Effect of Sarsaponin on Ruminal Fermentation with Particular Reference to Methane Production in Vitro

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ABSTRACT

This experiment was designed to investigate the effects of different concentrations (0, 1.2, 1.8, 2.4, and 3.2 g/L) of sarsaponin on ruminal microbial methane production using the substrates soluble potato starch, cornstarch, or hay plus concentrate (1.5:1). Ruminal fluid was collected from a dairy cow, mixed with phosphate buffer (1:2) and incubated (30 ml) anaerobically at 38°C for 6 and 24 h with or without sarsaponin. Excluding the lower level of sarsaponin, pH of the medium was slightly decreased. Ammonia-N concentration and numbers of protozoa were decreased in a dose-dependent manner. Total volatile fatty acids and total gas production were increased. Molar proportion of acetate was decreased and propionate was increased with a corresponding decrease in acetate:propionate ratio. Hydrogen production was decreased. As the concentration of sarsaponin increased from 1.2 to 3.2 g/L, fermentation of soluble potato starch, cornstarch, or hay plus concentrate decreased methane production from 20 to 60% (6 h) and 17 to 50% (24 h), 21 to 58% (6 h) and 18 to 52% (24 h), and 23 to 53% (6 h) and 15 to 44% (24 h), respectively. Excluding the lower dose concentration (1.2 g/L) of sarsaponin, in vitro disappearance of dry matter of hay plus concentrate was decreased after 24 h. In conclusion, these results show that sarsaponin stimulated the mixed ruminal microorganism fermentation as well as to inhibit methane production in vitro. (**Key words:** *Yucca schidigera*, saponin, methane, rumen fermentation)

Abbreviation key: IVDDM = in vitro disappearance of DM.

INTRODUCTION

Methane produced during anaerobic fermentation in the rumen represents 2 to 12% gross energy loss to the host animal and contributes to emissions of greenhouse

gases into the environment (Moss, 1993). Because methane production has a negative correlation with energy utilization in ruminants (Ørskov et al., 1968), there have been many efforts to inhibit its production and to rechannel hydrogen to produce more VFA and microbial mass. Many compounds have been tested in vitro and in vivo as methane inhibitors (Czerkawski and Breckenridge, 1972; Martin and Macy, 1985). However, ruminal microbial populations in vivo adapt (Clapperton, 1977) or in vitro degrade (Martin and Macy, 1985) many of these compounds, and favorable effects on animal performance have rarely been observed (Davies et al., 1982; Demeyer et al., 1986).

Sarsaponin is a group of steroidal glycosides extracted from the *Yucca schidigera* plant. The addition of sarsaponin to diets improved the growth of steers (Goodall and Matsushima, 1979; Goodall et al., 1979), reduced ammonia, and increased propionate concentrations in continuous flow fermentors (Grobner et al., 1982) and in vivo (Hristov et al., 1999). In subsequent studies, Goetsch and Owens (1985) reported that sarsaponin also had beneficial effects on ruminal fermentation with low-concentrate diets, improved ruminal OM digestion (Goetsch and Owens, 1985; Valdez et al., 1986), and did not affect animal performance (Goetsch and Owens, 1985; Wu et al., 1994). Saponins have antimicrobial properties, particularly in suppressing ciliate protozoa (Wallace et al., 1994; Hristov et al., 1999), peptidase-producing bacteria (Wallace et al., 1994; Wang et al., 2000), and cellulolytic bacteria (Wang et al., 2000). Methanogenic bacteria were metabolically correlated with ciliate protozoa (Stumm et al., 1982; Newbold et al., 1995) and elimination of ciliate protozoa from the rumen reduced methane emission by 30 to 45% (Jouany et al., 1981; Itabashi et al., 1984; Ushida et al., 1986). Keeping the above facts in view, the present study was conducted to observe the effects of sarsaponin on ruminal microorganism fermentation in vitro.

MATERIALS AND METHODS

Diet and Additives

Soluble potato starch and cornstarch (Wako Pure Chemical Industries, Ltd., Tokyo, Japan) and Su-

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Table 1. Composition of experimental diets.

