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Effect of Allicin and Illite Supplementation on the Methane Production and Growth Performance of the Beef Cattle

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ABSTRACT

Background: Methane reduction technology has not yet been put into practical use because of problems such as reduced digestibility, increased costs, additives remaining and microbial adaptations. The objectives of this study were to evaluate the effects of allicin and illite supplementation on ruminal methane production, growth performance and carcass characteristics of the Hanwoo steers.

Methods: For the *in vitro* experiment, 3 Hanwoo cows equipped with rumen fistula were used. The 28 Hanwoo steers were randomly assigned to one of the four dietary groups: control (CON), T1 (1.0% illite), T2 (0.1% allicin), or T3 (1.0% illite and 0.1% allicin). All data were analyzed using the generalized linear model procedure.

Result: The methane production was rapidly increased for all the treatments after 8 h, but decreased compared to the control after 24 h ($P<0.05$) of incubation. There was no difference in the growth performance and carcass characteristics of steers among the treatments. Thus, allicin 0.1% and/or illite 1.0% have the potential to be used as natural feed additives to reduce methane production.

Key words: Allicin, Growth performance, Hanwoo steers, Illite, Methane.

INTRODUCTION

Anaerobic microorganisms in the rumen hydrolyze high molecular organic matter into low molecular organic matter and produce volatile fatty acids (VFAs), carbon dioxide (CO_2), hydrogen (H_2) and ethanol. Rumen methanogens use various substrates as electron acceptors to produce methane (CH_4) as the final metabolite (Martin *et al.,* 2010). Of the total gas generated from ruminants, 25-30% is CH_4 (Ellis *et al.,* 2007) and it has a negative impact on rumen energy loss and global warming (Sarkar *et al.,* 2018).

Studies of feeds and additives that aim to reduce the CH_4 generated from ruminants, have previously been conducted (Jafari *et al.,* 2020). However, CH_4 reduction technology has not yet been put into practical use because of problems such as reduced digestibility, increased costs, additives remaining and microbial adaptations to the additives (Jordan *et al.,* 2006; Osita *et al.,* 2019). Natural substances that can reduce CH_4 production under conditions that maintain or increase the existing productivity of ruminants without negative effects on livestock products or the environment are thus required. Among various other natural substances, allicin and illite have been suggested for this purpose, as previous studies have found that they are capable of reducing CH_4 released by ruminant (Liu *et al.,* 2013). However, further research is required to verify and better understand this and there have not yet been any relevant field trials.

The objectives of this study were to evaluate the effects of allicin and illite supplementation on *in vitro* ruminal CH_4 production, growth performance and the carcass characteristics of the Hanwoo steers.

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MATERIALS AND METHODS

Study area

The study was conducted in the Hanwoo Research Institute and Gangwon Livestock Techcology Institute during May 2019 to April 2020.

Animals, treatments and management

For the *in vitro* experiment, 3 Hanwoo cows (average weight 450.4±22.6 kg) were equipped with rumen fistula and 28 late fattening Hanwoo steers (average weight: 636.3±50.7 kg) were used in the field trial. The steers were randomly assigned to one of the four dietary groups: control (CON), T1 (1.0% illite), T2 (0.1% allicin), or T3 (1.0% illite and 0.1% allicin).

Concentrate was provided three times daily using an automatic feeding system, at volumes of approximately 2.0% (as-fed basis) of the body weight (BW) for the entire experimental period. Rice straw and water could be accessed freely. The chemical compositions of experimental diets are presented in Table 1.

In vitro ruminal fermentable characteristics

Ruminal fluid was collected from the ruminal fistulas of the Hanwoo cows before feeding in the morning and filtered through four layers of cheese cloth and was then used as an inoculum for *in vitro* incubation. *In vitro* cultures were established by mixing rumen inoculum with a previously prepared artificial buffer solution in accordance. 100 mL of the prepared *in vitro* culture solution was placed in a 160 mL bottle containing concentrate and additives (allicin and/ or illite) and O_2-free CO_2 gas was infused for 5 s to eliminate the air. The bottles were then incubated for 2, 4, 8, 12 and 24 h in a shaking incubator (HB-201SLI, Hanbaek Scientific Co., Bucheon, Korea) at 39°C.

Measurements and analyses

The *in vitro* ruminal pH was measured using a pH meter (Corning Glass Works, Medfield, MA, USA). The ammonia concentration was calculated by measuring the absorbance at 630 nm using a spectrophotometer (Spectronics 21D, B&L, NY, USA).

