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Impacts of rumen fluid modified by feeding *Yucca schidigera* to lactating dairy cows on *in vitro* gas production of 11 common dairy feedstuffs, as well as animal performance

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Abstract

The objective was to determine effects of feeding increasing levels of a *Yucca schidigera* extract (YSE) to dairy cows on 24 h *in vitro* gas production and 27 h *in vitro* neutral detergent fibre (aNDFom) digestion of 11 common dairy feedstuffs, as well as *in vivo* rumen fermentation and performance of the cows to which the YSE was fed. The principle was to use YSE to potentially modify the rumen microbial population *in vivo* and measure subsequent impacts of the adapted rumen fluid on feedstuff fermentation *in vitro*. Four rumen cannulated late lactation Holstein cows (810 ± 54.7 kg body weight) were used in a 4×4 Latin Square design experiment with 14 d periods. Cows were housed in pens with individual feeding gates and had *ad libitum* access to water while fed a total mixed ration (TMR) of alfalfa hay, corn grain, barley grain, dried distillers grains, whole cottonseed, beet pulp, soybean meal, almond hulls, rumen inert fat and a mineral/salt mixture. Based upon sarsaponin assay of four commercial YSE products, Monterey Sarsaponin 15® was selected and added to the TMR to provide 0, 5, 10 or 15 g of sarsaponin/cow/d. Rumen fluid from each cow in each period was utilized for *in vitro* gas determinations to measure gas production and aNDFom digestion from the

Abbreviations: ADFom, acid detergent fibre; BW, body weight; aNDFom, neutral detergent fibre; dNDF30, NDFom digestible at 30 h of *in vitro* fermentation; CP, crude protein; NE, net energy; TMR, total mixed ration; VFA, volatile fatty acid; YSE, *Yucca schidigera* extract.

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test feeds. There was a strong linear effect (P=0.002), at an increasing rate (quadratic P=0.08), to increased extent of gas production with increased feeding of YSE. There was a quadratic effect to maximum rate of gas production (P=0.01) at the 5 g sarsaponin level. At 4 h of fermentation, gas production increased linearly (P<0.05), at an increasing rate (P<0.002), for almond hulls, barley grain and soybean meal with increasing levels of YSE. Gas production from barley grain had a quadratic effect (P<0.01), suggesting a maximum at about the 5 g sarsaponin feeding level. Gas production at 24 h of fermentation increased linearly (P=0.03), at a decreasing rate (P<0.03), but only soybean meal had a quadratic tendency (P=0.08) to minimum gas production at about the 5 g level of sarsaponin. *In vitro* fermentation of aNDFom at 27 h was not impacted by treatment. *In vivo* rumen pH, concentrations of total volatile fatty acids and rumen protozoal counts were not impacted by YSE feeding level, as were milk production, milk components and net energy (NE) balance. However, correlations between NE output and the proportional increases in 4 h gas production with increasing levels of YSE in the diet suggest that this measure may be predictive of animal responses to this YSE. Finally, multivariate analysis, used to create equations to predict impacts of the nutrients in the 11 feedstuffs on their proportional increase in 4 h gas production, suggests that the increase in 4 h gas production of any feed may be predicted from its organic nutrient profile, offering the potential to determine the optimal feeding level of sarsaponin in any TMR based on its nutrient profile.

Keywords: Fermentation; Sarsaponin; Volatile fatty acids; Ammonia

1. Introduction

Yucca schidigera is a desert plant native to the arid deserts of the Mexican states of Baja California, Guerrero and Huajaca. It averages 4.5 m in height with 1 m leaves and is also known as "Spanish Dagger" or "Mohave Yucca" (Cheeke, 2000). Most commercial production of *Y. schidigera* is in the arid Mojave and Sonoran deserts of the southwestern United States and northwestern Mexico. In most processes, the trunk and root of the plant are harvested, mechanically macerated, ground and dried to produce a yucca powder, or squeezed in a press to produce a yucca juice, which is then concentrated by evaporation to create a yucca extract (Cheeke, 2000; Øleszek et al., 2001). The major active components of the *Y. schidigera* plant used as animal feed additives are the steroidal saponins (Cheeke, 1998). According to Wang et al. (2000b), steroidal saponins form complexes with cell walls of cellulolytic and amylolytic bacteria, which disrupt membrane function and cell growth of some bacterial genera, thereby reducing their numbers in the rumen. According to Wina et al. (2005), raw yucca extract contained 44 g/kg of DM of steroidal saponins, which are the secondary plant glycosides with attached sugars (Wang et al., 2000a). However, since yucca extracts are produced to company specification, individual products contain different concentrations of sarsaponin and, therefore, may have varying anti-microbial impacts in the rumen.

