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# Comparison of *in vitro* ruminal fermentation incubated with different levels of Korean corn grains with total mixed ration as a basal

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## Abstract

The present study was conducted to investigate the effect of different levels of Korean corn grain on *in vitro* ruminal fermentation with total mixed ration (TMR) as a basal feed. Three ruminal cannulated Holstein steers (Body Weight 479 ± 33.0 kg) were used as rumen fluid donors. Treatments for *in vitro* fermentation were TMR only (control, 3.0 g), TMR substituted partially with high level (HC, TMR 1.5 and corn 1.5 g), and with low level of Korean corn grain (LC, TMR 2.25 and corn 0.75 g), respectively. To measure *in vitro* ruminal pH, gas production, ammonia N and volatile fatty acids (VFA), the *in vitro* fermentation incubation was triplicated at 39°C, 120 rpm for 0, 1, 3, 6, 12, 24 and 48 h, respectively. Mean ruminal pH was significantly lower ($p < 0.05$) for HC than control. Changes in rumen pH was rather similar between the groups till 6 h after incubation, but the lowest pH for HC (pH 5.10) appeared at 48 h compared with control and LC. Total gas production was tended ($p < 0.09$) to be higher and ammonia N was significantly lower ($p < 0.05$) for HC than control and LC. Total VFA was higher ($p < 0.05$) for HC and LC than control but no differences appeared between HC and LC. Overall, the present data indicate that feeding different levels of Korean domestic corn grain may lead to high and sustainable starch degradation in the rumen.

**Keywords:** different level, *in vitro* rumen fermentation, Korean corn grain, ruminal pH

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## Introduction

Corn grains are considered to be the most adaptable feed energy sources for livestock but the optimization of corn feeding levels as an energy source remains under discussion amongst the ruminant nutritionists (Huntington, 1997; Lee et al., 2002; Van Zwieten et al., 2008; Kim et al., 2017a). Acceptability of corn grain as an energy source is because of its chemical composition, which has 72% dry matter (DM) as starch as compared to barley and oats are approximately 57% to 58% (Huntington, 1997).

By increasing rumen available energy contents in the diets of cattle has the potential for improved efficiency of nutrient utilization in the rumen and also increased microbial activity along with metabolizable nutrient supply (Gozho and Mutsvangwa, 2008). Generally in high-producing ruminants, energy supplied via starch is useful for them and major sources are maize, barley, wheat, rye, triticale, sorghum and oats. All of the cereal grains show large variations in their amounts of ruminally degradable protein and starch (Seifried et al., 2016; Seifried et al., 2017). Although, highly fermentable diets are quickly converted to organic acids within the rumen and they can easily dissociate to decrease rumen pH. It can increase the risk of sub-acute and acute acidosis inhibiting fiber utilization by bacteria (Plaizier et al., 2001).

In Korea, imported concentrates is estimated to be more than 11 million metric tons (MT) per year; specifically corn (Seo, 2005). Over 99% of corn used in Korea is imported so that only below 0.1% of Korean domestic corn is used as a feed. For the year 2016, corn production was 75,550 MT, though it is more than mean of the previous 10 years but needs attention (ROKGFAR, 2017).

For increased production of high marbling beef and milk yield, corn is the main feed ingredient for energy supply. Although many studies in terms of digestibility, rumen metabolism and reviews of grains including corn were reported (Galyean et al., 1981; Huntington, 1997; Van Zwieten et al., 2008; Kim et al., 2017a). However, few data from rumen studies including rumen fermentation characteristics of Korean domestic corn as a main feedstuff are available. *In vitro* systems have provided less expensive, more rapid alternatives and valuable data on kinetic aspects of starch digestion (Huntington, 1997). Therefore, this study conducted to investigate the effect of different levels of Korean corn grain on *in vitro* ruminal fermentation with total mixed ration (TMR), providing fundamental data of Korean corn in terms of rumen nutrition.

# Material and Methods

## Experimental design and treatments

Three ruminally cannulated Holstein steers (Body Weight 479 ± 33.0 kg) were used as rumen fluid donors and adapted to TMR diet for 21 days. TMR was being fed (09:00 and 17:00) daily. Water and mineral-vitamin block were allowed ad libitum. For *in vitro* rumen fermentation, treatments were TMR only (control, 3.0 g), TMR substituted partially with high level (HC, TMR 1.5 and corn 1.5 g), and low level of Korean corn grain (LC, TMR 2.25 and corn 0.75 g), respectively.

