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Effects of the Combined Odor Reducing Additive on Fecal Fermentation Characteristics and Growth Performance in Hanwoo Steers

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ABSTRACT

Background: Odors from livestock facilities are mainly caused by livestock manure. This causes deterioration of the health of the farm workers and the productivity of livestock. Complex additives with different actions are required to effectively reduce fecal contaminants. This study was conducted to investigate the effects of combined odor reducing additive (CORA) supplementation on the odor emission of feces and the growth performance of Hanwoo steers.

Methods: Ninety-six Hanwoo steers were randomly assigned to the following four groups: control group fed with CORA-unsupplemented formula feed; T0.05 group fed with 0.05% CORA-supplemented formula feed; T0.1 group fed with 0.1% CORA-supplemented formula feed and T0.2 group fed with 0.2% CORA-supplemented formula feed. The CORA comprised 88% zeolite, 3% Bacillus licheniformis, 3% Bacillus polyfermenticus and 6% saponin.

Result: The fecal NH_3-N gas emission was significantly lower in the T0.2 group than in the control group ($P<0.05$). The fecal H_2S gas emission at 20 d of incubation was lower in the T0.2 group than in the control group ($P<0.05$). The fecal NH_3-N concentration was lower in proportion in all the groups with different supplementation levels of CORA than in the control group after 7 d of incubation ($P<0.05$). The number of fungi in feces was lower in the treatment groups than in the control group and the lowest was for T0.2 group ($P<0.05$). The results of this study indicated that CORA supplementation can reduce the emission of harmful gases (NH_3-N and H_2S) and odor-causing substances in feces and inhibit mold growth.

Key words: CORA supplementation level, Fecal, Gas emission, Odor-causing substances.

INTRODUCTION

The industrialization and the large-scale local congestion of livestock farms not only generate a large amount of odor derived from manure but also pose a social problem. Bad odors from livestock facilities are mainly caused by livestock manure and this causes deterioration of the health of the farm workers and the productivity of livestock (Donham *et al*., 2000).

In general, the odor of livestock manure is affected by various factors, such as feed ingredients, livestock conditions, excretion amounts, treatment conditions and environmental factors (Le *et al*., 2005). Most of the substances that results in odor in livestock manure are derived from the decomposition of specific components contained in the excrement, rather than the time of excretion. In particular, carbohydrates and proteins in the feed are known to be precursors to odorous substances. The odor is caused by incomplete anaerobic fermentation by microorganisms (Mackie *et al*., 1998). Odor-causing substances in livestock manure include volatile fatty acids (VFAs), alcohols, aromatic substances, amides, ammonia and sulfides (Hartung and Phillips, 1994; Zahn *et al*., 2001).

Bio-scrubbers, bio-filters and feed additives have been studied as methods for removing odor from livestock feces (Pagans *et al*., 2007). Among them, supplementation with microorganisms have been reported to improve the livestock environment by reducing nitrogen excretion and ammonia gas emission by inhibiting the growth of harmful microorganisms as they settle in the intestines of animals and help the digestion and absorption of feed (Colina *et al*., 2001). In addition, it has been reported that clay minerals such as zeolite are composed of fine porous matter. Therefore, they are capable of adsorbing ammonia and hydrogen sulfide because of their excellent physical adsorption and chemical cation substitution (Venglovsky *et al*., 2005).

Complex additives with different actions are required to effectively reduce fecal contaminants. However, most of them have used a single additive. In addition, various studies have been conducted on pigs and chickens concerning odor reduction, but relatively few studies have been conducted on cattle. Thus, this study was conducted to investigate the

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effect of supplementation of zeolite and microorganisms (*Bacillus licheniformis* and *Bacillus polyfermenticus*) on the odor emission of feces and the growth performance of Hanwoo steers.

MATERIALS AND METHODS

Ethics statement

All procedures on animals were carried out in compliance with South Korea regulations (Animal and Plant Quarantine Agency·Ministry of Food and Drug Safety Joint Animal Testing and/or Laboratory Animal Related Committee (IACUC; 2020) Standard Operating Guidelines).

Study area

The study was conducted in the Livestock Research Center, Nonghyup Co., Ltd. during August 2020 to September 2021.

