased proportion of acetate and decreased propionate fermentation in rumen. The soluble NDF such as pectin generate mainly acetate meanwhile propionate is decreased as NDF fermented (Alamouti et al., 2014). And also, Mansfield et al (1994) determine the nutrition intake and production capacity in dairy cattle which divided two groups fed with containing 15% corn and 15% beet pulp or 30% corn. The experimenters conducted culture for 10 days to evaluate fermentation and digestibility. As results, there no any effects on the degradability of DM, organic matter, non-structural carbohydrates, NDF, and ADF. The concentration of acetate was higher in the BP diet group meanwhile the concentrations of butyrate and branched-chain VFA were decreased in high-corn diet. However, propionate concentrations and total VFA fermentation were not different between two groups. However, there are very few papers and research about the effects of beet pulp when replaced corn grain on growth performance, rumen fermentation and especially carcass characteristics and marbling fineness of beef cattle *in vivo.*

Grain feeds as energy source in ruminants

Grains are an important feedstuff for ruminant animals, including beef cattle. They provide essential energy, protein, and other nutrients required for growth, production, and maintenance of the animals. Grains commonly used in ruminant diets include corn (maize), barley, wheat, oats, sorghum, and triticale. These grains are rich in carbohydrates and provide readily available energy for cattle (Owens et al., 1997). Grains are primarily used as a source of energy for ruminants. They contain starches, sugars, and fibers that can be digested by the rumen microbes. The energy content of grains is typically measured in terms of Total Digestible Nutrients (TDN) or Net Energy (NE) values. These values help in formulating balanced diets for cattle. In general, feeding a lot of amount of grain-based diets is usual strategy to increase IMF content of beef cattle (Pethick et al., 2004). However, continued feeding high level of starch content diets such as corn flake have risk which cause decrease of ruminal pH and rumen acidosis (Enemark et al., 2002). Moreover, excessive starch and sugar contents in grains can affect adversely to fiber digestibility, which may in turn decrease intake (Hoover, 1986). Grains have a lower fiber content compared to forages. Fiber is crucial for rumen health and proper digestion in ruminant animals. Although grains are relatively low in fiber, they provide readily fermentable carbohydrates that stimulate the growth of fiber-digesting

microbes in the rumen. Thus, the proper amount of forage has to be contained in ruminant diet.

2. Intramuscular fat deposition

Adipocyte growth

The marbling namely intramuscular fat is most important factor deciding beef quality (Jeong et al., 2022). Especially, in Korea and Japan beef industry, IMF content is considered more important than other countries (Wheeler et al., 1994). To compete with imported beef that has a relatively low marbling content and cheap price, producing highly marbled beef is the essential countermeasure. There are a lot of factors affecting beef quality, the intramuscular fat deposition has to be preceded than other factors. IMF deposition influences sensory quality and palatability like tenderness, flavour and juiciness (Bottema et al., 2020). Adipocyte deposition, or the accumulation of fat cells, is an important process in the development of beef cattle. Adipose tissue plays a critical role in energy metabolism and body composition, and it affects both the quality and quantity of meat produced by the animal. Most of intramuscular fat is deposited between muscle fibre bundles in the perimysial connective tissue in beef cattle (Moody and Cassens, 1968). In beef cattle, adipocyte deposition occurs in two main stages: pre-natal and post-natal (Park et al., 2018). During the pre-natal stage, adipocyte differentiation and proliferation take place in the developing fetus. Adipogenesis proceeds through several stages. First, commitment of

mesenchymal stem cells to preadipocytes. Second, determination and proliferation of preadipocytes. Third, differentiation and proliferation of preadipocytes. Fourth, differentiation of preadipocytes into adipocyte (Hausman et al., 2009). This process is influenced by genetic factors, as well as maternal nutrition during gestation. In the pre-natal stage, increasing the number of adipocytes (hyperplasia) derived from adipogenic-fibrogenic progenitor cells is main process (Uezumi et al., 2011). Du et al (2013) reported that adipocyte hyperplasia in muscle occurs approximately between conception and 250 d after birth in cattle. In contrast, there was a suggestion that hyperplasia of adipocytes is occurred for whole life of beef cattle (Robelin, 1981). Thus, the number of adipocytes did not different between two groups, there was no difference in hyperplasia of adipocytes. After birth, post-natal adipocyte deposition occurs primarily through the expansion of existing adipocytes, rather than the creation of new ones. This process is influenced by several factors, including diet, age, sex, and breed. It has been common strategies that animals fed a high-energy diet for greater adipocyte deposition than those fed a low-energy diet, while older animals tend to have larger adipocytes than younger ones (Park et al., 2018). Adipocyte hypertrophy in beef cattle refers to the enlargement of fat cells within the animal's body. Hypertrophy mainly occur during fattening stage from excessive energy intake. Du et al (2010) suggested that hypertrophy is a greater contributor to IMF deposition than is hyperplasia from late growth stage of age to slaughter. On the other hand, in case of German Holstein and Charolais crossbreed bulls, hyperplasia plays more important role in the growth of adipose

tissue (Yang et al., 2006). In overall, formation of marbling derived from the interaction of hyperplasia and hypertrophy (Figure 1).

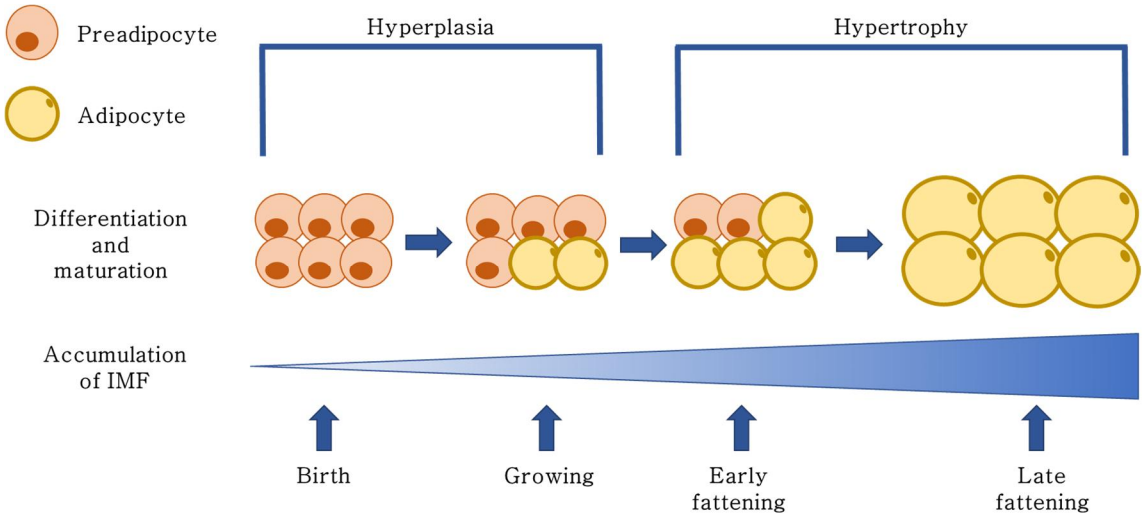

Figure 1. The timelines of adipocyte formation in beef cattle life cycle (Du et al., 2015).

Therefore, strategies to develop the hyperplasia and hypertrophy of adipocyte are most important to produce highly marbled beef. However, the research about whether hyperplasia or hypertrophy is more important on high marbled beef production is insufficient in current situation. Triglyceride synthesis is a major factor for intramuscular fat deposition (Pethick et al., 2004). NEFA and glycerol are required for triglyceride synthesis. In addition, the location of adipocyte deposition in beef cattle also plays a role in meat quality. Intramuscular adipocytes, or marbling, are highly valued in the meat industry as they contribute to tenderness, juiciness, and flavor. On the other hand, subcutaneous adipocytes can negatively affect meat quality by increasing fat content and decreasing the proportion of edible part of meat. By understanding

the mechanisms of adipocyte deposition, producers can implement strategies to produce high-quality beef that consumer demands.

Factors affecting IMF deposition

Adipocyte deposition is a complex process that affects both the quantity and quality of meat produced by beef cattle. Factors such as genetics, nutrition, age, and sex can all influence this process, as can the location of adipocytes within the animal's body.

Genetic factors are most influent factor affecting IMF deposition. Especially, depending on breed, marbling percentage can be changed greatly. Some breeds of beef cattle are more prone to fat deposition than others. Different breeds of cattle have different genetic backgrounds that can influence the development of marbling in beef. Some breeds are known for their ability to produce high levels of marbling, while others may have lower levels of intramuscular fat. The breed with the highest fat deposition in the world is Japanese black cattle (Wagyu). According to Irei et al (2011), Japanese Wagyu contains about 36.5% marbling content. Wagyu cattle have a unique ability to deposit fat within their muscle fibers, creating a distinctive marbling pattern. They also have a genetic predisposition for producing unsaturated fatty acids, which contribute to the tenderness and flavor of the meat (Motoyama et al., 2016). Korean cattle steer (Hanwoo) is the breed with the second highest marbling content in the world. Hanwoo contains average 13.7% marbling content (Jung et al., 2013). Hanwoo

is a breed of cattle that is native to South Korea and is highly valued for its meat quality, particularly for its marbling. Hanwoo has a distinct genetic makeup that sets it apart from other breeds of cattle (Jo et al., 2012). Furthermore, feeding strategies, slaughter age and management practices affect producing of high-quality beef (Lee et al., 2014). Overall, breed can have a significant impact on marbling formation in beef cattle. A combination of genetics and environmental factors are required to produce beef with high levels of marbling and excellent eating quality. Genetic factors affecting marbling formation in beef cattle are complex and involve multiple genes. One of the most important genes involved in marbling is the fatty acid binding protein 4 (FABP4) gene, which is involved in the uptake and transport of fatty acids within muscle cells (Pannier et al., 2010). Other genes involved in lipid metabolism, such as fatty acid synthase (FASN) and acetyl-CoA carboxylase (ACC), also play a role in marbling formation (Morris et al., 2007 and Zhang et al., 2010). However, it is important to note that other factors such as nutrition, management, and age also play a role in determining the quality of the meat.

Nutrition plays a crucial role in the formation of marbling in beef cattle. The amount and type of nutrients that cattle receive can impact the quality and quantity of intramuscular fat, which contributes to the flavor, tenderness, and juiciness of the meat. One of the most important nutritional factors affecting marbling formation in beef cattle is energy intake (Baik et al., 2023). Cattle require a certain amount of energy to maintain their body weight and to support muscle growth and development. If they receive an excess of energy in their

diet, it can be stored as intramuscular fat, leading to marbling (Pethick et al., 2004). However, if they do not receive enough energy, their growth and development can be stunted, leading to lower meat quality. Feeding a large amount energy including rich of starch content like corn grain to increase fat deposition is a common practice in late fattening period of beef cattle. According to Smith and Crouse (1984), intramuscular adipocytes prefer glucose as an adipogenic precursor, while subcutaneous adipocytes prefer acetate. However, more recent studies have suggested that management practices can influence the preference of different adipose tissue depots for acetate and glucose (Nayananjalie et al., 2015). Thus, promoting acetate fermentation could potentially stimulate intramuscular fat deposition by providing a substrate for lipogenesis. The synthesis of fatty acids in ruminants involves the fermentation of polysaccharides by microbes in the rumen, which produces volatile fatty acids such as acetate, propionate, and butyrate. Acetate and propionate can both contribute to fatty acid synthesis, with acetate being converted to acetyl-CoA in the cytoplasm of ruminant cells for use in synthesis, while propionate can enter the tricarboxylic acid cycle via succinyl-CoA and be utilized as a substrate for gluconeogenesis, the production of glucose (Hanson and Ballard 1967). Glucose can then generate citrate in the tricarboxylic acid cycle, which can be transported to the cytosol and degraded into oxaloacetate and acetyl-CoA by ATP-citrate lyase, with the resulting acetyl-CoA used for fatty acid synthesis (Smith and Prior, 1981). The summary of synthesis pathway of fatty acids in ruminants is presented in Figure 2.