Item	Sudangrass hay	Concentrate mixture ¹
Feed composition, % of DM basis	60.5	39.5
Chemical composition, % of DM basis		
OM	88.8	93.9
CP	10.2	20.4
NDF	65.3	20.2
Ash	11.2	6.1

¹Contained 41.3% corn, 23.4% wheat bran, 8.0% rapeseed oil, 7.4% soybean meal, 6.0% corn germ meal, 5.6% corn gluten feed, 4.8% grains, 0.6% molasses, 1.7% CaCO₃, 1.0% NaCl, 0.2% vitamins (A and D) and trace minerals premix, which contained 5.6% Mg, 0.4% Zn, 0.2% Mn, 0.08% Fe, 0.01% I, 0.002% Co, and (per gram) 1500 IU of vitamin A, 500 IU of vitamin D, 9 mg of water-soluble vitamins, and 0.5 mg of α -tocopherol.

dangrass hay plus concentrate mixture were used as the components of the diet for in vitro incubation. Sudangrass hay plus concentrate mixture were ground by a high-speed grinder (Retsch ZM 100, Tokyo) and passed through a 1-mm screen automatically. Sarsaponin (DK Sarsaponin 35) was supplied from Desert King International (Chula Vista, CA), and it contained 6% moisture, 2.4% CP, 0.8% crude fat, 24.7% crude fiber, 4.9% ash, and 61.1% carbohydrate. The chemical composition of the Sudangrass hay and concentrate mixture are shown in Table 1.

In Vitro Batch Fermentation

Short-term in vitro incubations were carried out with ruminal fluid from a 600-kg lactating Holstein dairy cow fed 7.5 kg of forage (Sudangrass hay) and 7.5 kg of concentrate supplement (47% grain, 27% chaff and bran, 22% oil seed meals and others 4%) per day. The dairy cow was cared for in accordance with the Guide for the Care and Use of Laboratory Animals prepared by Tokyo University of Agriculture and Technology. Ruminal fluid was withdrawn by a flexible stainless steel stomach-tube before the morning feeding into a vacuum flask that was flushed with CO₂, and the initial characterization of ruminal fluid were shown in Table 2. The rumen liquor was squeezed through four layers of surgical gauze into an Erlenmeyer flask under continuous flushing with CO₂, and efforts were made to maintain the temperature at 38 to 39°C. The fluid was then mixed with buffer (pH 6.9) (containing, per liter, 292 mg of K₂HPO₄, 240 mg of KH₂PO₄, 480 mg of (NH₄)₂SO₄, 480 mg of NaCl, 100 mg of MgSO₄·7H₂O, 64 mg of CaCl₂·2H₂O, 4000 mg of Na₂CO₃, and 600 mg of cysteine hydrochloride) in a ratio of 1:2 (Russell and Martin, 1984). After mixing, 30 ml of diluted rumen fluid was anaerobically transferred to 60-ml serum bottles con-

Table 2. Characteristics of ruminal fluid collected by oral tube.

Characteristic	Mean $\pm$ SD
pH	6.72 $\pm$ 0.20
Protozoa, $\times 10^5$ /ml	8.95 $\pm$ 0.29
Ammonia-N, mg/dl	11.31 $\pm$ 1.88
Total VFA, mM	92.65 $\pm$ 10.35
Acetate, mol %	62.8 $\pm$ 2.54
Propionate, mol %	21.7 $\pm$ 1.39
Butyrate, mol %	12.9 $\pm$ 1.06
<i>iso</i> -Butyrate, mol %	0.4 $\pm$ 0.12
Valerate, mol %	1.3 $\pm$ 0.28
<i>iso</i> -Valerate, mol %	0.9 $\pm$ 0.19