Increasing rumen digestibility of carbohydrates is important to the dairy industry because it affects the amount of feed energy that is released to meet animal metabolic needs. Supplementation of anti-microbial ionophores was effective in increasing efficiency of utilization of structural carbohydrates. However the dairy industry is increasingly looking for 'natural' products that mimic the beneficial actions of ionophores, particularly in the European Union due to the recent ban of feeding ionophores to ruminants.

In the 1980s, there was interest in use of *Y. schidigera* as a means of defaunating the rumen, which has now become a renewed subject of research because of the positive effects of defaunation on rumen metabolism. In addition, *Y. schidigera* increased ruminal volatile fatty acid (VFA) production, while decreasing methane production (Lila et al., 2003), suggesting that it may be effective in changing microbial populations and shifting the rumen carbon balance to increase carbohydrate digestion. Indeed, Goetsch and Owens (1985) reported increased ruminal digestion of medium and low concentrate diets that were supplemented with sarsaponin at 0.13 g/100 kg BW/d, the principle saponin in *Y. schidigera* extracts (YSEs) (Kaneda et al., 1987). A current focus of *Y. schidigera* research is to better understand the ruminal carbon shift among fermentation end-products caused by feeding it, and identify diets that maximize this effect.

Our objectives were to determine the sarsaponin concentration in four commercial sources of *Y. schidigera*, and utilize one at increasing dietary levels in an *in vivo* study with lactating dairy cows to create rumen fluid adapted to those increasing levels of the YSE. These adapted rumen fluids were then used in an *in vitro* gas production study to determine how impacts of increasing feeding levels of YSE on rumen fluid impacted gas production and aNDFom digestion among feeds. The hypothesis was that supplementation of YSE could potentially alter the rumen microbial population, perhaps to impact digestion of nutrients differently, thereby increasing or decreasing gas production to differing extents *in vitro*, which could impact digestion of feeds differently based upon their nutrient profiles. This could allow identification and selection of diets that would optimize positive effects of YSE supplementation. Efficacy of the selected YSE product was simultaneously evaluated in an *in vivo* study with lactating dairy cows to determine its effects on rumen ammonia N levels, rumen VFA concentrations and molar proportions, rumen pH, rumen protozoal counts as well as milk production and composition, and BW change, when fed at increasing levels to the cows.

2. Materials and methods

2.1. Yucca schidigera products

The *Y. schidigera* sarsaponin products Micro-Aid® (Distributors Processing Inc., Porterville, CA) De-Odorase® (Alltech Inc., Nicholasville, KY) Monterey Sarsaponin 15® (Monterey Ag Resources, Fresno, CA) and DK Sarsaponin 30® (Desert King International, San Diego, CA) were assayed for sarsaponin content as outlined in Section 2.5.1.

2.2. Cows

Four ruminally cannulated (Bar Diamond, Parma, ID, USA; plastisol) late lactation (298 ± 32.4 DIM) Holstein cows (810 ± 54.7 kg BW) were used in a 4×4 Latin Square design experiment with 14-d periods and housed in a sand bedded free stall pen equipped with Calan gates (American Calan Inc., Northwood, NH, USA). There was a 7-d adaptation period to the basal ration prior to the start of period 1. The study took place in June/August of 2006 when daytime high temperatures averaged 35–40 °C. Cows were fed a total mixed

ration (TMR) twice daily in equal amounts at 06:30 and 18:30 h just after milking at 06:00 and 18:00 h.