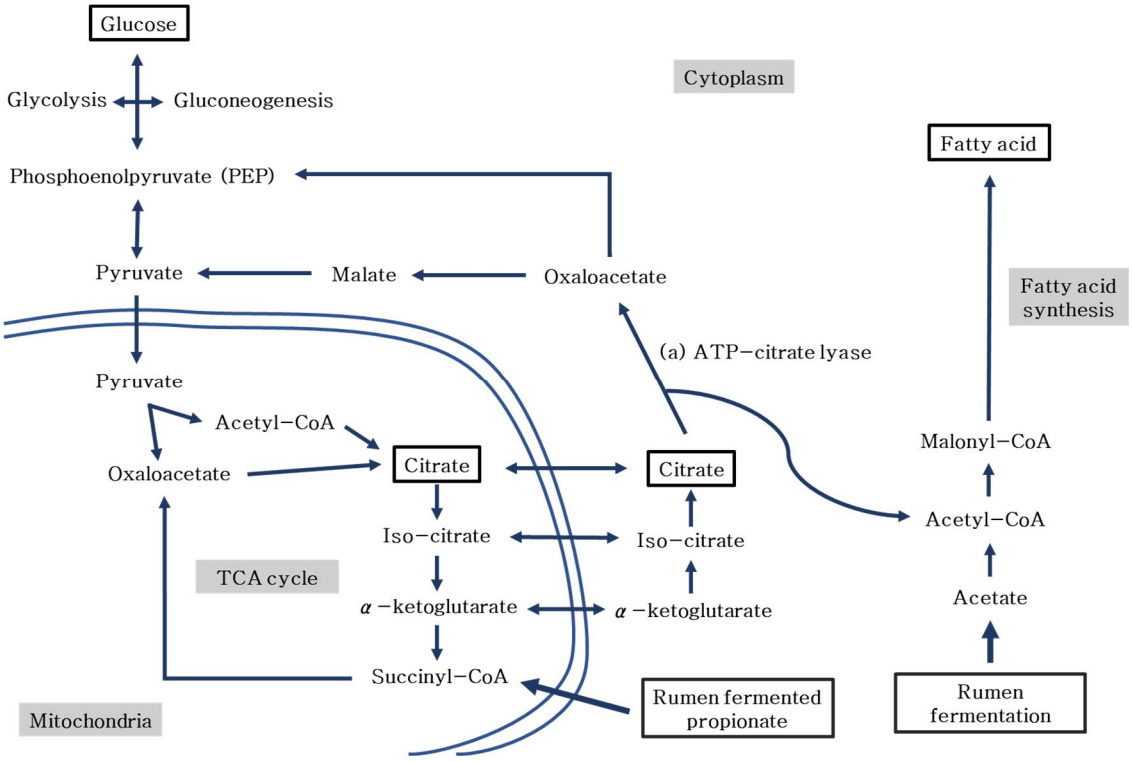

Figure 2. The pathway of fatty acid (FA) synthesis in ruminants. (Modified from Bauman et al., 1973).

Protein is also essential for muscle growth and development in beef cattle. Adequate protein intake is necessary to support the formation of muscle fibers, but excessive protein can lead to reduced intramuscular fat deposition. In addition, cattle require certain vitamins and minerals to support growth and development. Adequate levels of vitamins A, D, and E, as well as minerals such as calcium, phosphorus, and selenium, are necessary to promote marbling formation and overall meat quality (Park et al., 2018).

Finally, many management factors, such as slaughter age, weaning age, castration, and environmental factors, can affect the IMF deposition in beef

cattle. Slaughter age is critical factors that affect IMF deposition in beef cattle. It has been reported that IMF deposition in beef cattle increases with age and reaches its peak at around 24–30 months of age (Mule et al., 2013 and Shackelford et al., 1995). Therefore, delaying slaughter beyond this age can lead to an increase in IMF deposition. On the other hand, early slaughter, before the animal reaches its peak IMF deposition, can result in a lower level of IMF in the meat (Pethick et al., 2004). Weaning age is another important factor that can affect IMF deposition in beef cattle. Early weaning of beef cattle has been suggested as a management practice to increase IMF deposition in beef cattle. The advantage of early weaning is that it allows for greater control over the calf's diet and can result in earlier and more rapid weight gain. This, in turn, can increase the deposition of IMF in the muscle tissue of the calf (Blanco et al., 2009 and Myers et al., 1999). Castration of beef cattle involves the removal of the testicles of male calves, typically before they reach puberty. This practice is widely used in the beef industry to improve the quality and safety of beef products. Castration has been shown to have several advantages, including improved meat quality, reduced aggression and stress in animals, and decreased risk of boar taint. In terms of IMF deposition, castration can have a positive effect on beef quality, but the degree of impact may vary depending on the breed and age of the animal (Lee et al., 2004). Environmental factors can have a significant impact on IMF deposition in beef cattle. Factors such as diet, management practices, and climate can affect the quality and quantity of IMF in the meat (Tume, 2004). Cattle raised in hot climates tend to have a lower level

of IMF compared to those raised in cooler climates (Chen et al., 2022). This is because heat stress can lead to a decrease in feed intake, which can result in a decrease in IMF deposition. Understanding these factors can help producers optimize beef production and improve beef quality.

3. Physiological changes by feeding beet pulp in ruminant

Methane release

Cattle farming has been linked to the release of methane, a potent greenhouse gas (GHG)that contributes to climate change. Methane is produced during the digestive process of cattle, and its release has become a significant concern for environmentalists, policymakers, and farmers alike (Karl et al., 2015). Livestock generate 14.5% of anthropogenic greenhouse gas and GHG emission from CH4 through enteric fermentation in ruminants occupies 39% of the livestock sector (Gerber et al., 2013). The production of methane in cattle is a complex process that involves different microbial populations in the rumen. The primary mechanism through which methane is produced enteric fermentation, which is the breakdown of feed in the rumen. During enteric fermentation, microbes such as methanogens convert the carbon in the feed into methane gas, which is then released through belching (Johnson and Johnson, 1995). The process of enteric fermentation is influenced by several factors, including the type and quality of feed, animal genetics, and the microbial populations present in the rumen. Several factors can affect the production of

methane in cattle, including diet, animal breed, and management practices. Research has shown that the type and quality of feed can significantly impact methane production in cattle. For instance, diets that are high in fiber and low in digestible energy are associated with higher methane emissions (Van Zijderveld et al., 2011). Additionally, the breed of cattle can also influence methane production, with some breeds being more efficient at digesting feed and producing less methane (Gerber et al., 2013). Management practices such as grazing management and feed supplementation can also affect methane production in cattle (Boadi et al., 2004). Several strategies have been proposed to reduce methane emissions from cattle, including dietary modifications, genetic selection, and feed additives. One promising approach is the use of feed additives such as ionophores, which can alter the microbial populations in the rumen and reduce methane production (Hristov et al., 2013). Another approach is genetic selection, which involves breeding cattle with lower methane emissions. Finally, dietary modifications such as the use of high-quality forage and the inclusion of legumes in the diet can also reduce methane production (Beauchemin et al., 2011). The available evidence suggests that feeding beet pulp to ruminants can increase methane production in the rumen, thus potentially exacerbating the impact of ruminant agriculture on climate change (Hatew et la., 2015). The mechanism behind this increase in methane production is thought to be due to the high soluble fiber content of beet pulp, which is more easily fermented in the rumen, leading to increased microbial activity and methane production (Ibáñez et al., 2014). While there is still some debate over the overall

impact of beet pulp on greenhouse gas emissions from ruminant production, it is clear that more research is needed to fully understand the potential implications of using beet pulp as a feed stuff.

Gene expression

Gene expression is the process by which the information encoded in a gene is used to synthesize a functional protein or RNA molecule. The expression of genes can be influenced by various factors, including diet, and can impact physiological processes and overall health. Several genes are involved in lipid metabolism in ruminants, such as fatty acid synthase (FASN), fatty acid binding protein 4 (FABP4), acetyl-CoA carboxylase (ACC), peroxisome proliferator activated receptor gamma (PPARG), and lipoprotein lipase (LPL). FASN encodes for the enzyme fatty acid synthase, which is responsible for synthesizing long-chain fatty acids (Raza et al., 2018). FABP4 encodes for the fatty acid binding protein, which transports fatty acids to different parts of the cell for utilization (Hoashi et al., 2008). ACC encodes for acetyl-CoA carboxylase, an enzyme that catalyzes the first step in fatty acid synthesis (Zhang et al., 2010). PPARG encodes for the peroxisome proliferator-activated receptor gamma, a transcription factor that plays a critical role in adipocyte differentiation and lipid metabolism (Goszczynski et la., 2016). LPL encodes for lipoprotein lipase, an enzyme that catalyzes the hydrolysis of triglycerides in lipoproteins (Oh et al., 2013). Studies have shown that depending on feeding

system to ruminants can alter the expression of genes involved in lipid metabolism (Dervishi et al., 2011). For example, feeding beet pulp has been shown to upregulate FASN and FABP4 gene expression in the rumen of dairy cows, which may result in increased production of milk fat (Marchitelli et al., 2013). However, Graugnard et al (2009) reported that PPARG gene expression was higher in high-starch diet feeding group than low-starch in Angus × Simmental steer calves. On the other hand, in holstein heifers, the solute carrier family 9-member A3 (SLC9A3) that play role in epithelial brush border Na/H exchanger that uses an inward sodium ion gradient to expel acids from the cell expression was increased by feeding beet pulp (Beckett et al., 2021). However, there are just few researches about gene expression change when sugar beet pulp was fed to ruminant. The further research in demanded.

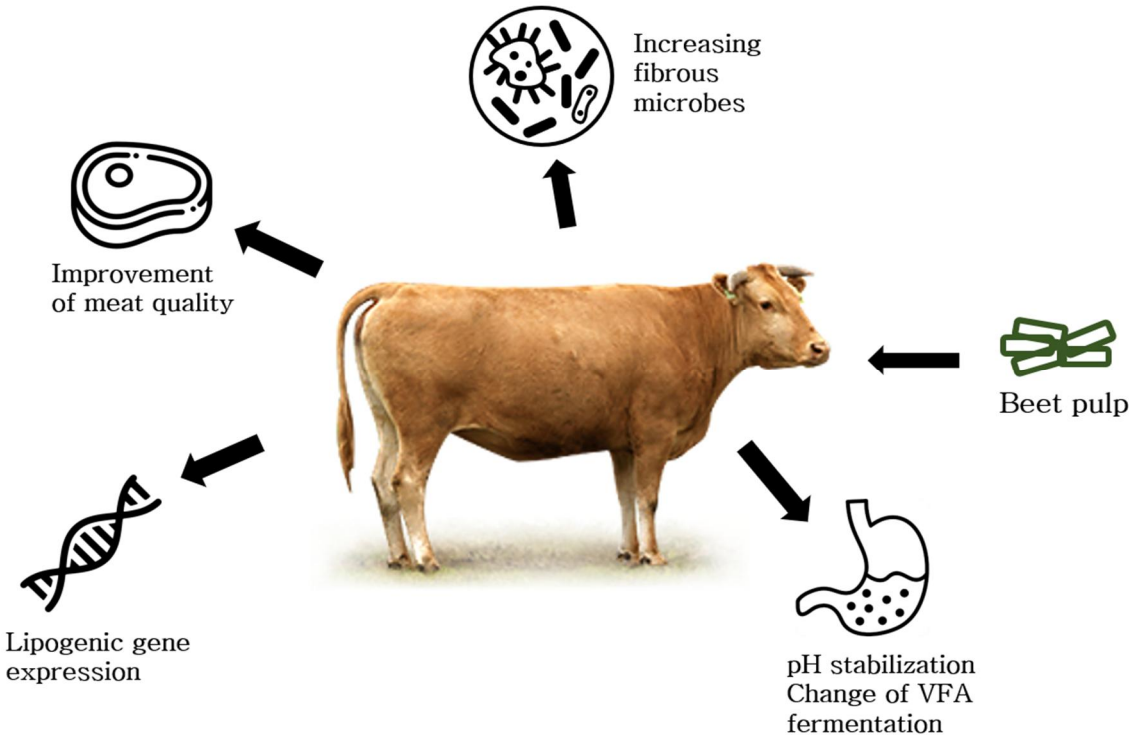

Figure 3. The overall effects of feeding beet pulp in beef cattle.