taining 200 mg of each substrate. Weighed amounts of sarsaponin were added to achieve final concentrations of 0, 1.2, 1.8, 2.4 and 3.2 g/L. Two sets of bottles were sealed anaerobically under CO₂ atmosphere with butyl rubber stoppers and aluminium caps, and placed in an incubator at 38°C for 6 and 24 h with gentle shaking. Total gas was measured after insertion of the glass syringe needle through the butyl rubber stopper in the headspace above the medium. The gas volume above the medium was transferred into the syringe barrel by withdrawal of the syringe plunger. The bottles were uncapped, and pH was determined in culture fluid. For analysis of ammonia and VFA, 1 ml of 25% meta-phosphoric acid was added to 5 ml of fermentation fluid, centrifuged (10,000 $\times g$ for 10 min at 4°C) and supernatants were stored at -30°C until analyzed. One milliliter of the incubated fluid was diluted with 4 ml of methylgreen-formalin-saline to count ruminal ciliate protozoa. In vitro fermentation experiments were separately conducted for each substrate with three replicates per day. Consequently, the fermentation was realized in each set of 45 fermentation bottles for 6 and 24 h. After 24 h of incubation with Sudangrass hay plus concentrate, in vitro disappearance of DM (IVDDM) was calculated as original weight of DM of hay plus concentrate (added in each incubation) minus dry residue weight (after incubation) divided by the original sample weight. These values were then multiplied by 100 to derive IVDDM percentage.

Analysis

At the end of the incubation period, 0.5 ml of gas was removed from each of the bottles with gas tight syringe, and methane and hydrogen were measured by a gas chromatograph (model GC-8A, Shimadzu Co. Ltd., Kyoto, Japan) using a Molecular Sieve 5A column (1.6 m $\times$ 3.2 mm I. D., 60-80 mesh, Shinwakako, Kyoto, Japan) and a thermal conductivity detector (column temperature = 60°C, injector and detector temperature = 80°C). The carrier gas (Ar) flow rate was 50 ml/min. The pH

Table 3. Effect of sarsaponin (S) on fermentation of soluble potato starch by mixed ruminal microorganisms.

Item	6-h Incubation						24-h Incubation					
	S, g/L						S, g/L					
	0	1.2	1.8	2.4	3.2	SEM	0	1.2	1.8	2.4	3.2	SEM
pH	6.09	6.10	6.09	6.05	6.02	0.02	5.78	5.79	5.77	5.76	5.73	0.04
Ammonia-N, mg/dl	6.2 ^a	4.9 ^b , L	4.2 ^c	3.6 ^d	3.1 ^d	0.12	9.8 ^a	8.2 ^b , L ¹	7.3 ^c	6.4 ^d	6.1 ^d	0.23
Total VFA, mM	40.8 ^a	42.4 ^b , L	42.8 ^b	43.7 ^c	45.2 ^d	0.77	56.8 ^a	58.3 ^b , L	59.0 ^c	60.4 ^d	62.4 ^e	1.44
Acetate, mol %	60.8 ^a	58.0 ^b , L	55.2 ^c	53.8 ^d	52.0 ^e	0.55	58.1 ^a	56.4 ^b , L	54.8 ^c	53.3 ^c	50.8 ^d	0.45
Propionate, mol %	22.7 ^a	25.3 ^b , L	27.1 ^c	28.1 ^d	29.2 ^e	0.66	28.1 ^a	29.5 ^b , L	30.2 ^{bc}	30.9 ^c	31.7 ^d	0.53
Butyrate, mol %	11.7 ^a	12.1 ^a , L	13.2 ^b	13.7 ^b	14.6 ^c	0.22	12.5 ^a	13.2 ^b , L	13.4 ^{bc}	13.9 ^c	14.7 ^d	0.18
<i>iso</i> -Butyrate, mol %	1.8	1.8	1.7	1.8	1.5	0.08	1.6	1.6	1.7	1.5	1.5	0.05
Valerate, mol %	1.7	1.3	1.4	1.4	1.4	0.05	1.6	1.6	1.6	1.4	1.5	0.04
<i>iso</i> -Valerate, mol %	1.3	1.5	1.4	1.2	1.3	0.04	1.4	1.5	1.3	1.4	1.1	0.04
Acetate:propionate	2.68 ^a	2.29 ^b , L	2.02 ^c	1.88 ^{cd}	1.76 ^{de}	0.03	1.95 ^a	1.79 ^b , L	1.71 ^{bc}	1.65 ^{cd}	1.55 ^d	0.05
Protozoa, $\times 10^4$ /ml	12.3 ^a	11.6 ^a , L	10.0 ^b	9.1 ^c	8.6 ^c	0.22	ND ²	ND	ND	ND	ND	ND
Total gas, ml/incubation	26.4 ^a	27.9 ^b , L	28.8 ^c	29.3 ^c	31.2 ^d	0.51	36.6 ^a	38.1 ^b , L	38.8 ^b	40.2 ^c	42.0 ^d	0.77
H ₂ , mM	0.184 ^a	0.170 ^a , L	0.170 ^a	0.148 ^b	0.141 ^b	0.003	0.037 ^a	0.032 ^b , L	0.030 ^b	0.030 ^b	0.030 ^b	0.004
CH ₄ , mM	13.87 ^a	10.96 ^b , L	9.57 ^c	7.25 ^d	5.82 ^d	0.02	12.86 ^a	10.56 ^b , L	8.48 ^c	7.69 ^c	6.18 ^d	0.03

