A THESIS

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# Effects of feeding beet pulp instead of corn on growth performance, rumen fermentation and microbial characteristics, and blood parameters in Korean cattle steers

비트 펄프가 *in vitro* 반추위 발효성상과 한우 거세우의 성장, 반추위 발효와 미생물 및 혈액 성상에 미치는 영향

by

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# Effects of feeding beet pulp instead of corn on growth performance, rumen fermentation and microbial characteristics, and blood parameters in Korean cattle steers

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# Abstract

This experiment evaluated the effects of feeding beet pulp (BP) instead of corn on growth performance, ruminal fermentation characteristics, microbial profiles and blood parameters in fattening steers. In experiment 1, two Holstein steers (1054 ± 0 kg) were used for *in vitro* experiment to compare dry matter (DM) degradability and ruminal fermentation characteristics between corn and BP. In experiment 2, Twelve Korean cattle steers (body weight, 485 ± 19 kg; age, 18.0 ± 0.17 months) were divided into control (corn) and treatment (BP) groups. Both groups were fed the same basal concentrate to meet 80% of the nutrient requirements of Korean cattle for 12 weeks. For the control group, corn (DM) was supplemented for the remaining 20% of the requirement by top dressing; the BP group was supplemented with 17.7% BP plus 2.3% protected-fat to provide the similar energy level as the corn group. In experiment 1, ruminal acetate proportion was higher ($P < 0.001$) in BP at 6, 12 and 24h compared to corn and concentrate. In experiment 2, dry matter intake (DMI), average daily gain (ADG), and feed conversion ratio (FCR) were not affected by feeding BP instead of corn; nor were iso-butyrate, iso-valerate, valerate proportion, or total volatile fatty acid (VFA) concentrations in the rumen fluid ($P > 0.05$). However, acetate proportion ($P = 0.001$) at $4^{th}$ week, insulin concentration ($P = 0.01$) at $12^{th}$ week and relative

abundances of cellulolytic bacteria including *Fibrobacter succinogenes* (P = 0.01) and *Ruminococcus albus* (P = 0.04) at 12$^{th}$ weeks were higher in BP group compared with corn group, whereas propionate and butyrate proportion, or non-esterified fatty acid (NEFA) at 12$^{th}$ week (P $\leq$ 0.04) was lower in BP group compared with corn group. Therefore, this experiment suggests that BP could be a good energy source for increasing the acetate proportion without impairing cattle performance in the fattening period.

**Keyword:** Beet pulp, Corn, Korean cattle, Rumen characteristics, Growth performance

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# List of abbreviations

ADF: Acid Detergent Fiber

BP: Beet Pulp

CP: Crude Protein

IMF: Intramuscular Fat

SCF: Subcutaneous Fat

BWG: Body Weight Gain

DM: Dry Matter

DMI: Dry Matter Intake

FCR: Feed Conversion Ratio

NDF: Neutral Detergent Fiber

NEFA: Non-Esterified Fatty Acids

ME: Metabolizable Energy

$NE_m$: Net Energy of Maintenance

VFA: Volatile Fatty Acids

qPCR: quantification real-time Polymerase Chain Reaction

# Units and marks

%: Percent

kg: Kilogram

d: daily

g: Gram

mg: Milligram

ml: milliliter

mM: Millimole

dL: Deciliter

h: Hour

# Ⅰ. Introduction

Intramuscular fat (IMF) which affects beef quality is one of the most critical traits for beef cattle. IMF is closely related with flavor, tenderness, juiciness, and palatability of beef (Wheeler et al., 1994) in the USA (Hunt et al., 2014).

The lipogenesis of ruminants generally happens in adipose tissue via the conversion of acetate (Vernon, 1980) and glucose to fatty acids (Nafikov and Beitz, 2007). The glucose is preferred as a precursor to improve the IMF in ruminants by several studies (Smith and Crouse, 1984; Schoonmaker et al., 2003). Hence, the corn producing high propionate via ruminal fermentation has been used as an ingredient for diet of beef cattle (Huntington, 1997). However, Nayananjalie et al (2015) suggested that acetate was more effective than glucose for the fat synthesis. Similarly, several studies reported that fat synthesis did not have any preferences in precursor which is acetate or glucose for fat synthesis depending on depot in Hanwoo steers (Lee et al., 2000; Song et al., 2001). From these previous studies, the farmer may improve the efficiency of high quality beef production using some fiber sources which mainly produce the acetate in the ruminal fermentation (Marounek et. al., 1985).

BP is a by-product of the sugar-extracted form from sugar beet. Dried BP contains digestible neutral detergent fiber (NDF) up to 40%, which is abundant in readily fermentable fiber (pectic material) compared with roughage (Abo-Zeid et al., 2017). Degradation of pectin is faster than NDF in roughage, producing more acetate and less propionate compared with starch (Marounek et al., 1985).

Additionally, BP reduces the problem of rumen acidosis (Voelker and Allen, 2003b; Mojtahedi and Mesgaran, 2011). The energy value of BP is approximately 85% of corn (Habeeb et al., 2017) and 83% of net energy in barley (Lofgreen et al., 1962). Therefore, BP can be used as an energy source of cattle for producing acetate in ruminal fermentation (Habeeb et al., 2017; Voelker and Allen, 2003a). In a previous study, pelleted BP can replace corn up to 24% of DM as an energy source in dairy cattle (Voelker and Allen, 2003a). However, limited information is available for utilization of BP as an energy source for producing acetate in beef cattle.

We hypothesized that feeding BP instead of corn for the fattening Korean steer has no negative effects on ruminant health and growth performance. It may increase the acetate production in ruminal fermentation affecting nutrient metabolism. Therefore, the objective of this experiment is to evaluate the effects of feeding BP instead of corn on degradability, growth performance, ruminal fermentation, microbial characteristics, and blood metabolites in fattening Korean steers.

# Ⅱ. Literature review

## 1. Energy sources and lipid metabolism in ruminants

### 1.1. Energy sources in ruminants

#### 1.1.1. Energy metabolism

In Korea, the per capita total meat consumption has increased from 11.3 kg (1980) to 51.3 kg (2014), with a notable increase of beef consumption from 2.6 kg to 11.6 kg. Korean cattle beef is similar to Japanese Wagyu which has the highly marbled fat, thin muscle fibers, specific flavor characteristic flavor, and minimal connective tissue (Kim et al., 1994). Although Korean cattle have lower subcutaneous fat (SCF) depth than Australian Angus, the Korean cattle have higher marbling scores than those of Australian Angus (Cho et al., 2005).

VFAs in the rumen meet about 60 to 75% of digestible energy requirements in cattle (Sutton, 1979). Carbohydrates such as cellulose, hemicellulose, pectin, starch, and sugars are the main substrates utilized by ruminal microbes. Approximately 90% of carbohydrate is digested in the rumen. Although this value can be much lower for diets containing highly processed forages which have slow rates of digestion. Therefore, these are not adequately processed before consumption (Sutton, 1979).

The approximately 75 to 85 % of feed energy is converted to VFAs with heat and $CH_4$ (Sutton, 1979). The main VFAs in the rumen are acetate, propionate, and butyrate. VFAs are produced by digestion of feed by rumen bacteria and serve as the main source for glucose and fat synthesis in ruminants. Carbohydrates in the rumen are prone to hydrolytic digestion by the microorganisms. The major end products of ruminal fermentation are VFA, $CH_4$, $CO_2$, and microbial cell (Wolin et al., 1997). Proteins are degraded to peptide and amino acids, which are incorporated into bacterial cells or deaminated to form $NH_3$, VFA, and $CO_2$.

The branched-chain VFAs such as iso-butyrate, iso-valerate are important sources for supporting the growth of some ruminal bacteria (Allison, 1969).

The relative proportion of VFAs produced in the rumen depends on the chemical composition of the diet including additives, and factors affecting the ruminal condition (Sutton, 1979). High-forage diet can increase the acetate concentration in rumen. Hence, the C2:C3 ratio is above 3:1 (Owens and Goetsch, 1988; Hobson, 1997). On the contrary, the high-concentrate diet can increase the propionate concentration. Hence, the C2:C3 ratio is lower than 2:1 (Orskov et al., 1991; Bevans et al., 2005; Wierenga et al., 2010; Li et al., 2011).

Propionate is the main precursor for gluconeogenesis in cattle. About 27 to 54% of the glucose in the ruminant is formed from propionate (Sano et al., 1995; Lindsay, 1970). The 80 to 95% of propionate is absorbed through the epithelium in the rumen and converted to phosphoenolpyruvate from methylmalonyl-CoA, succinate, and oxaloacetate (Fahey and Berger, 1988; Kristensen and Harmon,

2004).

### 1.1.2. Grain

Typical grains are wheat, corn, barley, oat, rye, and sorghum for diet of beef cattle. The corn is very important ingredient in cattle diet, due to its low cost and high energy value. Soy and barley are normally used to complement corn and provide various nutrients. Contents of grain source should be increased gradually in cattle diet, because overconsumption can cause the health problems. Also, grains are normally processed to minimize the amount of undigested waste.

Since the 1940s, the beef industry has used corn as a feed ingredient in fattening cattle diet (Corah, 2008). Corn used for cattle (yellow dent corn) normally consists of 5 components (endosperm; 82.9%, germ; 11%, bran coat; 5.3%, and tip cap; 0.8%). Corn contains approximately 72% starch (Huntington, 1997), which is mainly located in the endosperm. The starch of corn is a glucan comprised of two main types of molecules: amylopectin which is α1-4 linkages and amylose which is α1-4 and α1-6 linkages. Amylopectin is a branched polymer which is the highest component of starch (Rooney and Pflugfelder, 1986). Hence, corn is selected as the main energy source by most of the animal nutritionists (Vasconcelos and Galyean, 2007). Since the 1980s, the use of corn for alcohol fuel has increased from 0.7% to 49.4%, leading to a decline from 86.5% to 41.1% as a source of animal feed, and the price has increased until now (ERS, 2012; ERS, 2018). Therefore, many researchers have found new ingredients for

substituting corn with an inexpensive and effective energy source, with byproducts which are normally high fiber and low starch such as dried distiller's grains with solubles.

### 1.1.3. Beet pulp (BP)

BP is a by-product of the extraction of sucrose from the sugar beet (*Beta vulgaris*; Bichsel, 1988). One ton of sugar beet produces 149kg sugar and 50kg by-product. The chemical composition of BP can be affected by location, year, and process. BP is utilized in some feeding experiments with ruminants. BP also includes a significant amount of gums (especially pectic materials), which also contains about 25% of dried BP (Walter al., 1991). Pectic materials are a session of polysaccharides that are variable and partly, soluble in water, and are particularly recognized for their gelling characteristics in the existence of acid or sugar. Pectin is located in the highest concentrations in the middle lamella of plant cells (Mauseth, 1988). The pectin has various molecular weight and chemical composition among sources, but the main constituent of pectin is D-galacturonic acid, fused by α1,4 linkages (Walter, 1991). The energy value of BP is approximately 85% energy of corn (Habeeb et al., 2017) or 83% net energy of barley (Lofgreen et al., 1962). Several previous studies reported that the substitution for corn with BP increased NDF digestibility and decreased DMI caused by ruminal filling effects. However, the fat-corrected milk yield is similar or greater compared to cattle fed corn-based diet. Thus, the authors concluded

that BP might increase the efficiency of conversion of feed to milk, also BP could be substituted for corn partially (Voelker and Allen, 2003a, b, c).

## 1.2. Energy and lipid metabolism

In ruminants, adipose tissue has been recognized as the main depot for synthesizing fatty acid, contrasting with non-ruminants which have the principal depot is the liver (Vernon, 1980; Christie, 1981). Adipocyte growth is dependent on adipogenesis (Du et al., 2013). Adipogenic cell is derived from mesenchymal progenitor cells (Du et al., 2013). Adipogenesis consist of several steps, including commitment of mesenchymal stem cells to preadipocytes, determination and proliferation of preadipocytes, and differentiation and proliferation of preadipocytes, and differentiation of preadipocytes into adipocyte (Hausman et al., 2009). The growth of fat cell normally occurs as the mass increase through hyperplasia and hypertrophy (Hood and Allen, 1973; Owen et al., 1993). After occurring hyperplasia until birth mainly, the hypertrophy occurs to fat deposition from 250 day of age to fattening periods. Both hyperplasia and hypertrophy contributed to fat deposition (Esteve, 2014). Thus, strategies to increase the hyperplasia and hypertrophy of adipocyte are important to improve fat deposition for producing high quality meat. Triglyceride synthesis is a main factor for IMF deposition (Pethick et al., 2004). NEFA and glycerol are required for triglyceride synthesis. Glucose and acetate are utilized for fatty acid synthesis.