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Ⅲ. Materials and methods

All of experiments were approved by the Seoul National University Institutional Animal Care and Use Committee (SNU-IACUC; SNU-230210-3), Republic of Korea, and were conducted according to the Animal Experimental Guidelines provided by SNUIACUC.

This experiment was conducted at the Animal Farm of the College of Agriculture and Life Sciences of Seoul National University, Pyeongchang Campus, South Korea.

1. Animals, Experimental Design, and Diets

A total of eighteen Hanwoo (Korean Native cattle) steers of 25.9 ± 0.25 months of ages were used for this experiment, and their initial body weights were 636 ± 10.9 kg. Steers were housed in an indoor barn with a sawdust bedded floor and covered with a roof. The steers were assigned to either the corn flake or beet pulp group considering body weight, age, ultrasonic predicted intramuscular fat. During the 5 weeks of adaptation period, 89% of DM requirement was provided as concentrate portion (1.16% of the body weight/day), and the remaining portion (11% of DM requirements) was supplemented with tall fescue hay (1 kg/day). Seventy-eight% of concentrate portion was given by pelleted basal concentrate, and the remaining 22% was offered in the form of corn flake. For 9 days of adaptation period, the portion of

corn flake was gradually increased from 7% to 22%. The concentrate was fed by automatic feeding machine (DeLaval ALPRO system; DeLaval, Tumba, Sweden) from 10:00 h to 21:00 h freely except before 1 h of morning and afternoon feeding to increase forage and supplement intake. The forage and supplement (corn flake and beet pulp) were fed separately in baskets for an hour each. Forage was fed two times at 08:00 h and 14:00 h, and supplement was fed two times at 09:00 h and 15:00 h.

After adaptation period, the steers (n = 9/group) were divided into CF group and BP group. For the 20 weeks experimental period, two groups were fed the same basal concentrate. For CF group, 78% of total DM was provided with commercial concentrate mixture, and the remaining 22% DM was corn flake. For BP group, 72% DM was provided with commercial concentrate mixture and 28% with beet pulp pellet. The commercial concentrate mixture was composed of 327 g/kg of corn flake, 150 g/kg of corn gluten feed and 107 g/kg of corn flour (Table 1). Thus, about one third (37.9%) of total corn in the CF diet was replaced with beet pulp. Portion of basal concentrate was adjusted from 1.17% to 1.07% of body weight per animal at 4 weeks intervals according to Cargill Agri Purina feeding program. The tall fescue hay was provided in the amount of 1.0 kg daily for all the animals throughout the experimental period. The chemical composition and ingredients of the feeds are in Table 1. Water was provided freely by automatic drinker. Any remains of feed stuffs were collected after every feeding time and weighed to calculate intake. The amount of feed intake was recorded every day. Most of animals consumed all of provided supplement

and hay. The amount of concentrate intake was controlled by the online ALPRO system (DeLaval, Tumba, Sweden) and automatically recorded every day. Bodyweight was recorded on every sampling day before the morning feeding time. The average daily gain (ADG) was calculated as body weight gain divided by the number of experimental days. Feed efficiency was calculated as kg of ADG divided by kg of daily total DM intake.

Table 2 Ingredients and chemical composition of dietary treatment and individual feeds.

Item	Dietary treatment		Individual feed			
	Corn flake	Beet pulp	Concentrate	Corn flake	Beet pulp	Tall fescue
Ingredients, g/kg dry matter (DM)						
Concentrate [1]	692	640				
Tall fescue hay	112	112				
Supplemented corn flake	197	0				
Supplemented beet pulp	0	249				
Total	1000	1000				
Chemical composition, g/kg						
Dry matter (DM)	883	887	884	863	885	910
Crude protein (CP)	149	150	182	85.7	107	60.2
Ether extract (EE)	39.3	31.1	43.0	42.4	8.4	13.9
Ash	61.3	65.5	73.8	11.4	41.3	72.1
Crude fiber (CF)	95.4	139	69.6	25.7	209	378
Neutral detergent fiber (NDF)	220	285	199	75.2	362	605
Acid detergent fiber (ADF)	115	168	92.9	24.4	253	408
Nitrogen free extract (NFE) [2]	654	615	632	835	634	476
Non-fiber carbohydrates (NFC) [3]	530	469	503	785	481	249
Starch	399	237	361	739	9.30	33.0
Calcium (Ca)	6.60	8.30	9.10	0.10	0.86	3.20
Phosphorus (P)	4.70	4.10	6.00	2.30	0.70	1.00
Energy values						
Total digestible nutrient (TDN), % [4]	79.6	76.8	80.7	89.7	76.8	54.5
Digestible energy (DE), Mcal/kg [5]	3.51	3.39	3.56	3.96	3.39	2.40
Metabolizable energy (ME), Mcal/kg [6]	2.88	2.78	2.92	3.24	2.78	1.97

[1] Ingredient of concentrate (g/kg) : corn flake 326, wheat flour 190, corn gluten feed 150, corn flour 106, palm kernel meal 60, copra meal 49.4, molasses 45, lupin seeds 30, limestone fine 12.6, rumen buffer mix 10, rice bran 5, linseed meal 5, urea 3.3, palm oil 2, salt 1.8, ammonium chloride 1.5, yeast fermentation product 0.5 and Mineral and vitamin premix contained

Vit. A, 100,000 IU; Vit. D, 3,800 IU; Vit. E, 44,000 IU; Co, 667 mg; Cu, 13,333 mg; I, 1,333; Fe, 33,333 mg; Mn, 66,6667 mg; Se, 266 mg; Zn, 100,000 mg.
[2] NFE (g/kg) = 1000 - (CP + EE + Ash + CF).
[3] NFC,(g/kg) = 1000 - (CP + EE + Ash + NDF).
[4] TDN (%) = ((NFE × NFE_dig) + (CP × CP_dig) + 2.25(EE × EE_dig) + (CF × CF_dig)) / 100. (All contents are %), (dig; digestibility), (NRC, 2016).
[5] Digestible energy (Mcal/kg) = 0.04409 × TDN (%) (NRC, 2016).
[6] Metabolizable energy (Mcal/kg) = 0.82DE (Mcal/kg) (NRC, 2016)

2. Analyses of chemical composition of feedstuffs and tissue physicochemical characteristics

The chemical contents of the concentrates and tall fescue were determined using analytical methods provided by the Association of Official Agricultural Chemists (AOAC, 2000); dry matter (method 930.15), crude protein (Kjeldahl N × 6.25, method 981.10), ether extract (method 920.39), ash (method 942.05), starch (method 948.02), calcium (method 927.02), phosphorus (method 965.17), and acid detergent fiber contents (method 973.18). The neutral detergent fiber contents of the concentrate and tall fescue were analyzed using the sequential method and the ANKOM200 Fiber Analyzer (Ankom Technology Corp., Macedon, NY, USA) according to the methods described by Mertens (2002). The lignin contents in feed and fecal samples were determined according to Van Soest (1973). The water holding capacity (WHC) was analyzed according to Kim et al. (2019). The pH was measured using a pH meter (SevenGo, Mettler Toledo Inti, Inc., Schwerzenbach,

Switzerland). Warner Bratzler shear force (WBSF) was measured by a Warner Bratzler shear attachment on a texture analyzer (CT3 10K, Brookfield Engineering Laboratories, Middleboro, MA, USA), as described by Piao et al. (2015).

3. Rumen fluid collection and analysis

Rumen fluid sample was collected 3 hours after morning feeding using the oral stomach tube method described by Shen et al. (2012). The first 50 mL of rumen fluid was discarded to prevent saliva contamination, and 150 mL of rumen fluid was collected. The pH of rumen fluid was measured immediately using a pH meter (Ohaus Corp., Parsippany, NJ, USA). For the analysis of volatile fatty acid, after filtration, 1 mL of rumen fluid was mixed with 0.2 mL of 25% metaphosphoric acid and stored at -70 ℃ until analysis. The VFA concentrations were determined with Agilent Tech 7890A gas chromatograph (Hewlett Packard, Waldbronn, Germany) using SUPELCOWAX 10 Capillary GC Column. The detailed method was described by Kang et al. (2020). Ammonia nitrogen was analyzed using a modified colorimetric method (Chaney and Marbach, 1962).

4. Blood collection and analysis

Blood was collected at 3 hours after morning feeding on the initial starting day and 4-week intervals thereafter. Blood samples were collected via jugular vein puncture by using non-heparinized vacutainer (20 mL; Becton Dickinson, Franklin Lakes, NJ, USA) and ethylenediaminetetraacetic acid (EDTA)-treated vacutainer (20 mL; Becton Dickinson). Serum and plasma were separated by centrifugation (1,500 × g at 4℃ for 15 min), and stored at −70℃ until analysis. Serum levels of albumin, glutamic pyruvic transaminase (SGPT, ALT), glutamic oxaloacetic transaminase (SGOT, AST), total cholesterol, blood urea nitrogen (BUN), glucose, total protein, triglyceride (TG), and non-esterified fatty acids (NEFA) were analyzed by using an automatic analyzer (Cobas 6000; Roche, Switzerland). The analytical reagents used for NEFA were purchased from Wako Pure Chemical (Osaka, Japan) and the other reagents were purchased from Roche (Basel, Switzerland). Plasma insulin was determined using the Bovine Insulin Kit (Mercodia AB, Uppsala, Sweden), and leptin was analyzed using the Bovine Leptin Kit (MyBioSource, San Diego, CA, USA). The intra and interassay coefficients of variation for the insulin kit were 3.83% and 7.10% based on bovine serum samples, and those of leptin were <10%. The analytical methods of blood were validated in our laboratory, as reported previously (Jeong et al., 2022).

5. Slaughter procedure, tissue sampling, carcass measurements, and meat physicochemical traits

Steers were transported to commercial slaughterhouse (Bucheon, Republic of Korea) and slaughtered by captivate bolt stunning, as previously described in our study (Piao et al., 2021). The LT hot carcass (~ 100 g) samples were obtained from between the 13th thoracic vertebra and 1st lumbar vertebra immediately after slaughter, frozen in liquid nitrogen, and stored at − 80 ℃ for mRNA expression analyses.