a,b,c,d,e Means within a row lacking a common superscript letter differ ($P < 0.05$).

¹Linear effect due to dose of sarsaponin ($P < 0.05$).

²Not determined.

was measured immediately with a pH meter. Volatile fatty acids were analyzed by a gas chromatograph (model GC-14B, Shimadzu Co. Ltd.) with a Thermo-3000 5% Shincarbon A column (1.6 m $\times$ 3.2 mm I. D., 60-80 mesh, Shinwakako) and flame-ionization detector (column temperature = 130°C, injector and detector temperature = 200°C). The carrier gas (N₂) flow rate was 50 ml/min. Ammonia-N was determined by the micro diffusion method (Conway, 1962). To determine the IVDDM, the content of the bottles were transferred into a tube and centrifuged at 11,000 $\times g$ for 15 min at 4°C. The supernatants were discarded and the residues were passed through a filter paper. The bottles were washed twice with distilled water and acetone, and dried to the constant weight at 105°C. Protozoa were counted using a Fuchs-Rosenthal counting chamber (Hausser Scientific Partnership, Horsham, PA) as described previously (Ogimoto and Imai, 1981).

Statistical Analysis

In vitro experiments were separately conducted for each substrate using a completely randomized design. Compounds were tested in triplicate, and fermentations were repeated on three separate days for each substrate in a 1-wk interval. The data were analyzed by a general linear model procedure of SAS (SAS, 1994). Orthogonal polynomial contrasts were used to test for linear or quadratic or cubic trends of sarsaponin. Treatment effects were tested using day $\times$ treatment interaction. The LSD method was used to determine significance between treatment means. Least squares means for

sarsaponin levels were reported and significance was tested at the $P < 0.05$.

RESULTS

Table 3 shows the effects of sarsaponin on microbial fermentation with soluble potato starch after 6 and 24 h of incubation. The pH of the medium numerically decreased at the high dose level of sarsaponin. Ammonia-N decreased ($P < 0.05$) with all levels of added sarsaponin. Total VFA concentration increased ($P < 0.05$) as the concentration of sarsaponin increased from 0 to 3.2 g/L. Molar proportion of acetate decreased ($P < 0.05$) and propionate increased ($P < 0.05$), whereas the molar proportion of *iso*-butyrate, valerate, and *iso*-valerate were unchanged. Molar proportion of butyrate increased as sarsaponin increased ($P < 0.05$). Total gas production increased ($P < 0.05$) in a dose-dependent manner. Numbers of protozoa decreased ($P < 0.05$) after 6 h of incubation and mostly disappeared after 24 h of incubation (data not shown). Increasing the concentration of sarsaponin resulted in a linear decrease ($P < 0.05$) in methane and hydrogen.