However, the acetate is more predominant as a substrate for fatty acid synthesis from ruminal fermentation. Glucose also contributes to fatty acid *de novo* synthesis in ruminants. Glucose is derived from gluconeogenesis using propionate/lactate or absorbed glucose in small intestine (Nafikov and Beitz, 2007).

Smith and Crouse (1984) reported fat synthesis has other processes depending on fat depots. The authors suggested that fat synthesis of the IMF uses glucose as the main precursor, but the fat synthesis of SCF uses the acetate as the main precursor. Recently, Nayananjalie et al (2015) suggested that palmitate synthesis rate from acetate was 13 times higher compared to glucose regardless of fat depot including IMF and SCF. The authors concluded that acetate is a major source for fat synthesis regardless of the fat depot. This result is in agreement with several studies (Lee et al., 2000; Song et al., 2001), but not with Smith and Crouse (1984). According to Miller et al (1991), different breeds could make the various unique adipose tissues that could be one of the reasons for different lipogenesis metabolism of IMF and SCF. However, if the acetate in the rumen can be used for improving IMF in ruminants like the previous study, the researchers and farmers can produce more marbled fat efficiently. Possibly, the different results among the previous studies might be the difference between *in vitro* and *in vivo* experiment. Thus, more researches about association of the acetate with IMF deposition in the beef cattle are required.

Normally, beef production needs higher cost and longer duration compared to

swine and broiler relatively. Thus, finding a precise and suitable indicator for expecting the degree of IMF before slaughter is important. Leptin, glucose, and insulin concentrations have been suggested as indicators of IMF (Trenkle and Topel, 1978; Matsuzaki et al., 1997; Wegner et al., 2001). Leptin released by fat cells contributes to control energy balance and feed intake, also its effects on fat accumulation in humans and animals (Xie et al., 1999). Some previous studies have proved the association between leptin and IMF in beef cattle (Bonnet et al., 2007; Geary et al 2003; Wegner et al., 2001)

Bonnet et al., 2007 studied the correlation between leptin and IMF using Angus (n = 10) steers, Limousin (n = 12) and Japanese Black × Angus (n = 10). IMF separated from the longissimus thoracis muscle and SCF were analyzed to estimate the degree of leptin mRNA expression. This study showed that Angus had higher leptin mRNA compared to the other breeds. Moreover, Angus had a higher amount of IMF. Thus, the authors concluded that leptin could be associated with IMF contents.

Geary et al (2003) used leptin as a marker of beef composition. Tarentaise (n = 22), Charolais (n = 22), and Red Angus (n = 44) were utilized under the feedlot condition. This study demonstrated that leptin concentration affected the degree of marbling positively. Thus, the authors suggested that leptin could be a valuable marker for measuring the degree of IMF.

However, Wegner et al., 2001 reported that the differences were shown in the leptin level among the groups of Wagyu crossbred with 0%, 50% (Wagyu / Angus) and 75% (Wagyu / Angus, Holstein, or Simmental) Wagyu. Here is a

positive correlation between leptin concentration and fat content in longissimus thoracis of pure Wagyu breed (0% Wagyu/Angus), while 50% Wagyu cattle almost didn't show the correlation. In contrast, the 75% Wagyu crossbred cattle showed a negative correlation between leptin level and fat content of longissimus thoracis muscle. The authors concluded that leptin and marbling were correlated, and negative correlation occurred with the genetic increase of Wagyu. It could be attributed to breed effect on factor about IMF deposition. In summary, leptin could be used as an important marker of an IMF previous slaughter.

Insulin and glucose are important factors in the growth of adipose tissues. Glucose is the main energy source and fragment for synthesizing the fat in muscle fibers (Shingu et al., 2001). Insulin is an important factor for regulating transcription of the lipogenic enzyme, which controls fat synthesis (Wong and Sul, 2010). High insulin concentration may cause hyperinsulinemia and lead to down-regulated insulin receptors in tissues such as adipocytes, hepatocytes, and myocytes; it could cause insulin insensitivity (Wellnitz, 2014). Thus, glucose in the blood is used for synthesizing fatty acids in the liver, forming triglycerides, and released it into circulation, then possibly deposited in the tissue such as IMF (Carmen et al., 2006).

Mir et al (2002) examined the glucose tolerance test from intravein and its connection to IMF using Limousin, Wagyu × Limousin, and Wagyu. The authors showed that Limousin steers had a lower insulin response to glucose than Wagyu steers that was not expected because the Wagyu breed normally shows the higher fat content than the Limousin. Finally, the authors concluded that the

intravenous glucose tolerance test was not a proper marker of IMF. Similarly, Shingu et al (2001) showed insulin concentration and glucose response to insulin using Japanese Black heifers (beef) and Holstein heifers (dairy). Normally, Japanese Black tends to show high IMF deposition. The insulin secretion was higher in Japanese Black heifers than Holstein heifers after sexual maturation, while glucose response to insulin was not different between breeds. Thus, insulin might be correlated with IMF, but not glucose response to insulin in cattle. Thus, insulin can be a proper indicator for estimating IMF.

## 2. Effects of BP on growth performance, ruminal fermentation, and lipid metabolism in ruminants

### 2.1. Effect of BP on growth performance.

The BP is generally used for energy sources to replace the grain ingredients in cattle (Abo-Zeid et al., 2017; Voelker and Allen, 2003; Mojtahedi and Mesgaran 2011). The energy value of BP is approximately 85% of corn (Habeeb et al., 2017).

The one group (Abo-zeid et al., 2017) examined the effects of BP on male buffalo calves (237.2 ± 24.46 kg, 12~14 months) with replacing corn in the diet with BP (0, 333, 667, 1000 g/kg). ADG, body weight gain (BWG), and FCR were more efficient in BP addition groups than corn group. Likewise, total VFA, the concentrations of acetate and butyrate, C2:C3 ratio, and protozoa were higher in BP addition groups than corn group, while propionate and iso-propionate were

lower in treatment groups compared to the corn group. Intake and total apparent digestibility of organic matter, NDF and acid detergent fiber (ADF) were higher partially in BP addition groups compared to corn group. The authors concluded that BP could improve ruminal fermentation. And then it might increase ADG.

Similarly, Voelker and Allen (2003) reported that BP could decrease DMI, when corn was replaced by 24% with BP in the dairy cow diet while DMI was not decreased by a 12% substitution group. The BP has not impact on body weight, total VFA, and milk yield in all groups. The authors concluded that BP might increase ruminal fermentation. The substitution of BP for highly fermentable grain could promote ruminal fermentation by increasing the chewing time and ruminating time (Mojtahedi and Mesgaran, 2011). The fermentation rate of pectin is normally faster than insoluble carbohydrates (cellulose and hemicellulose), while it is slower than soluble carbohydrates (sugar and starch). In *in vitro* study using rumen microorganism from goats fed concentrates and hay, the rate of VFA formation from pectin was faster than hemicellulose at the normal range of ruminal pH (Marounek et al., 1985). Pectin is fermented faster than insoluble fiber such as cellulose and hemicellulose. Pectin can stimulate the degradability of cellulose and hemicellulose due to postponed greatest fermentation of pectin for some hours after feeding, which can make the stability of ruminal pH concentration. And slow fermentation of pectin can increase flow rate time in rumen that can increase the physiological activation of rumen bacteria. Thus, increased pectin such as BP may offer and improvement

in ruminal fermentation of carbohydrate in diets for beef cattle without negatively affecting the digestion of cellulose and hemicellulose.

Therefore, the replacement of corn with BP can increase the rumnal fermentation in cattle from increasing NDF, chewing time, retention time in rumen.

## 2.2. Effect of BP on ruminal fermentation and microbial population

Among components of dairy cow feed, BP has abundant pectin approximately 25%, DM (Walter, 1991). Insoluble fiber constituents of pectin are digested broadly and quickly (Torrent et al., 1994; Bhatti and Firkins, 1995). BP was attractive as a substitute ingredient to roughage and grain to increase the digestibility of the diet. Some evaluations have been conducted to check the fermentation rate among BP, other by-products, forages, and grains on *in vitro* and *in situ* (Chester-Jones et al., 1991; Sanson, 1993; Mansfield et al., 1994; Sunvold et al., 1995; Bach et al., 1999).

Mansfield et al (1994) examined intake and production using dairy cattle which were fed with diet containing 15% corn plus 15% BP or 30% com group. The authors used continuous culture for 10 days to evaluate the features of fermentation and digestion. BP has no impact on the degradability of DM, organic matter, non-structural carbohydrates, NDF, and ADF. The concentration of acetate was higher, while the concentrations of butyrate and branched-chain

VFA were decreased in the BP diet compared to the high-corn diet. However, propionate concentrations and total VFA were not different. Similarly, Chester-Jones et al (1991) substituted rolled maize with dried BP at 0%, 15%, and 30% of diet DM to evaluate the features of fermentation and digestion using continuous culture for 8 days. BP addition increased the acetate concentration, but decreased butyrate and iso-butyrate concentration. However, the BP did not affect propionate concentration.

Voelker and Allen (2003) examined the effect of BP on the dairy cow on *in vivo*. They replaced corn with BP at 0%, 6%, 12% and 24% of diet DM. The correlation between the ruminal pH and the fiber digestion rate was shown. Higher pH concentration was correlated with the rate of digestion of NDF with increased minimum pH level. It can lead to reducing the risk of acidosis in the rumen. Moreover, increasing the addition of pelleted BP increased C2:C3 ratio without any changes in total VFA. In another *in vivo* study, Abo-Zeid et al, (2017) reported that the substitution of corn with BP at 0 g/kg, 333 g/kg, 667 g/kg, and 1000 g/kg, respectively increased the total VFA, acetate and butyrate concentrations, C2:C3 ratio, and protozoa, while propionate and iso-butyrate concentrations decreased due to pectin levels increased. This result is consistent in the Holstein steer study (Mojtahedi and Mesgaran, 2011).

The previous studies in the 1950s and 1960s discovered *Prevotella ruminicola, Butyrivibrio fibrisolvens, Succinivibrio dextrinosolvens, Lachnospira mulitparus*, and *Fibrobacter succinogenes* which could ferment pectin (Bryant and Doetsch, 1954;

Bryant and Small, 1956a; Bryant and Small, 1956b; Bryant et al., 1958). Pectin fermentation in rumen produced less propionate and lactate (Strobel and Russell, 1986; Alamouti et al., 2014) while produced more acetate (Marounek et al 1985). Hence, as mentioned above, BP addition increases C2/C3 ratio in the rumen.

Increasing the rate of fermentation of feed can decrease the ruminal pH level and it could lead to a reduction of ruminal fermentation (Strobel and Russell, 1986). A fast passage flow is a characteristic of grain ingredient such as corn, which means short retention time in the rumen and subsequently reduced rumination time (Poorkasegaran and Yansari, 2014). In contrast, physically effective fiber (peNDF) increased by BP encourages saliva secretion, chewing and rumination activity (Yang and Beauchemin, 2006) ensuring a suitable ruminal condition for microbial growth (especially fibrolytic bacteria such as *Fibrobacter succinogenes, Ruminococcus albus, and Ruminicoccus flavefaciens).* Thus, replacing the corn with BP can increase the stability of pH, rumen condition for microorganism, and availability of fiber source.

## 2.3. Effect of BP on lipid metabolism

As mentioned above, BP tends to increase acetate concentration in ruminal fermentation (Mansfield et al., 1994; Abo-Zeid et al., 2017). However, propionate was the main source for IMF (Smith and Crouse, 1984). On the contrary, the acetate contributes to about 80 percent of the acetyl unit for lipogenesis in SCF,

while providing 10 to 25 percent contribution in IMF on *in vitro*. As mentioned above, Nayananjalie et al (2015) suggested that the acetate was more efficient than glucose. Therefore, further study is required to define role of BP on lipogenesis in the muscle of beef cattle.

