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RESEARCH ARTICLE

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Effects of *Liriope platyphylla* and Organic Acids in Hanwoo Feed on Greenhouse Gas Reduction

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ABSTRACT

Background: *Liriope platyphylla* and organic acids (humic and fulvic acids) as additives in the feedstock may aid in decreasing CH_4 formation in the rumen. Based on previous studies, Hanwoo steers were raised with feedstock added with *Liriope platyphylla* and organic acids and their fecal samples were analyzed to determine the changes in their organic matter and nitrogen content determining the GHG emissions from feces in the form of CH_4 and N_2O.

Methods: Ten Hanwoo cattle were provided with experimental diet and their weights were measured to determine harmful effects of experimental diet and measure fecal composition. To measure CH_4 emission from the rumen *in vitro*, rumen fluid with *Liriope platyphylla* 1.0% and organic acids 0.05% added to the feed was prepared and incubated in triplicate.

Result: The addition of *Liriope platyphylla* had a positive effect on immune function and blood composition, as well as CH_4 reduction and further the addition of organic acids resulted in the lowering of CH_4 emissions when compared with that of the control. The addition of *Liriope platyphylla* and organic acids is a potential additive of reducing CH_4 emissions from ruminants without disrupting cattle growth.

Key words: Greenhouse gas, Hanwoo, *Liriope platyphylla*, Organic acids.

INTRODUCTION

Greenhouse gas (GHG) emissions from ruminants have been greatly highlighted because the livestock industry produces 14.5% of the anthropogenic GHG emission. In addition to GHG emissions from livestock manure, enteric GHG emissions from ruminants are an unavoidable by-product of beef production, because anaerobic digestion in the rumen of cattle, which releases GHG emissions, is inevitable. In the entire livestock farming system, including transportation, feed production and use of fertilizers, enteric fermentation accounts for 39% of GHG emission produced (Grossi *et al*., 2019). Many studies from each country have reported how to decrease CH_4 and N_2O produced by the agricultural sector.

Similar to CH_4 emissions, GHG emissions from enteric fermentation are influenced by the type of feedstock provided and the components of feed for ruminants are divers and depend on the climatic conditions of the area. Rossi *et al*. (2001) reported CH_4 emissions from ruminants being 3.29 and 3.74 mM/g dry matter (DM) with corn and barley, respectively, as feedstock via an *in vitro* test. Chaudhry *et al*. (2012), in an *in vitro* study, described a higher production of CH_4 from wheat fermentation than hay fermentation due to greater hydrogen transfer in wheat fermentation. As ruminal digestibility, pH, VFAs, CO_2, H_2 and CH_4 emissions have been shown to vary with livestock feed, further studies are required on the variations with different feed rations. Hence, many studies to reduce CH_4 emission via feedstock have been widely carried out. Crops with high tannin content were found to induce relatively low CH_4 emissions without hindering digestibility (Carulla *et al*., 2005). Additionally, Oliveira *et al*. (2007) reported that there were no effects on CH_4 reduction with a high tannin concentration in sorghum silage. On the other hand, saponins are known to synthesize microbial proteins and biogas and decrease hydrogen availability for methane evolution in the rumen by reducing protein degradation (Makkar *et al*., 1998). Mao *et al*. (2010) also showed that an experiment with lamb for 60 days in a respiration chamber resulted in a 27.7% reduction in CH_4 emissions. In recent years, *Liriope platyphylla* has become known for its antidiabetic, mnemonic and anti-inflammatory effects (Cho *et al*., 2013). However, the articles using *Liriope platyphylla* containing saponins for GHG reduction from ruminants have rarely been found so far.

Humus materials have also been reported to potentially lower GHG emissions from ruminants (Terry *et al*., 2018). The addition of humus materials lowered CH_4 production by ruminants *in vitro* (Tan *et al*., 2018). Martinez *et al*. (2013) showed that the reduction in CH_4 production from ruminants was caused by the addition of humus had no effects on CH_4

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reduction due to the physico-chemical differences in the humus materials studied. Additionally, CH_4 productivity might be recovered by microbial floc changes was observed with humus addition to feed (McMurphy *et al.*, 2011).