## Ruminal inoculation and *in vitro* incubation

Rumen digesta was collected after two hours of morning feeding and filtered with 4 layers of cheesecloth. Rumen fluid buffer mixture, comprising McDougall buffer (McDougall, 1948) and the rumen liquor in the ratio of 2 to 1, was dispensed anaerobically into 150 mL glass bottle containing one of the treatments as substrate. All bottles were filled and capped with a rubber stopper and held in a shaking incubator (Jeio Tech, SI-900R, Daejeon, Korea). Each of the *in vitro* fermentation incubation was triplicated at 39°C, 120 rpm for 0, 1, 3, 6, 12, 24 and 48 h, respectively. Incubated samples were collected and gas production measurement was done according to the assay outlined by (Theodorou et al., 1994). Gas pressure in the headspace was read from the display unit after insertion of the hypodermic syringe needle through the butyl rubber stopper above the culture medium. After that samples were filtered through four-layer cheesecloth and then liquor was used to measure pH using digital pH meter. For ammonia-N determination 0.3 mL of 50% $H_2SO_4$ was added to 15.0 mL of rumen fluid. For volatile fatty acid (VFA) determination, 1.0 mL of saturated $HgCl_2$ solution and 4.0 mL of 1 M NaOH solution were added to 10.0 mL of rumen fluid. Samples were stored frozen at - 20°C until analysed. Ammonia-N and VFA were determined according to (Choi and Oh, 2011).

### Feed composition analysis

All the treatment samples were frozen and dried in a 60°C forced air oven. Samples were ground to pass a 6 mm sieve in a Wiley mill (Model 4; Thomas scientific, Swedesboro, NJ, USA) and analyzed for DM, crude protein (CP), ether extract, crude fiber and crude ash according to the procedure of (AOAC, 1990). The concentration of neutral detergent fiber corrected for residual ash was determined with heat-stable amylase and sodium sulfate according to the method of (Van Soest et al., 1991). While the concentration of acid detergent fiber corrected for residual ash was determined according to the procedure of (AOAC, 1990). The chemical composition of experimental feeds is given in Table 1.

### Statistical analysis

Data for pH, gas production, ammonia N and VFA were analyzed using the general linear model procedure of the Statistical Analysis System Institute, Inc. (SAS, 2002). Differences among means were tested for significance ($p < 0.05$) using the least significant difference.

## Results and Discussion

Differences in ruminal pH were observed among different levels of Korean corn grains (Table 2

**Table 1.** Chemical composition of experimental feeds (% of dry matter basis).

| Items | Total mixed ration[z] | Domestic corn |
|---|---|---|
| Dry matter (%) | 62.75 | 81.21 |
| Crude protein | 17.24 | 10.95 |
| Ether extract | 5.85 | 5.62 |
| Crude fiber | 13.85 | 2.23 |
| Crude Ash | 7.92 | 1.31 |
| Calcium | 0.62 | 0.0070 |
| Phosphorus | 0.53 | 0.32 |
| Neutral detergent fiber | 32.30 | 7.81 |
| Acid detergent fiber | 15.75 | 2.13 |
| Calorie (kcal/g of dry matter) | 4.41 | 4.49 |

[z]Total mixed ration consisted of commercial corn based concentrate (48.5%), barley brewer's grain (25.5%), rice straw silage (13.4%), perilla meal (6.7%), corn flake (2.8%), soybean curd process (2.8%) and limestone (0.3%).

and Fig. 1). Mean ruminal pH was significantly lower ($p < 0.05$) for HC than control. Changes in rumen pH was rather similar between the groups till 6 h after incubation, but the lowest pH for HC (pH 5.10) appeared at 48 h compared with control and LC. Generally low ruminal pH can affect feed intake, microbial metabolism and feed digestion (Dijkstra et al., 2012). Previous study

