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Bloat Mitigation Potential of Plant Tannins and Yucca Extracts based on in Vitro Ruminal Fermentation and Methane Gas Production from Wheat Forage

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Abstract: *Three commercial plant tannin and a yucca extract having different secondary compound profiles were simultaneously evaluated in vitro for their effects on ruminal methane gas production and rumen fermentation patterns of wheat forage. Overall objective was to quantify in vitro, the sources of tannins and dose levels of tannins and yucca extract on rate of gas production, ruminal fermentation, foam height and foam strength from wheat forage. In vitro gas and methane production were measured from 0 to 6 h rumen incubation periods. In vitro rumen volatile fatty acids (VFA) was measured after 6 h rumen incubation. Pine bark was used as condensed tannins sources to serve as a non-extracted plant tannin source. In vitro rate of gas and potential gas production linearly decreased (P < 0.01) in a dose dependent manner for quebracho, mimosa, chestnut tannins, and pine bark addition. In the presence of quebracho (P < 0.03), mimosa (P < 0.02), chestnut (P < 0.02), yucca (P = 0.06), and pine bark (P = 0.11), methane production linearly decreased. Cumulative hourly total in vitro ruminal gas production was similar between control, yucca and pine bark at 5 mg extracts/ml after 6 h rumen fermentation. Cumulative ruminal gas production was lower for quebracho (P < 0.01), mimosa (P < 0.001), and chestnut tannins (P < 0.001) after 3-h fermentation. Total average VFA concentration was not affected by chestnut and yucca extracts treatments, but total VFA linearly decreased for quebracho (P < 0.04), mimosa (P < 0.01) and pine bark (P < 0.01) tannins treatments. Acetate and propionate (A/P ratio) molar ratios for quebracho (P <0 .01), mimosa (P < 0.01), yucca (P = 0.11), and pine bark (P = 0.001) decreased linearly in a dose dependent manner. Foam production and foam strength were lower (P < 0.01) from quebracho, mimosa, chestnut, and pine bark tannins than control treatment, but foam production and foam strength were higher (P < 0.01) for yucca extracts than for control treatment. It is concluded that addition of commercial tannins and yucca extracts changed in vitro rumen fermentations and VFA profiles. Except for chestnut tannins, addition of tannins and yucca extracts, decreased acetate/propionate (A/P) ratios, suggesting that plant tannins and yucca extracts may be nutritionally beneficial in terms of altering VFA ratios to favor improved animal growth.*

Keywords: *Gas production; Plant tannins; Rumen*

1. INTRODUCTION

Winter wheat is a dual-use crop in the Southern Great Plains, where stocker cattle can be grazed on wheat pastures from fall through February, removed and a grain crop harvested. Alternatively the wheat can be harvested for grain only or grazed out. Winter wheat forage in the vegetative stage of growth has high nutritive value, typically containing 17 to 31% crude protein and in vitro digestibility >80 % (Pinchak et al., 1989). Rumen microbial populations rapidly digest nutrients in wheat. From 45 to 62 % of the nitrogen (N) in wheat forage is highly soluble (Horn et al., 1977; Vogel, 1988; Min et al., 2005b) and as much as 30 to 47 % of soluble N can be lost before entering the small intestine as a result of rapid degradation to ammonia-N that exceeds the capacity for microbial protein synthesis (MacRae and Ulyatt, 1974; Vogel, 1988; Min et al., 2000). The total digestible nutrients (TDN) to crude protein (CP) ratio of wheat forage in the vegetative stage of 2.5 to 4 suggests there is a deficit in available energy to available N (Moore et al., 1999). The greater levels of soluble N in wheat forages likely exacerbate the imbalance. Beever and Siddons (1986) presented data to suggest that

performance of cattle on wheat pasture was limited by inadequate non-ammonia N arriving at the small intestine. Vogel (1988) counter that the lack of performance response to supplementation with rumen escape protein suggests metabolizable energy (ME) intake is the first-limiting nutrient with respect to animal gain. Collectively, these reports suggest that 1) decreasing rapid ruminal fermentation N losses and increasing forage N flow to the small intestine and/or 2) increasing the ME yield from wheat forage diets will increase cattle performance on wheat pasture. The primary route to increase in vivo ME would be to decrease methane production from wheat.

Secondary plant compounds are known to alter the extent, rate and site nutrient utilization (Barry and McNabb, 1999; Min et al., 2003) and methane production (Puchala et al., 2005). Condensed tannins are plant polyphenolic compounds that precipitate dietary proteins, with the extent of this reaction dependent on the concentration and sources of tannins (Min et al., 2003). The effects of plant tannins could be beneficial or detrimental to the ruminant, depending upon whether the undegraded nitrogeneous compounds are available for absorption in the small intestine. Min et al. (2005a, 2007a) reported that low level of quebracho-condensed tannins (1-2%/kg DMI) supplementation reduced in vitro ruminal gas production (including methane gas) and frothy bloat potential, but simultaneously increased average daily gain by 25% in steers grazing winter wheat forage (Min et al., 2006b). Generally, however, no information is given as to whether there is a difference between the effects of condensed and hydrolysable tannins.