The carcass characteristics (back fat thickness, rib eye area, marbling score and quality grade, yield grade) were evaluated by an official meat grader 24 h post−mortem at 4 ℃ in a cold room (Piao et al., 2017), using the Korean carcass grading system of the Korea Institute of Animal Products Quality Evaluation (KAPE, 2013). The LT cold carcass samples (~ 1.0 kg) were taken from the upper part of the graded area (1st lumbar vertebra) of the left carcass, vacuum−packaged, transported on ice pack to the laboratory, and stored at 4 ℃ for a day. The packages containing the LT samples were opened, and the external fat was trimmed away. The LT samples (~ 100 g) were minced using a mini chopper (CH180, Kenwood, Shanghai, China) for 30 s, and portions of the minced LT samples were used immediately to analyze pH and water holding capacity, and remaining parts were stored at − 70 ℃ for the meat composition analysis. Samples for shear force were prepared from a center part of the LT without mixing, and shear force was determined immediately.

Physiochemical traits of the LT were analyzed, as described previously (Fassah et al., 2023). The pH of the beef samples was measured using a pH meter (SevenGo, Mettler-Toledo, Inc., Schwerzenbach, Switzerland). Maximum shear force (N, Newton) was measured using a Warner-Bratzler shear attached to a texture analyzer (CT3 10 K, Brookfield Engineering Laboratories, Middleboro, MA, USA). Water holding capacity was measured using a filter paper (Whatman No. 4, Whatman International Ltd., Kent, UK).

6. Computer image analysis (CIA) of the marbling characteristics

Immediately after beef grading, cross-sectional photographs of the ribeye area were taken along the side of the 13th thoracic vertebra using the beef carcass photography equipment, HK 333 (Hayasaka Ricoh Co., Ltd., Sapporo, Japan) developed by Kuchida et al. (2001). The CIA characteristics were analyzed with Beef Analyzer II software (Hayasaka Ricoh Co., Ltd., Sapporo, Japan) as reported in our laboratory by Beak et al. (2021). Briefly, the digital image of the LT cut surface was converted to binarized images and segmented into lean muscle and fat images. The marbling percentage was calculated by dividing the number of pixels with marbling by the total number of pixels in the LT. The total number of marbling particles (MPs) was counted from the binarized fat image. Fine MPs were defined as those with an area between 0.01 and 0.5 cm2. The fineness index was obtained by dividing the number of fine

MPs by the rib eye area (cm2). The MPs in highly marbled beef tend to be connected and the thinning process is a way to separate the MPs (Nakahashi et al., 2008). The image of the coarse MPs was obtained after thinning the binarized fat image with 15 rounds and removing the hairlines. We counted the coarse MPs that were larger than 0.5 cm2. The coarseness index was obtained by dividing the total area of the coarse MPs (> 0.5 cm2) by the total area of MPs (cm2) in the binarized fat image.

7. Histological analysis

For the cellularity analysis of marbling adipocytes, three of LT samples (2×3×0.5 cm) from each animal were collected, and fixed in 10% neutral buffered formaldehyde solution. The LT samples were dehydrated in a graded series of ethanol, and embedded in paraffin blocks. The paraffin blocks were cut into 4-um sections using a microtome (Leica, Wetzlar, Germany). Tissue sections were stained with haematoxylin and eosin in the Austostainer XL (Leica). Briefly, the sections were deparaffinized four times with xylene for 10 min each, and washed twice in 100% ethanol for 1 min and 95% ethanol for 1 min. The sections were stained in hematoxylin and eosin, and mounted on slides. Three slides of each LT sample were scanned by a Leica SCN400 F (Leica, Wetzlar, Germany) and random five spots in the scanned image of each slide were captured at 7 × magnification and saved as tiff image files. The adipocyte diameter was measured from total forty five (3X3X5 spots) captured images

(2.12mm2) per animal by using Adiposoft image analysis software (MathWorks, Portola Valley, CA, USA) with ImageJ v1.53 (National Institutes of Health, Stapleton, NY, USA) (Galarraga et al., 2012).

The adipocyte diameter in the LT was measured from 40 to 200 μm with 10 μm− intervals. Mean value of adipocyte size (diameter) was calculated as the sum of all individual adipocyte size in the 2.12−mm2 cross sectional area divided by the number of adipocyte cells. The distribution frequency of adipocyte size was calculated by dividing the number of adipocytes in the range of 10μm by the total number of adipocytes.

8. Quantitative polymerase chain reaction (qPCR) analysis

Total RNA extraction from LT and qPCR were performed as previously described by Park et al. (2018). Briefly, total RNA was extracted using Tirol Reagent (Molecular Research Center, Cincinnati, OH, USA). The RNA was quantified using a NanoPhotometer (Implen, Munich, Germany), and RNA quality was checked using ethidium bromide staining of the 28S and 18S bands of agarose gel electrophoresis and a Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The RNA was stored at −70 ℃ until analysis. Total RNA was transcribed into cDNA using an iScript cDNA synthesis kit (ENZO, Farmingdale, NY, USA). Gene expression was evaluated by qPCR using the AMPIGENE®MqPCR Green Mix Lo−Rox (Dong In Biotech Co., Ltd., Seoul, Republic of Korea). The qPCR was conducted using a Rotor−Gene Q instrument

(Qiagen, Hilden, Germany). Primer information for qPCR is presented in Table 3. The ΔΔCT method was used to determine relative fold changes, as previously described (Livak & Schmittgen, 2001), and the qPCR data were normalized to the β-actin housekeeping gene.

Table 3 Primer sequences for real time PCR analysis

Gene name (Symbol)	Gene bank accession no.	Primer	Sequence (5' 3')	Length (bp)
Fatty acid uptake & muscle growth				
Lipoprotein lipase (LPL)	NM_001075120.1	Forward	GGACATTTACCCTAACGGAGGC	130
		Reverse	ATGAACGGAACGTTCGTGGG	
Fatty acid translocase (CD36)	NM_001278621.1	Forward	ACTGTCCTCTGTGTCTAAGC	124
		Reverse	GTTGTGTCTGCCTCAAGTGC	
Fatty acid binding protein 4 (FABP4)	NM_174314.2	Forward	GCTGCACTTCTTTCTCACCT	140
		Reverse	TTCCTGGTAGCAAAGCCCAC	
Lipolysis				
Patatin like phospholipase domain containing 2 (PNPLA2)	NM_001046005.2	Forward	CCAACGCCACGCACATCTA	103
		Reverse	ACCTCGATGATGTTGGCACC	
Monoglyceride lipase (MGLL)	NM_001206681.1	Forward	AGGACTCGGCGCTCGT	146
		Reverse	CAGTACCGGCAGAAGAGGTG	
Lipase E, hormone sensitive type (LIPE)	NM_001080220.1	Forward	TATCTGAAGAGGCCTGGGAGG	103
		Reverse	AGAAGGCCATGTTGTCCTCTG	
Adipogenesis & lipogenesis				
Peroxisome proliferator activated receptor gamma (PPARG)	NM_181024.2	Forward	AATCCCTGTTCCGTGCTGTG	149
		Reverse	AAAGTTGGTGGGCCAAAACG	
Acetyl CoA carboxylase alpha (ACC)	NM_174224.2	Forward	AGGAGGGAAGGGAATCAGAA	69
		Reverse	GCTTGAACCTGTCGGAAGAG	

Fatty acid synthase (FASN)	NM_001012669.1	Forward	AAGGCGTGCGTGACACTT	92
		Reverse	GGGATGCGGGAATACAGTTG	
Acyl-CoA synthetase short chain family member 2 (ACSS2)	NM_001105339.1	Forward	CTATGGGAACCACGAACGCT	125
		Reverse	TGTCATCGATCCTGCCAGTG	
Diacylglycerol acyltransferase 2 (DGAT2)	NM_205793.2	Forward	CAGCTCCAAGTCATCTCGGT	121
		Reverse	AGTAGAGCACGGCAATGAGC	
Glycerol-3-phosphate acyltransferase (GPAM)	NM_001012282.1	Forward	TGTGCTATCTGCTCTCCAATG	116
		Reverse	CTCCGCCACTATAAGAATG	
Muscle growth and stress response				
Insulin like growth factor 1 (IGF1)	NM_001077828.1	Forward	GCACTTCAGAAGCAATGGGA	104
		Reverse	GGAGGATGTGATGGGCATCTTC	
Signal transducer and activator of transcription 5a (STAT5a)	NM_001012673.1	Forward	GATTTCTCCATCCGATCCCT	136
		Reverse	CGTATCCGTCCACTGCTTTA	
Growth hormone receptor (GHR)	NM_176608.1	Forward	GTGAAGCCACACCAGCTTTC	116
		Reverse	TTCAGGTGAACGGCACTTGG	
Myostatin (MSTN)	NM_001001525.3	Forward	AAGACGATGACTACCACGCC	86
		Reverse	GGGTTTTCCTTCCACTTGCG	
Haptoglobin (HP)	NM_001040470.2	Forward	TGGGTCGTGTGGGTTATGTG	90
		Reverse	CAGCCACAGGTAGCATGACA	
Serum amyloid A2 (SAA2)	NM_001075260.2	Forward	TCACAGGCCTCATTCTCTGC	139
		Reverse	TCTGCACCCTTGTAGTTGGC	
β actin[1]	NM_173979	Forward	AGCAAGCAGGAGTACGATGA	120
		Reverse	ATCCAACCGACTGCTGTCA	

[1] Housekeeping gene.

9. The CH_4 measurement by CO_2 method

To measure methane generation from Hanwoo steers, the background air samples in the barn were collected for 30 min before and after each experiment and analyzed background concentration of CO_2 and CH_4. Gas samples were collected consecutively from breath every 5 s for 10 min for each steer when morning and afternoon feeding times. At the same time, the CO_2 gas concentrations were analyzed by a gas analyzer VA−3000 (Horiba, Kyoto, Japan) that was equipped with non−dispersive infrared CO2 (0 - 10,000 ppm) gas sensor. The CH_4 gas concentrations were analyzed by a gas analyzer Airwell+7 (KINSCO technology, Seoul, Republic of Korea) equipped with tunable diode laser absorption spectroscopy CH_4 gas sensor (0 - 1,000 ppm). All steers were fixed by stanchion during sampling. The breath gas was collected through a pipe fixed to the forage feeder, and we covered the feeder with plastic sheet during sample collection to prevent dilution with atmospheric air. The detailed methods of calibration and CH4 production calculation are described in previous report (Haque et al., 2017). Briefly, Calculate the CH_4 : CO_2 ratio in samples, and then Calculate total CO_2 and CH_4 production per day. The total CO2 and CH4 production were calculated by the following equations:

1. HP (watt) = $7.64 \times BW^{0.69} + Y\left[\frac{23}{M} - 1\right]\left[\frac{57.27 + 0.302 \times BW}{1 - 0.171 \times Y}\right]$
2. CO_2 (L/d) = $\mathrm{HPU} \times 180 \times 24$
3. CH_4 (L/d) = $\mathrm{CO2} \times \frac{CH4}{CO2}$

Where, HP; heat production of the animal, BW; body weight of the animal, Y; average daily gain of the animal, M; energy content of the feed, MJ/kg dry matter, HPU; heat producing unit, HP/1000.

10. Economic efficiency analysis

For comparison of economic efficiency, the import price information of all the feedstuffs was provided from Cowin AS (Gwangju, Korea) and Cargill Agri Purina (Seongnam, Korea). All of monetary unit was in US dollars at the time of purchase. The average price of BP, CF and concentrate basal diets were 0.43, 0.50 and 0.75 $/kg respectively. Daily feed cost was calculated based on daily DMI and feed cost per body weight gain was calculated as daily feed cost divided by ADG. Total revenue was calculated as total meat price - total feed cost. The average meat price on auction of CF group was 17.05 $/kg and that of BP group was 19.02 $/kg. Total economic efficiency was calculated as total meat price / total feed cost.