Table 4 shows the effects of sarsaponin on microbial fermentation of cornstarch after 6 and 24 h of incubation. Similar to results with soluble potato starch, medium pH numerically decreased at the high dose level. Ammonia-N decreased ($P < 0.05$) by all treatment. Total VFA concentration increased ($P < 0.05$) with sarsaponin. Molar proportion of acetate decreased ($P < 0.05$), and molar proportions of propionate and butyrate increased ($P < 0.05$). Other VFA were unchanged. Num-

Table 4. Effect of sarsaponin (S) on fermentation of cornstarch by mixed ruminal microorganisms.

Item	6-h Incubation						24-h Incubation					
	S (g/L)						S (g/L)					
	0	1.2	1.8	2.4	3.2	SEM	0	1.2	1.8	2.4	3.2	SEM
pH	6.14	6.15	6.15	6.09	6.07	0.02	5.85	5.86	5.86	5.80	5.79	0.02
Ammonia-N, mg/dl	5.1 ^a	4.1 ^b , L	3.8 ^{bc}	3.3 ^d	3.1 ^d	0.11	4.3 ^a	3.9 ^{ab} , L ¹	3.6 ^b	2.7 ^c	2.0 ^d	0.15
Total VFA, mM	52.9 ^a	54.8 ^b , L	55.6 ^{bc}	56.9 ^d	57.7 ^d	1.11	60.7 ^a	62.2 ^b , L	63.1 ^{bc}	64.8 ^d	66.6 ^e	1.05
Acetate, mol %	56.1 ^a	53.7 ^b , L	52.0 ^c	51.2 ^{cd}	50.4 ^d	0.58	57.0 ^a	55.6 ^b , L	52.8 ^c	51.0 ^d	49.5 ^e	0.77
Propionate, mol %	28.5 ^a	29.9 ^b , L	31.4 ^c	32.4 ^d	32.6 ^d	0.45	27.9 ^a	29.1 ^b , L	30.8 ^c	31.9 ^d	33.1 ^e	0.48
Butyrate, mol %	10.7 ^a	11.7 ^b , L	12.2 ^{bc}	12.5 ^{cd}	12.8 ^{de}	0.23	10.7 ^a	11.3 ^a , L	12.4 ^b	12.9 ^{bc}	12.3 ^d	0.26
iso-Butyrate, mol %	1.5	1.5	1.4	1.2	1.2	0.20	1.2	1.3	1.1	1.2	1.3	0.13
Valerate, mol %	1.7	1.7	1.6	1.4	1.5	0.25	1.5	1.6	1.4	1.4	1.5	0.31
iso-Valerate, mol %	1.5	1.5	1.4	1.3	1.5	0.24	1.7	1.5	1.5	1.6	1.5	0.29
Acetate:propionate	1.98 ^a	1.80 ^b , L	1.67 ^{cd}	1.59 ^{de}	1.55 ^e	0.04	2.05 ^a	1.91 ^a , L	1.72 ^b	1.61 ^{bc}	1.50 ^d	0.05
Protozoa, ×10 ⁴ /ml	13.4 ^a	11.7 ^b , L	10.4 ^c	9.8 ^{cd}	9.1 ^e	0.21	ND ²	ND	ND	ND	ND	ND
Total gas, ml/incubation	20.8 ^a	22.7 ^b , L	24.1 ^c	24.9 ^{cd}	25.9 ^e	0.77	31.1 ^a	33.8 ^b , L	36.2 ^c	40.5 ^d	43.4 ^e	2.04
H ₂ , mM	0.325 ^a	0.289 ^b , L	0.274 ^b	0.252 ^c	0.238 ^c	0.005	0.034 ^a	0.032 ^{ab} , L	0.029 ^b	0.029 ^b	0.027 ^{bc}	0.005
CH ₄ , mM	15.43 ^a	12.33 ^b , L	11.41 ^c	9.04 ^d	6.21 ^d	0.15	13.84 ^a	11.48 ^b , L	9.63 ^c	8.98 ^{cd}	6.94 ^d	0.036

^{a,b,c,d,e}Means within a row lacking a common superscript letter differ ($P < 0.05$).

¹Linear effect due to dose of sarsaponin ($P < 0.05$).

