Effects of polyphenols (mixed tannins) and saponin (*Yucca schidigera*), with or without a direct fed microbial (DFM) on in vitro rumen fermentation

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ABSTRACT

Rumen fluid from fistulated steers receiving a high concentrate diet was utilized to examine the impact of polyphenols and saponin with or without a direct fed microbial (DFM) on in vitro fermentation characteristics. Treatments consisted of: Control (no polyphenols, saponin or DFM); 2) Polyphenols (Mixed Tannins = 15 g/hd/d); 3) Saponin (*Y. schidigera* = 2 g/hd/d); 4) Polyphenols + DFM (DFM = 1E+7 *Lactobacillus animalis* + 1E+8 *Propionibacteria acidilactici* cfu/hd/d); and 5) Saponin + DFM. Rumen fluid was collected and combined in equal amounts from 3 rumen fistulated steers and mixed at a 1:1 ratio of artificial saliva to rumen fluid. Fermentation substrate consisted of 0.5 g of the high concentrate diet. Fermentation bottles were capped with an air-tight rubber stopper and incubated in a water bath for 12 and 18h (7 replicates/treatment/time point). After incubation, the total volume of gas produced was measured and a subsample analyzed for N, CH4 and CO2 concentrations. After gas sampling, pH, VFA concentrations, and DMD were determined. In vitro fermentation parameters were analyzed using a mixed effects model repeated measures analysis for a completely randomized block (day) design. Acetic acid was decreased while valeric acid was increased (P <0.05) by the Saponin + DFM treatment vs. Control. At 12h DMD was greater in Saponin, Saponin + DFM and Polyphenols + DFM (P <0.001) treatments compared to Control or Polyphenols alone. Polyphenols produced greater amounts of CH_4 /DMD than all other treatments (P <0.01). Microbial protein production and efficiency were greater (P <0.001), Saponin + DFM compared to other treatments. Other fermentation parameters measured were not impacted by treatments. Under the conditions of this experiment these data suggest combining DFM with Saponin or Polyphenols produces different ruminal effects from when they are fed alone.

Table 1. Ingredients and chemical composition of the basal diet (DM basis).

Item	Percentage
Ingredient Composition (% DM)	
Steam Flaked Corn	61
Corn Silage	10
Alfalfa Hay	10
Dry Distillers Grain (DDG)	10
Fat (Tallow)	5
Supplement*	4
Chemical Composition	
Dry Matter, %	69.86
Crude Protein, %	12.9
ADF, %[1]	9.45
NDF, %[2]	16.27
NEg, Mcal/kg[3]	1.43

*Macro- and microminerals included in diet: calcium = 0.71%, phosphorus = 0.33%, salt 0.51%, potassium 0.62%, sulfur 0.16%, magnesium 0.18%, zinc 19.50 ppm, iron 169.42 ppm, copper 6.04 ppm, manganese 11.80 ppm, cobalt 0.12 ppm, iodine 0.50 ppm, selenium 0.13 ppm, sodium 0.24%, and chlorine 0.44%
[1] ADF = Acid detergent fiber
[2] NDF = Neutral detergent fiber
[3] NEg = Net energy for growth

BACKGROUND

The public concern surrounding antibiotic resistance has led to investigation of alternative technologies for improving production efficiency. Tannins are phenolic compounds found in plants that can potentially modulate the rumen microbial ecosystem (Van Soest, 1994). When fed at low concentrations, tannins can reduce ruminal gas production and protein degradation, resulting in increased metabolizable amino acid flow to the small intestine (Min et al., 2003). *Yucca schidigera* saponin has been reported to alter rumen VFA, total gas production and microbial protein in ruminants (Wang, Y., T. A. McAllister, L. J. Yanke, Z. J. Xu, P. R. Cheeke, and K.-J. Cheng. 2000). The use of direct-fed microbials (DFM) has been reported to enhance feedlot efficiency by altering ruminal bacterial communities (Krehbiel et al., 2003). Even though DFM and plant compounds have been separately shown to positively benefit ruminant animals, the combined impacts of DFM and plant compounds on ruminal fermentation or feedlot performance are difficult to elucidate. We hypothesize that saponin and tannins without or with DFM are capable of altering rumen fermentation. Therefore, the objective of this study was to evaluate the effects of saponin, tannins, saponin + DFM and tannins + DFM on ruminant volatile fatty acids, rumen gas production and ruminal microbial protein *in vitro,* as possible substitutes for dietary antimicrobials.