2.3. Rations fed

The TMR was the same throughout the study and consisted of 361 g/kg alfalfa hay, 166 g/kg steam-flaked corn grain, 111 g/kg steam-rolled barley grain, 34 g/kg dried distillers grains, 95.6 g/kg whole cottonseed, 55 g/kg beet pulp, 20.7 g/kg soybean meal, 120 g/kg almond hulls, 8 g/kg of a rumen inert fat, 3.5 g/kg salt and 17.3 g/kg mineral mixture. The YSE was added to the basal TMR to provide 0, 50, 100 or 150 g of Monterey Sarsaponin 15®/cow/d, or approximately 0, 5, 10, or 15 g of sarsaponin/cow/d.

2.4. Measurements

2.4.1. Milk production and composition

Cows were milked twice daily at 06:00 and 18:00 h in a single six herringbone style milking parlor. Milk weights were measured daily by Westfalia milk meters and samplers (Westfalia, Naperville, IL, USA). Milk samples were preserved with a bronopol/natamycin preservative and transported to the UC Davis Nutrition Lab to be analyzed by infrared spectroscopy (Foss, Eden Prairie, MN, USA) for fat, crude protein (CP) and lactose.

2.4.2. Rumen fluid

Rumen fluid was collected by inserting an 80-cm fixed tube with numerous 5-mm holes at 2-h post-feeding on the 9th and 14th d of each experimental period and the pH was immediately determined using a Cole Parmer pH meter (Cole Parmer Instrument Company, Chicago, IL, USA). Rumen fluid was immediately transported to the laboratory in a pre-warmed thermos bottle, strained through two sheets of cheesecloth, and centrifuged at $500 \times g$ for 10 min at 26 °C, and supernatant was stored at −11 °C for later determination of VFA and ammonia N. A separate sample (8 mL) of strained rumen fluid was collected and added to 2 mL of 250 g/kg NaCl formaldehyde (Fenn and Leng, 1990) and stored at 4 °C for later determination of protozoal counts by a Bright-line Neubaur hemacytometer (Hausser Scientific, Horsham, PA, USA).

On the 12th and 13th d of each period, rumen fluid was separately collected, as described above, at 2 h post-feeding from two of the four cows such that each of the four cows was collected once in the 2 d. Each sample was filtered through two sheets of cheesecloth and utilized, by cow, for 27 h in an *in vitro* gas production procedure (as described by Menke and Steingass, 1988) using 11 feedstuffs that included samples of a low and high aNDFom alfalfa hay, corn silage, beet pulp, corn grain, dried corn distillers grains, soybean meal, almond hulls, barley grain, whole crop wheat silage and whole linted cottonseed. Each feed utilized in the *in vitro* gas production technique was ground to pass a 1-mm screen on a model 4 Wiley Mill. An aNDFom analysis was also completed on each feedstuff after the *in vitro* gas production procedure to determine total digestible aNDFom of each feedstuff and the impact of rumen fluid from cows fed increasing levels of the YSE on it.

2.5. Analytical procedures and calculations

2.5.1. Basal TMR, Orts, YSE analysis

Feeds were ground to pass a 1-mm screen on a model 4 Wiley Mill, and DM was determined by gravimetric loss of free water from heating to 105 °C for 2 h (Reuter et al., 1986). Acid detergent fibre (ADFom) and lignin(sa) were determined using Ankom Technology (AOAC, 1997, Method #973.18). Neutral detergent fibre analyses included a heat-stable amylase (Van Soest et al., 1991) and are expressed both inclusive (aNDF) and exclusive (aNDFom) of residual ash. Ash was determined by gravimetric determination by incineration at 550 °C for 3 h. Total N was determined with a N gas analyzer utilizing an induction furnace and thermal conductivity (LECO FP-528, AOAC, 1997, Method #990.03). P, Na, Cl, Zn, Mn, Fe, Co, S, Ca, and Mg levels were determined by microwave acid digestion and dissolution of the sample and quantitative determination by AAS or ICP-AES (Sah and Miller, 1992; Meyer and Keliher, 1992). Total K was determined using a 2 g/kg acetic acid extraction, then quantitatively by AES (Johnson and Ulrich, 1959). Total N insoluble in AD was determined in AD residue by N Gas Analyzer using an induction furnace and thermal conductivity (LECO FP-528, AOAC, 1997, Method #990.03). Total Se content was determined by nitric/perchloric acid digestion and dissolution and vapor generation by ICP-AES (Tracy and Moeller, 1990). Glucose, fructose, and sucrose were determined by HPLC (Johansen et al., 1996), and starch was determined by enzymatic hydrolysis, then HPLC of the resultant glucose (Smith, 1969). Sarsaponin content of all four YSE products (Table 1) was determined by multiple methanol extractions, evaporated and partitioned between *n*-butanol and water (Salem et al., 2006).