Yucca extract has been used manipulate the nutrient digestion and performance of ruminant animals (Goetsch and Owens 1985; Wang et al. 1998; Hristov et al. 1999), but the responses observed have been inconsistent (Wang et al. 1998; Pen et al. 2006). Based on our earlier work with quebracho tannin extracts, we decided to conduct experiments designed to determine the influence of mimosa and chestnut tannin extracts, ground pine bark and yucca extract on in vitro gas production and rumen fermentation characteristics from wheat forage. Our overall objective was to quantify effects of secondary compound extract and dose levels on in vitro rate of gas production, ruminal fermentation characteristics, and foam height and foam strength from wheat forage.

2. MATERIALS AND METHODS

2.1. Experimental Design

An in vitro study was conducted to compare different secondary constituents (plant tannins and saponin-containing yucca extract; *Yucca schidigera*) on in vitro gas production system, rumen fermentation profiles and foam dynamics from wheat forage. Quebracho (*Schinopsis* spp.), mimosa (*Acacia mearnsii***;** black wattle**)** and chestnut tannins **(***Castanea sativa* Mill.**)** were used as representatives of condensed (quebracho, 76%) and hydrolysable (chestnut tannins, 80%) tannins or mixed tannins (mimosa tannins; contains condensed (70%) as well as some of hydrolysable tannins; Viviers et al., 1983; Krisper et al., 1992; Zimmer and Cordesse, 1996; Hervas et al., 2003b; Romani et al., 2006). Pine bark (*Pinus radiate*; 35% condensed tannins) was chosen as a condensed tannin-containing plant source (Jerez et al., 2007) to serve as a non-extracted tannin source for indicator.

2.2. Sample Preparation

Fresh wheat forage was minced (blender Model DS-7; Warning Products Co., Winsted, CT) at 500 rpm prior to all in vitro Experiments (Min et al., 2005a). Vegetative stage of forage samples were randomly selected within pasture and cut to ground level (February, 2005) and stored at –20^{O}C for in vitro ruminal gas and foam production analyses. Pine bark was ornamental mulch grade purchased from a garden center. Commercial tannins were obtained from Chemtan Company (INC, Exeter, NH) and yucca extract was purchased from DPI (Distributers Processing Inc., Micro-Aid feed grade concentrate; Porterville, CA). The yucca extract concentration selected for this study (0, 5 and 10 mg/ml of total volume) was calculated from the amount determined to be necessary to substantially reduce rumen fermentation and methane gas in the rumen (Lila et al., 2003; Pen et al., 2006). The amount of all other tannins extracts were used as same as yucca extracts.

2.3. In Vitro Gas Production

In vitro gas production was measured as plunger displacement (ml) at 0, 1, 2, 3, 4, 5, and 6 h incubation periods with or without tannins supplementation (0.0, 5.0, and 10.0 mg/ml; Min et al., 2005a). In vitro incubation was under taken in duplicate. Total in vitro gas produced was corrected to blank incubations (i.e. no ruminal fluid). The rumen fluid was collected from two cannulated steers

receiving Bermuda grass hay (*Cynodon dactylon*) diet, mixed and strained through four layers of cheesecloth and flushed with CO_2 gas for in vitro rumen incubation.

The in vitro rumen incubation procedure consisted of 5 g minced fresh forage being placed in 250 ml volumetric flasks containing 20 ml of rumen fluid, 30 ml of artificial saliva, buffered to pH 6.8, saturated with CO_2 gas and maintained at 39 ^{o}C (Min et al., 2005a). Flask stoppers were equipped with rubber tubing connected to 60 ml syringes (Tyco Health Care Ltd., Mansfield, MA). All gases were collected from the in vitro rumen incubation for total gas and methane gas production analyses (Puchala et al., 2005).

2.4. In Vitro foam Production and Strength

The effect of plant extracts (0.0, 5.0, and 10.0 mg/ml) on rumen foam production and strength was measured (pH 6.8) with minced wheat forage, according to Okine et al. (1989) and Min et al. (2005a). The time for the foam column to collapse through itself to original fluid volume was used as an index of foam strength.

2.5. Chemical Analysis

Experimental culture tubes were analyzed for VFA by the gas chromatography (GC)-gas proportional counting method of Nelson and Zeikus (1974). Culture gas phase was injected directly into the GC using a 1-cm^3-glass hypodermic syringe and a pressure-lock fitting (Supelco). Standard gas and VFA mixtures adjusted all gas and VFA quantities. Methane gas was determined from 6 h in vitro incubation gas samples in an open-circuit respiration calorimetric system (Sable Systems; Henderson, NV; Puchala et al. 2005). Analyzers were calibrated with gases of known concentrations.