11. Statistical analysis

The power analysis indicated that at least six animals per group were needed to detect a significant difference with a power of 0.8 and a type III error rate of 0.05. The steers were assigned to either the corn flake or beet pulp group considering body weight, age, ultrasonic predicted intramuscular fat. The data on ruminal fermentation, blood metabolites and methane release were subjected to repeated measures analysis of variance using the MIXED procedure (PROC MIXED) to investigate changes over time (weeks). The model included treatment (diet), period (sampling date), and the treatment × time interaction as the fixed effects, with animal as the random effect within a diet group. Three variance-covariance structures (autoregressive type 1, compound symmetry, and Toeplitz) were tested, and the covariance structure that minimized the Schwarz' s Bayesian information criterion was chosen. When a dietary treatment was found to have a significant effect, pairwise differences between means were assessed using the PDIFF procedure. Differences between treatment means were tested by student' s t test at the significance level of 0.05 in growth performance parameters, carcass characteristics, histological measures of *longissimus dorsi* area, gene expression and economic efficiency. All the statistical analyses were conducted using SAS (ver. 9.4; SAS Institute Inc., Cary, NC, USA). A p value 0.05 was considered to indicate significance, and 0.05 < p 0.10 indicated a tendency.

Ⅳ. Results

1. Growth performance and feed intake

There was no statistical difference between the BP and CF groups in average daily gain and feed efficiency ($p \geq 0.83$) by feeding beet pulp (Table 4). Partial substitution of corn flake with beet pulp did not affect ($p \geq 0.06$) DM intake/day of concentrate, forage, or supplement. On the other hand, DM intake/body weight (BW) of concentrate and supplement were different ($p \leq 0.02$) between the two groups. Total intake of CF, NDF and ADF (both kg/day and kg/BW) were significantly higher (all $P < 0.001$) in the BP group. Meanwhile, total EE, NFC, starch intake (both kg/day and kg/BW) were significantly lower ($P \leq 0.01$) in the BP group than CF group.

Table 4 Effects of partial replacement of corn flake with beet pulp on growth performance of Korean cattle steers.

Items	Dietary treatment			
	Corn flake	Beet pulp	SEM	*p* value
Initial body weight, kg	669	668	10.9	0.97
Final body weight, kg	775	775	14.6	1.00
Average daily gain, kg	0.74	0.75	0.04	0.96
Feed efficiency, gain/feed	0.09	0.09	0.005	0.79
Total feed intake [1], kg/day				

Dry matter (DM)	8.52	8.53	0.17	0.99
Crude protein (CP)	1.27	1.29	0.03	0.81
Ether extract (EE)	0.34	0.27	0.01	< 0.001
Ash	0.56	0.52	0.01	0.08
Crude fiber (CF)	0.79	1.16	0.05	< 0.001
Neutral detergent fiber (NDF)	1.84	2.40	0.08	< 0.001
Acid detergent fiber (ADF)	0.95	1.41	0.06	< 0.001
Nitrogen free extract (NFE)	5.60	5.25	0.12	0.13
Non fiber carbohydrates (NFC)	4.55	4.01	0.11	0.01
Starch	3.45	2.06	0.18	< 0.001
Concentrate DM intake	5.89	5.57	0.13	0.21
Supplement DM intake	1.74	2.04	0.07	0.02
Forage DM intake	0.89	0.92	0.02	0.56
Total feed intake, g/kg of body weight				
Dry matter (DM)	10.5	10.6	0.09	0.65
Crude protein (CP)	1.57	1.60	0.01	0.36
Ether extract (EE)	0.42	0.33	0.01	< 0.001
Ash	0.64	0.70	0.009	< 0.001
Crude fiber (CF)	0.99	1.45	0.06	< 0.001
Neutral detergent fiber (NDF)	2.29	2.99	0.09	< 0.001
Acid detergent fiber (ADF)	1.19	1.76	0.07	< 0.001
Nitrogen free extract (NFE)	6.90	6.52	0.07	0.004
Non fiber carbohydrates (NFC)	5.59	4.98	0.09	< 0.001

Starch	4.22	2.56	0.20	< 0.001
Concentrate DM intake	7.28	6.91	0.08	0.01
Supplement DM intake	2.10	2.52	0.07	0.002
Forage DM intake	1.14	1.17	0.02	0.40

[1] Total feed intake = concentrate + supplement (corn flake or beet pulp) + forage intake.

2. Ruminal fermentation characteristics

The pH, iso butyrate, butyrate, valerate and ruminal ammonia in the ruminal fluid were not affected ($p \geq 0.93$) by feeding of beet pulp (Table 5). However, there were difference of period effect in pH, iso butyrate and valerate ($p \leq 0.004$). In total VFA tendency of treatment effect was observed ($p = 0.10$). The iso valerate fermentation was significantly higher in CF group than BP group ($p = 0.002$).

Table 5 Effects of partial replacement of corn flake with beet pulp on ruminal fermentation profile in Korean cattle steers.

Items	Dietary treatment				*p* value		
	Corn flake	Beet pulp	Mean	SEM	Treat	Period	Interaction
pH					0.93	< 0.001	0.22
week 0	6.72	6.73	6.72 ^{xy}	0.09			
week 4	6.52	6.53	6.52 ^{x}	0.07			
week 8	6.81	6.66	6.72 ^{y}	0.09			
week 12	6.17	6.29	6.23 ^{z}	0.08			
week 20	6.33	6.56	6.45 ^{xz}	0.12			
Total VFA, mM					0.10	0.01	0.54
week 0	100	101	101 ^{xy}	4.02			

week 4	93.2	113	103 [x]	6.16			
week 8	97.3	114	106 [x]	5.24			
week 12	113	115	114 [x]	4.04			
week 20	87.0	87.7	87.3 [y]	6.46			
Acetate, mol/100 mol					< 0.001	< 0.001	< 0.001
week 0	58.8	59.9	59.3 [x]	0.73			
week 4	55.1 [b]	59.9 [a]	57.5 [xy]	0.89			
week 8	56.4 [b]	60.4 [a]	58.4 [xy]	0.88			
week 12	51.6 [b]	61.1 [a]	56.4 [y]	1.51			
week 20	56.5 [b]	62.2 [a]	59.4 [x]	0.93			
Propionate, mol/100 mol					< 0.001	< 0.001	0.01
week 0	21.6	20.6	21.1 [x]	0.64			
week 4	28.1 [a]	21.7 [b]	24.9 [y]	1.24			
week 8	25.2	21.9	23.5 [xy]	1.18			
week 12	30.9 [a]	20.9 [b]	25.9 [y]	1.76			
week 20	24.4	20.5	22.4 [xy]	1.07			
Iso butyrate, mol/100 mol					0.38	0.004	0.84
week 0	1.03	1.00	1.02 [x]	0.04			
week 4	0.86	0.77	0.81 [yz]	0.04			
week 8	1.03	0.91	0.97 [wx]	0.08			
week 12	0.87	0.83	0.85 [wz]	0.05			
week 20	0.91	0.85	0.88 [xyz]	0.04			
Butyrate, mol/100 mol					0.47	0.20	0.002
week 0	14.2	14.2	14.2	0.43			
week 4	12.5	14.5	13.5	0.66			
week 8	13.2	13.6	13.4	0.68			
week 12	12.6	14.2	13.4	0.59			
week 20	14.3	13.7	14.0	0.68			
Iso valerate, mol/100 mol					0.002	< 0.001	0.15
week 0	2.81	2.53	2.67 [x]	0.13			
week 4	1.74	1.47	1.60 [y]	0.11			
week 8	2.56 [a]	1.56 [b]	2.06 [z]	0.21			
week 12	1.98 [a]	1.30 [b]	1.64 [yz]	0.16			
week 20	2.04 [a]	1.21 [b]	1.63 [yz]	0.14			

Valerate, mol/100 mol					0.20	0.004	0.004
week 0	1.62	1.74	1.68 ^{xy}	0.06			
week 4	1.74	1.69	1.71 ^{xy}	0.05			
week 8	1.65	1.55	1.60 ^{x}	0.05			
week 12	2.02 ^{a}	1.66 ^{b}	1.84 ^{y}	0.07			
week 20	1.79	1.61	1.70 ^{xy}	0.07			
Acetate : Propionate ratio					< 0.001	0.002	0.01
week 0	2.80	2.93	2.86 ^{x}	0.11			
week 4	2.06 ^{b}	2.77 ^{a}	2.41 ^{y}	0.13			
week 8	2.39	2.79	2.59 ^{xy}	0.13			
week 12	1.81 ^{b}	2.94 ^{a}	2.37 ^{yz}	0.17			
week 20	2.44 ^{b}	3.06 ^{a}	2.75 ^{xy}	0.14			
Ammonia, mg/dL					0.46	0.15	0.08
week 0	12.7	12.5	12.6	1.72			
week 4	11.4	10.2	10.8	1.49			
week 8	12.6	9.15	10.9	1.27			
week 12	10.1	10.4	10.2	1.27			
week 20	13.6 ^{a}	8.6 ^{b}	11.1	1.21			

SEM, standard error of the means; $^{a, b}$ mean values with different letters in the same row differ ($p < 0.05$); $^{w, x, y, z}$ mean values with different letters in the same column differ ($p < 0.05$).

The period effect was observed in iso valerate ($p < 0.001$) and in valerate, period and interaction effect were observed (both $p = 0.004$). The acetate fermentation and C2:C3 ratio were significantly higher in BP group than CF group (all $p < 0.001$) and show the period and interaction effects also ($p < 0.002$). In propionate fermentation, all of effects (treatment, period, interaction) were observed also ($p < 0.01$), however higher in CF group than BP group ($p < 0.001$).

3. Blood metabolites and hormones

There were no any treatment effects in all of metabolites and hormones (Table 6). In glutamic pyruvic transaminase, total Cholesterol, blood urea nitrogen, glucose, total protein, triglyceride, non esterified fatty acids, leptin and insulin, only period effect was observed ($p \leq 0.01$) and tendency of period was observed in albumin, glutamic oxaloacetic transaminase and insulin like growth factor 1 ($p \leq 0.10$).

Table 6
Effects of partial replacement of corn flake with beet pulp on blood metabolites and hormones in Korean cattle steers.