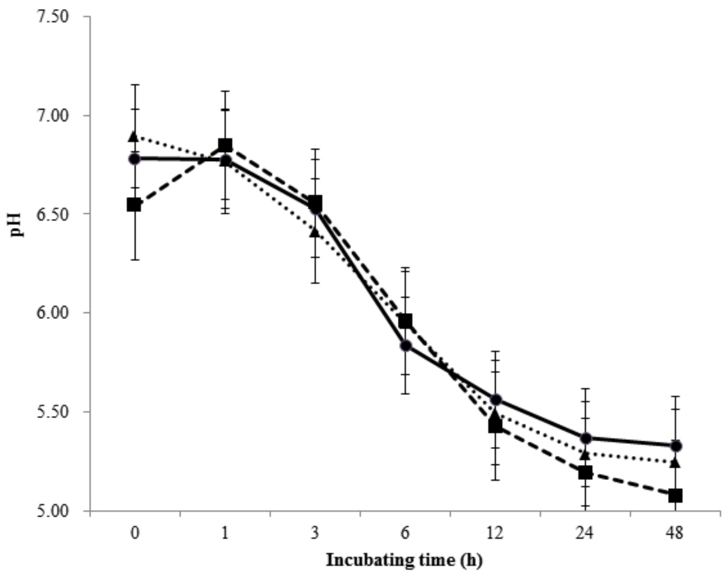


**Fig. 1.** Changes in rumen pH after ruminal fermentation incubated with different levels of Korean corn grains with total mixed ration (TMR) as a basal. Treatments were Control ● (TMR only, 3.0 g), HC ■ (TMR 1.5 + corn 1.5 g) and LC ▲ (TMR 2.25 + corn 0.75 g), respectively.

**Table 2.** Effect of different levels of Korean corn grains with total mixed ration on *in vitro* rumen fermentation parameters.

| Items | Treatments[z] | | | |
|---|---|---|---|---|
| | Control | HC | LC | SEM |
| Ruminal pH | 6.03a | 5.95b | 6.01ab | 0.026 |
| Gas production (mL/g DM) | 92.9 | 98.9 | | 2.370 |
| Ammonia N (mg/l) | 101.6a | 74.7b | 100.3a | 4.750 |
| Total VFA (mmoles/100mL) | 89.1b | 124.6a | 121.3a | 6.200 |
| Individual VFA (mmoles/100mL) | | | | |
| Acetate | 51.2b | 50.9b | 52.1a | 0.210 |
| Propionate | 25.7a | 25.3b | 25.09b | 0.104 |
| Iso-butyrate | 0.91a | 0.86b | 0.86b | 0.009 |
| Butyrate | 17.4c | 18.9a | 17.9b | 0.120 |
| Iso-valerate | 1.51b | 1.58a | 1.55b | 0.020 |
| Valerate | 1.72a | 1.57b | 1.64ab | 0.010 |
| Capronic acid | 0.73 | 0.72 | 0.76 | 0.009 |
| Acetate/Propionate | 2.02b | 2.05b | | 0.015 |

SEM, Standard error of the mean; VFA, Volatile fatty acid; TMR, total mixed ration.
[z]Treatments were Control (3.0 g TMR only), HC (TMR 1.5 + corn 1.5 g) and LC (TMR 2.25 + corn 0.75 g), respectively.
a - c: Means in a row with different letters are significantly different ($p < 0.05$).

presented by Lee et al. (2002) also stated that increased levels of grinding processed treatments of corn can cause a linear reduction in pH and it also implies that, up to 12 h of incubation VFA and lactic acids are rapidly produced. In the present study, the pH gradually decreased among all the treatments but significant difference ($p < 0.05$) appeared at 48 h after incubation mentioned above, in particular the pH for HC at 48 h was 5.10. Critical pH which degradation of fiber is impaired is approximately 6.0 - 6.3 (Mourino et al., 2001). Although the present data was produced from *in vitro* trial and the pH for the other groups was rather low (below pH 5.5), the TMR substituted with high level of corn may induce negative microbial environment for fiber degradation in the rumen (Mourino et al., 2001; Kim et al., 2017b). However, ruminal pH from *in vitro* rumen fermentation incubated with higher level of corn grain have often decreased during the entire incubation period, in particular at late incubation times (Kim et al., 2017b) due to faster and sustainable starch degradation with increased microbial availability and lactic acid production as compared to ruminal degradation incubated with other types of carbohydrates including fiber (Van Zwieten et al., 2008).