2.6. Statistical Analysis

Data are presented as mean values, together with the standard error of the mean (SEM). The variables in experiment included in vitro ruminal gas production, VFA, foam production and strength. The model included sources of plant extracts (quebracho, mimosa, chestnut tannins, yucca, and pine bark) and dose levels (0.0, 5.0, and 10.0 mg/ml) of plant extract addition. An in vitro gas production rate was measured repeatedly and calculated using the exponential equation of ∅rskov and McDonald (1979): $Y = a + b(1-e^{-ct})$.

Where Y was defined as gas production in time *t*; a, b, and c being constants of the exponential equation where a = the gas production at time 0, b = the proportion of gas production during time (*t*), and c = the rate of gas production of the 'b' fraction. The constants b and c for each treatment were calculated with the method described by Min et al. (2000) using the Non-Linear Regression (NLIN) procedure from SAS Institute (1990). Cumulative in vitro gas production in each time point was analyzed using the MIXED procedure of SAS Institute (1990). The dose level effects were tested by an orthogonal contrast for equally spaced treatments estimated by the MIXIED procedure of SAS. The *F*-test-protected least squares means procedure of SAS was used to separate treatment means.

3. RESULTS

In vitro gas, methane gas and cumulative gas productions during in vitro fermentation of wheat forage displayed extract and dose responses that were always linear and generally quadratic in nature (Table 1). Differential extract and dose specific responses led to an extract x dose level interaction for rate of gas production ($P < 0.002$) and potential gas production ($P < 0.001$), while tending ($P < 0.12$) to effect methane production (Table 1 and Fig. 1 A, B, C). Extract x dose interaction for rate of gas production resulted from chestnut and mimosa extracts exhibiting large linear and quadratic responses while dose levels in the remaining extracts were linear responses only. Rate of gas production was less at 10 mg/ml for all but chestnut extracts. The extract x dose interaction for potential gas production resulted from mixed responses at the level and a combination of linear and quadratic responses at the 10mg/ml dose. Methane production was decreased more by commercial tannin extracts at 5mg/ml than by yucca extract and pine bark. Increasing dosage to 10 mg/ml dose of commercial tannin extracts did not further decrease methane, where as yucca extract and pine bark dosed at that level led to dramatic ($P < 0.05$) decrease in methane production.

Acetate: propionate ratio

Means in a row with different superscripts (a,b) are different ($P < 0.05$).

Column means that do not have common letters (A,B,C) in their superscripts differ ($P < 0.05$). S.E.M. is standard error of the mean.