To analyze the VFAs concentrations, 10 mL of the culture solution was collected in a 160 mL bottle for each incubation time, followed by the addition of 1 mL of 20% HPO_3 and 0.5 mL of saturated $HgCl_2$ and then centrifuged at 4°C at 1,000 × g for 15 min. The supernatant was discarded and the VFA concentration was measured via gas chromatography (Shimadzu-17A, Shimadzu, Kyoto, Japan).

Total gas production (TGP) was measured using a pressure sensor gauge (EA-6, SunBee Instrument, Seoul, Korea). To measure CH_4 and CO_2 production after TGP, the gas generated using a vacuum tube was collected and analyzed using gas chromatography (Agilent Technologies HP 5890, Agilent, CA, USA). CH_4 production was estimated using the formula of Qrskov and McDonald (1979) with the CH_4 obtained from the TGP for each incubation time.

Average daily gain (ADG) was calculated by measuring BW at 10 am every 2 months. Dry matter intake (DMI) was measured weekly and the amount of DMI was determined by measuring the orts present before the morning feeding. The feed conversion ratio (FCR) was calculated using the DMI and ADG.

At the end of the experimental period, all steers were slaughtered at the local slaughterhouse. Yield and quality grades were determined as the evaluation criteria of the Korean carcass grading system (MAFRA, 2018).

Statistical analyses

All data were analyzed using the generalized linear model procedure in the SAS package 9.1 software program (SAS Institute Inc.; Carey, NC, USA). Significant differences between the treatments were analyzed using Tukey's test at the 95% significance level.

RESULTS AND DISCUSSION

Ruminant pH and ammonia concentration

The effects of the allicin and illite supplementations on the *in vitro* ruminal pH and ammonia concentrations are summarized in Table 2. Ruminal pH was significantly lower in T2 and T3 compared to control at 4 h of incubation ($P<0.02$), but overall there was little difference among the treatment groups. Ammonia concentrations were not affected by the addition of allicin and illite.

Similar to our study, Soriano (2014) reported a transient decrease in rumen pH in the 0.5% garlic powder treatment only at the early incubation (2 h). However, in this study, as reported by Wanapat *et al.* (2008) and Biswas *et al.* (2018), illite and allicin do not appear to affect rumen pH and ammonia production.

VFA concentrations

The effects of allicin and illite supplementation on the *in vitro* ruminal VFA concentrations are shown in Table 3. Acetate concentration was lowest in T3 after 4 and 8 h of incubation, but the acetate concentration after 24 h was significantly higher in T3 than in the control and T1 ($P<0.05$). Propionate concentrations tended to increase in all the treatment groups as the incubation time increased and were higher in the control than in the T1 and T3 after 4 h of incubation ($P<0.01$). The total VFAs concentration was significantly ($P<0.05$) higher in the control than the T3 after 4 h of incubation, but there was no difference between the treatments at different incubation times.

Table 1: Chemical composition of experimental diets.

Item	Treatments				Rice straw
	Control	T1	T2	T3	
			Chemical composition (%)		
TDN[1]	73.00	73.16	73.44	73.14	41.50
Dry matter	88.68	88.64	88.69	88.66	8.21
Crude protein	12.80	12.93	12.87	12.90	4.05
Ether extract	3.76	3.58	3.76	3.64	1.89
Crude fiber	6.10	5.25	6.10	5.26	30.42
Crude ash	6.62	6.35	6.51	6.60	12.38

[1]TDN-total digestible nutrients (calculated value).

When CH_4 production is reduced in the rumen, it is converted into reactions that consume H_2, such as the production of propionate and butyrate due to the H_2 sink (Busquet *et al.,* 2005). In the present study, allicin supplementation increased the concentrations of propionate and butyrate at the partial incubation time and therefore, allicin supplementation was considered to affect the reduction in CH_4. This finding is concurrent with a previous investigation (Kongmun *et al.,* 2011). Biswas *et al.* (2018) reported that the concentrations of propionate and butyrate

Table 2: Effects of illite and allicin supplementation on *in vitro* ruminal pH and ammonia concentration of experimental diets.

Item (hrs)	Treatment					
	Control	T1	T2	T3	SEM[1]	Pr> |t|
pH						
2	5.56	5.56	5.56	5.55	0.02	0.993
4	5.41[a]	5.37[ab]	5.23[c]	5.33[b]	0.02	0.002
8	5.25	5.24	5.26	5.30	0.02	0.244
12	5.17	5.16	5.19	5.19	0.02	0.783
24	5.16	5.18	5.12	5.16	0.03	0.794
Ammonia-N, mg/100 mL						
2	10.55	10.08	10.03	11.45	0.51	0.395
4	10.98	11.38	10.89	12.18	0.66	0.525
8	12.80	13.49	12.52	7.91	2.19	0.449
12	13.69	14.16	15.92	16.89	0.93	0.208
24	17.12	17.61	16.29	18.65	0.42	0.068

[1]SEM- Standard error of the mean.