Items	Dietary treatment		Mean	SEM	*p* value		
	Corn flake	Beet pulp			Treat	Period	Interaction
Albumin, g/dL					0.93	0.10	0.16
week 0	3.79	3.72	3.76	0.03			
week 4	3.74	3.81	3.73	0.04			
week 8	3.86	3.91	3.88	0.05			
week 12	3.90	3.76	3.83	0.07			
week 16	3.80	3.83	3.82	0.04			
week 20	3.71	3.80	3.76	0.04			
Glutamic pyruvic transaminase, U/L					0.98	< 0.001	0.01
week 0	15.3	13.9	14.6 ^{x}	0.55			
week 4	16.4	17.9	15.2 ^{y}	0.75			
week 8	16.2	15.8	16.0 ^{xy}	0.78			
week 12	16.1	16.1	16.1 ^{xy}	0.69			
week 16	16.9	16.6	16.7 ^{y}	0.69			
week 20	17.1	17.7	17.4 ^{y}	0.69			
Glutamic oxaloacetic					0.30	0.06	0.76

transaminase, U/L							
week 0	81.1	71.6	76.3	6.06			
week 4	79.2	72.9	75.4	5.78			
week 8	77.0	66.2	71.6	5.56			
week 12	72.8	62.4	67.6	4.27			
week 16	77.4	65.3	71.4	4.74			
week 20	74.3	62.8	68.6	3.73			
Total cholesterol, mg/dL					0.60	< 0.001	0.73
week 0	144	141	143 [x]	5.32			
week 4	151	159	146 [xy]	7.25			
week 8	161	169	165 [y]	7.81			
week 12	161	168	165 [y]	7.40			
week 16	149	161	155 [xy]	6.16			
week 20	138	144	141 [x]	4.69			
Blood urea nitrogen, mg/dL					0.58	< 0.001	0.08
week 0	16.0	16.6	16.3 [x]	0.61			
week 4	14.9	14.6	15.7 [xz]	0.48			
week 8	16.7	14.8	15.7 [xz]	0.49			
week 12	15.4	14.9	15.2 [xz]	0.67			
week 16	16.0	16.1	16.1 [x]	0.40			
week 20	14.9	13.7	14.3 [yz]	0.65			
Glucose, mg/dL					0.53	< 0.001	0.04
week 0	74.8	75.7	75.2 [w]	1.26			
week 4	78.6	80.8	77.1 [x]	1.20			
week 8	77.6	78.4	78.0 [wx]	1.32			
week 12	74.6 [a]	68.1 [b]	71.3 [y]	1.31			
week 16	77.3	74.2	75.8 [w]	0.83			
week 20	48.3	47.7	48.0 [z]	1.52			
Total protein, g/dL					0.42	0.01	0.02
week 0	6.81	6.72	6.77 [xy]	0.07			
week 4	6.67	6.84	6.69 [y]	0.06			
week 8	6.62	6.90	6.76 [xy]	0.08			
week 12	6.78	6.88	6.83 [xy]	0.12			
week 16	6.78	6.83	6.81 [x]	0.08			
week 20	6.88	7.02	6.95 [x]	0.08			

Triglyceride, mg/dL					0.30	< 0.001	0.58
week 0	21.2	25.4	23.3 [x]	1.23			
week 4	19.7	24.7	22.6 [y]	1.80			
week 8	25.6	26.2	25.9 [xy]	1.87			
week 12	22.8	26.2	24.5 [yz]	1.85			
week 16	27.3	28.6	27.9 [x]	2.01			
week 20	25.2	29.7	27.4 [xy]	1.60			
Non−esterified fatty acids, μEq/L					0.89	< 0.001	0.48
week 0	174	166	170 [x]	16.4			
week 4	102	117	134 [y]	11.3			
week 8	131	140	136 [xy]	18.9			
week 12	85.2	109	97.2 [y]	6.72			
week 16	169	135	152 [x]	15.1			
week 20	129	139	134 [xy]	10.2			
Leptin, ng/mL					0.52	< 0.001	0.24
week 0	35.7	34.6	35.2 [x]	2.21			
week 4	28.1	26.0	37.1 [yz]	1.69			
week 8	22.6	24.5	23.6 [y]	1.24			
week 12	22.0	26.7	24.4 [yz]	1.57			
week 16	26.8	26.7	26.7 [yz]	1.56			
week 20	25.8	31.7	28.7 [z]	1.78			
Insulin, ug/L					0.40	0.001	0.54
week 0	0.81	0.70	0.75 [x]	0.07			
week 4	1.06	0.87	0.96 [xz]	0.09			
week 8	1.25	0.89	1.07 [xz]	0.10			
week 12	1.50	1.75	1.62 [y]	0.20			
week 16	1.21	0.99	1.10 [z]	0.09			
week 20	1.08	0.90	0.99 [xz]	0.09			
Insulin like growth factor 1, ng/ml					0.22	0.10	0.07
week 0	15.1	13.7	14.3 [x]	1.64			
week 4	12.2 [b]	28.5 [a]	20.3 [xy]	3.77			
week 8	13.8	16.5	15.2 [xy]	3.60			
week 12	14.2	16.6	15.4 [xy]	1.84			
week 16	11.5	18.6	15.0 [xy]	2.01			
week 20	27.6	19.0	25.4 [y]	4.98			
Growth hormone							

week 0
week 4
week 8
week 12
week 16
week 20

SEM, pooled standard error of the means; a, b mean values with different letters in the same row differ ($p < 0.05$); x, y, z mean values with different letters in the same column differ ($p < 0.05$).

4. Adipose cellularity

Fat cell size distribution of the LT was measured by image analysis of histological sections (Figure 4, 5; Table 7). Average adipocyte size (diameter) of the LT was bigger ($p = 0.007$) in the BP group than in the CF group. The distribution frequency of adipocyte size was calculated by dividing the number of adipocytes by the total number of adipocytes from 40 to 200 μm with the range of 10μm-intervals (Figure 5). Adipocyte size frequency (%) from 40μm to 50μm were lower in the BP group than in the CF group ($P = 0.02$). On the other hand, adipocyte size frequency (%) from 100μm to 130μm, from 160μm to 170μm, and from 180μm to 190μm were higher in the BP group than in the CF group ($P < 0.04$).

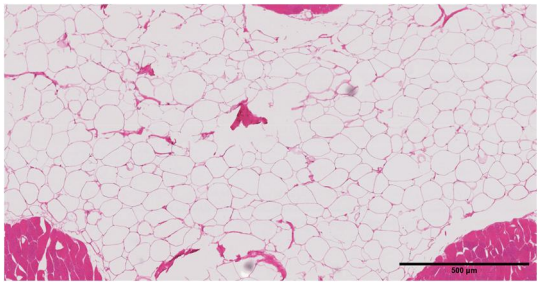
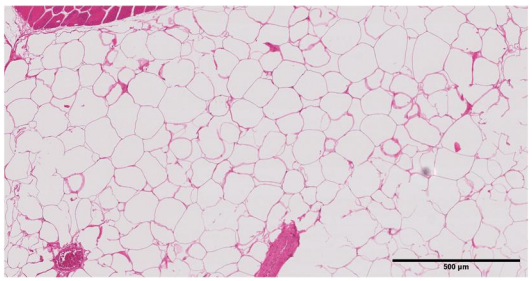
Corn flake	Beet pulp
	

Figure 4. Representative adipocyte images of longissimus thoracis in Hanwoo steer. Area of a captured image is 2.12mm2. Black scale bar, 500μm.

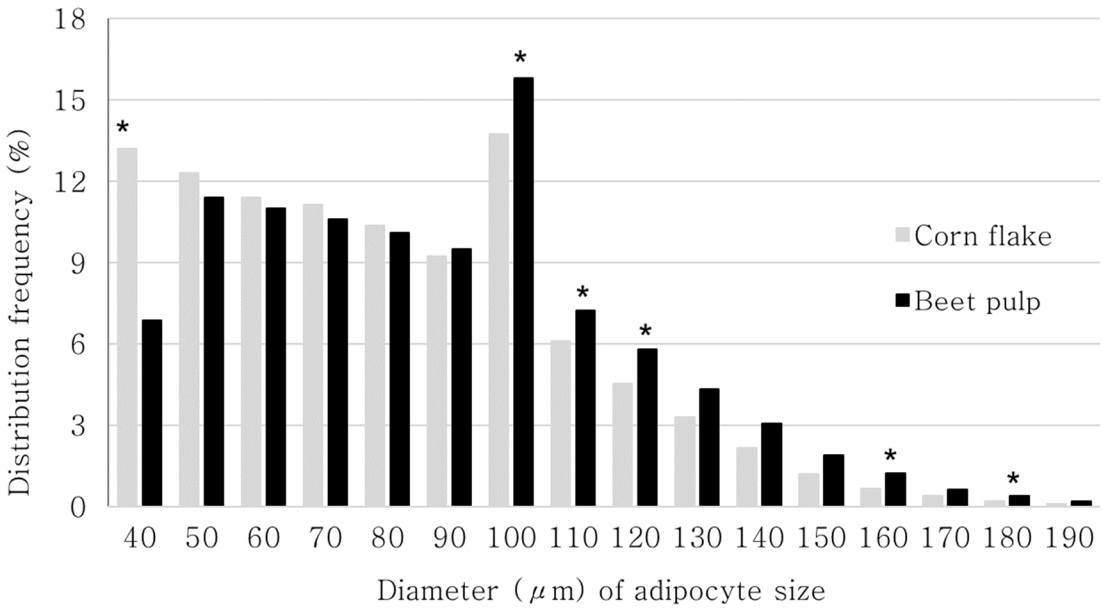


Figure 5. Effects of partial replacement of corn flake with beet pulp on intramuscular adipocyte size distribution in the longissimus thoracis (LT) of Hanwoo steer. The adipocyte diameter was measured from total forty five (3X 3X5 spots) captured images (2.12mm2) per animal by using Adiposoft image analysis software. The distribution frequency of adipocyte size was calculated by dividing the number of adipocytes by the total number of adipocytes from 40 to 200 μm with the range of 10μm-intervals. * $P < 0.05$.

Table 7
Effects of partial replacement of corn flake with beet pulp on intramuscular adipocyte size (diameter) of *longissimus thoracis* (LT) in Korean cattle steers.

Item	Dietary treatment			
	Corn flake	Beet pulp	SEM	*P* value
Size (diameter), μm	82.3	92.9	0.42	< 0.001

Mean value of adipocyte diameter was calculated as the sum of all individual adipocyte sizes in the 2.12−mm^2 cross−sectional area of the LT divided by the number of adipocytes.

5. Carcass, CIA, and physicochemical characteristics

The backfat thickness, rib eye area and marbling score were not different between the BP and CF groups (Table 8). However, yield index and yield grade tended to be higher (both p = 0.1) in BP group than in CF group. The quality grade and total auction price also tended to be higher (both p = 0.08) in BP group. The meat price was significantly affected (P < 0.001) by substitution of corn flake with beet pulp. CIA characteristics including coarseness index and fineness of marbling, and physicochemical traits including water holding capacity, pH, Warner Bratzler shear force, and fat percentage were not statistically different between two groups.

Table 8
Effects of partial replacement of corn flake with beet pulp on carcass, computer image analysis, physicochemical traits of *longissimus thoracis* in Korean cattle steers.

Items	Dietary treatment			
	Corn flake	Beet pulp	SEM	*p* value
Carcass traits				
Back fat thickness, mm	17.3	13.1	1.49	0.20
Rib eye area, cm^2	96.4	101	2.73	0.43
Carcass weight, kg	447	441	10.1	0.78
Yield index [1]	60.6	62.1	0.43	0.10
Yield grade [2]	16.3	22.2	1.71	0.10
Marbling score [3]	6.25	7.11	0.31	0.20
Quality grade [4]	40.0	46.7	1.81	0.08
Meat price, $/kg	16.0	17.9	0.30	< 0.001
Total auction price, $	7,407	8,219	213	0.07
Computer image analysis traits				
Rib eye area, cm^2	92.3	97.7	3.28	0.44
marbling percentage, % [5]	0.21	0.23	0.01	0.46
number of marbling particles	2,113	2,445	139	0.27
number of coarse marbling particles [6]	66.4	79.6	5.15	0.24
coarseness index [7]	0.17	0.16	0.01	0.79
number of fine marbling particles [6]	216	238	13.5	0.46
fineness of marbling [8]	2.33	2.40	0.09	0.72

Physicochemical traits				
Moisture, %	60.5	59.9	0.93	0.78
Water holding capacity	62.2	58.1	1.81	0.29
pH	5.45	5.47	0.02	0.54
Warner Bratzler shear force, neuton (N)	28.4	27.7	2.05	0.88
Ether extract (EE), %	20.1	21.1	0.93	0.59
Crude protein (CP), %	19.4	19.9	0.26	0.32

[1] Yield index = [11.06398 - 1.25149 × back fat thickness (mm) + 0.28293 × rib eye area (cm^2) + 0.56781 × carcass weight (kg)] / carcass weight (kg) × 100 (Korea institute for animal products quality evaluation, 2020 and Cha et al., 2022).
[2] Yield grade : C = 10; B = 20; A = 30.
[3] Marbling score : 1 = trace marbling; 9 = highly abundant marbling.
[4] Quality grade : 1 = 30; 1+ = 40; 1++ = 50.
[5] Marbling percentage (%) = marbling area (cm^2) / rib eye area (cm^2) × 100.
[6] Coarse marbling particle : > 0.5 cm^2, fine marbling particle : 0.01cm^2 ≤ marbling particle ≤ 0.5cm^2.
[7] Coarseness index = total area of coarse marbling particles (cm^2) / total area marbling particles (cm^2).
[8] Fineness index = the number of fine marbling particles / rib eye area (cm^2).