²Not determined.

bers of protozoa decreased ($P < 0.05$) after 6 h of incubation. Total gas production increased ($P < 0.05$) in a dose-dependent manner. In contrast, increasing the dose of sarsaponin resulted in a linear reduction ($P < 0.05$) of methane and hydrogen.

Table 5 shows the effects of sarsaponin on the fermentation of hay plus concentrate after 6 and 24 h. In the case of incubation with hay plus concentrate, ammonia-N and total VFA was higher than soluble potato starch

and cornstarch. Similar to results described above, the pH of the medium numerically decreased at the high dose level of sarsaponin. Ammonia-N decreased ($P < 0.05$) by all treatments. Total gas production increased ($P < 0.05$) in a dose-dependent manner. Total VFA concentration increased ($P < 0.05$) as concentration of sarsaponin increased from 0 to 3.2 g/L. The molar proportion of acetate decreased ($P < 0.05$), and propionate and butyrate increased ($P < 0.05$) with sarsaponin. Test

Table 5. Effect of sarsaponin (S) on fermentation of hay plus concentrate by mixed ruminal microorganisms.

Item	6-h Incubation						24-h Incubation					
	S, g/L						S, g/L					
	0	1.2	1.8	2.4	3.2	SEM	0	1.2	1.8	2.4	3.2	SEM
pH	6.25	6.24	6.23	6.20	6.12	0.08	5.95	5.88	5.87	5.83	5.82	0.06
Ammonia-N, mg/dl	14.1 ^a	11.6 ^b , L	10.5 ^c	9.4 ^d	8.8 ^d	0.39	16.5 ^a	14.6 ^b , L ¹	13.0 ^c	12.6 ^{cd}	11.3 ^e	0.72
Total VFA, mM	56.3 ^a	57.2 ^b , L	62.7 ^c	63.0 ^{cd}	64.7 ^e	0.83	73.3 ^a	76.4 ^b , L	77.8 ^c	79.8 ^d	81.7 ^d	1.32
Acetate, mol %	63.0 ^a	57.8 ^b , L	53.6 ^c	52.1 ^d	48.5 ^e	0.65	60.2 ^a	57.1 ^b , L	55.1 ^c	52.3 ^d	50.2 ^e	0.75
Propionate, mol %	24.3 ^a	26.8 ^b , L	30.3 ^c	30.9 ^{cd}	33.3 ^e	0.61	23.9 ^a	25.4 ^b , L	27.1 ^c	29.7 ^d	30.7 ^e	0.53
Butyrate, mol %	9.6 ^a	12.0 ^b , L	12.2 ^b	13.4 ^c	14.0 ^d	0.16	12.3 ^a	13.5 ^b , L	13.7 ^b	14.7 ^c	14.9 ^c	0.34
iso-Butyrate, mol %	1.2	1.2	1.4	1.3	1.3	0.01	1.0	1.0	1.0	0.9	0.9	0.08
Valerate, mol %	0.9	1.0	0.8	1.1	1.3	0.17	1.6 ^a	1.8 ^b	2.0 ^{bc}	1.5 ^a	1.5 ^a	0.11
iso-Valerate, mol %	0.5 ^a	0.6 ^b	1.1 ^c	0.8 ^b	1.0 ^c	0.04	1.0 ^a	1.2 ^a	1.1 ^a	1.1 ^a	2.1 ^b	0.37
Acetate:propionate	2.59 ^a	2.16 ^b , L	1.77 ^c	1.68 ^d	1.46 ^e	0.54	2.52 ^a	2.25 ^b , L	2.03 ^c	1.76 ^d	1.64 ^e	0.09
Protozoa, ×10 ⁴ /ml	11.6 ^a	9.5 ^b , L	8.9 ^c	6.7 ^d	6.6 ^d	0.37	ND	ND	ND	ND	ND	ND
IVDDM, ³ %	ND ³	ND	ND	ND	ND	ND	51.6 ^a	48.1 ^{ab} , L	47.1 ^{bc}	45.8 ^{cd}	43.5 ^d	0.44
Total gas, ml/incubation	11.3 ^a	14.3 ^b , L	16.3 ^c	17.5 ^d	18.8 ^e	0.62	42.5 ^a	43.0 ^{ab} , L	43.5 ^c	45.0 ^d	46.0 ^e	1.06
H ₂ , mM	0.325 ^a	0.288 ^b , L	0.269 ^b	0.260 ^b	0.232 ^c	0.005	0.031 ^a	0.029 ^{ab} , L	0.026 ^b	0.026 ^b	0.026 ^b	0.003
CH ₄ , mM	18.87 ^a	14.65 ^b , L	13.69 ^{bc}	11.79 ^d	8.93 ^e	0.02	17.36 ^a	15.06 ^b , L	13.60 ^c	12.03 ^d	9.83 ^e	0.34