MATERIALS AND METHODS

Three crossbred feedlot steers (450 kg; ~ 3.0 years of age) fitted with ruminal fistulas were adjusted to a high energy finishing grain-based diet (1.43 NEg, Mcal/kg DM) for 21 d (Table 1). On d 22 rumen fluid (~ 4 L) was collected from all three steers, was filtered twice through four layers of cheesecloth and combined into a pre-warmed (39°C) thermos. A modified McDougall's buffer solution was mixed with rumen fluid at a 1:1 ratio (Tilley and Terry, 1963). The dried ground basal diet was weighed and dispensed (1.000 ± 0.005 g) into pre-labeled 100 mL vaccine bottles containing the appropriate dose of each treatment. Treatments consisted of: Control (no polyphenols, saponin or DFM); 2) Polyphenols (Mixed Tannins = 15 g/hd/d); 3) Saponin (*Y. schidigera* = 2 g/hd/d); 4) Polyphenols + DFM (DFM = 1E+7 *Lactobacillus animalis* + 1E+8 *Propionibacteria acidilactici* cfu/hd/d); and 5) Saponin + DFM; same doses as described above. Vaccine bottles were sampled to evaluate rumen fermentation characteristics (VFA concentrations, gas composition, and microbial protein) at two time points (12 h and 18 h) and this process was repeated twice on two different days (run 2). In vitro fermentation parameters were analyzed using a mixed effects model repeated measures analysis for a completely randomized block (block=day) design (Mixed Procedure of SAS version 9.4, SAS Institute Inc., Cary, NC). Least squares means (LSM) and pooled standard errors of the means (SEM) were reported for all response variables.

Table 2. Least square means results of rumen fermentation effects due to polyphenol, saponin, polyphenol +DFM & saponin +DFM additions to a high concentrate finishing diet.

Item	Con[1]	Poly[2]	Sap[3]	Poly[2]+ DFM[4]	Sap[3]+ DFM[4]	SEM[5]	P < Treatment	P < Time	P < Treatment x Time
pH	5.56[a]	5.60[a]	5.64[a]	5.67[a]	5.55[a]	0.08	0.825	0.001	0.21
TVFA, mM	79.98[a]	84.03[a]	81.96[a]	82.17[a]	84.39[a]	3.37	0.891	0.001	0.944
Acetate, M%	30.98[a]	28.54[a]	30.06[a]	28.57[a]	25.04[b]	1.9	0.239	0.004	0.724
Propionate, M%	27.27[a]	28.59[a]	27.57[a]	27.32[a]	29.22[a]	1.78	0.916	0.356	0.349
Butyrate, M%	15.65[a]	17.37[a]	15.67[a]	15.50[a]	15.94[a]	3.05	0.992	0.996	0.998
Valerate, M%	5.62[a]	7.82[ab]	6.26[ab]	5.72[a]	12.18[b]	1.97	0.114	0.694	0.056
CH4/GP, ml/psi	5.22[a]	6.21[b]	5.31[ac]	5.26[ac]	4.69[ac]	0.25	0.002	0.001	0.114
GP/DMD, psi/%	22.54	25.14	11.95	8.55	9.91	2.27	0.001	0.001	0.001
CO2/DMD, ml/%	55.03	61.57	53.67	51.64	51.82	2.2	0.022	0.001	0.005
DMD, %	47.87	44.72	57.45	59.99	62.74	1.89	0.001	0.001	0.001
MicProt, ug/ml	2.63	2.95	3.01	2.94	4.27	0.37	0.029	0.001	0.025
MicProt/DMD, ug/%	2.76	3.15	2.96	2.84	4.08	0.49	0.183	0.014	0.026