6. Lipid metabolism and muscle growth gene expression in the LT

The mRNA levels related to lipid metabolism and muscle growth genes were examined in the LT (Figure 6). mRNA levels of fatty acid uptake and transport genes (lipoprotein lipase, cluster of differentiation 36, fatty acid binding protein 4) were not different ($P \geq 0.41$) between two groups. mRNA levels of lipogenic fatty acid synthase (FASN) gene were higher ($P = 0.03$) in the BP group than in the CF group (Figure 1), whereas mRNA levels of lipogenesis including peroxisome proliferator activated receptor gamma and acetyl CoA carboxylase genes were not different ($P \geq 0.16$) between two groups. mRNA levels of patatin like phospholipase domain containing 2 and lipase E, hormone sensitive type gene were higher ($P = 0.02$ and 0.08 each) in the CF group than in the BP group. On the other hand, mRNA level of monoglyceride lipase was not different ($P = 0.51$) between two groups. mRNA levels of muscle growth and stress response like insulin-like growth factor 1 gene were not different ($P \geq 0.34$) between two groups.

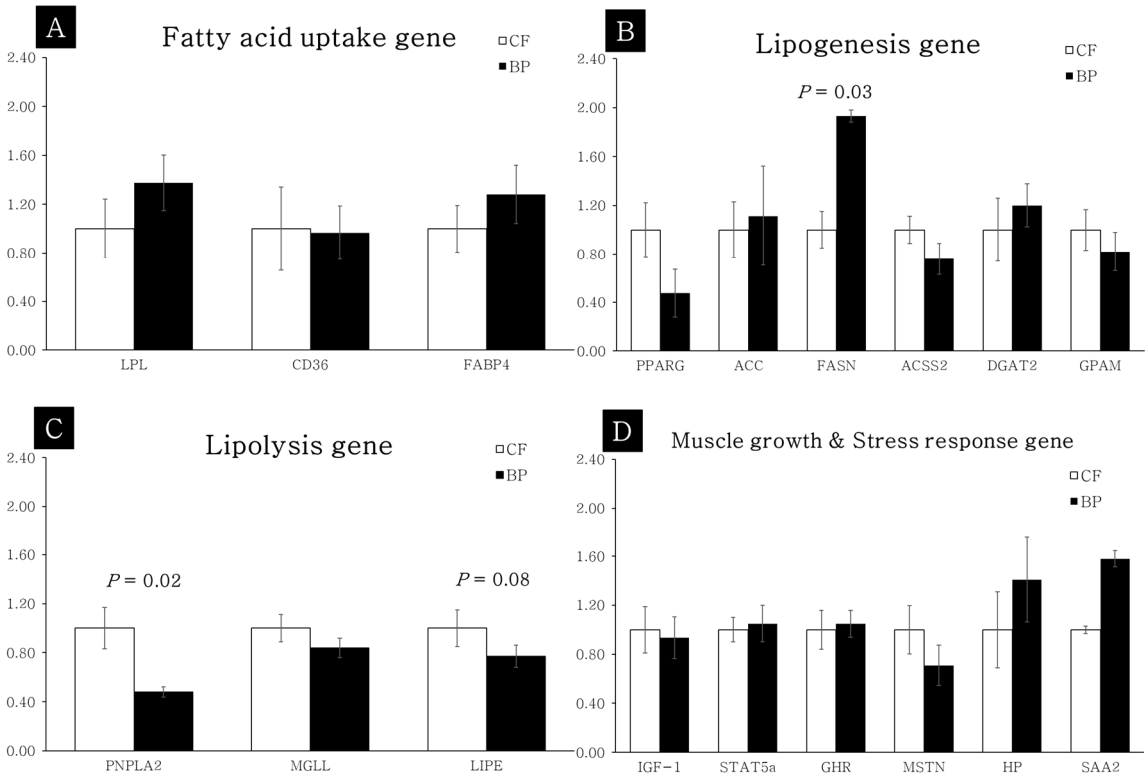


Figure 6. Effects of partial replacement of corn flake with beet pulp on mRNA levels associated with fatty acid uptake and transport (A), lipogenesis (B), Lipolysis (C), and muscle growth & stress response (D) in the longissimus thoracis of Hanwoo steers. mRNA levels were determined by quantitative polymerase chain reaction and results were normalized with the β actin gene. mRNA levels of the control group were normalized to 1.0. Values are the means $\pm$ standard errors of the mean (n = 9). PPARG; peroxisome proliferator activated receptor gamma, ACC; acetyl CoA carboxylase, FASN; fatty acid synthase, ACSS2; acyl-CoA synthetase short chain family member 2, DGAT2; diacylglycerol acyltransferase 2, GPAM; glycerol-3-phosphate acyltransferase, LPL; lipoprotein lipase, CD36; cluster of differentiation 36, FABP4; fatty acid binding protein 4, PNPLA2; patatin like phospholipase domain containing 2, MGLL; monoglyceride lipase, LIPE; lipase E, hormone sensitive type, IGF 1; insulin like growth factor 1, STAT5a; signal transducer and activator of transcription 5a, GHR; growth hormone receptor, MSTN; myostatin, HP; haptoglobin, SAA2; serum amyloid A2.

7. Methanogenesis

The amount of methane release was not affected by partial replacement of beet pulp. There was no difference in treatment, period and interaction effects between BP group and CF group.

Table 9
Comparison of methanogenesis of partial replacement of corn flake with beet pulp in Korean cattle steers

Item	Dietary treatment		SEM	*p*−value		
	Corn flake	Beet pulp		Treat	Period	Interaction
Methane release (Litre/day)				0.16	0.28	0.83
week 0	173	189	5.73	0.17		
week 14	186	196	7.32	0.27		
week 20	188	199	5.68	0.58		

[1] SEM, standard error of mean.

8. Economic efficiency

The partial replacement of corn flake with beet pulp could not statistically change the feed cost (head per day and kg gain) (p = 0.18 and 0.36) (Table 10). However, reduced from 6.20 $ to 5.91 $ and from 8.74 $ to 7.93 $ each. The total revenue showed higher tendency in BP group than CF group (p = 0.06). The total economic efficiency was significantly affected (p = 0.001) by feeding of beet pulp.

Table 10
Effects of partial replacement of corn flake with beet pulp on economic efficiency in Korean cattle steers.

Item	Dietary treatment			
	Corn flake	Beet pulp	SEM	p value
Feed price, $/kg DM	0.76	0.72		
Feed cost, $/head/day	6.20	5.91	0.11	0.18
Feed cost, $/kg gain	8.74	7.93	0.43	0.36
Total revenue, $/head [1]	6,764	7,556	212	0.06
Total economic efficiency [2]	8.83	10.2	0.24	0.001

[1] Total revenue : meat price - feed cost.
[2] Economic efficiency : meat price/feed cost.

Ⅴ. Discussion

1. Growth performance and feed intake

In this study, the 27.6% replacement of total corn in the concentrate portion of the diet with beet pulp did not affect total DM intake, average daily gain, and feed efficiency. Likewise, feeding beet pulp did not affect feed intake in Hanwoo steer (Jeong et al., 2022) and in Egyptian buffalo calves (Abo Zeid et al., 2017). Taken together, beet pulp could be a good energy source for beef cattle without negative effects on growth performance and feed intake.

In late fattening period, feeding a large amount of corn containing abundant starch is a common practice in order to increase fat deposition of Hanwoo steer (Park et al., 2018b; Baik et al., 2023). Feeding a large amount of corn in the diet may provide relatively high amount of starch and thus non-fiber carbohydrate but less amount of neutral detergent fiber and acid detergent fiber. In this study, the partial replacement of corn with beet pulp decreased starch consumption and thus non-fiber carbohydrate but increased neutral detergent fiber and acid detergent fiber consumption. These changes in consumption of carbohydrate types in the diet may affect rumen fermentation characteristics including volatile fatty acid proportions.

2. Ruminal fermentation characteristics and blood parameters

Beet pulp contains abundant pectin and hemicelluloses, which are less acidotic, and may have favorable effects on ruminal pH (Yang et al., 2006). In the present study, feeding beet pulp did not affect ruminal pH. Similarly, feeding beet pulp did not affect ruminal pH in dairy cow (Petri et al., 2019) and in Hanwoo steer (Jeong et al., 2022). In this study, the ruminal pH was within the normal range (6.17 ~ 6.81) in both groups, as reported in NASEM (2016). Our basal concentrate contained rumen buffering mix including sodium bicarbonate, and the buffering materials may maintain a normal range of ruminal pH.

Ruminal total VFA production tended to be higher in the BP group compared with CF group. The increased trend of total VFA production may be related to increased NDF degradability, as previously reported by Münnich et al. (2018a). In this study, feeding beep pulp increased ruminal acetate proportion and decreased propionate proportion. Similarly, feeding beet pulp promoted ruminal acetate proportion and reduced propionate proportion in lactating dairy cows (Voelker and Allen, 2003b), Egyptian buffalo calves (Abo Zeid et al., 2017), and Hanwoo steer (Jeong et al., 2022). The beet pulp contains abundant carbohydrates including NDF and pectin (Abo-Zeid et al., 2017; Habeeb et al., 2017), and acetate in the rumen is produced primarily from the fermentation of these structural carbohydrates (Feng et al., 1995; Alamouti et al., 2014). Thus, the increased neutral detergent fiber and acid detergent fiber consumption and the decreased starch consumption and thus non-fiber carbohydrate in the BP

group is likely contributed to the increased acetate proportion and the decreased propionate proportion, resulting in the increased acetate to propionate ratio. In this study, feeding beet pulp decreased the iso-valerate proportion. Similarly, substitution of beet pulp for corn in the diet decreased iso-valerate concentrations. Likewise, iso-valerate fermentation was lower in BP group in in vitro condition (Mansfield et al., 1994). Meanwhile, the butyrate, iso butyrate, and valerate proportions were not affected by beet pulp feeding. Similarly, partial substitution of beet pulp for corn in the diet did not affect butyrate and iso-butyrate proportions in dairy cow (Nemati et al., 2020). The ruminal ammonia concentrations were not affected by beet pulp feeding.