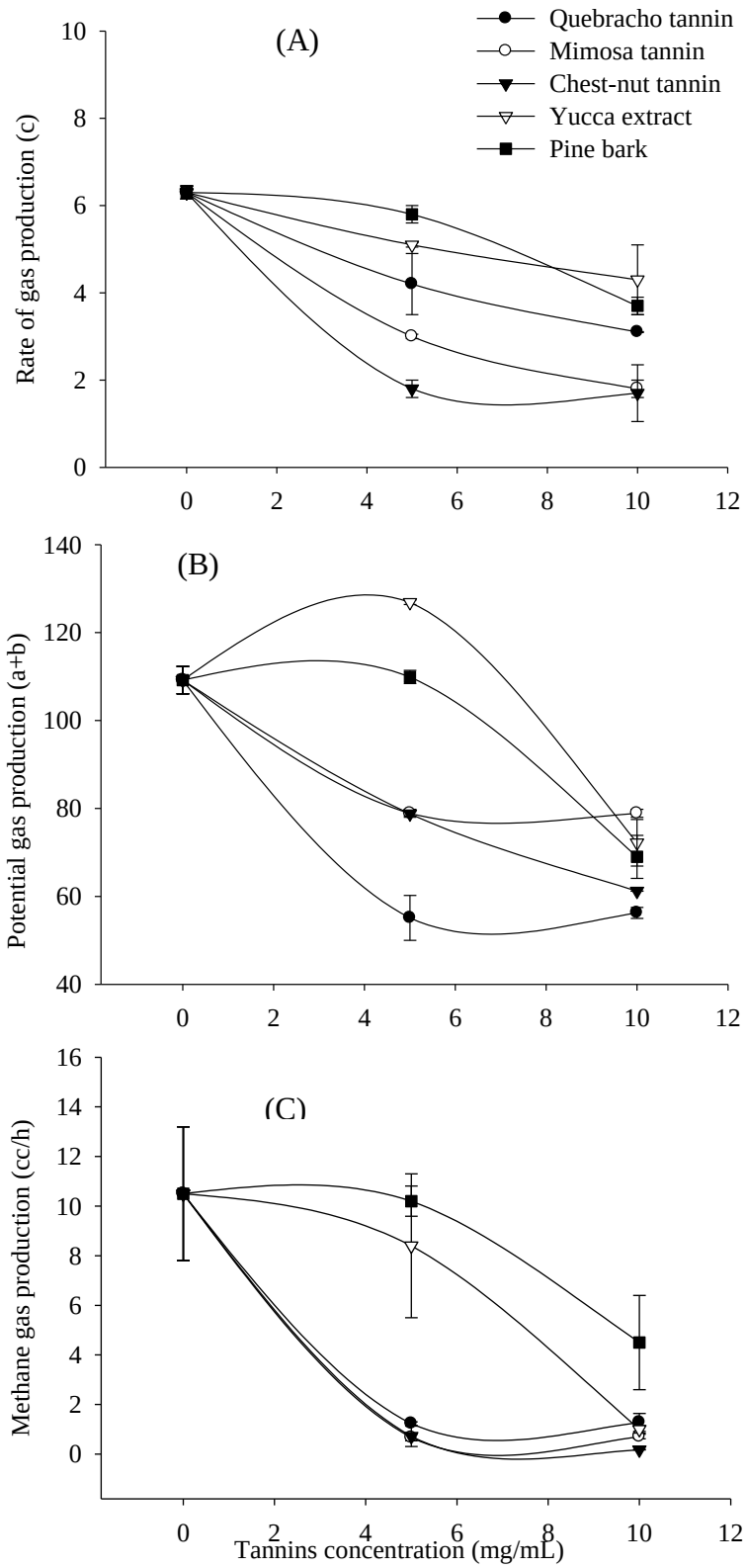

Fig1. *The effect addition of tannins and yucca extracts on in vitro rate of ruminal gas (A), potential gas (B) and methane gas (C) production. Results are the mean of duplicate determinations, and error bars represent standard error of the means.*

Table1. *The effect of plant tannins and yucca extracts on the ruminal gas production[1] by mixed rumen microorganisms in vitro.*

Item (mg/ml)	Rate of gas Production c, ml/h	Potential gas Production a + b, ml/6h	Methane gas ml/h
Quebracho			
0.0	6.4	109.2	10.5
5.0	4.2	55.1	1.24
10.0	3.1	56.3	1.28
SEM	0.32	3.18	1.05
Mimosa			
0.0	6.4	109.2	10.5
5.0	3.0	78.9	0.7
10.0	1.8	79.0	0.7
SEM	0.12	1.67	0.97
Chestnut			
0.0	6.4	109.2	10.5
5.0	1.8	78.8	0.7
10.0	1.7	61.2	0.2
SEM	0.33	1.24	1.07
Yucca			
0.0	6.4	109.2	10.5
5.0	5.1	126.5	8.4
10.0	4.3	72.2	1.0
SEM	0.33	3.02	1.92
Pine bark			
0.0	6.4	109.2	10.5
5.0	5.8	109.9	10.1
10.0	3.7	68.9	4.5
SEM	0.20	2.77	1.73
ANOVA			
Quebracho			
Linear	0.02	0.001	0.03
Quadratic	0.32	0.01	0.09
Mimosa			
Linear	0.001	0.01	0.02
Quadratic	0.01	0.01	0.08
Chestnut			
Linear	0.01	0.001	0.02
Quadratic	0.02	0.07	0.09
Yucca			
Linear	0.08	0.01	0.06
Quadratic	0.66	0.01	0.41
Pine bark			
Linear	0.01	0.01	0.11
Quadratic	0.06	0.03	0.34
Interactions			
Source of tannins (ST)		0.001	0.02
Dose level (DL)		0.001	0.001
ST x DL		0.001	0.12

Cumulative in vitro ruminal gas production was similar at hourly time steps between control, yucca and pine bark after 6 h of fermentation at 5 mg extracts/ml (Fig. 2 A). In contrast, when tannin extracts were dosed at 5 mg extract/ml cumulative, in vitro ruminal gas production was lower for quebracho ($P < 0.01$), mimosa ($P < 0.001$), and chestnut tannins ($P < 0.001$) after 3-h fermentation from control treatment. Cumulative hourly in vitro gas production for all tannins extracts, yucca, and pine bark treatments dosed at 10 mg extracts/ml was less than controls ($P < 0.01$) after 3-h in vitro rumen fermentation (Fig. 2B).