[abcd] means with different superscripts differ significantly (P<0.05)
[1]Con = finishing diet with no additives
[2]Poly = polyphenols derived from condensed and hydrolyzed tannins (15 g/hd/d of ByPro)
[3]Sap = saponin derived from *Yucca schidigera* (2 g/hd/d of MicrobSapp)
[4]DFM = direct fed microbial (50 mg/hd/d of Direct =1E+7 *Lactobacillus animalis* and 1E+8 *Propionibacteria acidpropionici*)
[5]SEM = standard error of the means

RESULTS

Least squares means (LSM) and standard errors (SEM) for the rumen fermentation main effects are presented in Table 2. Rumen pH, total VFA (mM), butyrate and propionate (molar %) main effects were similar across treatments. However, acetic acid was lesser (*P* <0.05) in Saponin +DFM treatment vs Control, while valeric acid was greater (*P* <0.05) in Saponin + DFM vs Control, Saponin and Polyphenol + DFM treatments. Total gas pressure (psi), N (ml), CH4 (ml) and CO2 (ml) were similar across treatments. A treatment x time interaction (*P* <0.001) was detected for DMD (Table 2). Dry matter digestibility was greater at 12 h post fermentation for Saponin (*P* <0.01), Saponin + DFM (P <0.001) and Polyphenols + DFM (P <0.001) when compared to Control or Polyphenol treatments (Table 3). Saponin +DFM and Polyphenols + DFM had the greatest 12 h DMD (70.95% and 75.61%, respectively). Adjusted CH_4 to a common GP resulted in polyphenols producing greater (*P* <0.01) CH_4 than all other treatments, while adjusted CH4 production was similar (*P* >0.10) among Control, Saponin, Polyphenols + DFM and Saponin +DFM treatments. Significant treatment x time interactions also occurred for MicProt production and MicProt adjusted to a common DMD (Table 2). At 18 h, Sap + DFM treatment had greater (*P* <0.001) MicProt production and efficiency of MicProt production (*P* <0.01), adjusted to a common DMD (Table 3). Although reports presenting the separate effects of Polyphenols, Saponin and DFM on rumen fermentation are available, little if any previous reports on combined effects of Polyphenols + DFM or Saponin + DFM are known to exist.

Table 3. Least square means for treatment x time interactions for rumen fermentation effects due to polyphenol, saponin, polyphenol +DFM & saponin +DFM additions to a high concentrate finishing diet.

Item	Con[1] 12 h	Con[1] 18 h	Poly[2] 12 h	Poly[2] 18 h	Sap[3] 12 h	Sap[3] 18 h	Poly[2]+ DFM[4] 12 h	Poly[2]+ DFM[4] 18 h	Sap[3]+ DFM[4] 12 h	Sap[3]+ DFM[4] 18 h	SEM[5]
DMD, %	49.62	46.12	42.11	47.33	63.81*	51.1	75.61**	44.37	70.95**	54.52	2.76
CO2/DMD, ml%	46.5	63.57	63.24	60.9	46.6	60.74	37.89	65.4	41.4	62.24	3.2
GP/DMD, psi/DMD	18.56	26.52	26.17	24.1	2.92	20.99	-9.38	26.48	-4.46	24.28	3.02
MicProt, ug/ml	3.35	1.52	4.67	1.22	4.48	1.54	4.35	1.53	4.33	4.21**	0.52
MicProt, g/ml/%	3.8	1.72	4.88	1.43	4.25	1.66	3.88	1.8	3.96	4.20*	0.74

*Means differing from Control (P <0.01)
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[1]Con = finishing diet with no additives
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[3]Sap = saponin derived from *Yucca schidigera* (2 g/hd/d of MicrobSapp)
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[5]SEM = standard error of the means

CONCLUSION

Under the conditions of this experiment our results suggest that when DFM was combined with Saponin or Polyphenols, different ruminal fermentation effects (VFA, DMD and MicProt) were obtained in comparison to Saponin or Polyphenols effects alone.

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