2.5.2. Calculations

The potential extent and rate of gas production were determined using the one-pool exponential model of Blümmel et al. (1997) as

$$\text{Gas} = E(1 - \mathrm{e}^{-kt})$$

where E is the potential cumulative gas production, k the rate of gas production and t is the time.

The gross energy concentration in milk was calculated based on measured production characteristics according to Tyrell and Reid (1965) as

$$\text{milk energy (MJ/kg)} = 4.184 \times \left(\frac{((41.63\text{Fat}) + (24.13\text{Protein}) + (21.60\text{Lactose})) - 11.72}{1000} \right) \times 2.204$$

where fat = milk fat g/kg milk, protein = milk protein g/kg milk, lactose = milk lactose g/kg milk. The milk energy output was then calculated as

$$\text{milk energy output (MJ/d)} = \text{NE milk (MJ/kg)} \times \text{milk yield (kg/d)}$$

The energy in body weight (BW) change was determined as

$$\text{BW energy (MJ/d)} = 4.184 \times (\text{BW change (kg/d)} \times 4.92 \text{ or } 5.12)$$

where BW change = daily BW change of the cow, and 4.92 or 5.12 were assigned to loss (4.92) *versus* gain (5.12) by cow within period (NRC, 1989).

The energy of maintenance was calculated as

$$\text{maintenance energy (MJ/d)} = 4.184 \times (\text{BW(kg/d)}^{0.75} \times 0.08)$$

where BW is in kg/d (NRC, 1989).

The NE of lactation was calculated as

$$\text{NE output (MJ/d)} = \text{milk energy (MJ/d)} + \text{BW change energy (MJ/d)} + \text{maintenance energy (MJ/d)}$$

Finally, the NE density of the TMR was calculated as

$$\text{NE (MJ/kg DM)} = \frac{\text{NE output (MJ/d)}}{\text{DM intake (kg/d)}}$$

2.6. Statistical analysis

Differences in *in vitro* measurements (*i.e.*, Table 3) were determined using the PROC GLM procedure of SAS (2002) with cow, period, YSE treatment level, test feed and the treatment (T) by feed (F) interaction as dependent variables in a factorial design within a 4×4 Latin Square experiment. In the absence of an F×T interaction, YSE effects were determined as linear and quadratic contrasts within SAS as defined by Steel and Torrie (1980). In the case of an F×T interaction, differences in *in vitro* measurements, within feed, were determined using the PROC GLM procedure of SAS (2002) with cow, period and YSE treatment level as dependent variables in a factorial design within a 4×4 Latin Square experiment. YSE effects were determined as linear and quadratic contrasts within SAS as defined by Steel and Torrie (1980). Significance was accepted if $P<0.05$.

Differences in *in vitro* measurements (*i.e.*, Tables 4 and 5) were determined using the PROC GLM procedure of SAS (2002) with cow, period and YSE treatment level as dependent variables in a factorial design within a 4×4 Latin Square experiment. The YSE effects were determined as linear and quadratic contrasts within SAS as defined by Steel and Torrie (1980). Significance was accepted if $P<0.05$.

Multiple regression analysis was used to predict increases in gas production (*i.e.*, Table 7) based on feedstuff chemical composition (*i.e.*, ash, fat, CP, aNDFom, *in vitro* aNDF digestible at 30 h (*i.e.*, dNDF30), iNDF, starch) and dose level of the YSE using the PROC STEPWISE (BACKWARD) procedure of SAS.