^{a,b,c,d,e}Means within a row lacking a common superscript letter differ ($P < 0.05$).

¹Linear effect due to dose of sarsaponin ($P < 0.05$).

²IVDDM = in vitro disappearance of dry matter of hay plus concentrate was determined after 24 h incubation.

³Not determined.

compound had little effect on other VFA. Numbers of protozoa decreased ($P < 0.05$) gradually as concentration of sarsaponin was increased from 1.2 to 3.2 g/L after 6 h of incubation. Similar to the results above, increasing concentration of sarsaponin resulted in a linear decrease ($P < 0.05$) of methane and hydrogen. In vitro disappearance of DM of hay plus concentrate was not affected at lower concentration of sarsaponin (1.2 g/L), but it was decreased ($P < 0.05$) by 8.7 to 15.6%, as the concentration of sarsaponin increased from 1.8 to 3.2 g/L after 24 h.

DISCUSSION

Decreased ruminal pH associated with sarsaponin supplementation has been reported previously (Grobner et al., 1982; Goetsch and Owens, 1985). Hristov et al. (1999) observed a slightly decreased ruminal pH in heifers receiving sarsaponin from 20 to 60 g/d (DM basis), but the results were not significant. In the present experiment, excluding lower dose level, the addition of sarsaponin numerically decreased the medium pH.

Reductions in ruminal ammonia concentration with sarsaponin supplementation have been varied. Sarsaponin reduced ammonia concentration in vitro (Grobner et al., 1982; Wallace et al., 1994). Grobner et al. (1982) found a 15% reduction in ammonia concentration when saponins were included at 60 mg/kg in the incubation medium. In studies with dairy cows, supplementation of sarsaponin at 77 mg/kg (dietary DM basis) (Goetsch and Owens, 1985; Valdez et al., 1986) or 8 g/d of sarsaponin (Wu et al., 1994) did not significantly affect ruminal ammonia concentrations. Hristov et al. (1999) included sarsaponin in diets for heifers and observed a decrease in ruminal ammonia concentration significantly. In the present experiment, ruminal ammonia concentrations were reduced ($P < 0.05$) by all treatment, compared with control.

Headon et al. (1991) explained that *Y. schidigera* extract has two components. The glyco component, which is separated from the saponin fraction, binds ammonia, and the saponin fraction may affect ammonia concentration indirectly via their toxicity to rumen ciliate protozoa. Reduced ammonia concentrations in the rumen are typical when protozoa are inhibited (Williams and Coleman, 1991). Wallace et al. (1994) reported species-dependent effects of saponin on pure cultures of carbohydrate fermenting ruminal bacteria (*Streptococcus bovis* ES1, *Butyrivibrio fibrisolvens* SH13, *Selenomonas ruminantium* Z108, and *Prevotella ruminicola*) and suggested that saponin may affect bacteria with gram-positive ultrastructure more than it does gram-negative organisms. In a subsequent study, Wang et al. (2000)

reported that steroidal saponins reduced the growth of *Strep. bovis*, *P. bryanti*, and *Ruminobacter amylophilus*, but the growth of *S. ruminantium* was enhanced. Similar results were obtained with ionophores that inhibit gram-positive carbohydrate fermenting bacteria and protozoa also decreased deamination (Van Nevel and Demeyer, 1977), but these bacteria have low specific activities of ammonia production. In a later study, Krause and Russell (1996) reported that monensin also decreased obligate amino acid fermenting bacteria *Peptostreptococcus anaerobius*, *Clostridium sticklandii*, and *Clostridium aminophilum*. Therefore, reduced ammonia concentrations by sarsaponin in this study may be due to the inhibition of gram-positive bacteria and protozoa.