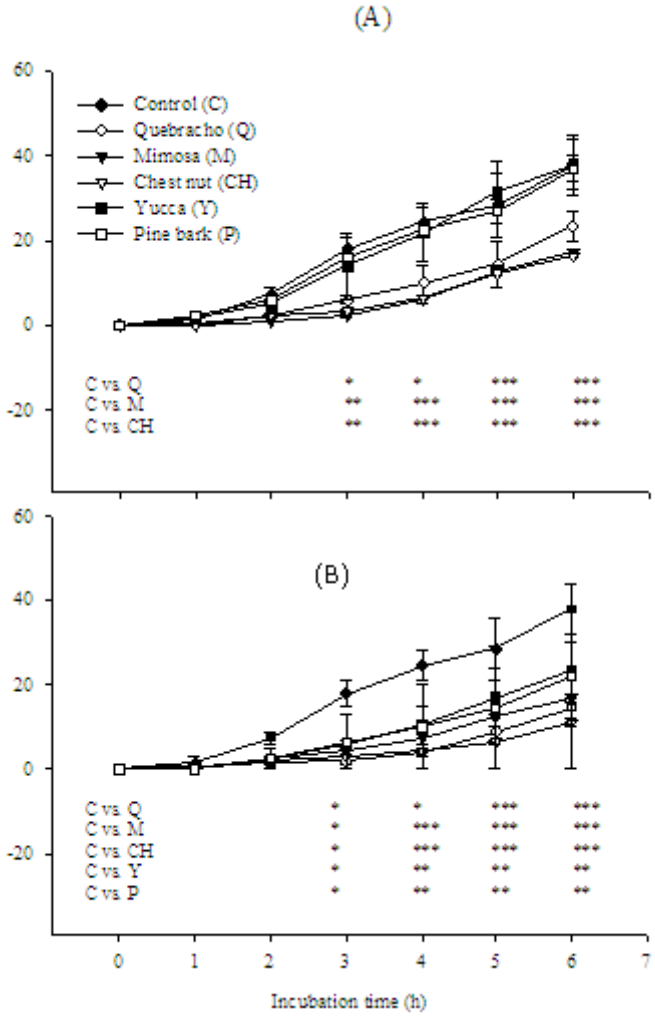

Fig2. *The effect addition of 5 (A) and 10 mg/ml (B) of tannins and yucca extracts on in vitro ruminal gas production. Results are the mean of duplicate determinations, and error bars represent standard error of the means.*

Total average VFA concentration was not affected by chestnut and yucca extracts, but linearly decreased for quebracho ($P < 0.04$), mimosa and pine bark tannins treatments ($P < 0.01$; Table 2). Total VFA concentration tended to be lower ($P < 0.07$) for pine bark than for other extracts at 5 mg of extracts/ml. The greatest reduction in total VFA's generally occurred at the 5 mg of extracts/ml dosage.

Table2. *The effect of plant tannins and yucca extracts on the ruminal volatile fatty acids (VFA) profiles by mixed rumen microorganisms in vitro.*

		Dose level (mg/ml)			Contrast	
Item	0	5	10	SEM	Linear	Quadratic
Ruminal VFA						
Total VFA (mM)						
Quebracho	66.1	53.1^{A}	42.9	3.61	0.04	66.1
Mimosa	66.1	43.9^{A}	36.5	2.35	0.01	0.11
Chestnut	66.1	41.0^{A}	58.5	9.23	0.69	0.25
Yucca	66.1	46.4^{A}	36.9	7.25	0.23	0.77
Pine bark	66.1	31.1^{B}	42.3	1.38	0.01	0.01
VFA composition (mol/100 mol)						
Acetate (A)						
Quebracho	37.6	30.2^{A}	22.8	2.28	0.04	0.99
Mimosa	37.6	24.3^{A}	19.1	1.57	0.01	0.14
Chestnut	37.6	21.7^{A}	34.1	7.07	0.84	0.30
Yucca	37.6	25.4^{A}	18.0	5.05	0.19	0.83

Pine bark	37.6	14.9^{B}	22.5	0.91	0.01	0.001
Propionate (P)						
Quebracho	15.6	13.6^{A}	12.6	0.99	0.16	0.71
Mimosa	15.6	12.3^{A}	10.4	0.34	0.01	0.23
Chestnut	15.6	11.6^{A}	14.8	1.77	0.78	0.25
Yucca	15.6	12.3^{A}	11.5	1.81	0.11	0.25
Pine bark	15.6	8.5^{B}	10.9	0.39	0.01	0.001
Butyrate (B)						
Quebracho	7.5	5.4	4.2	0.33	0.01	0.37
Mimosa	7.5	4.4	3.7	0.24	0.01	0.05
Chestnut	7.5	4.2	1.4	0.68	0.22	0.11
Yucca	7.5	5.1	4.0	0.92	0.05	0.21
Pine bark	7.5	4.2	5.2	0.41	0.001	0.001
Isobutyrate (IB)						
Quebracho	1.6	1.3^{A}	1.2	0.07	0.05	0.37
Mimosa	1.6	0.6^{B}	1.1	0.25	0.41	0.21
Chestnut	1.6	1.4^{A}	1.5	0.43	0.44	0.20
Yucca	1.6	1.2^{A}	1.1	0.07	0.02	0.06
Pine bark	1.6	1.1^{AB}	1.2	0.05	0.001	0.04
5-methylbutyrate (MB)						
Quebracho	0.6	0.3	0.2	0.05	0.05	0.45
Mimosa	0.6	0.2	0.1	0.03	0.01	0.07
Chestnut	0.6	0.2	0.3	0.04	0.06	0.06
Yucca	0.6	0.3	0.2	0.06	0.01	0.09
Pine bark	0.6	0.2	0.3	0.03	0.01	0.001
Valerate (V)						
Quebracho	1.8	1.4	1.1	0.05	0.01	0.65
Mimosa	1.8	1.1	1.1	0.05	0.03	0.02
Chestnut	1.8	1.0	1.2	0.09	0.04	0.04
Yucca	1.8	1.3	1.1	0.13	0.11	0.54
Pine bark	1.8	1.1	1.2	0.05	0.01	0.02
Isovalerate (IV)						
Quebracho	1.3	1.1	0.9	0.08	0.09	0.59
Mimosa	1.3	0.9	0.9	0.06	0.05	0.11
Chestnut	1.3	0.9	1.0	0.08	0.04	0.07
Yucca	1.3	1.0	0.9	0.09	0.10	0.62
Pine bark	1.3	1.0	0.9	0.05	0.02	0.26
A:P ratio[1]						
Quebracho	2.4^{a}	2.2^{ab}	1.8^{b}	0.08	0.01	0.19
Mimosa	2.4^{a}	1.9^{ab}	1.8^{b}	0.11	0.01	0.01
Chestnut	2.4^{a}	1.8^{b}	2.2^{ab}	0.27	0.73	0.39
Yucca	2.4^{a}	1.9^{ab}	1.6^{b}	0.21	0.11	0.80
Pine bark	2.4^{a}	1.8^{b}	2.0^{ab}	0.05	0.001	0.01