3. Results

3.1. Y. schidigera products

As this study was designed to evaluate the bioactivity of sarsaponin, it was important to select a product that was a pure yucca extract and not one that was blended with other

Table 1
Sarsaponin composition (DM basis, g/kg) of four *Yucca schidigera* extracts

Monterey Sarsaponin 15®	95.4
Alltech De-Odorase®	170.6
Desert King, DK Sarsaponin 30®	189.1
DPI Micro-Aid®	181.6
SEM	0.097

potentially bioactive constituents. Of the four major commercially available *Y. schidigera* products, two claim their products to be "100% Natural Pure *Y. schidigera* powder," being DK Sarsaponin 30® and Monterey Sarsaponin 15®. As the Monterey Sarsaponin 15® product was much less concentrated than the other three products (Table 1), and could be added to TMR of the cows at higher levels thereby increasing allocation and mixing accuracy, it was used for the *in vivo* study. A more detailed analysis for secondary compounds was completed on the Monterey Sarsaponin 15® product supplement (Table 2).

The TMR (Table 2) had a chemical composition that met or exceeded recommended nutrient requirements NRC (1989) of late lactation dairy cows.

3.2. In vitro study

There was no effect of treatment on digestibility of aNDFom at 27 h of *in vitro* fermentation. However, there was a strong linear effect (P=0.002) for increased extent of gas production (Table 3), at an increasing rate (P=0.08). There was a quadratic effect (P=0.01) on rate of gas production, with the 50 g feeding level of YSE being near the maximum.

At 4 h of fermentation (Table 3), gas production increased linearly (P<0.001) at a decreasing rate (P=0.002). However this differed among feeds (F×T: P<0.001) with gas production increasing linearly for almond hulls (P=0.04), corn silage (P=0.06), soybean meal (P=0.02) and wheat silage (P=0.09), while increasing linearly (P=0.04), at a decreasing rate (quadratic P=0.01) for barley grain where maximum gas production occurred at about the 50 g YSE feeding level, similar to beet pulp (P=0.08).

At 24 h of incubation, no feed showed convincing impacts of YSE addition, in spite of a feed by treatment interaction (P<0.001), although overall gas production increased linearly (P=0.03).

3.3. In vivo study

Rumen pH was not influenced by YSE supplementation (Table 4), but molar proportions of propionate had a linear tendency (P=0.08) and quadratic effect (P=0.04) to maximum production at intermediate YSE levels. The acetate:propionate ratio also had a tendency to minimum values at intermediate YSE feeding levels (quadratic P=0.06). Rumen fluid ammonia N concentrations had a tendency (P=0.06) to decrease linearly, but protozoal counts were not influenced by YSE supplementation.

Neither DM intake nor milk production, or its components (Table 5), were influenced by YSE supplementation. Only BW change and maintenance energy had a linear tendency (P=0.07) to increase with increasing levels of YSE supplementation.

Table 2
Chemical composition (g/kg 105 °C DM basis) except trace minerals as noted of the basal TMR and Monterey Sarsaponin 15®

	Total mixed ration	S.E.	Monterey Sarsaponin 15®[a]	S.E.
Dry matter (g/kg)	921	2.4	958	1.5
Organic matter	930	2.4	888	1.2
aNDF	289	5.7	272	0.8
aNDFom	280	5.8	290	0.9
ADFom	205	4.2	NA[b]	
Lignin(sa)	53	0.9	NA	
Starch	139	9.1	5	<0.1
Glucose (free)	16.4	1.38	5	<0.1
CP	183.1	0.57	NA	
AD-insoluble CP	13.1	0.07	NA	
Ca	8.5	0.27	22.6	0.09
P	4.2	0.10	0.8	<0.10
K	14.7	1.00	7.5	<0.10
Mg	5.0	0.14	2.0	<0.10
S	2.8	0.06	0.7	<0.10
Na	4.3	0.12	0.5	<0.10
Cl	5.2	0.36	NA	
(mg/kg)				
Zn	82	2.7	25	0.5
Mn	66	1.9	22	0.3
Fe	311	12.8	1150	9.1
Cu	16.0	1.43	16.7	0.17
Se	0.56	0.041	0.05	<0.01
Secondary compound fractions (g/kg)				
Total phenolics			20.5	
Saponins			95.4	
Alkaloids			<0.0	
Aqueous fraction			20.4	

[a] The Monterey Sarsaponin 15® was added at increasing levels to the basal diet as described in the text.
[b] NA, not assayed.