Animals, treatments and management

The study was performed using 48 (398.1±34.5 kg; aged 18 months) early fattening and 48 (642.5±52.5 kg; aged 29 months) late fattening Hanwoo steers. Hanwoo steers (n=96) were randomly assigned to the following four groups: control group fed with combined odor-reducing additive (CORA)-unsupplemented formula feed; T0.05 group fed with 0.05% CORA-supplemented formula feed; T0.1 group fed with 0.1% CORA-supplemented formula feed; and T0.2 group fed with 0.2% CORA-supplemented formula feed. The CORA comprises 88% zeolite (73.96% SiO_2, 15.24% Al_2O_3, 0.66% Fe_2O_3, 0.46% CaO, 4.78% Na_2O, 4.67% K_2O and 0.01% MgO), 3% *Bacillus licheniformis* (3.0×10^8 cfu/g), 3% *Bacillus polyfermenticus* (3.0×10^8 cfu/g) and 6% saponin.

The steers were housed in 16 pens (5 × 10 m), where the floor was covered with 20 cm of sawdust. The formula feed was provided twice daily (08:00 and 17:00) using an automatic feeding system (SEOCHANG 65M/M, Seochang Co. Ltd., Cheonan, Korea). Steers had free access to rice straw, water and mineral blocks. Other feeding management procedures were conducted as per the practices of the experimental farm. The ingredients and chemical composition of the experimental diets are listed in Table 1.

Gas emission and fermentation characteristics

To evaluate the change in the characteristics of feces through *in vitro* incubation based on the supplementation with CORA, feces collected from the early fattening Hanwoo steers in the control group were used. Feces collected from the pen and CORA (0, 0.05, 0.1 and 0.2%) were sufficiently mixed for each treatment group. The feces mixed with CORA were transferred to a gas collection container at 600 g in three repetitions for each treatment group.

After incubating the feces in an incubator at 30°C for 10 and 20 d, gas was collected in a gas collection container, using a gas measuring glass syringe and transferred to a gas collection bag. Next, it was diluted 100 times using the same mixed gas (78% nitrogen and 22% oxygen), as the atmospheric gas standard. NH_3-N and H_2S concentrations were measured using a gas meter (Gas Alert Micro 5, BW Technologies, Honeywell, Mexico).

The pH was measured using a pH meter (Thermo Sci, Korea) by mixing 4 g of incubated feces with 16 mL of distilled water (Miller and Varel, 2001). NH_3-N concentration was determined using the method described by Chaney and Marbach (1962) and VFAs concentration was measured using a gas chromatograph (Agilent 7890A, Agilent Technology, CA, USA).

Evaluation of the fecal properties

The *in vitro* ruminal pH was measured using a pH meter (Corning Glass Works, Medfield, MA, USA) in a 160 mL bottle for each incubation time. The ammonia concentration was calculated according to the method of Chany and Marbach (1962). VFAs concentrations was measured *via* gas chromatography (Shimadzu-17A, Shimadzu, Kyoto, Japan).

Growth performance

Body weight (BW) was measured at the beginning and end of the experimental period. Average daily gain (ADG), dry matter intake (DMI) and feed conversion ratio (FCR) was also calculated.

Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS)/Windows 24 (SPSS Inc., Chicago, IL, USA). The means of different groups were compared using a one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Differences were considered statistically significant at $P<0.05$.

RESULTS AND DISCUSSION

Gas emission and fermentation properties of feces during *in vitro* incubation

The fecal NH_3-N gas emission showed a linearly decreasing tendency as the supplementation level of CORA increased at 10 d of *in vitro* incubation and was significantly lower in the T0.2 group than in the control group ($P<0.05$). The fecal H_2S gas emission at 10 d of incubation was lower in the T0.1 and T0.2 groups than in the control group ($P<0.05$). The fecal H_2S gas emission at 20 d of incubation was lower in the T0.2 group than in the control group ($P<0.05$, Table 2).

The fecal pH and NH_3-N concentration at 10 d of incubation were not affected by CORA levels. The fecal acetate concentration showed a linearly decreasing tendency as the supplementation level of CORA increased and was particularly effective in T0.1 and T0.2 groups ($P<0.05$). There was no difference between treatment groups in the fecal propionate, isobutyrate and butyrate concentrations (Table 3).