Several studies reported that triglyceride, cholesterol, NEFA, and other blood parameters were not modified by BP (O'Mara et al., 1997; Mojtahedi and Mesgaran, 2011; Abo-Zeid et al., 2017). Abo-zeid et al., (2017) showed that glucose was not changed by BP despite decreased propionate in ruminal fermentation. The propionate in ruminal fermentation is the main glucogenic precursor in ruminant (Hanmmon et al., 2010). The author concluded that the flux of propionate conversion to glucose in the liver could cause no difference in spite of decreased propionate. Mojtahedi and Mesgaran (2011) reported similar results with Abo-zeid et al., (2017). However, blood urea nitrogen, triglyceride, insulin-like growth factor-1, cortisol, and $T_4$ (thyroxine) were increased partially in the BP addition group compared to corn group. On the contrary, cholesterol and creatinine were decreased. The authors concluded that decreased triglyceride and cholesterol in blood could stimulate fat synthesis and inhibit lipolysis. O'Mara et al (1997) examined effects of replacing unmolassed BP with ground corn, wheat, and wheat treated with sodium hydroxide in the dairy cow. Glucose and NEFA did not show differences between ground corn and BP addition group. Mandebvu and Galbraith (1999) reported that replacing barley grain with BP quadratically increased glucose and insulin, but decreased propionate

concentration. However, the reasons for increased glucose and insulin were not clear. These results indicate that replacing the BP with the grain such as corn and barley has no impact on blood glucose concentration, but it sometimes increases the insulin. However, pectin which is the fermentable NDF in BP tends to produce less propionate in ruminal fermentation (Strobel and Russell, 1986). Propionate is the main glucogenic precursor in ruminant (Hanmmon et al., 2010). Possibly, the fiber source including pectic materials in BP may improve the ruminal fermentation.

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# Ⅲ. Materials and Methods

All experimental procedures involving animals were approved by the Seoul National University Institutional Animal Care and Use Committee (SNUIACUC: SNU-180717-3), Republic of Korea, and conducted in accordance with the Animal Experimental Guidelines provided by SNUIACUC.

The experiments were conducted at the University Animal Farm of the College of Agriculture and Life Sciences, Pyeongchang Campus of Seoul National University, South Korea.

## *1. In vitro experiment*

Ruminal fluid was acquired from two cannulated Holstein steer (average body weight:1054 kg) 1h before morning feeding and pressed using four layers of cheesecloth into a vessel flushed with $O_2$ – free $CO_2$. The cattle were supplied with a basal diet *ad libitum* twice a day (8:00 and 16:00). The rumen fluid was diluted with buffer (Miyazaki et al., 1989) at a ratio of 1:2 (v/v). The samples (Table 2) were added to 30mL of rumen fluid-buffer solution in 60mL serum bottles. The serum bottles were capped with rubber stoppers and aluminum cap. Samples were incubated in a 39℃ incubator for 0, 6, 12, 24, and 48h. After incubating the samples, the pH of samples was measured with pH meter (SevenEasy pH model

AG 8603; Mettler-Toledo, Schwerzenbach, Switzerland). The samples were centrifuged (3600rpm, 30min). The supernatant was used for analyzing the VFA by gas chromatography Agilent Technologies 7890A (Hewlett Packard, Waldbronn, Germany) with a Supelco fused silica capillary column (30 m × 0.25 mm × 0.25μm). And pellet was dried in 65℃ for 24 h for analyzing the DM degradability.

Table 1**.** Chemical compositions (%, DM) of corn, beet pulp, and concentrate used for *in vitro* fermentation.

| Items | Corn | Beet pulp | Concentrate |
|---|---|---|---|
| NDF | 15.9 | 40.3 | 18.2 |
| ADF | 2.77 | 22.5 | 5.66 |
| Crude protein | 7.13 | 9.33 | 14.8 |
| Ether extract | 3.44 | 0.86 | 3.72 |
| Ash | 1.36 | 3.58 | 6.33 |

Table 2**.** Preparation (mg DM / bottle) of corn (C), beet pulp (BP), concentrate (Con) or mixtures used for *in vitro* fermentation.

| Items | Corn | Beet pulp | Concentrate | Con90 + C10 | Con90 + BP10 | Con80 + C20 | Con80 + BP20 |
|---|---|---|---|---|---|---|---|
| Corn | 214 | - | - | 18.0 | - | 36.0 | - |
| Beet pulp | - | 222 | - | - | 22.0 | - | 44.0 |
| Concentrate | | | 222 | 203 | 199 | 203 | 199 |

Con90 + C10; concentrate (90%) + corn (10%), Con80 + C20; concentrate (80%) + corn (20%), Con90 + BP10; concentrate (90%) + beet pulp (10%), Con80 + BP20; concentrate (80%) + beet pulp (20%).

## 2. *In vivo feeding trials*

### *2.1 Animals, diet, and experimental design*

Twelve Korean cattle steer with body weight 485 ± 19 kg and the age of 18.0 ± 0.17 months were used. All steers were fed with a fattening period concentrate diet using an automatic feeding system (DeLaval Alpro system, DeLaval, Sweden) and oat grass. Steers were divided into control (corn) and treatment (BP) groups. Before the experiment, all steers were fed the same basal concentrate to meet 80% of the nutrient requirements, and then corn (DM) was supplemented for the remaining 20% of the requirement by top dressing on oat grass (2kg as fed) during 2 weeks. Both groups were fed the same basal concentrate to meet 80% of the nutrient requirements of Korean cattle for 12 weeks. The chemical compositions of experimental diets are provided in Table 3. For the control group, corn (DM) was supplemented for the remaining 20% of the requirement by top dressing on roughage. The BP group was supplemented with 17.7% BP plus 2.3% fat (DM: 99.0, Ether extract: 82.3, Ca: 6.70) as DM basis to provide the similar energy level as the corn group. The chemical compositions of protected-fat were supplied by company (EUNJIN Int'l Bio-Technology USA CO, INC). Water was supplied *ad libitum*. Daily feed intake of a concentrate diet was measured automatically online using the DeLaval Alpro system. The equal volumes of forage and supplements such as corn or BP were supplied twice a day at 08:00 and 16:00. The residue of forage and ingredients were measured. The body weight was

recorded at 9 am before feeding at the beginning date and at $4^{th}$, $8^{th}$ and $12^{th}$ weeks. The diets were monthly collected and stored in - 20℃ until analysis.

Table 3. Feed ingredients and chemical composition of experimental diets on DM basis.

| Items | Percentage (%) | | | |
|---|---|---|---|---|
| Group | Concentrate | Oat grass | Corn | Beet pulp |
| Ingredients, % DM | | | | |
| Ground corn | 6.37 | - | - | - |
| Ground wheat | 13.1 | - | - | - |
| Sodium bicarbonate | 0.66 | - | - | - |
| Wheat flour | 10.8 | - | - | - |
| Salt | 0.21 | - | - | - |
| Molasses | 2.83 | - | - | - |
| Ammonium chloride | 0.16 | - | - | - |
| Palm oil | 0.22 | - | - | - |
| Condensed molasses solubles | 1.35 | - | - | - |
| Whole cottonseed | 0.55 | - | - | - |
| Steamed flaked corn | 29.4 | - | - | - |
| Distiller's dried grains with solubles | 1.00 | - | - | - |
| Rice bran | 1.97 | - | - | - |
| Corn gluten feed | 26.8 | - | - | - |
| Limestone | 3.00 | - | - | - |
| Palm kernel meal | 1.40 | - | - | - |
| Mineral-vitamin premix[1] | 0.21 | - | - | - |
| Total | 100.0 | - | - | - |
| Chemical composition, % DM | | | | |
| DM | 87.8 | 91.1 | 86.7 | 89.4 |
| Crude protein, CP | 16.0 | 5.39 | 7.73 | 9.09 |
| Ether extract, EE | 4.14 | 1.94 | 2.99 | 0.86 |
| Ash | 7.22 | 3.51 | 1.11 | 3.61 |
| NDF | 23.8 | 51.5 | 15.0 | 42.5 |

| | | | | |
|---|---|---|---|---|
| ADF | 8.64 | 28.0 | 2.79 | 23.1 |
| Non fiber carbohydrate, NFC[2] | 48.8 | 37.7 | 73.2 | 44.0 |
| Ca | 1.39 | 0.21 | 0.02 | 0.85 |
| P | 0.58 | 0.15 | 0.23 | 0.07 |
| ME[3], Mcal/kg | 3.51 | 3.54 | 3.71 | 3.65 |

[1] Mineral and vitamin premix contained Vit. A, 2,650,000 IU; Vit. D3, 530,000 IU; Vit. E, 1,050 IU; Niacin, 10,000 mg; Mn, 4,400 mg; Zn, 4,400 mg; Fe, 13,200 mg; Cu, 2,200 mg; I, 440 mg; Co, 440 mg (Grobic-DC, Bayer Health Care, Leverkusen, Germany).

[2] NFC (%) = 100 – (CP + EE + ash + NDF).

[3] ME (Mcal/kg) = (1.01 x (DE) – 0.45) + 0.0046 x (EE-3) (NRC, 2016).

Table 4. Supplement amounts of corn and beet pulp group

| Items | Supplement group | |
|---|---|---|
| | Corn | Beet pulp |
| Substitutions, % of concentrate | | |
| DM | | |
| Corn | 20.0 | |
| Beet pulp | - | 17.7 |
| Rumen protected fat | - | 2.30 |
| Energy value of supplement | | |
| ME[1], Mcal/kg of DM | 3.71 | 3.65[2] |

[1] ME (Mcal/kg) = (1.01 x (DE) – 0.45) + 0.0046 x (EE-3) (NRC, 2016).

[2] Beet pulp; 3.47 (Mcal/kg), Rumen protected fat; 7.82 (Mcal/kg). The ME of beet pulp group was calculated with beet pulp and rumen protected fat.

### *2.2 Blood collection*

Blood samples were collected 4 times (at $0^{th}$, $4^{th}$, $8^{th}$ and $12^{th}$ weeks) after fast during 9 hours by jugular venipuncture with both a non-heparinized vacutainer (20 mL; Becton-Dickinson, Franklin Lakes, NJ, USA) and ethylenediaminetetra acetic acid-treated vacutainer (20 mL). Serum and plasma were centrifuged at 1,500 g at 4℃ for 15 min and stored at -80℃ until analysis.

### *2.3 Blood analysis*

The blood serum was used for metabolite analysis. Reagents to analyze triglycerides, glucose, total cholesterol and NEFA, were purchased from JW Medical (Seoul, Korea). The analytical reagents for NEFA were obtained from WAKO (Osaka, Japan). All of these items were analyzed using an automated chemistry analyzer (Hitachi 7180; Hitachi, Tokyo, Japan). All of the analysis methods were verified in our laboratory, as previously reported (Kang et al., 2016). The serum leptin and insulin concentrations were quantified using enzyme-linked immnunosorbent assays (ELISA) according to the manufacturer's instructions. Leptin was analyzed using a Bovine Leptin ELISA kit (MyBioSource, San Diego, CA, USA) and insulin was analyzed using a Mercodia Bovine Insulin ELISA (Mercodia AB, Uppsala, Sweden). The intra- and inter-assay coefficients of variation for the insulin kit were 3.83 and 7.10% based on bovine serum samples. And those of leptin were less than 10% based on bovine serum samples.

### *2.4 Analysis of chemical composition of diets*

The chemical composition (DM, crude protein, EE, NDF, ADF, ash, calcium, and phosphorus) of the concentrate diet and oat grass was determined using the AOAC (1996). The NDF and ADF contents were analyzed using the sequential method with the ANKOM200 Fiber Analyzer (Ankom Technology Corp., Macedon, NY, USA) and reagents.

### *2.5 Rumen fluid collection and analysis*

After collecting blood, rumen fluid was extracted using oral stomach tube method (Shen et al., 2012) after 3h feeding (maintaining the fasting for more than 9 hours). The pH of rumen fluid was measured directly using a pH meter (Ohaus Corp., Parsippany, NJ, USA). For analyzing VFA, 1 mL of rumen fluid was added with 0.2 mL of 25% meta-phosphoric acid and kept at -20℃ until analysis. A total of 30 mL rumen fluid was kept at -20℃ for the ruminal ammonia nitrogen ($NH_3$-N) measurement. $NH_3$-N was analyzed using a modified colorimetric method.