Despite lack of significances, mean gas production for HC tended ($p < 0.09$, data not shown) to be higher than the other groups (Table 2). Throughout the incubation period, similar patterns in gas production appeared between the groups (Fig. 2), but accumulated gas production value was highest for HC followed by LC compared with control. Consistent with the present result, during *in vitro* incubation at increasing level of corn grains, increased gas production was reported (Lee et al., 2002). Amount of gas produced during *in vitro* incubation is proportional to acid production (Getachew et al., 2004). In the present study, the high gas production for HC in control was due presumably to

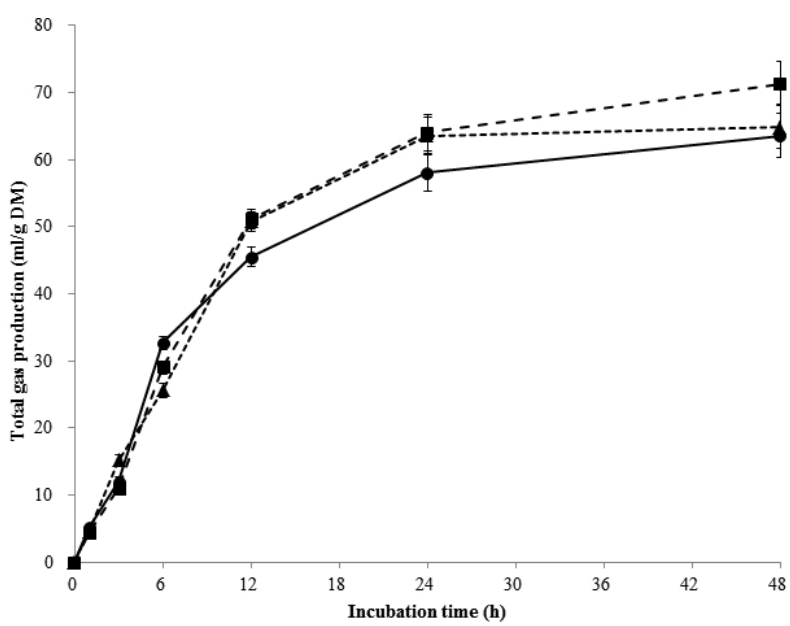


**Fig. 2.** Changes in gas production after ruminal fermentation incubated with different levels of Korean corn grains with total mixed ration (TMR) as a basal. Treatments were Control ● (TMR only, 3.0 g), HC ■ (TMR 1.5 + corn 1.5 g) and LC ▲ (TMR 2.25 + corn 0.75 g), respectively.

sustainable ruminal starch degradation as stated in ruminal pH above. The present observation may be correct because higher gas production is performed during *in vitro* incubation of energy feedstuffs due to higher microbial activity (Kim et al., 2015).

Mean ammonia N concentration was significantly higher ($p < 0.05$) for control and LC than HC (Table 2). No differences in ammonia-N between control and LC were rather acceptable due to slight different CP between the two groups (i.e., CP for control and LC was 17.2 and 15.7 %, data not shown). However, ammonia-N for HC (CP 14.1%, data not shown) was extremely low ($p < 0.05$) compared with the other groups. Up to 6 h of *in vitro* incubation, ammonia-N patterns appeared to be similar among the groups (Fig. 3). From the 12 h after incubation, ammonia-N concentration increased but its pattern for HC with the lowest spot at 12 h was lower than the other groups. The extremely low in mean ammonia N for HC was derived partially from the lowest ammonia N at 12 h, which could be analyzing error. Assuming that the unexpected error was accepted, the low ammonia-N pattern for HC was rather acceptable due to low CP content discussed above. Consistent with the present study, Kim et al. (2017b) recently reported the similar *in vitro* result that ammonia-N concentration in corn treated groups was significantly lower as compared to TMR group with no corn. When high CP content are available in the rumen, microbial activity generally increases and lead to increased ammonia-N concentration (Erdman et al., 1986). Due to low rumen degradable protein contents of corn grains as energy sources, ammonia-N concentration generally appears to be low during the beginning of *in vitro* rumen fermentation incubation with corn grains (Lee et al., 2002; Kim et al., 2017b). Present observation in terms of ammonia-N is supported by previous in vivo study conducted by Tamminga (2006) in which lower protein contents in the diet leads to lower ammonia-N