As mentioned above, since it has been reported that the component of saponins and humus matter potentially reduced GHGs such as CH_4, the addition of humus matter and *Liriope platyphylla* being distributed widely in Korea containing a large amount of saponins may be hypothesized for GHG reduction in this study. Besides, *Liriope platyphylla* among diverse raw materials for cattle feed was finally selected with consideration of its supply and demand, feed process and economic feasibility with respect to the application for the industrialization. Thus, our study aimed to determine the effects of adding 1.0% *Liriope platyphylla* and 0.05% humus matter to feed stock *in vitro* on ruminant fecal characteristics and GHG emissions.

MATERIALS AND METHODS

The study was conducted for 6 months from July to December in 2021 at Gyeongbuk Livestock Research Institute in Yeongju and Daegu University in Gyeongsan, South Korea. To determine the effects of additives in feedstock on livestock GHG emission reduction, 10 Hanwoo were prepared for final fattening. This experiment was approved by the Laboratory animal use and management committee with the approval number of GAEC/130/20. The effects of commercially sold feed (as the control (K)) were compared with those of two different basal diets (T1 and T2) that were each supplemented with *Liriope platyphylla* (1.0%) and 0.05% organic acid (humic and fulvic acids). The organic acids were composed of 1330 and 8622 mg/kg humic and fulvic acids, respectively, in the liquid phase. The basal diets for treatments T1 and T2 with *Liriope platyphylla* were indicated as T1M and T2M and T1MH and T2MH with the addition of humus to them (Table 1).

Hence, the effects of *Liriope platyphylla* and humus matter on GHG emissions and fecal composition were investigated from the two different basal diet and commercially selling feed (Table 2).

Preparation of feed for hanwoo

The total digestible nutrients (TDN) in the commercial feed, T1 and T2 were 85.1, 84.9 and 85.3%, respectively. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents of T2 were the lowest at 7.2 and 21.3%, respectively. Hanwoo were fed daily with a concentrate feed of 12 kg per steer and forage of 2 kg per steer with tall fescue (original basis), twice a day at 9 am and 5 pm, respectively. The steers were housed in a stanchion barn with sufficient space during the experiment and were given feed according to the Korean feeding standard program. Mineral blocks and water were fed ad libitum. To avoid transition effects from the previous feeding, each test feed was supplied to Hanwoo for at least two weeks and samples were collected on the last day of the assigned feed.

Blood analysis

At the end of the test due to feed change, blood samples were collected from all steers at 8:00 am. Serum was used for blood samples used in immunoglobulin G (Cytation i3; BioTek Instruments Inc., USA) and biochemistry (DRI-CHEM NX500i; FUJIFILM Corporation., JAPAN) analysis equipment. Complete blood cell count were analyzed with an automated hematology analyzer (ProCyte Dx Hematology Analyzer Vet; IDEXX Laboratories Inc., USA).

Determination of GHG emission potential from feces

Cattle feces were collected to analyze any changes in their compositions with feed changes. The samples were picked from the ruminants' rectum directly to minimize exposure to oxygen and stored at <-4°C until further analysis. The analyses were carried out for total solids (TS), total volatile solids (TVS), total nitrogen (T-N), NH_4-N, NOx-N, total phosphorus (T-P) and ortho-phosphate (O-P), following Standard methods for the examination of water and wastewater (APHA, 2005).

The contents of TVS and T-N in feces can influence the emission of GHGs while being treated in barns or composting lot. Furthermore, the potential of CH_4 and N_2O emissions from manure to act as GHGs were estimated in terms of TVS and T-N, respectively, according to the international panel on climate change (IPCC) guidelines (2006). The default values of CH_4 and N_2O emissions were suggested to be 0.1 m^3/kg VS and 0.02 kg N_2O/kg T-N in Asia.