Other major effects of sarsaponin on ruminal fermentation were the increased ($P < 0.05$) concentration of propionate and butyrate, and decreased ($P < 0.05$) acetate and acetate:propionate ratio. A decrease in molar proportion of acetate and increase butyrate was consistent with the results of the in vivo study (Christopher and Neal, 1987). Reported effect of sarsaponin on ruminal propionate has been varied. Grobner et al. (1982) reported a significant increase in propionate production in vitro. Valdez et al. (1986) found no significant change in propionate production in vivo. Hristov et al. (1999) found the effect of sarsaponin on propionate concentration in the rumen was persistent over the course of sampling and was evident even before the daily dose of sarsaponin was introduced in the rumen. Increased propionate in the rumen is often found in studies with defaunated sheep (Williams and Coleman, 1991). Similar results were obtained with monensin, which alters the proportions of VFA in the rumen contents towards higher propionate and decreased acetate in vitro and in vivo (Richardson et al., 1976). Total gas was increased with sarsaponin. This may be due to the increased production of propionate, because carbon dioxide is produced when propionate is made via the succinate:propionate pathway by some ruminal bacteria.

In the present experiment, excluding lower dose level, IVDDM was decreased. Hristov et al. (1999) reported that the extent of ruminal degradability of dietary DM was not affected by saponin treatment, although the rate of degradation of insoluble DM was increased with sarsaponin. They also explained that a portion of the ruminally administered sarsaponin could pass out of the rumen with the liquid phase of ruminal contents and, upon reaching the intestine, could affect the post-ruminal digestibility of nutrients. In a subsequent study, Wang et al. (2000) reported that steroidal saponins inhibited the ruminal cellulolytic bacteria (*Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *Ruminococcus albus*) and fungi (*Neocallimastix fron-*

talis and *Piromyces rhizinflata*). Similar results were also observed with monensin, which also inhibited the cellulolytic bacteria (Chen and Wolin, 1979; Helaszek and White, 1991) and cellulose digestion (Baldwin et al., 1992). Fiber degradability in the rumen was also observed to decrease in defaunated animals (Williams and Coleman, 1991).

The main reason for methane suppressing effects of sarsaponin may be due to the inhibition of H₂-producing bacteria such as cellulolytic bacteria (Wang et al., 2000) as well as other bacteria that use pyruvate-ferredoxin oxidoreductase to metabolize pyruvate to acetyl-SCoA. Rumen ciliates also provide hydrogen as a substrate for methanogens (Stumm and Zwart, 1986; Ushida et al., 1997) and reduced number of rumen ciliates by saponin partially inhibited methane production in this study. Hydrogen production was also decreased ($P < 0.05$) due to the inhibition of H₂-producing bacteria and ciliate by saponin. Based on the stoichiometric relationships (Demeyer and Van Nevel, 1975) between end products formed, we always found a lower hydrogen recovery in treatment compared with the control, also indicating accumulation of a reduced end product other than methane, hydrogen, propionate, and butyrate, since these are involved in the calculation of a hydrogen balance. Lower hydrogen recovery was also obtained due to the effect of monensin on rumen metabolism in vitro (Van Nevel and Demeyer, 1977).

IMPLICATIONS

We have shown that sarsaponin has the ability to partially inhibit methane production in in vitro microbial fermentations. This compound stimulated mixed ruminal microorganism fermentation, and the change in fermentation products, and decreased methane, hydrogen, and ammonia concentration were similar to the effects of ionophores on the ruminal microbial fermentation. Further research is necessary to establish the long-term efficacy of sarsaponin to inhibit methanogenesis and to improve animal performance.

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