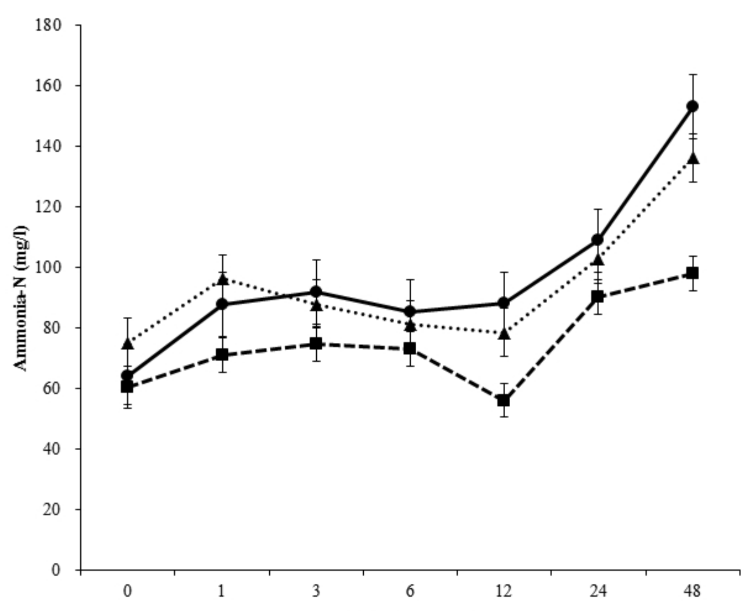


**Fig. 3.** Changes in ammonia N after ruminal fermentation incubated with different levels of Korean corn grains with total mixed ration (TMR) as a basal. Treatments were Control ● (TMR only, 3.0 g), HC ■ (TMR 1.5 + corn 1.5 g) and LC ▲ (TMR 2.25 + corn 0.75 g), respectively.

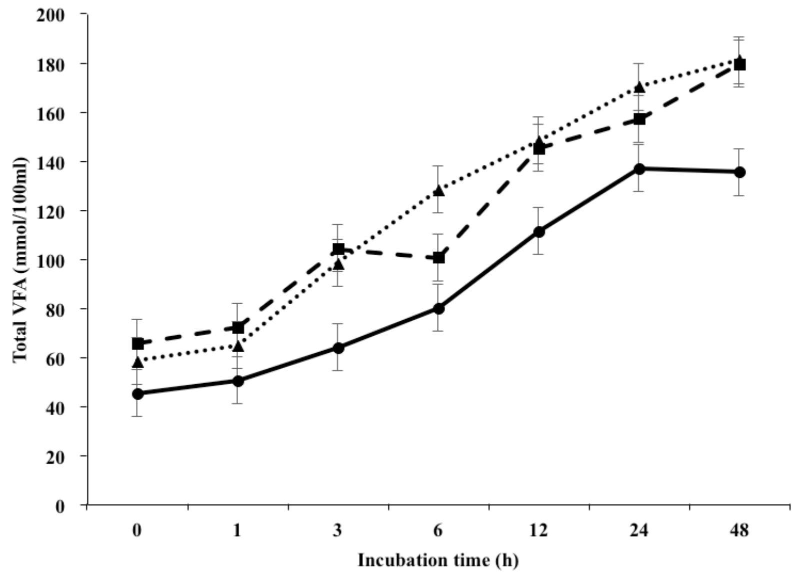


**Fig. 4.** Changes in volatile fatty acid (VFA) after ruminal fermentation incubated with different levels of Korean corn grains with total mixed ration (TMR) as a basal. Treatments were Control ● (TMR only, 3.0 g), HC ■ (TMR 1.5 + corn 1.5 g) and LC ▲ (TMR 2.25 + corn 0.75 g), respectively.

concentration. High soluble N content in TMR rather than corn grain may also cause high ammonia-N concentration for control and LC because high soluble N in feeds was closely related to high ammonia-N in the rumen (Armentano et al., 1993).

Incubation with corn grains statistically increased ($p < 0.05$) mean total VFA as compared to control (Table 2), and VFA patterns showed to be clearly higher for HC and LC than control during the entire trial except for the beginning of incubation at 0 and 1 h after incubation (Fig. 4). At 6 h after incubation, VFA for HC decreased, but it may be derived from analyzing error because each VFA was accumulated value. Present mean VFA concentration was in negative relationship to ruminal pH (Table 2). Generally, when ruminal pH decreases, increased ruminal VFA concentration was observed during rumen fermentation (Lee et al., 2002). VFA production parameters during ruminal fermentation has much importance because it provides 70% of the ruminant's energy supply (Dijkstra et al., 2012). Consistent with the present study, total VFA increased and ratio of acetate to propionate decreased when rumen fluid was incubated with ground corn grain for 48 h (Lee et al., 2002).

# Conclusion

The results of present study clearly implies that feeding different levels of Korean domestic corn grain may lead to high and sustainable starch degradation in the rumen. It also shows that high level of Korean corn grain may induce unstable rumen fermentation characteristics.

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