Gas emission and fermentation properties of feces

The fecal NH_3-N gas emission was lower in proportion to the supplementation level of CORA in all treatment groups than in the control group at 7 d of incubation ($P<0.05$). The fecal H_2S

Table 1: Ingredients and chemical compositions of experimental diets.

Item	Formula feed		Rice straw
	Early fattening	Late fattening	
	Ingredients composition		
Corn grain (%)	26.32	29.02	-
Wheat grain (%)	17.00	17.00	-
Cane molasses (%)	4.00	5.00	-
Tapioca residue (%)	3.00	6.00	-
Wheat flour (%)	3.00	3.00	-
Corn gluten feed (%)	20.00	20.00	-
Rapeseed meal (%)	4.00	4.00	-
Coconut meal (%)	3.78	-	-
Palm kernel meal (%)	11.00	8.82	-
Cottonseed hull (%)	1.00	1.00	-
Lupin (%)	3.00	3.00	-
Salt dehydrated (%)	0.50	0.50	-
Limestone (%)	2.50	1.96	-
Sodium bicarbonate (%)	0.50	0.50	-
Calcium sulfate (%)	0.20	-	-
Vitamin premix[1] (%)	0.10	0.10	-
Mineral premix[2] (%)	0.10	0.10	-
	Chemical composition (DM basis)		
Dry matter (%)	88.95	88.68	89.51
Crude protein (%)	15.63	14.55	4.05
Ether extract (%)	3.72	4.24	1.81
Crude fiber (%)	7.13	6.88	36.20
Neutral detergent fiber (%)	28.05	25.18	71.33
Acid detergent fiber (%)	11.04	9.55	21.79
Crude ash (%)	8.35	7.47	10.28
Ca (%)	1.34	1.03	0.29
P (%)	0.61	0.46	0.10
Total digestible nutrients (%)	71.00	73.00	38.31

[1]Vitamin premix: Vitamin premix provided the following quantities of vitamins per kilogram of diet: Vitamin A, 10,000 IU; Vitamin D3, 1,500 IU; Vitamin E, 25 IU.

[2]Mineral premix: Mineral premix provided the following quantities of minerals per kilogram of diet: Fe, 50 mg; Cu, 7 mg; Zn, 30 mg; Mn, 24 mg; I, 0.6 mg; Co, 0.15 mg; Se, 0.15 mg.

gas emission occurred from the 7 d of incubation, but there was no difference between the treatment groups (Table 4).

In early fattening Hanwoo steers, fecal acetate and isobutyrate concentrations were lower in T0.1 and T0.2 groups than in control and T0.05 groups ($P<0.05$). In late fattening Hanwoo steers, NH_3-N, acetate and propionate concentrations in feces were lower in the treatment groups than in the control group and the reduction effect increased as the level of supplementation increased ($P<0.05$). However pH and isobutyrate concentration was not different (Table 5).

Chemical compositions and microbial properties of feces

The supplementation levels of CORA did not have any effect on the moisture, nitrogen and crude ash contents in early and late fattening Hanwoo steers. Furthermore, there was no difference in compost maturity between the treatment groups (Table 6).

In both early and late fattening Hanwoo steers, the numbers of Bacillus, Coli form, lactic acid bacteria and yeast in feces were not affected by CORA, but the number of fungi was lower in the treatment groups than in the control group and the lowest in T0.20 ($P<0.05$, Table 7).

Growth performance

In early fattening Hanwoo steers, the ADG was significantly higher in the treatment groups than in the control group. CORA did not affect DMI. Supplementation with CORA did not affect ADG and DMI in late fattening Hanwoo steers (Table 8).

The results of this study showed that CORA (zeolite and probiotics) can effectively reduce the amount of odor-causing pollutants. This is because the concentration of odorous substances in feces tended to decrease in a dose-dependent manner in CORA. These results suggest that CORA may have a direct effect on the source of odor through two different mechanisms of action or may effectively remove the generated odor substance.

Clay minerals included in silicates have a high ion-exchange capacity, so they can adsorb harmful gases and toxic substances (Volzone, 2007). In particular, the aluminosilicate type is a clay mineral with a three-

Table 2: Effects of the CORA supplementation levels on concentrations of fecal NH_3-N and H_2S gases during *in vitro* incubation.