### *2.6 gDNA extraction and microbial population analysis by quantitative real-time PCR*

The primer information was shown in Table 5. Genomic DNA was extracted from pellets of rumen contents collected in $8^{th}$ and $12^{th}$ weeks, using DNeasy PowerSoil kit (Qiagen) following manufacture's protocol. qPCR use the

QunatiTest SYBR Green RT-PCR Master Kit (Qiagen, Valencia, CA, USA) for comparing fold difference of microbes between corn and BP group. gDNA was used 10ul ranged from 1 to 20ng. SYBR Green RT-PCR Master Mix was used 12.5ul, and each forward and reverse primer was used 1.25ul. Total solution was 25ul for total reaction. Temperature conditions of qPCR were 95℃ for 10min, 95℃ for 15 sec (denaturation), annealing and extension (60℃, 1min), desaturation and annealing and extension ware processed 40 cycle. And final melting was processed in from 55℃ to 95℃. For primer efficiency test, genomic DNA was serially diluted 10-fold from 20,000 pg to 0.020 pg. A PCR reaction was then carried out with each primer pair using the templates of serially diluted genomic DNA. The PCR was performed in same conditions previous mentioned. The PCR efficiency was calculated: E = 10(-1/slope)-1.

Table 5. Primer efficiency (%) and primers of selected microbes for the quantification real-time polymerase chain reaction (qPCR) assay

| | Forward / Reverse | Primer sequence | Reference | Efficiency (%) |
|---|---|---|---|---|
| Total bacteria | F | CGGCAACGAGCGCAACCC | Denman and McSweeney (2006) | 108.1 |
| | R | CCATTGTAGCACGTGTGTAGCC | | |
| Total protozoa | F | GCTTTCGWTGGTAGTGTATT | Sylvester et al. (2004) | 80.3 |
| | R | CTTGCCCTCYAATCGTWCT | | |
| Total fungi | F | GAGGAAGTAAAAGTCGTAACAAGGTTTC | Denman and McSweeney (2006) | 96.1 |
| | R | CAAATTCACAAAGGGTAGGATGATT | | |
| Methanogen | F | GAGGAAGGAGTGGACGACGGTA | Ohene-Adjei et al., (2007) | 95.5 |
| | R | ACGGGCGGTGTGTGCAAG | | |
| *Anaerovibrio lipolytica* | F | TGGGTGTTAGAAATGGATTCTAGTG | Khafipour et al., (2009) | 108.8 |
| | R | GCACGTCATTCGGTATTAGCAT | | |
| *Fibrobacter succinogenes* | F | GGAGCGTAGGCGGAGATTCA | Denman and McSweeney (2006) | 98.5 |
| | R | GCCTGCCCCTGAACTATCCA | | |
| *Ruminobacter amylophilus* | F | CTGGGGAGCTGCCTGAAT | Stevenson and Weimer (2006) | 91.8 |
| | R | CATCTGAATGCGACTGGTTG | | |
| *Ruminococcus albus* | F | CCCTAAAAGCAGTCTTAGTTCG | Koike and Kobayashi (2001) | 98.0 |
| | R | CCTCCTTGCGGTTAGAACA | | |
| *Ruminococcus flavefaciens* | F | CGAACGGAGATAATTTGAGTTTACTTAGG | Denman and McSweeney (2006) | 92.4 |
| | R | CGGTCTCTGTATGTTATGAGGTATTACC | | |
| *Streptococcus bovis* | F | TTCCTAGAGATAGGAAGTTTCTTCGG | Stevenson and Weimer (2006) | 112.0 |
| | R | ATGATGGCAACTAACAATAGGGGT | | |
| *Succinimonas amylolytica* | F | CGTTGGGCGGTCATTTGAAAC | Khafipour et al., (2009) | 85.8 |
| | R | CCTGAGCGTCAGTTACTATCCAGA | | |

| | | | | |
|---|---|---|---|---|
| *Methanobrevibacter* | F<br>R | CCTCCGCAATGTGAGAAATCGC<br>TCWCCAGCAATTCCCACAGTT | Ramírez-Restrepo et al., (2016) | 94.6 |

### *3. Statistical analysis*

Statistical analyses were done with R studio for Window software (R Studio, Boston, MA, USA). All data are expressed as the mean ± standard error. Statistical significance of all data were analyzed with one-way analysis of variance (ANOVA). The threshold for significance was $P \leq 0.05$, and tendencies are indicated by $0.05 < P \leq 0.10$

# Ⅳ. Results

## 1. Experiment 1: *In vitro* study

### *1.1 DM degradability and ruminal fermentation characteristics*

There was no difference in DM degradability among the corn, BP, and concentrate group (Table 6). BP addition did not affect DM degradability.

There was no difference in ruminal pH among the groups (Table 7). Ruminal acetate proportion was higher ($P < 0.001$) in BP at 6, 12 and 24h compared to corn and concentrate (Table 8). Additionally, ruminal acetate proportion was higher ($P < 0.001$) in concentrate 80% + beet pulp 20% (Con80 + BP20) group at 6, 12, and 24h compared to concentrate 80% + corn 20% (Con80 + C20). Ruminal propionate proportion was higher ($P < 0.001$) in corn and concentrate at 6, 12, and 24h compared to BP, but not mixture groups. Ruminal iso-butyrate proportion was higher ($P < 0.001$) in corn and concentrate at 12 and 24h compared to BP, but not mixture group. Ruminal butyrate proportion was higher ($P < 0.001$) in corn at 6, 12, and 24h compared to BP. Additionally, ruminal butyrate proportion was higher ($P < 0.001$) in concentrate 90% + corn 10% (Con90 + C10) at 12h compared to concentrate 90% + beet pulp 10% (Con90 + BP10) and was higher ($P < 0.001$) in Con80 + C20 at 6 and 12h compared to

Con80 + BP20. Ruminal iso-valerate proportion was higher ($P < 0.001$) in corn at 6, 12, 24, and 48 compared to BP. Additionally, ruminal iso-valerate proportion was higher ($P < 0.001$) in Con90 + C10 at 12 and 24h compared to Con90 + BP10 and was higher ($P < 0.001$) in Con80 + C20 at 12h compared to Con80 + BP20. Ruminal valerate proportion was higher ($P < 0.001$) in concentrate at 12, 24, and 48h compared to BP and corn. Ruminal total VFA concentration was higher ($P < 0.001$) in BP at 12 and 24h compared to corn, but not mixture groups. Ruminal C2/C3 ratio was higher ($P < 0.001$) at 6, 12 and 24h in BP compared to corn and concentrate, but not mixture groups.

Table 6. I*n vitro* DM degradability (%) of corn (C), beet pulp (BP), concentrate (Con) or mixtures at various time

| Incubation time(h) | Groups | | | | | | | SEM[1] | P-value |
|---|---|---|---|---|---|---|---|---|---|
| | Corn | Beet pulp | Concentrate | Con90 + C10 | Con90 + BP10 | Con80 + C20 | Con80 + BP20 | | |
| 0 | 1.53 | 1.68 | 1.08 | 1.50 | 1.30 | 1.18 | 1.39 | 0.24 | 0.32 |
| 6 | 47.3 | 46.4 | 45.5 | 45.5 | 46.3 | 43.9 | 46.0 | 0.69 | 0.16 |
| 12 | 67.0 | 68.5 | 68.9 | 69.5 | 72.8 | 68.1 | 68.4 | 3.26 | 0.51 |
| 24 | 82.0 | 81.3 | 81.2 | 79.2 | 79.5 | 83.2 | 83.8 | 3.82 | 0.46 |
| 48 | 87.2 | 88.8 | 90.5 | 92.9 | 92.5 | 92.9 | 92.9 | 4.28 | 0.21 |

[1] standard error of means

Con90 + C10; concentrate (90%) + corn (10%), Con80 + C20; concentrate (80%) + corn (20%), Con90 + BP10; concentrate (90%) + beet pulp (10%), Con80 + BP20; concentrate (80%) + beet pulp (20%)

Table 7. *In vitro* pH of corn (C), beet pulp (BP), concentrate (Con) or mixtures at various time

| Incubation time (h) | Groups | | | | | | | SEM[1] | P-value |
|---|---|---|---|---|---|---|---|---|---|
| | Corn | Beet pulp | Concentrate | Con90 + C10 | Con90 + BP10 | Con80 + C20 | Con80 + BP20 | | |
| 0 | 7.00 | 6.99 | 6.99 | 6.99 | 7.00 | 6.99 | 6.99 | 0.00 | 0.49 |
| 6 | 6.93 | 6.96 | 6.82 | 6.83 | 6.83 | 6.85 | 6.82 | 0.02 | 0.67 |
| 12 | 6.72 | 6.80 | 6.69 | 6.69 | 6.70 | 6.73 | 6.71 | 0.01 | 0.48 |
| 24 | 6.59 | 6.63 | 6.65 | 6.64 | 6.64 | 6.65 | 6.61 | 0.01 | 0.50 |
| 48 | 6.57 | 6.57 | 6.68 | 6.65 | 6.63 | 6.58 | 6.63 | 0.01 | 0.56 |

[1] standard error of means

Con90 + C10; concentrate (90%) + corn (10%), Con80 + C20; concentrate (80%) + corn (20%), Con90 + BP10; concentrate (90%) + beet pulp (10%), Con80 + BP20; concentrate (80%) + beet pulp (20%).

Table 8. *In vitro* VFA of corn (C), beet pulp (BP), concentrate (Con) or mixtures at various time.