Determination of GHG emissions from enteric digestion

Methane is produced as the end-product when the test feeds are digested in the rumen. To confirm the effects of feed additives on GHG emissions, approximately 2 L of rumen fluid was obtained from 30-month-old Hanwoo steers that weighted approximately 650 kg. The rumen fluid was collected after over 2 h of feeding and stored under anaerobic conditions at 29°C. The rumen fluid was filtered with three layers of cheese-cloth in the laboratory and the filtrate was sealed with N_2 gas to induce anaerobic conditions. McDougall's buffer has been widely used to avoid acidification

Table 1: Experimental design.

Positive control (K)	T1	T1 M	T1 MH
Commercial feed	-	T1 + *Liriope platypylla* (1.0%)	T1 + *Liriope platypylla* (1.0%) + Organic acid (0.05%)
	T2	T2 M	T2 MH
	-	T2 + *Liriope platypylla* (1.0%)	T2 + *Liriope platypylla* (1.0%) + Organic acid (0.05%)

of rumen fluid during fermentation (McDougall, 1948), the composition of which is tabulated in Table 3. The prepared buffer solution was mixed with the filtrate of rumen fluid in a 4 to 1 ratio and made to a volume of 250 mL; further, 2 g of the testing feed was applied to each bottle in triplicate.

Feed degradability and GHG emissions from rumen fluid were measured with 500 mL gas sealed bottles, which were connected to Tedlar bags and placed in a shaking incubator set at 40°C. The fermentation period was maintained for 24 h. After incubation, the gas samples were analyzed by gas chromatography (GC, Varian CP-3800, USA) equipped with a flame ionization detector. A Poraplot Q column was prepared and the temperatures of the injector, oven and detector were set to 240, 100 and 250°C, respectively.

Rumen microorganisms produce volatile fatty acids (VFAs) such as acetate (HAc), propionate (HPr) and butyrate (HBu), which may influence the degradability of each feedstock. To analyze the VFA contents, the same GC with gas analysis was used at a different channel equipped with a DB-FFAP 30 m column. The internal standard was heptanoic acid (100 mg/L).

Statistical analysis

Statistical analysis was carried out using the MIXED procedure of SAS (Version 9.1; SAS Institute Inc., Cary, NC) with treatment as fixed variable effect. Calculate method of least squares for each treatment, Average values were calculated through PDIFF option. The unit of experiment was Hanwoo. Statistical differences were considered significantly at $P<0.05$.

RESULTS AND DISCUSSION

Physiological changes

During the experiment of 185 days, four weight measurements were carried out. The average weight gain of the Hanwoo steer was 753.5±148.3 g/d, which was maintained independent of the test feedstock and there was no refusal to change the feedstock. Through blood tests, the levels of immunoglobulin G (IgG), which plays a role in the supply of antibodies to repress disease infection from pathogenic microorganisms, were measured, as the test additives in feedstock contained β-sitosterol, stigmasterol, steroid saponin, *etc*., which have been known to have properties conducive to respiratory disease treatment and the improvement of respiratory and immune function. *Liriope platyphylla* has been widely used as a medicinal herb in Korea and steroids derived from *Liriope platyphylla* are safe, because their mechanisms differ from those of other steroid hormones that show anti-inflammatory effects. All treatments, except T1 and T2 treatments, showed significantly higher IgG levels ($p<0.01$) and T1M and T2M with only Liriope platypylla presented higher IgG levels than treatments T1MH and T2MH. Because the IgG level in T1M showed the greatest difference from the IgG level in the control, it is clear that the addition of 1.0% *Liriope platyphylla* positively impacted the immune function of the cattle (Table 4).

Table 5 shows the results of blood analyses. There were no significant differences between the levels of most parameters ($p>0.05$), but monocytes in leukocytes were significantly lower ($p<0.05$). Although lymphocytes are responsible for immune responses, complementary pathogenic defense microorganisms are supplied by monocytes; these were lower in level in T1M, T1MH, T2M and T2MH, which may have been caused by the increase in IgG and consequent decrease in the need to activate defense functions. The results of the serum chemistry examinations are tabulated in Table 5. When the level of cholesterol is higher than normal, angiosis, such as arteriosclerosis, can occur, but the treatments of T1M, T1MH, T2M and T2MH showed significantly lower levels of total

Table 2: Composition of basal diet formula for treatments.