Items	Treatments				SEM
	Control	T0.05	T0.10	T0.20	
			10 d		
NH_3-N (ppm)	333[a]	300[a]	250[ab]	200[b]	21.34
H_2S (ppm)	1.467[a]	1.300[a]	1.100[b]	900[c]	81.65
			20 d		
NH_3-N (ppm)	533[a]	500[a]	450[ab]	400[b]	18.60
H_2S (ppm)	2300[a]	2100[a]	2050[ab]	1633[b]	37.86

SEM- Standard error of the mean.

[a,b,c]Means followed by different letters in the same row are significantly different ($P<0.05$).

dimensional crystal structure, large surface area, excellent thermal/hydrothermal stability and has been used as an important adsorbent owing to its high ion exchange capacity (Lopes *et al*., 2014). Zeolite is a representative aluminosilicate hydrated with alkali and has high cation exchange, water retention and adsorption capacities (Mumpton, 1999). Lefcourt and Meisinger (2001) reported that the supplementation of dairy sludge with 6.25% zeolite adsorbed ammonium lowered the dissolved ammonia gas, reducing ammonia emissions by approximately 50%. Islam *et al*. (2014) also reported that ammonia, sulfur dioxide and hydrogen sulfide gas in fecal were reduced by artificial zeolite supplementation.

Bacillus genus is the most beneficial microorganism, has an excellent production capacity for α-amylase and protease and can suppress harmful microorganisms by generating antibacterial substances (Ushida *et al*., 2003). It has been reported that microbial supplementation changes the intestinal microbial balance and produces lactic acid and antibiotics to reduce odor-causing gas due to the inhibition of the growth of harmful microorganisms (Smith and Jones, 1963). Similar to previous studies, in this study, the emissions of fecal ammonia and hydrogen sulfide gas decreased in proportion to the CORA supplementation level. This is considered to be due to the zeolite adsorption capacity. Moreover, microorganisms decreased the fecal NH_4^+ and affected harmful microorganisms in feces. In particular, direct feeding of *Bacillus* spp. to livestock is considered more effective in reducing ammonia gas by reducing fecal ammonia concentration. The results of this study are supported by previous studies (Chen *et al*., 2006) In some studies, it has been reported that supplementation with *Bacillus* spp. reduced ammonia production by improving the nitrogen availability of feed (Payling *et al*., 2017) and

Table 3: Effects of the CORA supplementation levels on fecal pH, NH_3-N and VFAs concentrations during *in vitro* incubations.

Item	Treatments				SEM
	Control	T0.05	T0.1	T0.2	
			10 d		
pH	7.92	7.82	7.89	7.79	0.03
NH_3-N (mg/dL)	16.20	17.68	17.57	18.52	0.30
Acetate (mM)	10.25[a]	9.25[ab]	8.46[b]	7.79[b]	0.36
Propionate (mM)	7.52	7.84	7.47	7.65	0.13
Isobutyrate (mM)	0.39	0.44	0.43	0.43	0.01
Butyrate (mM)	1.07	1.11	1.04	1.10	0.02
			20 d		
pH	8.03	8.00	8.07	7.86	0.03
NH_3-N (mg/dL)	19.92	20.67	18.57	20.08	0.36
Acetate (mM)	1.06	1.15	1.13	1.11	0.03
Propionate (mM)	9.85	10.26	8.72	10.04	0.26
Isobutyrate (mM)	0.67	0.64	0.61	0.62	0.01
Butyrate (mM)	0.28	0.21	0.13	0.19	0.03

SEM- Standard error of the mean.

[a,b,c]Means followed by different letters in the same row are significantly different (P<0.05).

Table 4: Effects of the CORA supplementation levels on concentrations of fecal NH_3-N and H_2S gases in Hanwoo steers.