| Incubation time(h) | Groups | | | | | | | SEM[1] | P-value |
|---|---|---|---|---|---|---|---|---|---|
| | Beet pulp | Corn | Concentrate | Con90 + C10 | Con90 + BP10 | Con80 + C20 | Con80 + BP20 | | |
| Total VFA (mM) | | | | | | | | | |
| 0 | 62.0 | 63.0 | 59.8 | 62.9 | 61.0 | 63.8 | 63.0 | 0.53 | 0.50 |
| 6 | 96.2$^{ab}$ | 82.4$^{b}$ | 103$^{a}$ | 102$^{a}$ | 91.7$^{ab}$ | 98.5$^{a}$ | 95.8$^{ab}$ | 2.65 | 0.003 |
| 12 | 121$^{a}$ | 106$^{b}$ | 122$^{a}$ | 129$^{a}$ | 126$^{a}$ | 119$^{a}$ | 123$^{a}$ | 2.79 | < 0.001 |
| 24 | 158$^{a}$ | 144$^{b}$ | 143$^{b}$ | 140$^{b}$ | 144$^{b}$ | 140$^{b}$ | 144$^{b}$ | 2.34 | < 0.001 |
| 48 | 158 | 153 | 148 | 145 | 142 | 138 | 140 | 2.73 | 0.11 |
| Acetate, mol/100mol | | | | | | | | | |
| 0 | 66.3 | 66.1 | 69.1 | 67.1 | 66.8 | 68.2 | 68.2 | 0.16 | 0.23 |
| 6 | 65.4$^{a}$ | 63.2$^{b}$ | 61.8$^{c}$ | 62.3$^{c}$ | 62.6$^{bc}$ | 62.5$^{c}$ | 64.8$^{b}$ | 0.20 | < 0.001 |
| 12 | 67.5$^{a}$ | 62.4$^{cd}$ | 62.2$^{cd}$ | 62.4$^{d}$ | 62.8$^{c}$ | 62.3$^{d}$ | 63.3$^{b}$ | 0.27 | < 0.001 |
| 24 | 67.3$^{a}$ | 60.8$^{c}$ | 62.0$^{c}$ | 61.1$^{c}$ | 62.4$^{bc}$ | 62.0$^{c}$ | 64.2$^{b}$ | 0.32 | < 0.001 |
| 48 | 65.3 | 62.5 | 61.1 | 61.3 | 61.5 | 61.4 | 62.2 | 0.21 | 0.09 |
| Propionate, mol/100mol | | | | | | | | | |
| 0 | 18.1 | 18.0 | 17.0 | 18.2 | 18.5 | 18.0 | 17.8 | 0.07 | 0.91 |
| 6 | 20.2$^{d}$ | 22.1$^{c}$ | 25.3$^{a}$ | 24.8$^{b}$ | 24.7$^{b}$ | 24.8$^{b}$ | 25.2$^{b}$ | 0.28 | < 0.001 |
| 12 | 20.4$^{c}$ | 23.8$^{b}$ | 25.0$^{a}$ | 23.9$^{b}$ | 24.3$^{b}$ | 24.8$^{a}$ | 24.7$^{a}$ | 0.22 | < 0.001 |
| 24 | 20.8$^{b}$ | 24.1$^{a}$ | 24.3$^{a}$ | 24.4$^{a}$ | 23.8$^{a}$ | 24.1$^{a}$ | 23.3$^{a}$ | 0.18 | < 0.001 |
| 48 | 21.6 | 22.3 | 23.6 | 23.4 | 23.5 | 23.8 | 23.8 | 0.12 | 0.20 |
| Iso-butyrate, mol/100mol | | | | | | | | | |
| 0 | 0.83 | 0.86 | 0.80 | 0.69 | 0.79 | 0.79 | 0.78 | 0.01 | 0.87 |
| 6 | 0.74 | 0.74 | 0.64 | 0.63 | 0.64 | 0.64 | 0.64 | 0.01 | 0.07 |
| 12 | 0.63$^{c}$ | 0.77$^{a}$ | 0.73$^{b}$ | 0.73$^{b}$ | 0.70$^{b}$ | 0.71$^{b}$ | 0.68$^{b}$ | 0.01 | < 0.001 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 24 | 0.69$^{c}$ | 0.81$^{b}$ | 0.91$^{a}$ | 0.92$^{a}$ | 0.86$^{ab}$ | 0.87$^{ab}$ | 0.82$^{b}$ | 0.01 | < 0.001 |
| 48 | 0.87$^{b}$ | 0.98$^{b}$ | 1.09$^{ab}$ | 1.14$^{a}$ | 1.14$^{a}$ | 1.13$^{a}$ | 1.09$^{ab}$ | 0.01 | < 0.001 |
| Butyrate, mol/100mol | | | | | | | | | |
| 0 | 11.7 | 11.8 | 9.7 | 10.6 | 10.6 | 10.0 | 10.4 | 0.11 | 0.24 |
| 6 | 10.8$^{b}$ | 11.8$^{a}$ | 10.2$^{c}$ | 10.3$^{c}$ | 10.2$^{c}$ | 10.1$^{c}$ | 9.47$^{d}$ | 0.19 | < 0.001 |
| 12 | 9.89$^{bc}$ | 10.8$^{a}$ | 9.93$^{bc}$ | 10.8$^{a}$ | 10.1$^{b}$ | 10.1$^{b}$ | 9.44$^{c}$ | 0.07 | < 0.001 |
| 24 | 9.47$^{c}$ | 12.0$^{a}$ | 10.1$^{c}$ | 10.9$^{b}$ | 10.5$^{bc}$ | 10.4$^{bc}$ | 9.35$^{c}$ | 0.13 | < 0.001 |
| 48 | 9.83 | 11.4 | 11.0 | 10.8 | 10.6 | 10.4 | 9.89 | 0.08 | 0.11 |
| Iso-valerate, mol/100mol | | | | | | | | | |
| 0 | 0.40 | 0.39 | 0.39 | 0.31 | 0.36 | 0.35 | 0.32 | 0.01 | 0.15 |
| 6 | 0.68$^{c}$ | 1.10$^{a}$ | 0.90$^{b}$ | 0.88$^{b}$ | 0.87$^{b}$ | 0.88$^{b}$ | 0.85$^{b}$ | 0.02 | < 0.001 |
| 12 | 1.05$^{bc}$ | 1.21$^{a}$ | 1.06$^{b}$ | 1.08$^{b}$ | 0.99$^{c}$ | 1.03$^{bc}$ | 0.92$^{d}$ | 0.01 | < 0.001 |
| 24 | 1.16$^{c}$ | 1.36$^{b}$ | 1.46$^{ab}$ | 1.48$^{a}$ | 1.36$^{b}$ | 1.39$^{ab}$ | 1.25$^{b}$ | 0.03 | < 0.001 |
| 48 | 1.29$^{b}$ | 1.67$^{a}$ | 1.92$^{a}$ | 1.95$^{a}$ | 1.89$^{a}$ | 1.86$^{a}$ | 1.75$^{a}$ | 0.03 | < 0.001 |
| Valerate, mol/100mol | | | | | | | | | |
| 0 | 1.21 | 1.29 | 1.36 | 1.27 | 1.19 | 1.14 | 1.04 | 0.02 | 0.28 |
| 6 | 1.07 | 0.99 | 1.10 | 1.07 | 1.06 | 1.05 | 1.05 | 0.00 | 0.80 |
| 12 | 0.91$^{c}$ | 0.92$^{c}$ | 1.10$^{a}$ | 1.10$^{a}$ | 1.07$^{a}$ | 1.06$^{b}$ | 1.02$^{b}$ | 0.01 | < 0.001 |
| 24 | 0.95$^{c}$ | 0.91$^{c}$ | 1.23$^{a}$ | 1.25$^{a}$ | 1.18$^{a}$ | 1.17$^{ab}$ | 1.08$^{ab}$ | 0.02 | < 0.001 |
| 48 | 1.10$^{b}$ | 1.11$^{b}$ | 1.30$^{a}$ | 1.38$^{a}$ | 1.36$^{a}$ | 1.32$^{a}$ | 1.29$^{a}$ | 0.02 | < 0.001 |
| C2/C3 | | | | | | | | | |
| 0 | 3.15 | 3.19 | 3.49 | 3.15 | 3.09 | 3.25 | 3.28 | 0.05 | 0.75 |
| 6 | 2.77$^{a}$ | 2.45$^{b}$ | 2.09$^{d}$ | 2.15$^{cd}$ | 2.18$^{c}$ | 2.16$^{cd}$ | 2.21$^{c}$ | 0.09 | < 0.001 |
| 12 | 2.83$^{a}$ | 2.25$^{b}$ | 2.13$^{c}$ | 2.24$^{bc}$ | 2.21$^{bc}$ | 2.15$^{c}$ | 2.20$^{bc}$ | 0.09 | < 0.001 |
| 24 | 2.78$^{a}$ | 2.16$^{b}$ | 2.18$^{b}$ | 2.15$^{b}$ | 2.25$^{b}$ | 2.20$^{b}$ | 2.36$^{b}$ | 0.08 | < 0.001 |
| 48 | 2.58 | 2.40 | 2.22 | 2.24 | 2.24 | 2.21 | 2.24 | 0.05 | 0.15 |

[1] Standard error of means.
Con90 + C10; concentrate (90%) + corn (10%), Con80 + C20; concentrate (80%) + corn (20%), Con90 + BP10; concentrate (90%) + beet pulp (10%), Con80 + BP20; concentrate (80%) + beet pulp (20%).
[a.b.c.d] Means within a same row with different superscripts differ at $P < 0.05$.

## 2. Experiment 2: Feeding trial

### *2.1 Growth Performance*

ADG and FCR were not different between corn and BP group (Table 9). Feeding BP instead of corn did not affect DM intake. However, BP group showed a higher intake ($P \leq 0.03$) of NDF, ADF, and EE in total feed and concentrate compared with corn group. In contrast, BP group showed a lower intake ($P \leq 0.04$) of NFC in total feed and concentrate compared with corn group.

### *2.2 Ruminal fermentation characteristics and microbial population*

The ruminal pH, iso-butyrate, valerate, iso-valerate proportions, total VFA concentrations and ammonia were not different between two diet groups (Table 10). Ruminal acetate proportion was higher ($P = 0.001$) in BP group at $4^{th}$ week compared with corn group. Ruminal propionate proportion was lower ($P = 0.01$) in BP group at $8^{th}$ and $12^{th}$ week compared with corn group. Ruminal butyrate proportion was lower ($P = 0.04$) in BP group at $4^{th}$ week compared with corn group. The C2:C3 ratios were higher ($P \leq 0.046$) in BP group at $4^{th}$, $8^{th}$, and $12^{th}$ weeks compared to corn group.

Relative abundances of cellulolytic bacteria including *Fibrobacter succinogenes* ($P = 0.01$) and *Ruminococcus albus* ($P = 0.04$) were higher in BP group at $12^{th}$ week

compared with corn group (Figure 1), but no difference was observed for *Ruminicoccus flavefaciens*. Relative abundances of amylolytic (*Succinimonas amylotica, Ruminobacter amylophilus, and Streptococcus bovis*), lipolytic (*Anaerovibrio lipolitica*), methanogenic (*Methanobrevibacter, and Methanogen*), fungi, and protozoa were not different in both groups.

Table 9. Effects of feeding corn or beet pulp (BP) on growth performance of Korean cattle steers.

| Items | Group | | SEM[1] | P-value |
|---|---|---|---|---|
| | Corn | BP | | |
| Initial body weight, kg | 484 | 485 | 5.36 | 0.98 |
| Final body weight, kg | 558 | 564 | 5.70 | 0.90 |
| Average daily gain, kg | 0.89 | 0.93 | 0.04 | 0.55 |
| Total feed intake, kg/d | | | | |
| DM | 7.89 | 7.49 | 0.20 | 0.57 |
| CP | 0.93 | 0.93 | 0.02 | 0.77 |
| EE | 0.25 | 0.34 | 0.02 | < 0.001 |
| NDF | 1.44 | 1.71 | 0.06 | 0.01 |
| ADF | 0.49 | 0.71 | 0.04 | < 0.001 |
| NFC | 3.49 | 3.04 | 0.12 | 0.01 |
| ME, Mcal/d | 26.4 | 25.9 | 0.58 | 0.60 |
| Concentrate intake, kg/d | | | | |
| DM | 6.07 | 5.67 | 0.20 | 0.58 |
| CP | 0.78 | 0.76 | 0.02 | 0.74 |
| EE | 0.21 | 0.28 | 0.01 | 0.002 |
| NDF | 1.20 | 1.41 | 0.05 | 0.03 |
| ADF | 0.41 | 0.58 | 0.03 | < 0.001 |
| NFC | 2.91 | 2.50 | 0.10 | 0.04 |
| ME, Mcal/d | 7.08 | 7.07 | 14.6 | 0.58 |
| Forage intake, kg/d | | | | |
| DM | 1.82 | 1.82 | 0.00001 | 0.45 |
| CP | 0.10 | 0.10 | 0.00001 | 0.34 |
| EE | 0.04 | 0.04 | 0.00002 | 0.34 |
| NDF | 0.94 | 0.94 | 0.001 | 0.34 |

| | | | | |
|---|---|---|---|---|
| ADF | 0.51 | 0.51 | 0.0003 | 0.34 |
| NFC | 0.69 | 0.69 | 0.004 | 0.34 |
| ME, Mcal/d | 19.3 | 18.8 | 0.58 | 0.57 |
| Total feed intake, g/kg of body weight | | | | |
| DM | 13.3 | 13.0 | 0.17 | 0.42 |
| CP | 1.91 | 1.88 | 0.02 | 0.52 |
| EE | 0.52 | 0.69 | 0.03 | < 0.001 |
| NDF | 2.94 | 3.46 | 0.09 | < 0.001 |
| ADF | 0.99 | 1.43 | 0.07 | < 0.001 |
| NFC | 7.15 | 6.16 | 0.17 | < 0.001 |
| Concentrate intake, g/kg of body weight | | | | |
| DM | 9.71 | 9.42 | 0.09 | 0.12 |
| CP | 1.39 | 1.36 | 0.01 | 0.22 |
| EE | 0.38 | 0.50 | 0.02 | < 0.001 |
| NDF | 2.15 | 2.50 | 0.06 | < 0.001 |
| ADF | 0.73 | 1.04 | 0.05 | < 0.001 |
| NFC | 5.21 | 4.46 | 0.12 | < 0.001 |
| Forage intake, g/kg of body weight | | | | |
| DM | 3.61 | 3.60 | 0.11 | 0.96 |
| CP | 0.19 | 0.19 | 0.01 | 0.80 |
| EE | 0.07 | 0.07 | 0.00 | 0.77 |
| NDF | 1.86 | 1.85 | 0.06 | 0.97 |
| ADF | 1.01 | 1.01 | 0.03 | 1.00 |
| NFC | 1.36 | 1.36 | 0.04 | 0.94 |
| Feed efficiency (G:F ratio) | 0.10 | 0.11 | 0.001 | 0.50 |

n = 6 per each group

[1]SEM = standard error.

