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Research Paper

Yucca schidigera extract depressed ammonia emission in manure incubation and green-house gases release in artificial rumen of cows

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ABSTRACT

The influences of ammonia (NH_3) and greenhouse gas (GHG) emissions from livestock on environment recently become a major concern of the worldwide. The objective of the study was to evaluate the effects of Yucca schidigera extract (YSE) on ammonia emission in manure and the release of green-house gases including methane (CH_4), nitrous oxide (N_2O) and carbon dioxide (CO_2) in artificial rumen of cows. Addition of YSE significantly decreased ammonia nitrogen (NH_3-N) concentration ($P < 0.01$) and increased the percentage of total nitrogen (N) ($P < 0.005$) in manure incubation. The populations of total aerobic bacteria were significantly decreased by the addition of YSE with 1% and 2% ($P < 0.05$), while the populations of Lactobacilli were significantly increased in dose-dependent manner ($P <0.001$) in manure. In 1-12 h and 13-24h of ruminal fermentation in vitro, the yields of CH_4, CO_2 and N_2O were dose-dependently reduced by the addition of YSE ($P < 0.05$). Results showed that YSE inhibit ammonia emission in manure and the release of green-house gases in artificial rumen of cows.

Keywords: Ammonia, green-house gas, manure, rumen, cows

A rapid climate change is probably the most challenging threat to mankind. Carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) are important biogenic greenhouse gases (GHGSs) from agricultural sector contributing to global warming. Agriculture sector is an important source of GHGSs emissions and AFLOU (Agriculture, Forestry and Other Land Use) sector contributed 24% of total emissions worldwide during 2010 (IPCC 2013). Livestock excretion plays a key role in the source of GHG emissions. It contributes up to about 45% and 70% of the total anthropogenic emissions of CH_4 and N_2O from manure. Ninety percent of livestock GHG emission is from ruminants mainly as dairy-based and meat-based cattle and small ruminant herd (Gerber *et al*., 2013). On the other hand global warming adversely affect the efficiency of livestock. In order to unwind the effect of heat load index suitable nutrient supplements are given to combat the adverse effect (Pankaj *et al.,* 2017)

Yucca schidigera is desert plant, prevalent in the south-western United States and northern Mexico. Yucca shidigera extract (YSE) is applied as food additive or medicine because of its biofunctional components as steroides, saponins and glycocomponents (Kaya *et al.,* 2003). In livestock diet, YSE is added for ammonia and odour control due to the presence of saponin (Ayasan, 2013; Piacente *et al.*, 2005; Wang *et al*., 2000).

MATERIALS AND METHODS

Experiment procedures

Manure incubation

Fresh manure sample was collected from dairy cows farm without pollution, mixed completely and filled in twenty four 500ml triangular flasks which processed asepsis, each triangular flask was filled with 200g manure sample. Twenty four samples were randomly divided in four treatments with six repetitions, four levels YSE was added into the triangular flasks in four treatments respectively: (1) the control treatment (0g YSE and 100ml distilled water added in the manure sample), (2) 0.5% YSE treatment (1.5g YSE and 100ml distilled water added in the manure sample), (3) 1% YSE treatment (3g YSE and 100ml distilled water added in the manure sample), and (4) 2% YSE treatment (6g YSE and 100ml distilled water added in the manure sample). Yucca schidigera extract was purchased from Shanxi Healthy Source Biotech Co., Ltd. Saponin content of YSE was not less than 40%. After YSE addition, the sample in each triangular flask was mixed fully with distilled water and the added YSE, each triangular flask was covered by double holes rubber plug. One hole of rubber plug was connected with inlet pipe and inlet pipe was ligatured. The other hole of rubber plug was connected with outlet pipe, and outlet pipe put into a triangular flask

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with 20ml 0.05% H_2SO_4 to collect ammonia produced from manure incubation. Then all triangular flasks with manure were placed and cultured in 37℃ thermostatic water bath shakers. The culture lasted for 5 days. After the beginning of manure incubation, every 2h injected 100ml fresh air into triangular flasks from inlet pipe and closed the pipe tightly. On day 4 of manure incubation, H_2SO_4 solution with the collected ammonia was removed from outlet pipe of each triangular flask and determined the amount of ammonia by indophenols blue colorimetric method. 5g manure sample from each triangular flask was collected and mixed with distilled water by 1:20 (wv^-1). The amount of NH_3-N was measured by indophenols blue colorimetric method after manure sample was filtered. On day 5 of manure incubation, 1g manure sample from each triangular flask was collected for bacteria count (total aerobic bacteria, E.coli and Lactobacilli) by plate count method, and 100g manure sample was used for the measurement of total N by kjeldahl method.