Raw material	Commercial feed	T1	T2
	Weight (%)		
Corn flakes	35.0	35.0	35.0
Corn	20.8	18.7	24.2
Wheat flour	4.0	2.0	2.0
Wheat bran	12.3	12.2	16.3
Rice bran	0.0	10.5	9.6
Corn gluten feed	7.58	5.41	2.3
Rapeseed meal	2	2	5.4
Palm meal	12	8	0.0
Limestone	1.4	1.3	1.3
Salt	0.5	0.5	0.5
Silicate	0.5	0.5	0.0
Molasses	3.0	3.0	3.0
MSG by product	0.5	0.5	0.0
Vitamin+mineral	0.2	0.2	0.2
Probiotics	0.2	0.2	0.2
Total	100.0	100.0	100.0
Nutrients	**Dry matter (%)**		
Crude protein	12.6	12.6	12.7
TDN*	85.1	84.9	85.3
Ca	0.7	0.7	0.7
P	0.5	0.6	0.6
ADF	11.3	10.1	7.2
NDF	28.4	26.0	21.3

*TDN: Total digestible nutrients; ADF: Acid detergent fiber; NDF: Neutral detergent fiber.

Table 3: Composition of McDougall's buffer.

Component	Values (g/L)
$NaHCO_3$	9.80
Anhydrous Na_2HPO_4	3.71
KCl	0.57
NaCl	0.47
$MgSO_4 \cdot 7H_2O$	0.12
$CaCl_2 \cdot 2H_2O$	1.0

cholesterol ($p<0.05$). Total cholesterol is a type of fat and is known to be a harmful component of blood. Cholesterol is an important component of cell membranes, myelin of nerve cells and lipoproteins, as well as a source of steroid hormones and bile acids. Glutamic pyruvic transaminase (GPT), mainly present in the liver, increases with liver inflammation or certain types of hepatonecrosis. The level of GPT in the treatments was significantly lower than that in the control ($p<0.05$), indicating that liver function might have improved with the treatments relative to the control.

During the fattening period, cattle were able to access feed at any time and blood samples were collected at the same time for all the cattle. Regardless of the additives, T2M, T2MH and T2 treatment diets showed significantly higher GLU levels ($p<0.01$) but lower ADF and NDF levels than the T1 basal diet (Table 6). Blood urea nitrogen (BUN) levels can increase with impaired renal function.

However, because the increase in BUN in a short period occurs with protein hyper ingestion due to the excess concentrated feed and BUN is rarely reflective of renal function impairment, the significant difference in BUN may not be meaningful. In contrast, the level of albumin (ALB) synthesized in the liver decreases with enteritis and nephritis but increases with dehydration and chronic infectious conditions. Deficiencies in protein and fiber and excessive starch in feed can lead to low levels of ALB. Although the ALB level in the treatment group was significantly lower ($p<0.05$) than that in the K, these levels did not influence disease and health within the normal range; however, the risk of a chronic disease might have been lower in the treatment groups.

Feces composition

According to changes in additives in the feed diets, it was expected that the contents in feces would be influenced, but no significant variation was found ($p<0.05$). Thus, significant differences in digestibility were not found among the treatments, including the control.

Table 4: Effect of feed types on IgG index of Hanwoo.

Item[1]	Group							SEM	P-value
	K	T1M	T1MH	T2M	T2MH	T1	T2		
IgG (mg/mL)	24.4[c,d]	39.1[a]	28.2[b,c]	31.8[b]	29.3[b]	19.5[e]	23.0[d,e]	1.61	<0.001

[a-e] Means within a row without a common superscript letter differ ($p<0.05$).

[1]Each least squares mean represents seven observations.

Table 5: Effects of feed types on changes of blood composition.