ANOVA	A	P	B	IB	MB	V	IV
Sources of tannins (ST)	0.69	0.38	0.81	0.28	0.74	0.91	0.97
Dose level (DL)	0.01	0.01	0.001	0.01	0.01	0.01	0.001
ST x DL	0.63	0.60	0.44	0.68	0.28	0.51	0.94

[1] Acetate:propionate ratio.

Means in a row with different superscripts (a,b) are different ($P < 0.05$).

Column means that do not have common letters (A,B,C) in their superscripts differ ($P < 0.05$). S.E.M. is standard error of the mean.

Average acetate concentration linearly decreased in the presence of quebracho ($P < 0.04$) and, mimosa tannins ($P < 0.01$), but increased from 5 mg/ml to 10 mg/ml dosages of pine bark ($P < 0.01$). Average propionate concentration linearly decreased in the presence of mimosa extracts ($P < 0.01$). Average butyrate, isobutyrate, 5-methylbutyrate, valerate, and isovalerate were varied among source

of extracts and dose levels (Table 2). Isobutyarte production decreased with yucca extract (saponins) but did not repond to quebracho, chestnut or mimosa extracts (tannins).

Acetate and propionate (A/P ratio) molar ratios for quebraco (P <0 .01), mimosa (P < 0.01), and pine bark (P = 0.001) decreased linearly in a dose dependent manner. Overall tannins and yucca extracts treatments reduced the A/P ratio compared with control treatment. There was no source of tannins and dose levels interaction for ruminal VFA concentration.

The in vitro ruminal foam production and foam strength in response to tannins and yucca extracts are summarized in Fig. 3A and B. Foam production (Fig. 3A) and foam strength (Fig. 3B) were lower (P < 0.01) from quebracho, mimosa, chestnut, and pine bark tannins addition at 5 to 10 mg/ml of forage than control treatment, but foam production and foam strength were higher (P < 0.01) for yucca extracts than for control treatment.