Item	Day	Treatments				SEM
		Control	T0.05	T0.1	T0.2	
				Early fattening		
NH_3-N (ppm)	0	7.33	8.02	10.17	10.83	0.51
	7	1.233[a]	600[b]	600[b]	400[c]	109
H_2S (ppm)	0	ND	ND	ND	ND	-
	7	5.434[a]	2.634[b]	1.567[c]	2.633[b]	499
				Late fattening		
NH_3-N (ppm)	0	21.04	12.01	7.67	7.33	1.92
	7	5.133[a]	3.800[b]	1.533[c]	1.333[c]	554
H_2S (ppm)	0	ND	ND	ND	ND	-
	7	25.933[a]	17.200[b]	6.533[c]	5.867[c]	2.884

SEM- Standard error of the mean.

[a,b,c]Means followed by different letters in the same row are significantly different (P<0.05).

Table 5: Effects of the CORA supplementation levels on concentrations of fecal fermentation parameters in Hanwoo steers.

Item	Treatments				SEM
	Control	T0.05	T0.1	T0.2	
			Early fattening		
pH	7.68	7.84	8.07	8.04	0.11
NH_3-N (mg/dL)	8.06	5.98	7.42	8.49	0.57
Acetate (mM)	6.95^{a}	6.63^{a}	5.54^{b}	5.37^{b}	0.07
Propionate (mM)	1.37	1.47	1.12	1.13	0.08
Isobutyrate (mM)	0.35^{a}	0.30^{a}	0.18^{b}	0.24^{b}	0.03
Butyrate (mM)	0.38	0.39	0.29	0.34	0.01
			Late fattening		
pH	7.80	8.20	8.10	8.34	0.070
NH_3-N	16.41^{a}	6.97^{bc}	8.02^{b}	5.45^{c}	0.48
Acetate (mM)	14.97^{a}	4.42^{b}	4.53^{b}	2.62^{c}	0.07
Propionate (mM)	3.97^{a}	1.34^{b}	1.34^{b}	0.19^{c}	0.10
Isobutyrate (mM)	0.38	0.31	0.29	0.38	0.01
Butyrate (mM)	0.49	ND	ND	ND	-

SEM- Standard error of the mean.

^{a,b,c}Means followed by different letter in the same row are singnificantly different ($p<0.05$).

Table 6: Effects of the CORA supplementation levels on fecal compositions in Hanwoo steers.

Items	Treatments				SEM
	Control	T0.05	T0.1	T0.2	
			Early fattening		
Moisture (%)	79.62	82.05	82.91	78.09	0.80
Nitrogen (%)	0.62	0.52	0.48	0.53	0.06
Crude ash (%)	3.49	3.09	3.21	3.60	0.16
Compost maturity (%)	85.00	90.00	80.00	75.00	3.72
			Late fattening		
Moisture (%)	72.68	69.87	75.00	72.80	1.43
Nitrogen (%)	0.74	0.71	0.50	0.75	0.09
Crude ash (%)	3.38	3.86	2.93	3.82	0.34
Compost maturity (%)	95.00	90.00	95.00	100.00	2.06

SEM- Standard error of the mean.

Table 7: Effects of the CORA supplementation levels on fecal microbial compositions in Hanwoo steers.

Items	Treatments				SEM
	Control	T0.05	T0.1	T0.2	
			Early fattening		
Bacillus (log_{10} CFU/g)	6.18	6.11	6.38	6.38	0.04
Coli form (log_{10} CFU/g)	5.39	4.44	6.26	5.09	0.23
Lactic acid bacteria (log_{10} CFU/g)	8.27	8.19	8.15	8.10	0.02
Yeast (log_{10} CFU/g)	6.03	5.48	5.28	4.47	0.20
Fungi (log_{10} CFU/g)	5.85^{a}	5.14^{b}	5.18^{b}	4.71^{b}	0.14
			Late fattening		
Bacillus (log_{10} CFU/g)	5.87	5.31	5.37	5.38	0.08
Coli form (log_{10} CFU/g)	5.51	5.29	4.99	5.16	0.07
Lactic acid bacteria (log_{10} CFU/g)	7.60	7.48	7.53	7.49	0.02
Yeast (log_{10} CFU/g)	4.52	3.60	3.70	3.70	0.13
Fungi (log_{10} CFU/g)	6.28^{a}	4.93^{b}	5.11^{b}	5.07^{b}	0.19

SEM- Standard error of the mean.

^{a,b,c}Means followed by different letters in the same row are significantly different ($P<0.05$).