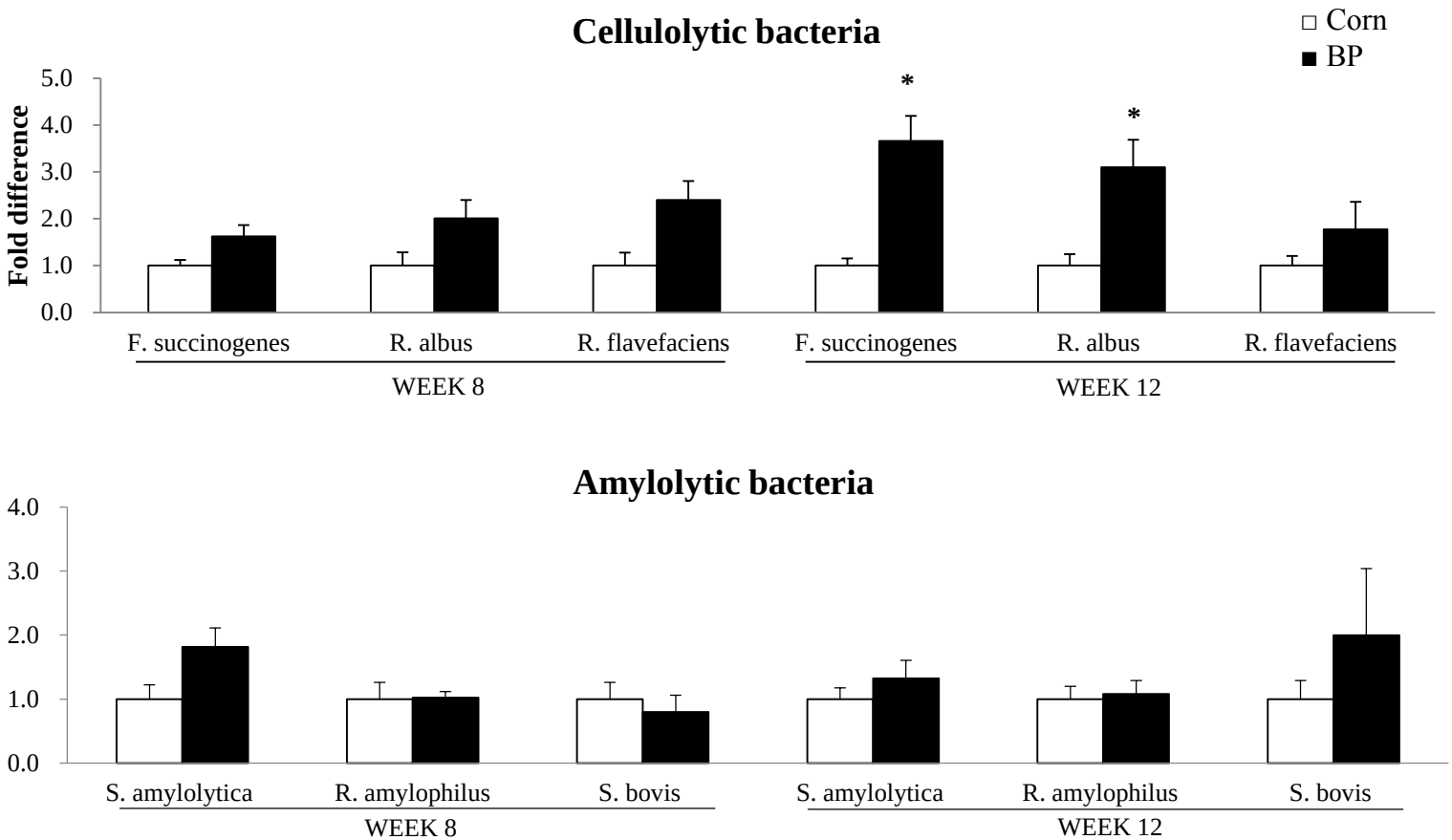
Table 10. Effects of feeding corn or beet pulp (BP) on ruminal fermentation characteristics of Korean cattle steers

| Items | Corn | BP | SEM[1] | *P*-value |
|---|---|---|---|---|
| pH | | | | |
| Week 0 | 6.54 | 6.50 | 0.06 | 0.76 |
| Week 4 | 6.74 | 6.89 | 0.11 | 0.53 |
| Week 8 | 6.43 | 6.57 | 0.09 | 0.48 |
| Week 12 | 6.91 | 6.99 | 0.09 | 0.67 |
| Ammonia (mg/dL) | | | | |
| Week 0 | 23.4 | 25.8 | 2.40 | 0.64 |
| Week 4 | 32.3 | 20.6 | 4.40 | 0.20 |
| Week 8 | 23.2 | 21.4 | 2.30 | 0.72 |
| Week 12 | 19.2 | 26.9 | 5.70 | 0.53 |
| Total VFA (mM) | | | | |
| Week 0 | 112 | 111 | 6.59 | 0.91 |
| Week 4 | 123 | 125 | 5.44 | 0.92 |
| Week 8 | 127 | 122 | 5.70 | 0.72 |
| Week 12 | 109 | 123 | 9.19 | 0.50 |
| Acetate (mol/100mol) | | | | |
| Week 0 | 59.2 | 58.8 | 0.53 | 0.77 |
| Week 4 | 60.0 | 63.4 | 0.62 | 0.001 |
| Week 8 | 62.8 | 64.2 | 0.51 | 0.19 |
| Week 12 | 62.9 | 64.1 | 0.51 | 0.24 |
| Propionate (mol/100mol) | | | | |
| Week 0 | 17.5 | 17.9 | 0.55 | 0.68 |

| | | | | |
|---|---|---|---|---|
| Week 4 | 17.9 | 16.2 | 0.57 | 0.13 |
| Week 8 | 22.2 | 19.7 | 0.55 | 0.01 |
| Week 12 | 21.8 | 19.7 | 0.45 | 0.01 |
| Iso-Butyrate (mol/100mol) | | | | |
| Week 0 | 0.88 | 1.08 | 0.001 | 0.25 |
| Week 4 | 0.64 | 0.72 | 0.0004 | 0.33 |
| Week 8 | 0.65 | 0.72 | 0.0004 | 0.42 |
| Week 12 | 0.71 | 0.64 | 0.0002 | 0.10 |
| Butyrate (mol/100mol) | | | | |
| Week 0 | 16.3 | 15.6 | 0.01 | 0.65 |
| Week 4 | 16.2 | 13.4 | 0.01 | 0.04 |
| Week 8 | 12.0 | 13.4 | 0.01 | 0.25 |
| Week 12 | 12.3 | 13.4 | 0.01 | 0.34 |
| Iso-Valerate (mol/100mol) | | | | |
| Week 0 | 1.43 | 1.48 | 0.001 | 0.86 |
| Week 4 | 0.89 | 1.18 | 0.001 | 0.30 |
| Week 8 | 0.96 | 0.78 | 0.001 | 0.27 |
| Week 12 | 1.06 | 0.45 | 0.001 | 0.09 |
| Valerate (mol/100mol) | | | | |
| Week 0 | 1.71 | 1.82 | 0.002 | 0.79 |
| Week 4 | 1.44 | 1.82 | 0.001 | 0.15 |
| Week 8 | 1.33 | 1.27 | 0.001 | 0.55 |
| Week 12 | 1.31 | 1.27 | 0.0003 | 0.55 |
| C2/C3 ratio | | | | |

| | | | | |
|---|---|---|---|---|
| Week 0 | 3.40 | 3.32 | 0.08 | 0.68 |
| Week 4 | 3.37 | 3.98 | 0.13 | 0.046 |
| Week 8 | 2.85 | 3.28 | 0.07 | 0.011 |
| Week 12 | 2.89 | 3.26 | 0.06 | 0.008 |

[1] Standard error of the mean


Cellulolytic bacteria
□ Corn
■ BP
Fold difference
5.0
4.0
3.0
2.0
1.0
0.0
*
*
F. succinogenes
R. albus
R. flavefaciens
F. succinogenes
R. albus
R. flavefaciens
WEEK 8
WEEK 12
Amylolytic bacteria
4.0
3.0
2.0
1.0
0.0
S. amylolytica
R. amylophilus
S. bovis
S. amylolytica
R. amylophilus
S. bovis
WEEK 8
WEEK 12

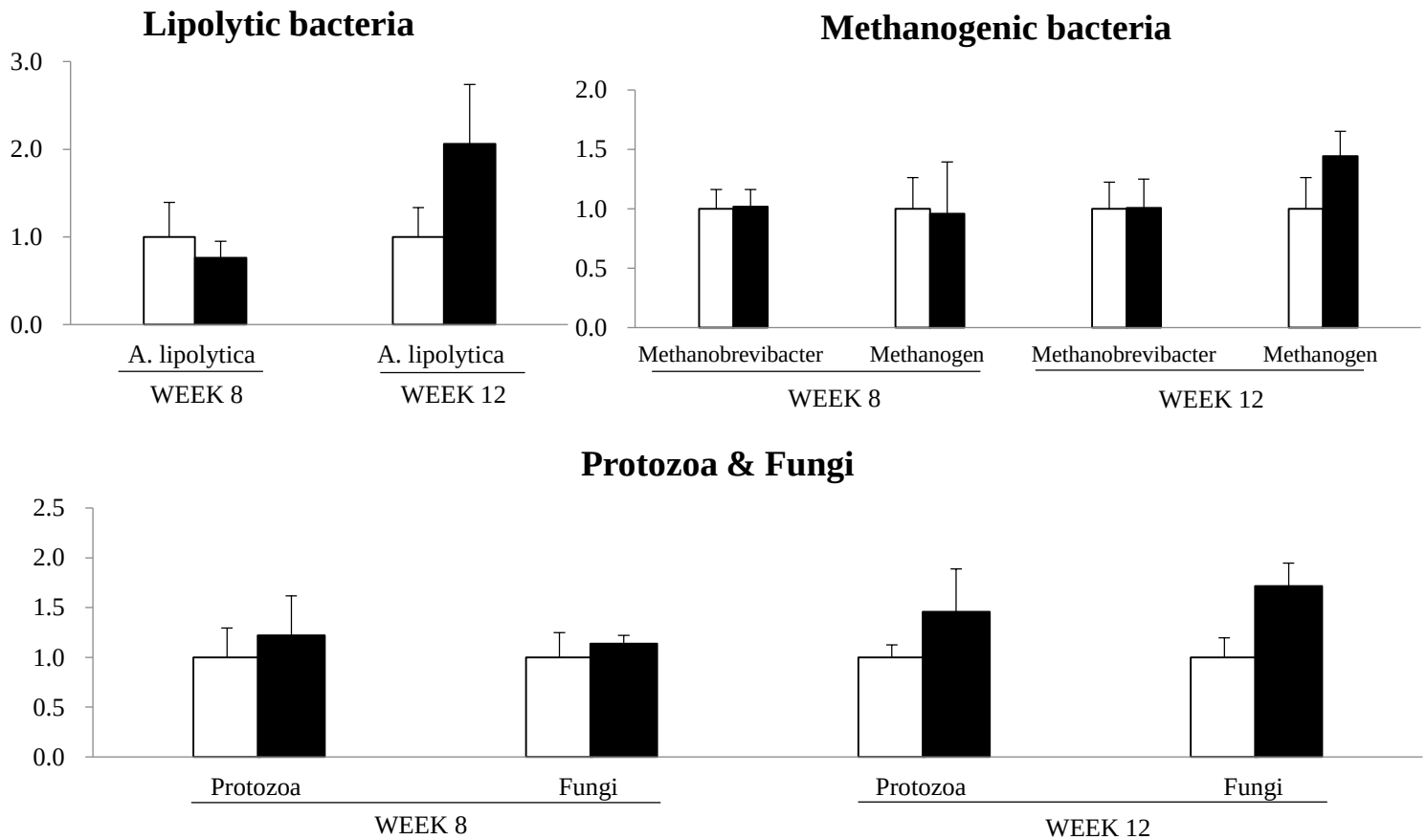



Figure 1. Effects of feeding corn or beet pulp (BP) on microbial population in the rumen of Korean cattle steers. Values display the mean fold difference ± SE. The values were measured using qPCR with value of total bacteria for

normalization. Values of corn group were normalized to 1.0 fold. “*” represents P-value < 0.05

### *2.3 Blood metabolites*

NEFA was lower (P = 0.004) in BP group at 12$^{th}$ week compared with corn group (Table 11). Serum insulin concentration was higher (P = 0.01) in BP group at 12$^{th}$ week. However, feeding the BP instead of corn did not affect the glucose, triglyceride, total cholesterol, and leptin concentrations.