Item[1]	Group							SEM	P-value
	K	T1M	T1MH	T2M	T2MH	T1	T2		
RBC[2] (M/iL)	9.5	8.7	8.2	8.7	8.3	8.5	8.1	0.38	0.163
HCT (%)	41.4	37.8	35.8	38.5	36.3	37.5	35.9	1.67	0.243
HGb (g/dL)	14.2	13.3	12.6	13.4	12.8	13.2	12.9	0.52	0.422
MCV (fL)	43.6	43.7	43.7	44.3	43.8	43.9	44.4	1.44	0.999
MCH (Pg)	14.9	15.3	15.3	15.4	15.4	15.5	15.9	0.38	0.69
MCHC (g/dL)	34.3	35.2	35.2	34.8	35.2	35.3	35.9	0.43	0.22
RDW (%)	36.4	35.8	34.5	34.4	32.9	33.2	32.4	1.42	0.376
RETIC (K/iL)	2.4	2.6	1.6	2.3	1.4	1.5	2	0.52	0.554
WBC (K/iL)	9.5	9	10.3	8.7	9.2	8.4	9.3	0.55	0.318
NEU (%)	37.8	39.1	40.6	37.9	37.4	37.2	37.6	1.78	0.823
LYM (%)	40.8	41.7	41.1	45	41.2	47.1	41.3	2.63	0.523
MONO (%)	6.6[a]	4.2[c]	6.1[ab]	4.7[bc]	5.5[abc]	6.4[a]	6.7[a]	0.59	0.023
EOS (%)	12.7	13.3	11.6	10.8	15	8.8	13.8	2.64	0.713
BASO (%)	2.1	1.7	0.6	1.6	0.9	0.5	0.7	0.59	0.302
PLT (K/iL)	364.3	357.9	356.9	328.6	389.1	343.4	371.3	25.78	0.754
MPV (fL)	9.7	9.7	9.7	10	9.8	9.8	9.9	0.2	0.945
PDW (fL)	8.2	8	8.1	8.3	7.9	8.1	7.4	0.31	0.525
PCT (%)	0.4	0.3	0.3	0.3	0.4	0.3	0.4	0.03	0.786

[a-c]Means within a row without a common superscript letter differ ($p<0.05$).

[1]Each least squares mean represents seven observations.

[2]RBC: Red blood cell; HCT: Hematocrit; HGb: Hemoglobin; MCV: Mean corpuscular volume; MCHC: Mean corpuscular hemoglobin concentration; RDW: Red cell distribution width; RETIC: Reticulocytet; WBC: White blood cell; NEU: Neutrophils; LYM: Lymphocytes; MONO: Mononucleosis; EOS: Eosinophil; BASO: Basophils; PLT: Platelet; MPV: Mean platelet volume; PDW: Platelet distribution width; PCT: Procalcitonin test.

The highest moisture content of 82.8% was found in T2M feces, which was not statistically significantly different from that of the other treatments. Furthermore, the index of organic compounds in feces, TVS/TS, was similar for all treatments (Table 6).

The 2006 IPCC guidelines suggested that the emission of methane and nitrous oxide from the agricultural sector be calculated based on the VS and T-N in manure. As seen in Table 6, VS values in all the treatments were significantly lower than those of the commercially sold control feed. In contrast, T-N in T2M was the highest at 5212.0 mg/kg, but T1MH and T2MH (which has organic acids) induced lower T-N in feces.

When comparing the global warming potentials (GWPs) of CO_2 equivalents based on the default values of IPCC 2006 for Asian cattle, the T2M treatment resulted in the lowest GWP of 25.5% of that of the control, taking into account both methane and nitrous oxide.

GHG emission *in vitro* test

Prepared feed supplemented with *Liriope platyphylla* and organic acids in the feedstock led to the reduction of CH_4 emissions from rumen fluid (Fig 1), the results of which are directly related to VFA production in rumen fluid during fermentation. As seen in Fig 1, the lowest CH_4 production rate and yield of 110.3 mg/kg C and 104.9 mg/L/h, respectively, were found in T2MH. T1MH induced production rate and yield values similar to those of T2MH, at 111.3 mg/kg C and 104.9 mg/L/h, respectively. The addition of *Liriope platyphylla* to T1M and T2M certainly led to the lowering of CH_4 production rate by 18.1 and 14.8%, respectively and compared with K (positive control), the addition of 0.05% organic acid to feedstock also decreased the CH_4 production rate of T1MH and T2MH by 22.5 and 23.2%, respectively.