4. Discussion

Increasing forestomach digestibility of carbohydrates in dairy rations as a way to increase NE level has been a long-term goal of dairy cattle farmers. However, due to concerns of consumers about feed supplements and treatments, particularly with respect to residues of antibiotics that dairy cattle have been fed impacting human antibiotic resistance (*e.g.*, the recent European Union feed ban of antibiotic growth promoters), dairy researchers have increased research efforts on ionophore mimicking compounds that have 'natural' origins, such as secondary compounds in plants. Interest in the use of sarsaponin, due to its potentially beneficial effects on rumen metabolism, has made supplementation of sarsaponin containing plants such as *Y. schidigera*, of renewed interest.

This study focused on establishing *in vitro* responsiveness of gas production of several common dairy feedstuffs to rumen fluid from cows fed a *Y. schidigera* extract at increasing

Table 3
Fibre digestion and gas production as impacted by YSE feeding level to the cows that produced rumen fluid inoculum

	Treatment (g YSE/cow/d)				S.E.M.	P		
	0	50	100	150		L	Q	F×T[a]
Fibre digestion								
$dNDF_{27}$ (mg NDF digested at 27 h/g NDF)	353	358	353	391	1.4	0.17	0.34	0.99
Gas parameters								
Extent (mL gas/g of substrate)	222.5	221.0	226.5	237.0	0.7	0.002	0.08	0.99
Rate (mL gas/(h g of substrate))	0.595	0.645	0.615	0.605	0.0022	0.96	0.01	0.78
Gas production (mL/g of substrate)								
At 4 h of incubation	78.5	87.5	89.0	89.0	0.27	<0.0001	0.002	<0.0001
Alfalfa (low NDF)	95.9	103.4	106.2	109.2	1.13	0.14	0.70	
Alfalfa (high NDF)	72.4	82.8	80.2	81.3	0.75	0.20	0.26	
Almond hulls	87.2	89.5	96.9	95.4	0.56	0.04	0.51	
Barley grain	107.4	122.8	120.3	117.0	0.45	0.04	0.01	
Beet pulp	105.5	124.0	120.8	113.5	1.2	0.47	0.08	
Corn grain	80.3	99.7	97.4	91.7	2.08	0.52	0.27	
Corn silage	68.5	77.4	84.9	80.4	0.83	0.06	0.16	
Dried distillers grains	37.0	41.2	42.2	43.3	0.57	0.17	0.62	
Soybean meal	71.8	73.9	80.8	89.5	0.82	0.02	0.45	
Wheat silage	77.9	81.8	84.9	88.3	0.76	0.09	0.95	
Whole cottonseed	60.0	63.8	67.2	68.9	0.74	0.12	0.80	
At 24 h of incubation	209.0	210.5	211.0	217.0	1.10	0.03	0.32	<0.0001
Alfalfa (low NDF)	185.7	194.7	195.3	185.4	1.51	0.99	0.26	
Alfalfa (high NDF)	174.0	181.2	176.5	182.9	1.20	0.44	0.95	
Almond hulls	196.5	202.5	204.8	201.5	0.99	0.47	0.39	
Barley grain	272.9	272.9	267.5	278.8	1.50	0.73	0.48	
Beet pulp	322.4	320.3	310.5	342.0	2.43	0.40	0.22	
Corn grain	288.7	285.7	294.8	304.9	1.37	0.11	0.38	
Corn silage	197.5	200.9	198.7	211.0	1.11	0.17	0.45	
Dried distillers grains	93.4	100.7	95.3	102.3	0.61	0.17	0.97	
Soybean meal	204.4	183.4	184.0	201.8	1.87	0.87	0.08	
Wheat silage	191.2	194.8	193.4	195.7	0.91	0.57	0.88	
Whole cottonseed	174.7	177.5	198.8	182.7	2.62	0.47	0.50	

[a] Feed (F)×YSE treatment (T) interaction.