Artificial rumen fermentation

Ruminal contents were collected immediately from the rumen after a Holstein cow was slaughtered, and were strained through four layers of cheesecloth under a continuous CO_2 stream. A simulated total mixed ration (STMR) consisted of 16% guinea grass, 44% alfalfa, 40% concentrate and grounded to pass a 1 mm screen. The incubation system consisted of a 100 ml fermentation vessels, equipped with rubber stopper, an input for nitrogen gas (CO_2) infusion, and an inlet for gas emission. Five hundred mg of the substrate and 20ml of strained rumen fluid were placed into each 100 ml fermentation vessel, which contained 40 ml of buffer solution (39℃). The buffer (Menke *et al.,* 1979) was freshly prepared and pre-gassed with carbon dioxide for 30 min. Each fermentation vessel was incubated 24 h at 39 ± 0.5℃in shaking bath. The experiment was designed that five treatments with six repetitions, five levels YSE was mixed with a simulated total mixed ration respectively to be used as the fermentation substrate: (1) the control treatment (STMR + 0% YSE), (2) 0.3% YSE treatment (STMR + 0.3% YSE), (3) 0.6% YSE treatment (STMR + 0.6% YSE), (4) 1.2% YE treatment (STMR + 1.2% YSE) and (5) 2.4% YSE treatment (STMR + 2.4% YSE). During the processes of in vitro incubation, gas emission were recorded and gas sample was collected from the inlet of all fermentation vessels at each 2 h, determined the concentration of GHG with a gas chromatograph (HP5890, Agilent 5890; Agilent Technologies Co. Ltd, Santa Clara, CA, USA). The yields of CH_4, CO_2 and N_2O were obtained by multiplying gas concentration with the gas emission at the corresponding incubation time. Immediately after incubation, pH value of incubation solution was measured with an EL20 pH meter.

Statistical analysis

The data from the trials were analyzed by ANOVA, using the General Linear Models (GLM) procedure of SAS software (Statistical Analysis System, Version 9.2, 2003). Means were compared by the Duncan's multiple range tests. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Effects of YSE on ammonia production and common bacterial population in manure incubation are shown in Table 1. Addition of YSE significantly decreased NH_3-N concentration ($P < 0.01$) and increased the percentage of total N ($P < 0.005$) in manure incubation. The populations of total aerobic bacteria were significantly raised by the addition of YSE with 1% and 2%. The populations of Lactobacilli were significantly increased in dose-dependent manners, while the populations of E. coli were unaffected by all levels of YSE addition. The effects of YSE on GHG production in artificial rumen fermentation are in Table 2. Total gas yields discharged from artificial rumen fermentation significantly declined by 1.2% and 2.4% levels of YSE additions, but were not significant different among all treatments at 12 h. In 1-12 h of in artificial rumen, the yields of CH_4, CO_2 and N_2O were dose-dependently reduced by the addition of YSE ($P < 0.05$). The yields of CH_4 were significantly reduced by 0.6%, 1.2% and 2.4% levels of YSE additions, CO_2 yields were significantly dropped by 1.2% and 2.4% levels of YSE additions, and N_2O yields of 2.4% YSE treatment was significantly lower than that of the control treatment. In 13-24 h of in artificial rumen, the yields of CH_4, CO_2 and N_2O were dose-dependently reduced by the addition of YSE ($P < 0.05$). The yields of CH_4 were significantly reduced by all levels of YSE additions, CO_2 yields were significantly dropped by 2.4% levels of YSE additions, and N_2O yields were significantly lowered by 1.2% and 2.4% levels of YSE addition.