Table 8: Effects of the CORA supplementation levels on growth performance in Hanwoo steers.

Items	Treatments				SEM
	Control	T0.05	T0.1	T0.2	
			Early fattening		
Initial BW (kg)	399.06	399.33	403.70	390.33	10.30
Final BW (kg)	454.94	461.41	465.02	452.14	11.87
ADG (kg/d)	0.93	1.04	1.02	1.03	0.04
Concentrate intake (DM, kg)	6.69	6.69	6.69	6.69	0.12
Rice straw intake (DM, kg)	1.78	1.78	1.78	1.78	0.08
DMI (kg)	8.47	8.47	8.47	8.47	0.17
			Late fattening		
Initial BW (kg)	638.43	621.84	677.76	639.17	15.17
Final BW(kg)	684.51	660.74	722.83	676.80	16.68
ADG (kg/d)	0.77	0.65	0.75	0.63	0.06
Concentrate intake (DM, kg)	7.78	7.78	7.78	7.78	0.15
Rice straw intake (DM, kg)	0.88	0.88	0.88	0.88	0.05
DMI (kg)	8.66	8.66	8.66	8.66	0.19

SEM- Standard error of the mean.

reducing fecal pH (Durand *et al*., 2015); however, no difference in fecal pH and nitrogen concentration was found in this study. Similarly, Wang *et al.* (2009) reported that 0.2% feeding of a mixture of *Bacillus subtilis*, *Bacillus licheniformis, aluminum silicate* and whey powder did not affect dry matter and nitrogen digestibility in pigs but reduced ammonia emissions from sludge. Therefore, 0.2% supplementation with a mixture of zeolite and microorganisms can reduce harmful gas emissions by increasing adsorption capacity and reducing fecal NH_4 concentration.

VFAs generated in barns is an important factor in evaluating odors and has been reported to cause odors (Miller and Varel, 2002). VFA are substances that stimulate the sense of smell even at very low concentrations. Pathogenic microorganisms (*Bacteroides, Propionibacterium, Clostridium, etc*.) decompose amino acids to produce acetic, butyric, propionic and isobutyric acids (Davila *et al*., 2013). In this study, CORA supplementation effectively reduced acetic acid and although there was a difference according to the supplementation level, it also reduced the concentrations of isobutyric, butyric and propionic acids. These results may have been influenced by the antibacterial effects of *Bacillus polyfermenticus* and *Bacillus licheniformis*. *Bacillus licheniformis* produces various types of surfactants and antibiotics (Grangemard *et al*., 2001). In this study, the number of fungi was significantly reduced in the feces of the CORA-supplemented groups, which is thought to be due to the decreased amount of acetic acid produced because CORA affected fiber decomposition. In addition, it is presumed that the antibacterial effect of *Bacillus polyfermenticus* and *Bacillus licheniformis* reduced VFAs concentration by inhibiting the growth of microorganisms.

Supplementation with clay minerals can improve the growth performance of calves and cattle and can have a positive effect on rumen health and fermentation by improving trace mineral supply and buffering capacity (Humer *et al*., 2019). In addition, *Bacillus* spp. can improve feed availability by producing carbohydrates and proteolytic enzymes. However, in this study, CORA supplementation did not affect the growth performance of early and late fattening Hanwoo steers. Although it cannot be concluded, these results may be due to the limited feeding of formula feed and the supplementation levels of CORA. Chesson (1994) reported that differences in growth performance may occur depending on several factors, including the age of the livestock, supplementation level, type of feed and interaction with other feed additives. Therefore, high-dose and long-term studies are needed to improve the growth performance of livestock as well as odor reduction.

CONCLUSION

In this study, CORA, composed of zeolite and microorganisms (*Bacillus polyfermenticus* and *Bacillus licheniformis*), reduced the emission of harmful gases (ammonia and hydrogen sulfide), odor-causing substances in feces and inhibited fungal growth. In addition, the treatment effect was proportional to the CORA supplementation level. Therefore, CORA is considered to have a high potential for use as an environmental improvement additive, which can effectively adsorb harmful gases from cattle feces and reduce the production of pollutants and odorous substances.

Conflict of interest

All authors declare that they have no conflict of interest.

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