[a,b,c] Means with different superscripts in the same row differ (P<0.05).

Table 3: Effects of illite and allicin supplementation on *in vitro* ruminal volatile fatty acids concentrations of experimental diets.

Item (hrs)	Treatment					
	Con	T1	T2	T3	SEM[1]	Pr> |t|
Acetic acid, mM/L						
2	25.69	25.74	26.19	24.64	0.51	0.592
4	20.86[a]	20.65[ab]	20.53[ab]	20.20[b]	0.11	0.042
8	23.20[a]	23.15[a]	22.78[ab]	22.43[b]	0.16	0.040
12	20.78	20.89	21.07	20.95	0.13	0.525
24	23.45[b]	23.77[b]	23.82[ab]	24.30[a]	0.14	0.027
Propionic acid, mM/L						
2	7.18	7.15	7.01	7.12	0.09	0.656
4	8.59[a]	8.25[c]	8.46[ab]	8.31[bc]	0.04	0.007
8	11.41	10.16	11.14	9.95	1.42	0.864
12	10.47	9.86	11.27	11.54	0.68	0.523
24	14.46	16.66	15.84	16.19	0.63	0.433
Butyric acid, mM/L						
2	10.08[b]	11.07[a]	11.24[a]	11.61[a]	0.27	0.031
4	11.34	9.99	10.33	8.73	0.65	0.144
8	7.84	7.69	7.99	8.79	0.23	0.057
12	11.73[b]	12.28[ab]	12.70[a]	12.73[a]	0.19	0.024
24	15.47[b]	16.07[ab]	16.03[ab]	16.55[a]	0.18	0.035
Total VFAs[2], mM/L						
2	42.95	43.96	44.44	43.37	0.57	0.501
4	40.80[a]	38.88[ab]	39.32[ab]	37.24[b]	0.65	0.041
8	42.45	41.00	41.91	41.18	1.52	0.903
12	42.98	43.03	45.04	45.22	0.71	0.112
24	53.37	56.51	55.69	57.04	0.86	0.204

[1]SEM- Standard error of the mean; [2]VFAs- volatile fatty acids.

[a,b,c] Means with different superscripts in the same row differ (P<0.05).

were increased by the supplementation of illite because the trace minerals contained in illite acted as enzyme components that could increase the VFAs by accelerating the metabolic pathway. However, further research is required to better understand the exact metabolic pathways and interactions.

Gas production

The effects of the allicin and illite supplementations on *in vitro* ruminal gas production are shown in Table 4. TGP did not differ among treatments after 12 h of incubation but was significantly lower in T2 than in the control and T1 after 24 h ($P<0.05$). CH_4 production was lower in all the treatments compared to the control after 24 h of incubation ($P<0.05$). The production of CO_2 was significantly lower in the control and T3 treatment than in T1 and T2 after 12 h of incubation ($P<0.01$).

Allicin been reported to reduce the production of CH_4 by reducing the number of methanogens (Kongmun *et al.,* 2011). Busquet *et al.* (2005) reported that CH_4 production was significantly reduced by allicin supplementation. They also found that the supplementation of allicin reduced the deoxyribonucleic acid (DNA) of methanogens. Meanwhile, Liu *et al.* (2013) suggested that illite had a high CH_4 adsorption capacity, which reduced CH_4 production in the intestine and Biswas *et al.* (2018) found that CH_4 production was reduced by 13% with 1% illite supplementations. As a result, it was presumed that allicin affected the methanogens, reduced CH_4 production and thereby increased the concentration of CO_2. In addition, it was also thought that illite may reduce the production of CH_4 gas through adsorption.

Growth performance

Table 4: Effects of illite and allicin supplementation on *in vitro* ruminal gas production of experimental diets.