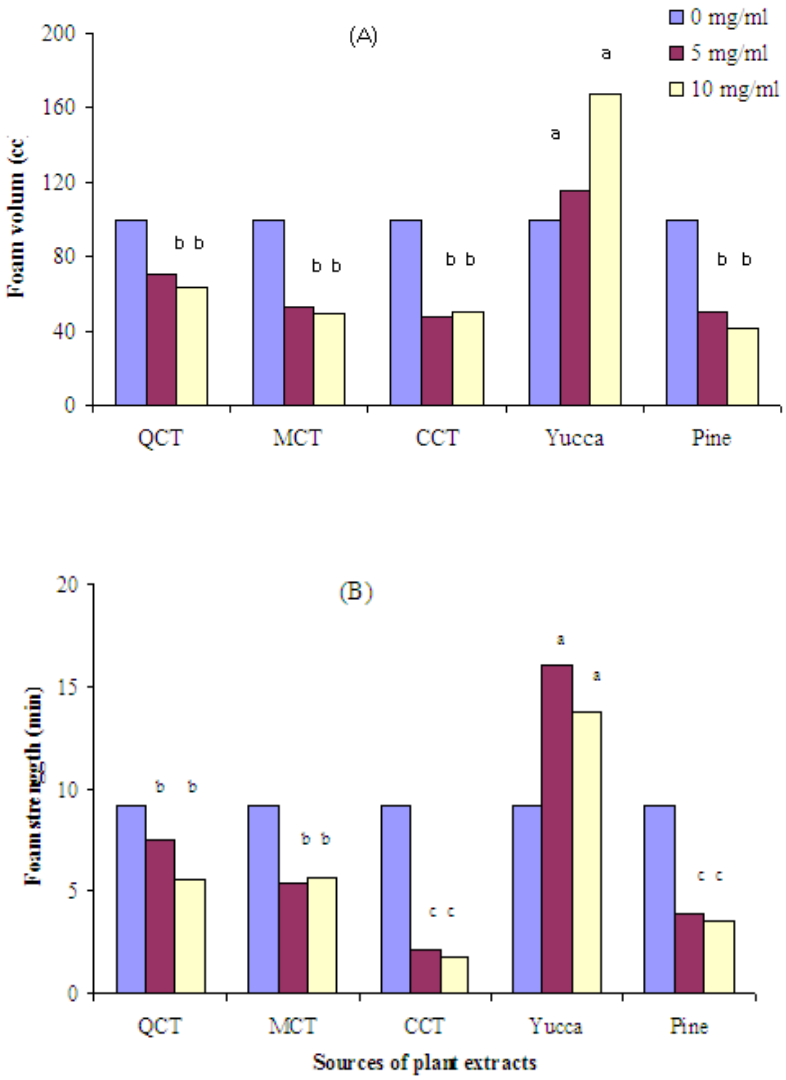

Fig3. *The effect of tannins and yucca extracts on in vitro ruminal foam height (mm; A) and strength (min; B). Means in a row with different superscripts (a, b, c) are different (P < 0.05).*

4. DISCUSSION

The rumen fermentation of fresh wheat forage was reduced in the presence of plant tannins and yucca extract, as revealed by in vitro gas production, and VFA concentration observed in this study. The effect of tannins and yucca extract in this aspect is dose level and type of extract-dependent. The variable reduction in ruminal fermentation activity among extracts apparently result from differential physical and biochemical interactions between plant extracts and rumen microorganisms.

4.1. Effects of Yucca Extract on Rumen Fermentation

A number of reports that array of responses in ruminal gas and methane gas production by supplementation of saponin-rich yucca extracts in vitro (Wang, et al. 1998; Takahashi et al., 2000; Lila et al., 2003; Pen et al. 2006) and in vivo (Santoso et al., 2004). Wang et al. (1998) reported that addition of yucca extract (0.5 mg/ml) did not affect in vitro total gas and methane gas production, or bacterial numbers. However, in the presence of yucca extract from 0 to 6 ml/l (Pen et al., 2006) or steroidal saponins from 0 to 0.23 mg/ml extracted from yucca (Wang et al., 2000a) rate of gas and methane gas production and ruminal protozoal numbers were reduced in a dose dependent manner (Pen et al., 2006). Addition of yucca extract from 0 to 3.2 mg/ml into in vitro incubator during 6 h incubation has shown to be effective in reducing methane gas (53%) production in cow's rumen fluid fed Sudan grass hay plus concentrate mixture (Lila et al., 2003). Santoso et al. (2004) reported suppression of in vivo ruminal methane gas production by 6% with yucca extract supplementation (0 vs. 3 mg/kg BW) in sheep. In the present study yucca extract dosed from 0 to 10 mg/ml reduced the rate of gas, potential gas and methane gas production in dose dependent manners up to 33, 34, and 91%, respectively. The reasons for dramatic suppression of methane gas production by yucca extract may be related to its inhibitory effects on ciliate protozoa (Pen et al., 2006) and on gram-positive bacterial population, especially H_2-producing cellulolytic bacteria (Wang et al., 2000b). Newbold et al. (1995) reported that ruminal protozoa are associated with ruminal methanogenesis (9 to 25%), due mainly to provide H_2 gas as substrate for methanogens (Stumm and Zwart, 1986).

Yucca extract decreased A/P ratios by 33%, consistent with the result of in vitro studies by Wang et al. (2000a), Lila et al. (2003), and Pen et al. (2006). In the present study, ruminal total VFA concentration was not affected by yucca supplementation, however, butyrate types of VFA (butyrate, iso-butyrate, and methyl-butyrate) concentration was decreased with increasing yucca addition, which is generally consistent with the in vitro study of Wang et al. (1998).

Legume and fresh wheat forages contain high concentrations of soluble proteins that are surface-active foaming agents (Coulman et al., 2000; Min et al., 2005b). Saponins are other naturally occurring surface-active foaming agents in legume forages that have been considered to be a potential cause of bloat (Lindahl et al., 1957; Sen et al., 1998). Our data also suggest that saponins-rich yucca extract increased foam volume and foam strength when incubated with minced wheat forage. Production of slime from alfalfa saponins by rumen bacteria and alteration of the surface tension by rumen content were suggested as factors contributing to bloat formation (Sen et al., 1998), but clear experimental proof for this is lacking in the literature In contrast, no significant differences in the occurrence of bloat and frothy rumen contents were found in animals fed high versus low saponin alfalfa (Majak et al., 1980), indicating that alfalfa saponins roll in bloat is unclear and variable (Sen et al., 1998).

4.2. Effect of Commercial Plant Tannins and Pine Bark on Rumen Fermentation

Plant tannins may have significant effects on all phases of in vitro rumen fermentation and metabolism. In vitro ruminal fermentation of minced wheat forage was reduced in the presence of condensed (quebracho and pine bark), hydrolysable (chestnut), and intermediate tannins (mimosa; contains both condensed and hydrolysable tannins), as evidenced by decreasing the rate and potential gas, methane gas and VFA concentration. The results indicate that the effect of tannins extracts in this expression is source of tannins and dose dependent.