Table 11. Effects of feeding corn or beet pulp (BP) on blood metabolites of Korean cattle steers

| Item | Corn | Treatment | SEM[1] | *P*-value |
|---|---|---|---|---|
| Glucose, mg/dL | | | | |
| Week 0 | 80.8 | 79.2 | 2.4 | 0.75 |
| Week 4 | 73.5 | 74.8 | 1.2 | 0.60 |
| Week 8 | 77.2 | 76.2 | 2.4 | 0.85 |
| Week 12 | 74.7 | 75.0 | 1.2 | 0.90 |
| Triglyceride, mg/dL | | | | |
| Week 0 | 23.3 | 20.7 | 1.29 | 0.33 |
| Week 4 | 24.3 | 19.7 | 1.71 | 0.18 |
| Week 8 | 23.0 | 20.3 | 1.70 | 0.46 |
| Week 12 | 18.8 | 16.0 | 1.00 | 0.18 |
| Total cholesterol, mg/dL | | | | |
| Week 0 | 130 | 123 | 6.21 | 0.62 |
| Week 4 | 158 | 145 | 10.9 | 0.58 |
| Week 8 | 175 | 209 | 11.7 | 0.16 |
| Week 12 | 170 | 202 | 9.61 | 0.10 |
| NEFA[2], mg/dL | | | | |
| Week 0 | 134 | 137 | 9.10 | 0.90 |
| Week 4 | 105 | 90.5 | 5.90 | 0.06 |
| Week 8 | 130 | 108 | 14.5 | 0.21 |
| Week 12 | 173 | 119 | 11.1 | 0.004 |
| Leptin, ng/mL | | | | |
| Week 0 | 9.79 | 9.01 | 0.45 | 0.43 |

| | | | | |
|---|---|---|---|---|
| Week 4 | 7.20 | 7.19 | 0.67 | 0.96 |
| Week 8 | 6.23 | 5.55 | 0.46 | 0.50 |
| Week 12 | 8.65 | 8.64 | 0.50 | 0.99 |
| Insulin, ng/mL | | | | |
| Week 0 | 1.08 | 1.27 | 0.14 | 0.54 |
| Week 4 | 1.16 | 1.54 | 0.14 | 0.17 |
| Week 8 | 0.55 | 0.84 | 0.08 | 0.08 |
| Week 12 | 0.79 | 1.52 | 0.15 | 0.01 |

[1] Standard error of the mean
[2] Non-esterified fatty acids

# Ⅴ. Discussion

### *1. In vitro DM degradability and fermentation characteristics*

In this study, DM degradability of corn, BP and mixtures did not exhibit any differences. BP contains abundant pectin up to 25% of dried BP (Walter al., 1991). Degradation of pectin is faster than NDF in roughage (Marounek et al., 1985). Similarly, BP addition up to 9.5% of the DM did not affect the disappearance rate of DM (Kim et al., 2012).

pH of corn, BP and mixtures did not exhibit any differences. Similarly, substitution of corn to unmolassed BP up to 50% of the DM did not affect the pH in dairy cow (O'Mara et al., 1997). Moreover, replacing the corn with BP up to 24% did not affect the pH in dairy cow (Voelker and Allen, 2003b).

The acetate proportion was higher in BP or BP mixture samples compared to corn or corn mixture samples. But, the propionate proportion was lower in BP or BP mixture samples compared to corn or corn mixture samples. These results could be related to the greater fiber content in BP which produces acetate in ruminal fermentation (Marounek et al., 1985). Furthermore, readily fermentable NDF (pectin) was reported to produce less propionate and lactate formation (Strobel and Russell, 1986; Alamouti et al., 2014). Hence, high fiber content in BP may contribute to higher acetate and lower the propionate proportion. Iso-butyrate, and iso-valerate proportion were lower in BP or BP mixture samples

compared to corn or corn mixture samples. Andries et al. (1987) reported that cellulolytic microorganisms used branched-VFA such as iso-butyrate and iso-valerate to make branched–chain amino acids for their proliferation. Therefore, high fiber source in BP might have favored the growth of cellulolytic bacteria in rumen fluid leading to lower proportions. Butyrate proportion was lower in BP or BP mixture samples compared to corn or corn mixture samples. This result may be attributed to low level of non-structural carbohydrate in BP that produces lower butyrate in ruminal fermentation (Stern et al., 1978) compared with corn which contains higher non-structural carbohydrate. Likewise, a continuous culture experiment showed lower butyrate proportion which may be resulted from the decreased NFC level with the increase of BP level up to 30% in Holstein steers. Hence, low non-structural carbohydrate in BP may contribute to lower butyrate proportion compared to corn.

C2/C3 ratio was higher in BP compared to corn. It may be attributed to high fiber contents that produce less propionate and lactate (Strobel and Russell, 1986; Alamouti et al., 2014) and that produce high acetate in BP (Voelker and Allen, 2003c). Our result was consistent with previous studies that showed higher C2:C3 ratio in BP addition compared to corn grain on *in vitro* experiment using Holstein (Getachew et al., 2003). Therefore, BP could contribute to higher C2/C3 ratio.

### *2. Growth Performance in feeding trial*

DMI, ADG, and FCR were not different in both groups, which could be

explained by the similar energy level of diets formed by feeding BP instead of corn. In a previous study, BP addition at 0, 333, 667 and 1000 g/kg did not affect DMI in Egyptian buffalo calves (Abo-Zeid et al., 2017). Another study showed a similar feed intake pattern in Arabian male lambs (Asadollahi et al., 2017). In the previous study, BP addition up to 32% of DM showed no difference in ADG, FCR using fattening bulls (Cuvelier et al. 2006). Thus, BP could be a good energy source in Korean steers.

### *3. Ruminal fermentation characteristics and microbial population*

In the current study, there were no differences in pH between the diets both *in vitro* and *in vivo*. However, according to Yang and Beauchemin (2006) physically effective fiber (peNDF) by BP encourages saliva secretion, chewing and rumination activity. It might make a suitable ruminal condition for ruminal fermentation. In the previous study, Egyptian buffaloes showed higher pH as increasing the BP addition up to 100% of corn (Abo-Zeid et al., 2017). In another study, the substitution of corn with BP up to 24% of DM could make the more stable condition of pH in dairy cattle (Voelker and Allen, 2003b). However, in this study, feeding BP instead of corn up to 17.7% of DM did not improve the stability of ruminal pH in Korean steers. This result might be attributed to our basal concentrate diet having enough buffers for stability of pH in rumen. Actually, the concentrate contains ammonium chloride as a pH buffer material.

Acetate proportion was higher in BP group at 4th week compared with corn group. In contrast, propionate proportion was lower in BP group at 8th and 12th weeks. Higher intake of fiber content and lower intake of NFC in BP group compared with corn group could make higher acetate and lower propionate production. This result is in agreement with several studies that showed that main VFA of fiber source is acetate (Marounek et al., 1985) and produces less propionate (Strobel and Russell, 1986; Alamouti et al., 2014). Butyrate proportion was lower in BP group at 4th week compared with corn group. Lower non-structural carbohydrate in BP produces lower butyrate in ruminal fermentation (Stern et al., 1978) compared with corn. This result is consistent with our *in vitro* study that showed higher C2/C3 ratio in BP group compared with corn group. Higher C2/C3 ratio in BP group could be explained by the fact that higher NDF of BP produces less propionate, lactate and more acetate production (Alamouti et al., 2014) compared with corn. Similarly, previous study reported that the use of BP as a substitute for barley of diet for Holstein steers increased C2:C3 ratio in ruminal fermentation (Mojtahedi and Mesgaran, 2011). In another study, substitution of corn with BP up to 24% increased C2:C3 ratio in dairy cattle (Voelker and Allen, 2003b). Consequently, the high fiber content in BP could contribute to higher C2:C3 ratio in ruminal fermentation compared with corn.

Population of *Fibrobacter succinogenes* and *Ruminococcus albus* were higher in BP group at 12th week. They are typical cellulolytic bacteria (Koike and Kobayashi,

2009) which have the various genes encoding enzymes which can degrade plant fiber (Flint et al., 1989; McGavin et al., 1989). Thus, high fiber content in BP may contribute to higher population of these cellulolytic bacteria compared with corn group. On the other hand, amylolytic bacteria (*amylolytica, Ruminobacter amylophilus*, and *Streptococcus bovis*), lipolytic bacteria (*Anaerovibrio lipolytica*), methanogenic bacteria (*Methanobrevibacter* and *Methanogen*), protozoa and fungi were not affected by BP addition up to 17.7% of DM. Therefore, higher acetate proportion in BP group compared with corn group could be explained by higher population of cellulolytic bacteria in this study..

### *4. Blood metabolites*

Insulin concentrations were higher in BP group at 12$^{th}$ week compared with corn group, whereas NEFA concentrations were lower in BP group. The insulin increases the lipogenesis and inhibits lipolysis (Saltiel and Kahn, 2001). Moreover, insulin has been suggested as a marker of IMF in cattle (Matsuzaki et al., 1997; Wegner et al., 2001). In a previous study, the high insulin concentration in dairy cows fed glycogenic diet showed low NEFA concentration compared with group fed lipogenic diet (Van Knegsel et al., 2005. Therefore, feeding BP instead of corn may have the potential which improve lipogenesis and IMF in Korean cattle. However, the reason about increased insulin in blood at fasting need to be more studied.

## Conclusion

The feeding BP instead of corn for 14 weeks in the fattening diet did not affect growth performance in Korean steers. Feeding BP instead of corn increased some cellulolytic bacteria at 12$^{th}$ weeks, acetate proportion at the 4$^{th}$ week, C2:C3 ratio at the 4$^{th}$, 8$^{th}$, and 12$^{th}$ weeks and insulin concentration at 12$^{th}$ week. In contrast, NEFA was decreased at 12$^{th}$ week. Therefore, BP could be used as an energy source for increasing acetate proportion. These results suggest that BP may be used for IMF deposition through providing acetate and higher circulating insulin levels during fattening period of beef cattle.

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# Ⅶ. Summary in Korean

본 연구에서는 비트 펄프가 *in vitro* 반추위 발효성상과 한우 거세우의 성장, 반추위 발효와 미생물 및 혈액 성상에 미치는 영향을 조사하였다. 실험 1 (*In vitro*)에서는 캐뉼라가 설치된 Holstein 거세우를 사용하여 발효성상을 비교하였다. 실험1 결과, 6, 12 및 24h에 옥수수보다 비트펄프에서 C2 농도와 C2:C3 비율이 높았다 (P < 0.001). 소화율 및 pH는 비트펄프와 옥수수 간에 차이가 없었다. 실험 2 (*In vivo*)에서는 한우 거세우(평균 485 ± 19kg, 18.0 ± 0.17개월령)를 대조구(Corn)와 처리구(BP)로 나누었다. 두 그룹 모두 관행적인 배합사료를 예정 섭취량의 80% 급여하였고, 나머지 20%를 대조구는 옥수수를, 처리구는 비트펄프 17.7%와 보호지방 2.3%를 12주동안 top-dressing으로 각각 급여하였다. 섭취량은 매일, 증체량, 반추위 발효성상 그리고 혈액 성상은 4주 간격으로 12주간 조사하였다. 실험 2 결과, 섭취량, 일당증체량, iso-C4, C5, iso-C5 비율과 total VFA 농도는 두 그룹간 차이가 없었지만, C2가 4주에 (P = 0.001), 혈중 인슐린 농도가 12주에 (P = 0.01) BP그룹에서 높았다. 반대로 혈중 NEFA는 12주에 BP그룹에서 낮았다 (P = 0.004). 그리고 12주차에 반추위

cellulolytic bacteria인 *Fibrobacter succinogenes* (P = 0.01)과 *Ruminococcus albus* (P = 0.04)의 분포가 BP 그룹에서 높았다. 그러나 total cholesterol, glucose, triglyceride, 및 leptin 농도는 차이가 없었다. 결론적으로 비트펄프 급여는 한우의 성장에는 영향을 미치지 않았으나, 반추위 C2의 비율을 높였고, 혈중 인슐린 농도의 증가를 통해 지방의 합성을 촉진하고 지방분해를 억제함으로써 지방축적 증진에 기여할 수 있는 가능성을 보였다.