Item (hrs)	Treatment					
	Control	T1	T2	T3	SEM[1]	Pr> |t|
Total gas, mM/L						
2	3.33	4.00	4.00	3.00	0.48	0.58
4	6.67	6.67	6.33	6.00	0.25	0.36
8	15.33	14.67	14.67	14.33	0.33	0.27
12	19.33	20.67	21.00	20.33	0.48	0.18
24	24.33[a]	24.33[a]	23.00[b]	23.67[ab]	0.25	0.03
Methane, mM/L						
2	0.62	0.67	0.56	0.64	0.07	0.908
4	0.90	0.82	0.85	0.88	0.10	0.942
8	2.47	2.53	2.35	2.03	0.26	0.520
12	2.72	3.13	3.35	2.50	0.41	0.638
24	4.60[a]	3.67[b]	3.41[b]	3.56[b]	0.06	0.029
Carbon dioxide, mM/L						
2	11.13	12.56	11.28	12.70	0.57	0.387
4	11.31	10.68	11.30	11.62	1.24	0.965
8	17.47	12.15	15.08	15.33	2.34	0.695
12	16.07[b]	18.12[ab]	18.75[a]	12.06[c]	0.64	0.004
24	18.12	21.53	24.40	22.79	2.14	0.319

[1]SEM- Standard error of the mean.

[a,b,c] Means with different superscripts in the same row differ ($P<0.05$).

Table 5: Effects of illite and allicin supplementation on growth performance of late fattening Hanwoo steers.

Item	Treatments					
	Control	T1	T2	T3	SEM[1]	Pr> |t|
Initial body weight, kg	643.17	628.03	630.14	643.92	8.12	0.232
Final body weight, kg	755.63	717.38	721.45	731.09	15.32	0.195
Average daily gain, kg	0.91	0.74	0.77	0.70	0.05	0.085
Feed intake (Dry matter)						
Concentrate intake, kg	10.17	9.43	10.20	9.24	0.15	0.517
Rice straw intake, kg	0.71	0.71	0.71	0.71	-	-
Total Dry matter intake, kg	10.88	10.14	10.91	9.96	0.19	0.653
Feed conversion ratio	11.96	13.70	14.17	14.23	0.21	0.263

[1]SEM- Standard error of the mean.

Table 6: Effects of illite and allicin supplementation on carcass traits of late fattening Hanwoo steers.

Item	Treatments					
	Control	T1	T2	T3	SEM[1]	Pr> |t|
Yield traits[2]						
Carcass weight, kg	440.76	427.28	420.31	432.78	6.32	0.682
Back fat thickness, mm	17.57	12.51	12.44	15.03	0.52	0.076
Rib eye area, cm^2	91.94	90.02	88.61	88.27	1.52	0.344
Yield index	61.79	65.03	65.06	63.10	0.41	0.154
Quality traits						
Marbling score	5.05	4.52	4.59	4.39	0.25	0.293
Meat color	5.01	4.99	4.98	5.15	0.03	0.525
Fat color	2.99	3.01	2.83	3.00	0.07	0.266
Texture	1.14	1.57	1.42	1.57	0.05	0.365
Maturity	2.00	2.00	2.00	2.00	-	-

[1]SEM- Standard error of the mean.

[2]Yield index was calculated using the following equation: [68.184 - (0.625 × back fat thickness (mm)) + (0.130 × rib eye area (cm^2)) - (0.024 × dressed weight amount (kg))] + 3.23.

The effects of the allicin and illite supplementation on the growth performance of the late fattening Hanwoo steers are shown in Table 5. There was little difference among the treatments for BW and ADG and the supplementation of allicin and illite had no effect on DMI or FCR.

Allicin has antibacterial activity and is known to improve the digestibility of organic matter in the rumen (Yang *et al.,* 2007). The supplementation of clay minerals such as illite has been reported to have a positive effect on ADG, feed efficiency and the prevention of rumen acidosis in cattle (Humer *et al.,* 2019). However, in this study, allicin and illite had no effect on growth performance. This may be because rumen or rumen microorganisms overcome and adapt to newly introduced compounds (Castillejos *et al.,* 2007).

Carcass characteristics

Table 6 shows the effect of allicin and illite supplementation on the carcass characteristics of the late fattening Hanwoo steers. In this study, carcass weight, Back fat thickness and Rib eye area were not affected by the addition of illite and allicin and there was no difference among treatments in quality traits.

Sung (2001) reported that, supplementation with 0.1% garlic powder reduced back fat thickness, but increased rib eye area and marbling score in Hanwoo steers. Kang *et al.* (2002) reported that the supplementation of 2% illite had the effect of increasing meat yield, meat quality and the farmer's income for Hanwoo steers. However, in this study, there was no difference in conductor characteristics and this might be due to the effect of the addition period and amount added.

CONCLUSION

Thus, allicin 0.1% and/or illite 1.0% could be used as natural feed additives to reduce CH_4 production without negatively affecting the *in vitro* ruminal fermentation, growth performance, or carcass characteristics of Hanwoo steers. In addition, we recommend that the supplementation of allicin alone is more efficient than the mixed supplementation of allicin and illite.

Conflict of interest: None.

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