The sum of VFAs in the commercial feed, K, was the highest compared to that in the other treatments (Fig 2). Metabolic pathways under anaerobic conditions were influenced by additives, *Liriope platyphylla* and organic acids. When their ratios were compared, the propionic acid content was higher in the treatments than that in the control, which probably affected CH_4 production induced by the treatments. The highest propionic acid content (17.7%) among C2, C3 and C4 (acetic, propionic and butyric acids) was observed in T1MH, which was consistent with the finding of Chen *et al*. (2020), who found that the active production of propionic acid during fermentation led to the improvement of feed degradation but not of CH_4 production.

In terms of the sums of VFAs, T1MH and T2MH with organic acids showed low conversion of feedstock to by-products (VFAs), which might indicate disruption of microbial activity in the rumen; however, the weight gain of cattle was not influenced by different feedstocks ($p<0.05$) during the test period of 6 months. The reduction in CH_4 production with the presence of organic acids was higher in the T2 group than that in the T1 group, which might be found in the basal diet (Table 7). NDF and ADF were lower in T2 than in

Table 6: Effect of feedstock changes on serum composition.

Item[1]	Group							SEM	P-value
	K	T1M	T1MH	T2M	T2MH	T1	T2		
TCHO (mg/dl)	196.1[ab]	188.9[abc]	170.3[bc]	161.7[c]	160.4[c]	203.4[a]	201.1[a]	10.44	0.012
GOT (U/l)	85.6	89.4	106.1	122.4	118.6	131.4	123.9	14.13	0.174
GPT (U/l)	29.6[a]	27.0[ab]	25.6[b]	27.0[ab]	24.6[b]	25.3[b]	26.3[b]	1.03	0.035
GLU (mg/dl)	58.1[d]	57.1[d]	65.7[c]	71.3[bc]	75.7[b]	70.6[bc]	83.4[a]	2.04	< 0.001
TG (mg/dl)	12.4	8.9	8.9	8.6	4.7	4.9	9.6	1.94	0.094
AMYL (U/l)	397.4	381.1	363.4	382.3	386.9	405.7	398.0	34.22	0.984
BUN (mg/dl)	16.8[b]	22.9[a]	10.2[cd]	9.5[d]	12.0[c]	15.8[b]	16.2[b]	0.76	< 0.001
ALB (g/dl)	4.1[a]	4.0[ab]	3.7[c]	3.8[bc]	3.6[c]	3.7[c]	3.8[bc]	0.08	0.003

[a-c]Means within a row without a common superscript letter differ ($p<0.05$).

[1]Each least squares mean represents seven observations.

[2]TCHO: Total cholesterol; GOT: Glutamic oxaloacetic transaminase; GPT: Glutamic pyruvic transaminase; GLU: Glucose; TG: Triglycerides; AMYL: Amylase; BUN: Blood urea nitrogen; ALB: Albumin.

Table 7: Feces composition with different additives in feedstock.

Treatment	TS*	TVS	NOx-N	NH_4-N	T-N	O-P	T-P	TVS/TS
		%	mg/kg					
K	23.4±1.9	20.7±1.7	17.1±5.2	142.6±188.7	4232.3±900.2	1007.4±245.3	2193.4±538.3	0.884
T1M	20.2±1.7	17.6±1.6	22.3±3.7	181.6±58.0	4136.3±421.0	958.8±141.9	2099.8±329.4	0.887
T1MH	19.0±2.2	16.8±2.1	34.1±5.1	97.7±40.2	3624.2±477.3	1016.5±96.8	1870.3±160.3	0.885
T2M	17.2±1.8	15.3±1.8	21.4±3.3	107.9±68.3	5212.9±471.4	1090.0±154.5	2237.2±359.7	0.863
T2MH	18.1±1.4	16.0±1.3	26.0±8.8	98.1±28.7	4401.0±597.4	1045.3±130.9	1488.8±1753.9	0.881

*TS: Total solids; TVS: Total volatile solids; T-N: Total nitrogen; O-P: Orthophosphate; T-P: Total phosphorus.