Decreases in rate of gas and methane production were more pronounced in chestnut tannins (hydrolysable tannins) and mimosa tannins (intermediate) than in quebracho tannins (condensed tannins), which may result from differential susceptibility to these tannins by rumen microorganisms and/or differential binding capacity with plant proteins (Min et al., 2007b). These findings agree with the data of Gonzalez et al. (2002), who reported that in vitro rumen fermentation activity (as measured by rumen ammonia and volatile fatty acids production) by rumen bacteria per unit of tannin added was higher for chestnut than for quebracho and mimosa tannins, indicating that chestnut tannins are more efficacious compared to quebracho and mimosa tannins. A decrease in rate of gas, potential gas and methane gas production by plant tannins addition was consistent with the result of other in vitro studies (Hervas et al., 2003a; Min et al., 2005a,b; Martinez et al., 2006). In addition, Bento et al. (2005) reported that the reduced gas production from mimosa tannins during in vitro incubation

reflects inhibition of cell walls, and its components, by mimosa tannins. As a result, mimosa tannins reduced microbial degradation of carbohydrates, and subsequently gas production (Akin et al., 1988; Markkar et al., 1995). It has been shown that plant tannins and its commercial extracts modified microbial population in the rumen, reduced microbial numbers and/or enzyme production from the rumen microorganisms available to ferment substrates (Min et al., 2002; Min et al., 2006a,b). These findings agree with our previous research that demonstrated quebracho tannins reduced ruminal gas and methane gas production (Min et al., 2005a,b).

The reduced ruminal VFA production and decreased A/P ratios with dosage of quebracho, mimosa and pine bark tannins may result from modified microbial fermentation of nutrients and enzyme activity associated with a shift in the microbial population (Min et al., 2007b). Other researchers also observed a similar trend in in vitro (Martinez et al., 2006) and in vivo (Zimmer and Cordesse, 1996). However, addition of chestnut tannins at 5 to 10 mg/ml did not significantly reduce total VFA, major VFA (acetate, propionate, and butyrate) and A/P ratios. These findings agree with the data of Zimmer and Cordesse (1996), who reported that ruminal VFA concentration in sheep and goats fed Mediterranean mixed hay with chestnut tannins supplementation (0 vs. 110 mg/kg hay) had similar results for total and individual VFA concentration, but chestnut tannins significantly reduced in situ dry matter digestibility, indicating that hydrolysable tannins like chestnut tannins may not directly alter carbohydrate digestion in ruminants as much as condensed tannins. One of the reasons may be explained that hydrolysable tannins degraded by rumen bacteria (Nelson et al., 1995). However, condensed tannins that are not hydrolyzed by rumen microorganisms (Markkar et al., 1995). Recently, Martinez et al. (2006) reported that both condensed (quebracho) and hydrolysable (tannic acid) tannins inhibited the microbial hydrolysis of grain protein. Furthermore, these same authors reported that these tannins did not prevent bacterial attachment to starch granules, but starch hydrolysis was slowed indirectly as a result of a tannin-mediated reduction in the degradation of the surrounding protein matrix.

Most fresh wheat forage contains high level of protein (over 20 to 27%) and in vitro dry matter digestibility (80 to 90%), but low level of fiber content (28%) during vegetative stage of growth (Pinchak et al., 1989; Min et al., 2005b). More than 53 to 59% of the nitrogen in wheat forage is highly soluble (Vogel, 1988; Min et al., 2005b). Wheat stocker cattle are frequently given ad libitum access to low quality roughages such as wheat straw or sorghum Sudan hay so that low quality roughages slows the rate of passage of wheat forage and, thereby, improves its utilization and reduces the incidence of bloat (Mader et al., 1983). Pine bark (*Pinus radiate*) contains high level of plant tannins (36% DM; Jerez et al., 2007) that are predominantly condensed tannins (catechin and epicatechin). Pine bark also contains high fiber content (79% DM as measured by neutral detergent fiber; data not shown in Table), but low level of in vitro dry matter digestibility (35%; data not shown in Table). In the presence of pine bark reduced the in vitro rumen fermentation as measured by rate of gas, methane gas production, VFA concentration, protease enzyme activity, as well as foam production due to high level of tannins.

5. CONCLUSIONS

Our results show that addition of commercial tannins extracts reduced in vitro gas production, methane gas and VFA production. In the presence of yucca extracts also reduced in vitro gas and methane production, but this trend was less magnitude than tannin extracts. Addition of yucca extracts increased foam production and foam strength in the rumen. Addition of tannins and yucca extracts, except chestnut tannins, decreased acetate and propionate ratios, indicated that plant tannins and yucca extracts may be nutritive benefit in terms of increased VFA efficiency with either dose level of those plant extracts. Further research is required to define animal growth responses to increasing lengths of feeding time on these plant extracts addition as feed additives.

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