Feeding beet pulp did not affect all of the blood metabolites including albumin, glutamic pyruvic transaminase, glutamic oxaloacetic transaminase, total cholesterol, blood urea nitrogen, glucose, total protein, triglyceride, and non-esterified fatty acids. Blood metabolic hormones including leptin, insulin, and growth hormone were not affected by beet pulp feeding. Feeding beet pulp increased insulin-like growth factor 1 contents at week 4 but not at other times. Increased IGF-1 can influence on tissue and muscle satellite cells number that plays an important role in skeletal muscles growth (Johnson et al., 1998). And it can be related with yield index and grade.

3. Carcass, CIA, and physicochemical traits of the LT

In this study, feeding beet pulp tended to improve beef quality grade. The marbling score or the intramuscular fat content is a major determinant of quality grade in Korean cattle steer and Japanese Black cattle (Baik et al., 2023). Numerical increase in marbling score in this study may in part contribute to the increased trend of beef quality grade by beet pulp feeding. Feeding beet pulp also tended to improve beef yield index and yield grade. Rib eye area and back fat thickness positively and negatively affect yield index and thus yield grade, respectively. The numerical increase in rib eye area and the numerical decrease in back fat thickness could be attributed to the increased trend of beef yield index and yield grade by beet pulp feeding. In this study, feeding beef pulp increased beef auction price. Beef auction price is mainly determined by marbling score and quality grade and yield grade in Korea (Beak et al., 2021; Baik et al., 2023). Thus, the increased auction price is primarily due to the increased trends of quality grade and yield grade by beet pulp feeding.

4. Adipocyte cellularity of the LT

The visible marbling and IMF deposition are regulated by the interaction of hyperplasia (cell number) and hypertrophy (cell size) (Cianzio et al., 1985 and Du et al., 2015). Hyperplasia of adipocytes occurs from late growth stage to early fattening stage about 12 ~ 18 month (Schiavetta et al., 1990 and Cianzio et al., 1985). However, there was suggestion that hyperplasia of adipocytes is occurred for whole life of beef cattle (Robelin, 1981). Since, the number of adipocytes did not different between two groups, there was no difference in hyperplasia of adipocytes. In present study, the adipocytes diameter in the LT was from 40 μm to 200 μm of range. The average size of adipocytes was 82.3 μm and 92.9 μm for CF and BP group, respectively. The adipocytes size and interaction were significantly higher in BP group than CF group. Propionate was considered as the main substrate of IMF deposition in ruminants through gluconeogenesis. On the other hands, Nayananjalie et al. (2015) reported that acetate play a larger role than propionate and glucose for IMF deposition in Angus × Simmental steers. In this study, ruminal acetate fermentation was stimulated by feeding beet pulp. Therefore, it can be considered that hypertrophy of adipocytes was more occurred in BP group by ruminal acetate. In addition, adipocytes hypertrophy occurs throughout beef cattle life but can be supposed that it mainly occurs in late fattening stage.

5. Lipid metabolism and muscle growth gene expression in the LT

Feeding beet pulp increased adipocyte cell size in this study. We have hypothesized that beet pulp feeding may change the expression of lipid uptake and transport and lipogenesis genes in the LT, inducing hypertrophy. Beet pulp feeding did not affect expression of lipid uptake and transport genes. Beet pulp feeding upregulated expression of lipogenic FASN gene, although it did not affect acetyl-CoA carboxylase gene expression. The FASN plays a central role in lipogenesis and promoting conversion of palmitic acid from acetyl-CoA and malonyl CoA (Roy et al., 2005). Hillgartner et al. (1995) argued FASN is candidate gene for marker-assisted selection and breeding IMF for improved beef quality and quality grade. Malonyl CoA is a rate-limiting enzyme for lipogenesis, and increased FASN gene expression could contribute to hypertropy of adipocytes. Moreover, there a study that FASN expression was increased in low starch diet in Angus (Graugnard et al., 2009). We found increased ruminal acetate proportion by beet pulp feeding. In ruminants, acetate or glucose are main precursors for lipogenesis (Smith and Crouse, 1984), and acetate was more effective than glucose as the primary substrate for intramuscular fat deposition in cattle (Nayananjalie et al., 2015). Furthermore, feeding beet pulp has a potential decreasing lipolysis gene like PNPLA2 and LIPE that promote conversion of diacylglycerol and monoacylglycerol from palmitoyl-CoA (Daddam et al., 2021) and it may have positive relation with high marbled beef (Ladeira et al., 2018). Gene expression for muscle growth was

not affected by beet pulp feeding, suggesting that activation of muscle growth gene expression is not related to increased tendency of yield grade.

6. Methanogenesis

Livestock generate 14.5% of anthropogenic greenhouse gas (GHG) and GHG emission from CH4 through enteric fermentation in ruminants occupies 39% of the livestock sector (Gerber et al., 2013). The production of methane was measured only in rumen. There a complex procedure producing methane in the rumen which uses diverse substrates such as CO2, formate and acetate. Especially, hydrogenotrophic methanogens are well known as the main methane producers in the rumen (Henderson et al., 2015). The methane generation could be affected by various treatment. Reinartz et al. (2018) reported that forage-based diets can increase the methanogenesis in the rumen. In addition, Acetate is the major precursor about two-thirds of the methane produced from anaerobic bioreactors (Jetten et al., 1992). Compared with corn flake, beet pulp contains rich fibber contents and high percentage of acetate is fermented. However, there was no any difference in methane release including treatment, period and interaction effects between BP group and CF group. The result appears that BP group has slightly higher methane generation. It may be derived from rich fibber contents in beet pulp and ruminal acetate. However, it was not significant.

7. Economic efficiency

Substitution of 28% beet pulp with corn flake reduced feed price about 0.04 $/kg in the BP group (0.76 $/kg) to CF group (0.72 $/kg). Feed cost of head of day and gain of body weight also decreased 6.20 $ to 5.91$ and 8.74 $ to 7.93 respectively. The decreased feed price and cost were derived from lower feed stuff cost and higher meat price in BP group. The average cost of beet pulp was 0.43 $/kg and corn flake was 0.50 $/kg. However, the feed stuff cost is variable depending on market situation. Thus, to replace of beet pulp with corn, the market condition and feed stuff cost stability must be considered in advance. In this study, beet pulp was cheaper than corn flake about 15%. Moreover, the meat price ($/kg) was 12% higher in BP group than CF group (Table 10). Therefore, increased total revenue and economic efficiency would be derived from the foregoing results. This study indicated that partial substitution of corn flake with beet pulp to late fattening Korean cattle steer was economically reasonable depending on market situation.

Ⅵ. Conclusion

The results of present study show that feeding beet pulp in late fattening stage stimulated ruminal acetate, C2/C3 ratio and some genes expression related lipogenesis without adverse effects of growth performance, feed efficiency, blood metabolites and hormones. On the other hand, lipolysis gene expression was decreased. Since above changes, adipocytes size might be enhanced and positively affect IMF deposition and high marbled meat production in Korean cattle steer. The economic efficiency was improved by increased meat price and current feed stuff market condition. However, the accurate breakeven point of feeding beet pulp is needed further research. Beet pulp could be used as a lipogenic energy source for improving beef quality grade without affecting growth performance of cattle.

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Ⅷ. Summary in Korean

현재 국내 사료산업 시장 상황과 축산업의 현황을 고려하였을 때, 인간과 동물의 영양적 경쟁을 줄이고 생산성을 높일 수 있는 사료가 꾸준히 요구되고 있다. 국내 쇠고기 시장에서 근내지방의 축적, 즉 마블링은 쇠고기의 풍미, 기호성 및 소비자의 수요에 가장 결정적인 영향을 미친다. 일반적으로 높은 수준의 전분을 함유하고 있는 곡물 기반 사료를 급여하는 것이 쇠고기의 마블링 함량을 높이는 일반적인 전략으로 적용되었다. 그러나 옥수수와 같이 전분 함량이 높은 사료를 과도하게 급여하면 반추위 pH가 감소하고 반추위 산과다증이 발생할 위험이 있다. 비트펄프는 사탕무에서 당분을 추출한 후 남는 식품 부산물로, 중성 세제 섬유 (NDF)와 펙틴을 비롯한 소화율이 높은 섬유소를 많이 함유하고 있으며, 반추위 안정화에 도움을 주고 Acetate 발효를 촉진하여 반추동물 사료로 적합하다. 또한 비트펄프는 옥수수의 약 85%의 에너지 가치를 가진 대체 에너지원으로 볼 수 있다.

본 연구는 한우 거세우의 비육 후기 사료 내 옥수수의 일부를 비트펄프 펠렛으로 대체 급여하여 한우 거세우의 성장능력, 반추위 발효 특징, 혈액 대사 물질 및 호르몬, 지방합성 관련 유전자 발현, 지방세포의 발달, 도체 특성 및 경제성에 미치는 영향을 분석하였다.

비육후기 한우 거세우 18마리(체중 636±10.9kg; 연령 25.9±0.25개월)를 체중, 연령, 초음파 예측 근내지방을 고려하여 옥수수 플레이크(CF) 그룹과 비트펄프(BP) 그룹으로 나누었다. 건물기준 영양소 요구량의 약 89%는 농후사료로 급여하였고 나머지 11%는 톨페스큐 건초로 급여 되었다. 총 농후사료 중 각각 78%와 72%는 CF 및 BP 그룹에 시중의 일반 농후사료로 제공되었고, 나머지 22%와 28%는 각각 옥수수 플레이크 또는 비트펄프 펠렛으로 급여되었다. 두 그룹의 사료내 총 조단백질과 에너지 수준은 동일하게 설정되었다.

실험은 5주간의 적응기간을 포함하여 총 25주간 진행되었다. 총 증체량, 일당 증체량 및 사료 효율은 비트 펄프의 대체 급여에 의해 영향을 받지 않았다 ($p \geq$

0.79). Ruminal acetate, C2/C3 비율 (둘 다 $p < 0.001$)은 CF 그룹보다 BP 그룹에서 더 높았다. 한편, Ruminal propionate ($p < 0.001$), iso-valerate ($p = 0.002$)의 비율은 CF 그룹보다 BP 그룹에서 더 낮았다. FASN (fatty acid synthase) mRNA 발현 수준은 CF 그룹보다 BP 그룹에서 더 높았다 ($P = 0.03$).

등심 내 근내지방의 세포 크기 ($p < 0.001$)는 CF 그룹보다 BP 그룹에서 더 높았다. 또한, 상대적으로 크기가 작은 근내지방구 세포의 비율은 CF 그룹이 BP 그룹보다 높았으며, 상대적으로 크기가 큰 (100μm 이상)근내지방구 세포의 비율은 BP 그룹이 CF 그룹보다 더 높았다 ($p < 0.04$). 육량지수, 육량등급, 품질등급은 BP 그룹에서 더 높은 경향을 보였다 ($p \leq 0.1$). 1Kg당 지육단가 ($p < 0.001$)는 CF 그룹보다 BP 그룹에서 더 높았으며, 총 수입 ($p = 0.06$)과 총 경제적 효율 ($p = 0.001$)은 BP그룹에서 더 높게 나타났다.

결론적으로, 비육 후기 한우의 사료내 옥수수 일부가 비트펄프로 대체되어도 성장 능력, 혈액 내 호르몬 및 메탄 발생량에 부정적인 영향을 미치지 않으면서, 일부 지방합성 관련 유전자 발현 량을 증가시킬 수 있고, 지방분해 관련 유전자의 발현량을 감소시킬 가능성이 등심의 지방 세포 크기 증가에 영향을 미칠 수 있다. 그 결과, 향상된 근내지방 합성으로 인하여 마블링의 함량이 증가하여 육질 등급의 긍정적인 영향을 미치고 이는 경제적인 효율의 증가로 이어질 수 있다.

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