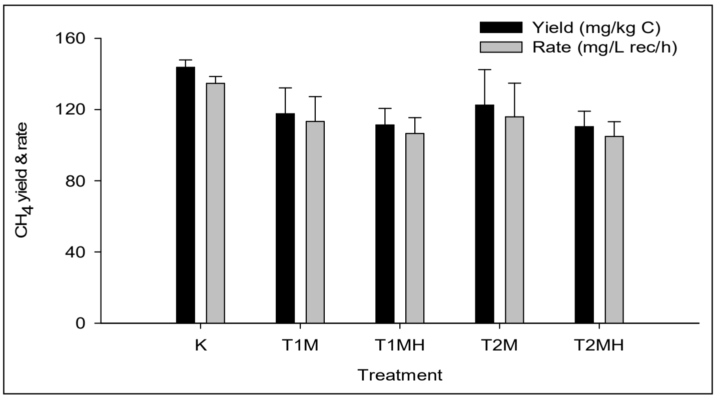

Fig 1: CH_4 production rate ($mg/L_{reactor}\cdot h$) and yield (mg/kg C) with feedstock changes.

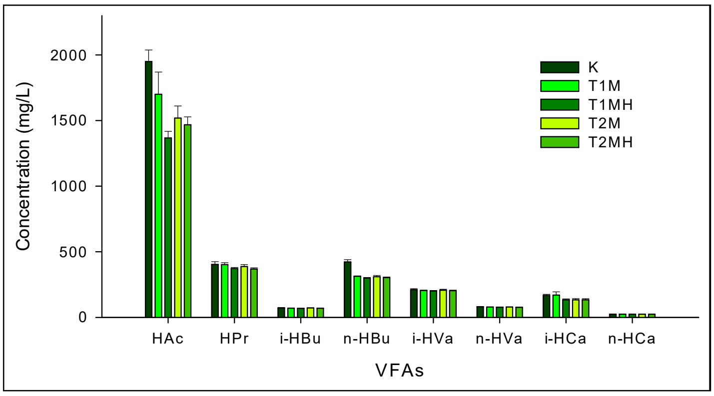

Fig 2: VFA concentration according to feedstock changes.

T1; T2 caused greater production of VFAs than T1 and the addition of organic acids induced an acidification of the rumen that was stronger than that induced by T1.

Overall, we found that *Liriope platyphylla* and organic acids caused reduction in CH_4 emissions during the test period *via* an *in vitro* test, but the optimal level of organic acids and its role to suppress GHG emissions is yet to be determined.

CONCLUSION

To maintain a sustainable beef industry, a reduction in GHG emissions from cattle is mandatory and the demand of GHG reduction has been continuously reinforced. Because cattle naturally emit CH_4, there is no straightforward method to reduce GHG emissions from livestock farms. Many materials have been reported to decrease the CH_4 emissions from the rumen in previous studies. Although *Liriope platyphylla* containing saponin as a feed additive for cattle might have high possibility to reduce GHG emissions from rumen, the effects of its addition have rarely been studied so far. Further, the humus was still unknown to confirm the GHG reduction from ruminal reaction. In this study, we investigated the efficacy of *Liriope platyphylla* and organic acid addition to cattle feed to reduce CH_4 emission. It was confirmed *via* blood analyses that the addition of *Liriope platyphylla* improved cattle health and the reduction in CH_4 emissions from rumen was found via *in vitro* test with ruminal microorganisms. In the case of the addition of organic acids, the ruminal circumstance was changed further to hinder CH_4 emissions, but this change might not be desirable. During the test period, the weight gain of cattle did not change and the addition of *Liriope platyphylla* certainly demonstrated its potential to reduce CH_4 emissions. Hence, further studies on organic acids in cattle feed may be required to determine their optimal amounts.

Conflict of interest: None.

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