# Ⅷ. Acknowledgement

가장 먼저 하나님 아버지께 감사의 말씀을 드리고 싶습니다. 부족한 자녀지만 항상 예쁘다고 기회라는 것을 끊임 없이 주시고, 그 기회를 잡을 수 있도록 능력도 항상 채워주시고, 또 그 능력 마음껏 활용하라고 늘 모든 조건과 환경을 최고로 맞춰주시는 아버지께 모든 영광을 올려 드립니다. 이 귀한 사실을 항상 잊지 않게 하시고 항상 모든 것이 아버지께로부터 나온다는 사실을 잊지 않게 해주신 것도 감사를 드립니다. 대학원 과정을 마쳐서 기쁜 것이 아니라 아버지께서 주신 일을 아버지의 능력을 받아 잘 마무리 할 수 있다는 귀함에 너무 행복합니다.

그리고 우리 식구들, 제가 가진 예민하고 날카로운 성격 탓에 잔소리도 많고 귀찮게 해서 항상 죄송하고 감사하게 생각합니다. 하지만, 그 모든 행동이 우리 가족을 진심으로 사랑하기 때문에 같이 잘살자는 마음에서 나오는 행동이라는 것을 우리 서로가 잘 알고 있을 것이라 생각합니다. 항상 고맙고 사랑합니다. 첫 번째로 우리 아버지와 어머니, 항상 부모님의 사랑으로부터 나오는 행동들에 존경을 표할 수 밖에 없습니다. 바보처럼 부모님의 희생을 당연한 것처럼 생각한 저를 돌아보고 아주 크게 반성하는 일이 많습니다. 표현이 어렵지만 항상 존경하고 있습니다. 부모님

처럼 살고 싶은 것이 제 목표입니다. 다만, 제 능력이 거기까지 도달할 수 있을지 모르겠지만 노력하겠습니다. 인생에서 소탐대실 하지 않고, 행복이 무엇인지를 항상 가르쳐 주시는 부모님의 교육과 철학에 자주 감명을 받습니다. 잊지 않겠습니다 감사합니다.

형과 형수님 그리고 사랑하는 우리 조카 라율이, 형은 잘 모르겠지만 제 인생에서 정말 중요한 선생님이셨습니다. 약 3~4세 정도에 제가 병원에서 큰 병으로 길게 입원하는 바람에, 형은 많은 관심이 필요한 어린 시절 힘든 시기를 보냈을 것이고 이로 인해 저 혼자만 이익을 보고 산 것 같아 항상 미안하고 고맙습니다. 하지만, 다행히 형의 희생 덕분에 저는 건강해 졌고, 형과 즐거운 시간을 보낼 수 있어서 하루하루 감사를 느끼고 있습니다. 어린 시절부터 많은 것을 가르쳐 주고 항상 동생이 잘 되길 바라는 마음에 귀한 이야기들을 해주는 형을 보면 나는 참 복이 많은 사람이구나 싶습니다. 밖에서는 어떤 사람인지 저는 잘 모르지만, 제가 보는 형은 재능도 많고 능력도 많은 사람입니다. 이 재능과 능력이 학창시절의 학업에 방해를 주었을지 몰라도 추후에 많은 노력과 사회 공부를 하였고, 지금 내 눈에는 열정적으로 움직이고, 적극적으로 배움을 가져가는 성공해 나가는 사람들 중 한 명이라고 생각합니다. 또 이러한 가치를 느끼고

우리 형과 백년가약을 맺어준 형수님께 감사하다는 말씀을 드립니다. 쉽지 않은 결정이었을 텐데 형의 가치를 믿고 따라와준 형수님 항상 고맙습니다. 가족이 되면서 크고 작은 갈등들이 생기고 앞으로도 생길 수 있겠지만, 우리는 '우리'이니까 가족이라는 힘을 통해 하나님 아버지 보시기에 좋았더라 라는 말이 나오도록 바르고 행복하게 살아갔으면 좋겠습니다. 그리고 우리 라율이 항상 삼촌이 바쁘고 공부하느라 신경을 쓴다고는 하지만 많이 부족한 거 같아요. 나중에 라율이가 크면 자랑스러워하는 삼촌이 되는 것이 삼촌의 또 다른 목표라서 조금 바쁘게 살았던 것 같아요. 앞으로도 그 꿈을 위해서 노력 할 것이지만 앞으로 조카를 위해서 희생하는 시간을 많이 가져보려고 해요. 라율이가 이 글을 볼 때쯤이면 초등학교 또는 중학교에 다니고 있을 텐데 자랑스러운 삼촌이 되었는지 평가해주고, 아니면 막 혼내주세요. 어쨌든 라율이가 바르게 성장하길 바라는 삼촌이니까 언제든지 찾아와서 고민상담하고 답을 얻어 갔으면 좋겠어요. 여기에 글을 모두 쓸 수는 없지만 우리 친가 할머니부터 민혁이까지, 그리고 외가 식구들 멀리 또는 가까이서 응원해주시고 지원해주셨기 때문에 오늘 이렇게 글을 쓸 수 있었다고 생각합니다. 항상 감사 드리고 또 감사 드립니다. 다른 말보다 진심이 담긴 감사 드린다는 말이 가장 제 입

장을 대변하기에 좋은 것 같습니다.

그리고 힘든 시기에 옆에서 항상 오빠한테 힘주고 응원해주는 지윤이 공동으로 대학원생활을 했다고 말할 수 있을 정도로 나에게 큰 힘이 되어주어서 고마웠어요. 지윤이가 있었기에 성공적으로 이루어 질 수 있었고, 항상 하나님 아버지 안에서 교제한다는 일념아래 참 행복하고 귀한 시간이 많아요. 앞으로도 아버지의 이끄심 속에 행복한 시간 보내길 서로 기도했으면 좋겠습니다. 임용준비 한다고 고생이 많은데, 아버지가 큰 선물 주시려고 귀한 시간 주시는 것이라고 믿어요. 그렇기 때문에 아버지의 능력으로 꼭 아버지께서 주신 사명을 이루었으면 좋겠어요. 항상 기도하고 고맙습니다.

우리 실험실 멤버들, 늦은 시간까지 매일 공부하고, 실험하고 주말 없이 계속 학교 나오면서도 항상 웃으면서 잘 지내는 모습이 너무 귀합니다. 우리의 노력이 절대 헛되이 되지 않을 것이라 생각합니다. 물론 요즘 노력 안 하는 사람들이 없을 정도로 다들 열심히 살지만, 우리 실험실 멤버들만큼 실험과 학업 그리고 잦은 출장을 같이 진행하면서도 웃으면서 잘 지낼 수 있다는 것이 나는 아주 큰 능력이라고 생각합니다. 또한 지금 능력이 부족할 수 있어도 학생이니까 당연한 부분이고, 부족한 부분은 시간

이 채워 줄 겁니다. 하지만 정말 중요한 전제 조건은 절대 포기하지 않는 힘이라고 생각합니다. 우리 멤버들은 이 능력을 기본적으로 갖추고 있다고 생각하기 때문에 걱정은 안되지만, 사람이 무슨일이 벌어질지 모르기 때문에, 아무리 힘들고 어려워도 포기 안 했으면 좋겠습니다. 지금은 졸업한 민우형, 혁중이형, 승주형(군인신분?) 그리고 상원이에게 항상 미안했어요. 끊임없이 귀찮게 물어보고, 실험디자인부터 내용 그리고 보고서까지 검토요청하고 그렇게 해서 미안했어요. 근데 제가 좀 적극적인 부분이 있고, 능력이 부족해서 어쩔 수가 없었어요. 그런 마음에서 항상 고맙게 생각하고 있습니다. 사회에 나가서 꼭 이루고자 하는 꿈 이루시길 바라겠습니다. 지금도 내 옆에서 유학을 준비하는 상원이는 고민이 많겠지만, 나는 그렇게 생각해. 후회 없는 인생을 살았으면 좋겠다고, 그래서 원래 이루고자 하는 꿈이 잘못된 길에 있는 꿈이 아니라면 힘들다고 포기하지 말고 일단 이루기 위해 최선을 다해서 시도하라고 말해주고 싶어. 분명히 열매가 있을 거라고 생각해요. 그리고 상엽이 사양실험 할 때부터 많은 이야기들을 해주고 공부시켜주고 신경 써줘서 너무 고마웠어 너 덕분에 졸업도 잘 마무리 할 수 있었고, 대학원생활도 원만하게 잘 할 수 있었던 것 같아 군대 다녀오느라고 고생했을 텐데 이제 대학원생활 남았

네? 이제 더욱 잘 할 수 있을 것이라 생각해. 도현이, 수종이, 선필이 우리 실험실의 유머담당 너희 덕분에 많이도 웃었고 즐거웠어. 자세한 이야기들을 여기 모두 적을 수는 없지만, 항상 고맙고 앞으로도 너희의 긍정적인 마음을 항상 유지하면서 행복하게 살아가길 기도할게.

그리고 우리 형들 진솔이형, 석현이형 장난끼 많은 동생이 들어와서 귀찮았을 수도 있다고 생각하지만 나는 개인적으로 두 형이 좋아서 그런 거고, 개인적인 생각이지만 난 형들이랑 친하다고 생각합니다 흐흐흐. 혹시 제가 서운하게 해드린 일이 있었다면, 정말 좋고 잘 지내고 싶어서 그렇게 한 것이니까 이해를 바라고 이 기회를 빌어서 용서를 구하고 싶어요. 매번 고마운 일들이 많아서 다 적을 순 없습니다. 만나서 밥 먹으면 그게 고마움의 표시이지 않을까 생각해요. 제가 취업하면 자주 학교 놀러 올 테니 시간 비워주세요. 그리고 재능이 넘치는 진오형 우리실험실 멤버들한테 호감도 1위일꺼 같은데 형만큼 성격 좋은 사람 많이 못 봤는데, 우리 멤버로 들어와 줘서 고마워요. 지금 힘든 과정을 겪고 계신 거 분명히 큰 열매로 돌아 올 것이라 믿습니다.

우리 막내 재성이와 인구, 실험실 들어와서 당황스러운 일이 많지? 다른 나라는 어떤지 모르겠지만, 원래 대학원 생활이라는 것이 예측불가의

상황이 빈번하고, 그러면서 또 현장경험을 쌓아가는 거니까 항상 긍정적으로 스트레스 받지 않고 원만하게 잘 졸업할 수 있기를 바랄게. 선배들이 항상 많이 고마워하고 있고, 뒤에서 도와주는 사람들이니까 모든지 자신감 있게 했으면 좋겠어. 힘내.

파트과정중인 가족 분들께, 희겸이 형님과의 이야기를 통해 귀한 아이디어를 확신 할 수 있었고, 이로 인해 제가 졸업을 하게 되었습니다. 형님도 꼭 좋은 아이디어로 실험하시고 무사히 졸업하시길 바라겠습니다. 국태형님과 종성형님, 우리 실험실 특성상, 파트과정이 쉽지 않고 어려운 부분이 많은 거 같아요. 하지만 형님들의 열정이 있기에 꼭 성공할 것이라고 믿습니다. 마지막까지 승리하시길 기도하겠습니다. 모든 것을 다 쓰지 못하지만 여기서 못다한 이야기는 만나서 이야기할 용도로 남겨두기로 하고 이 정도만 작성하겠습니다.

그리고 우리 백 명기 교수님, 부족한 제자 받아 주시고 큰 실험도 할 수 있게 이끌어 주셔서 항상 감사 드리고 있습니다. 처음 해보는 것이 많아서 교수님께 답답한 행동을 한적도 많고 실수도 많았습니다. 하지만 그때마다 끝까지 포기하지 않고 이끌어 주시는 행동에 저도 포기 하지 않고 할 수 있었습니다. 학업 외에도 인생을 살아가는 자세나 행동들에 대

해 지적해 주신 부분 잊지 않고 살아 가겠습니다. 많은 가르침에 감사를 드립니다.

이 곳에 적지 못한 분들 을 포함해서 제 대학원생활에 도움주신 모든 분들께 진심으로 감사의 말씀을 드립니다.

감사합니다.

정 인혁 올림.