To keep ideal milk production, crude protein in the feed ration of dairy cows is recommended in high levels. A big amount of N in feed is passed the gastrointestinal tract of dairy cows, emits gas as NH_3, N_2O or nitrogen oxides (N_XO) from the gastrointestinal tract directly or from manure indirectly to release in atmosphere. Manure from dairy cows operates the majority accounts of NH_3 emission. In this study, the addition of YSE decreased NH_3-N, while increased total N in manure. The glyco-fraction of Yucca schidigera binds NH_3 effectively (Santoso *et al.,* 2004; Takahashi *et al.,* 2000). The bindings release N to maintain a lower NH_3 concentration, enhance microbial utilization of N in rumen in previous studies (Singer *et al.,* 2008). Saponins fraction of YS has an antiprotozoal activity, which may direct N source fermented by certain bacteria species, but not by protozoal or Gram-positive bacteria. The effect of YSE on microorganism has discrepancy between in manure and in rumen. The population of Lactobacilli was increased by the addition of YS saponins, but the population of E.coli was not impacted by the addition of YS saponins in manure in this study. As the most noticeable bacteria in animal intestinal tract, L. plantarum, L. rhamnosus, B. thermophilum and B. longum grew well on the media containing 10% YS saponins, E.coli was not inhibited by the high concentrations of YSE and saponins, While the growth of other 13 strains was inhibited by YSE and saponins, (Katsunuma *et al*., 2000). Selcuk and Tuncer (2010) indicated that yucca inhibits urease activity and reduces urea degradation in rumen. Ammonia is an end-product resulting of microbiological hydrolysis of urea and uric acid by urease to form NH_4^+ and volatilize as NH_3 (Bouwman *et al.,* 1997). Urease is produced by microorganisms present in feces, while urea and uric acid are ubiquitous in urine. Yucca schidigera extract has antibacterial activity against urease producing bacteria (Yasmeen *et al.,* 2012). Thus, inhibiting effect of YSE on urease in manure was another reason for lower production of NH_3 in this study. From our result, YSE suppressed the release of

Table 1: Effects of YSE on NH_3 Production and Common Bacterial Population in Manure Incubation

Item	YSE level				SEM	*P*
	0	0.5%	1%	2%		
NH_3-N, mgg ^-1	0.74[a]	0.59[b]	0.57[b]	0.54[b]	0.050	0.008
Total N, %	2.68[b]	2.84[a]	2.91[a]	2.89[a]	0.058	0.004
Total aerobic bacteria, lgcfug^-1	8.36[a]	7.88[ab]	7.59[b]	7.59[b]	0.237	0.048
E. coli, lgcfug^-1	7.42	6.81	6.60	7.22	0.268	0.160
Lactobacilli, lgcfug^-1	7.40[b]	10.08[a]	10.45[a]	10.53[a]	0.581	<0.001

Table 2: Effects of YSE on the yield of GHG in artificial rumen

Item	YSE Level					SEM	*P*
	0	0.3%	0.6%	1.2%	2.4%		
1-12h							
CH_4, mL	10.69[a]	9.64[ab]	7.35[b]	7.47[b]	7.23[b]	0.966	0.042
CO_2, mL	23.41[a]	24.02[ab]	17.24[ab]	19.7[b]9	15.27[b]	2.478	0.046
N_2O, 10^{-6}mL	43.01[a]	35.92[ab]	38.64[ab]	34.36[ab]	31.62[b]	3.193	0.049
13-24h							
CH_4, mL	4.83[a]	3.22[b]	3.26[b]	3.19[b]	2.73[b]	0.448	0.018
CO_2, mL	4.82[a]	4.13[a]	4.59[ab]	3.67[ab]	3.06[b]	0.565	0.039
N_2O, 10^{-6}mL	9.39[a]	8.26[a]	7.80[a]	5.54[b]	5.33[b]	0.896	0.004

CH_4, CO_2 and N_2O. In a previous study, the glyco-fraction of YSE showed a binding activity not only to ammonia but also to other detrimental nitrous gases, reduced their emission in atmosphere (Lynos, 1992). Takahashi *et al.,* (2000) reported that yucca extract led the depressed production of CH_4 and CO_2 in ruminal incubation in vitro. The symbiotic relationship between methanogens and protozoa in ruminal environment was response for the depression of methanogenesis when YSE included in diets (van Zijderveld *et al.,* 2011). It is also produced by the reaction of ammonium carbonate $[(NH_4)_2CO_3]$ with H^+ ion during urea hydrolysis. Urea hydrolysis was control during N metabolism in rumen with the presence of YSE, assuming that low N production is associated with low CO_2 production as well.

CONCLUSION

YSE effectively depressed NH_3 emission in manure and GHG (CH_4, CO_2 and N_2O) release in artificial rumen in cow in this study. Since the efficacy of controlling gas emission of YSE was complex, further researches are needed in vivo or in vitro to be associated with the involved factors, such as the dosage and dietary composition, management and so on.

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