Table 4
Rumen parameters as influenced by YSE feeding level

	Treatment (g YSE/cow/d)				S.E.M.	P	
	0	50	100	150		L	Q
Rumen pH	6.11	6.08	6.10	6.16	0.033	0.44	0.41
Ammonia N (mg/L)	126.1	113.1	97.6	88.2	0.83	0.06	0.90
Total VFA (mM/L)	119.3	114.2	99.7	106.4	6.83	0.28	0.58
VFA (mM/L)							
Acetate	63.5	62.7	62.0	63.1	0.45	0.56	0.22
Butyrate	13.4	13.4	13.7	13.1	0.28	0.83	0.49
Propionate	20.3	21.0	21.3	20.9	0.14	0.08	0.04
Valerate	16.8	17.1	17.9	17.9	0.63	0.40	0.80
Isovalerate	10.6	10.9	10.0	10.1	0.55	0.56	0.89
A:P ratio	3.12	2.98	2.91	3.02	0.037	0.17	0.06
Protozoa (million cells/cm^3)	0.54	0.53	0.45	0.47	0.030	0.19	0.72

Table 5
Animal production parameters as influenced by YSE feeding level

	Treatment (g YSE/cow/d)				S.E.M.	P	
	0	50	100	150		L	Q
Intake							
DM (kg/d)	24.34	24.88	24.49	24.09	0.620	0.70	0.48
aNDFOM (kg/d)	6.82	6.97	6.86	6.75	0.116	0.69	0.48
aNDFOM (g/kg BW)	36.0	35.3	36.3	36.5	0.750	0.62	0.72
Crude protein (kg/d)	4.46	4.56	4.48	4.41	0.076	0.69	0.48
N efficiency (g milk N/N intake)	0.238	0.230	0.217	0.235	0.0129	0.70	0.34
NE (MJ/kg)[a]	6.02	6.49	7.53	7.15	0.161	0.37	0.68
Yield (kg/d)							
Milk	33.48	33.54	33.85	33.35	1.048	0.99	0.80
Fat	1.18	1.24	1.28	1.17	0.096	0.98	0.42
Crude protein	1.09	1.08	1.07	1.06	0.037	0.62	0.96
Lactose	1.50	1.60	1.60	1.55	0.084	0.73	0.46
Components (g/kg)							
Fat	34.9	36.7	37.1	35.1	0.15	0.86	0.26
Crude protein	32.3	31.9	31.4	31.6	0.03	0.13	0.46
Lactose	44.5	46.5	46.3	45.4	0.12	0.67	0.28
Milk energy (MJ/kg)	2.89	3.01	3.01	2.89	0.013	0.93	0.25
Body weight							
Mean (kg)	814	821	818	831	4.9	0.07	0.56
Change (kg/d)	−0.07	0.61	1.27	0.91	1.169	0.51	0.67
Net energy balance (MJ/d)							
Milk	97.7	101.3	103.0	97.5	4.01	0.97	0.48
BW change	−0.6	13.5	27.9	20.4	16.40	0.51	0.68
Maintenance	51.0	51.3	51.2	51.8	0.02	0.07	0.59
Total NE output	148.2	182.1	182.1	169.7	17.47	0.52	0.58

[a] Calculated from measured animal NE outputs and measured animal DM intake.

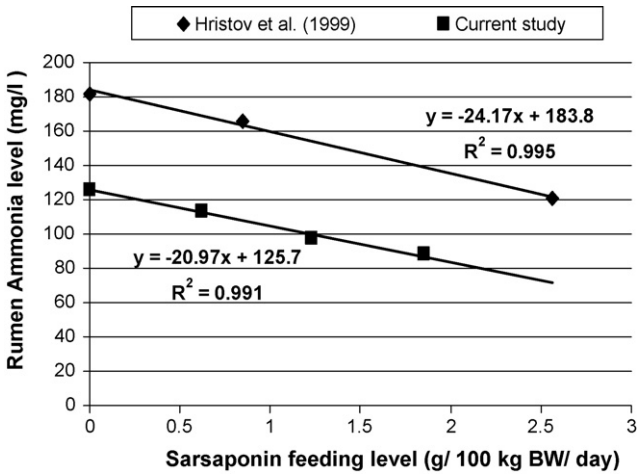

Fig. 1. Comparison of rumen ammonia level *vs.* dose of sarsaponin.

levels, and whether this responsiveness could be predicted from their chemical composition in order to predict the response to sarsaponin addition in dairy rations based upon the chemical composition of feedstuffs.

4.1. Impacts of YSE on rumen fermentation

Changes in ruminal ammonia N concentrations varied amongst studies that fed YSE, although the majority report a reduction. For example, YSE supplementation decreased ruminal ammonia N concentration in the *in vivo* studies of Hristov et al. (1999), Gibson et al. (1985), Goetsch and Owens (1985), Hussain and Cheeke (1995) and Pen et al. (2006). In the current study, rumen ammonia N levels tended (P=0.06) to decrease with increased YSE level in a similar way to Hristov et al. (1999), one of only two studies known to have fed sarsaponin from YSE at the high levels utilized in the current study (Fig. 1).

According to Wallace et al. (1994), the glyco-component of the sarsaponin molecule in YSE binds with ammonia. However this binding may be limited, and Wallace et al. (1994) suggested that most of the depression in rumen ammonia was actually due to decreased proteolysis in the rumen caused by anti-protozoal activity of sarsaponin in the rumen, rather than the affinity of glyco-components for ammonia. In the current study, very high levels of YSE were fed and, since it was suggested by Wang et al. (1997) that protease activity may be higher at high saponin feeding levels (*i.e.*, >5 mg/mL), while deaminase activity is stable preventing complete degradation of dietary protein to ammonia. Although deaminase activity was not measured in our study, a similar study (Hristov et al., 1999) utilizing doses of YSE in our range showed no change in deaminase activity at 4 h post-feeding, which suggests a similar response to our study, particularly since Hristov et al. (1999) measured rumen ammonia at similar times of 2–3 h post-feeding.

As rumen ammonia N levels were measured 2–3 h post-feeding in the current study, and because rumen ammonia N concentrations tend to be maximum at about 2–4 h post-feeding,

it is likely that the levels measured were relatively close to the daily maximum. According to Satter and Slyter (1974), rumen bacterial growth is negatively affected by N supplies once levels reach about 50 mg/L. Considering the relatively low levels of ammonia N in the rumen at the two highest feeding levels of YSE (*i.e.*, 88 and 98 mg/L), bacterial growth may have been negatively affected by low ruminal ammonia levels at other times of the day.

Bacterial species may react differently to low levels of ammonia in the rumen. According to Hungate (1966), immediately after ingestion of forage, when fibre-digesting bacteria have not yet had the opportunity to bind with newly ingested fibre particles, the feed amino acids that are soluble in rumen fluid that are not bound to carbohydrate have already been degraded. Hungate (1966) further suggested that fibre-digesting rumen bacteria utilize rumen ammonia as an N source thus, if the ammonia levels were to be reduced in the rumen, it would reduce ruminal fibrolytic bacterial population size and/or activity. According to Bryant (1973), amylolytic bacteria are not as dependent on ammonia as an N source, and may continue to grow despite a decrease in rumen ammonia levels. Thus changes in rumen bacterial populations may have occurred due to amylolytic bacteria filling niches created where fibre-digesting bacteria decreased in numbers due to reduced rumen ammonia N levels caused by higher YSE feeding.

4.2. Impacts of YSE modified rumen fluid on gas production in vitro

If amylolytic bacterial numbers and/or activity increased due to a decrease in fibre-digesting bacteria, then gas produced among feedstuffs would likely change differently relative to their chemical composition. Indeed gas production from fermentation of starch containing feedstuffs seemed to increase at 4 h (Table 3) as YSE dose level increased, which may be consistent with the hypothesis that amylolytic bacteria in the rumen fluid increased in numbers.

Inspection of differences in gas production at 4 h *versus* 24 h of fermentation (Table 3) suggests that the linear effect ($P<0.0001$) at 4 h is mostly due to gas production increases between the 0 and the 50 g feeding level of YSE, whereas the linear effect ($P=0.03$) at 24 h appears to be largely driven by higher levels of YSE feeding and may have been due to rumen microbial adaptation to these YSE feeding levels (Newbold et al., 1997). According to Segal et al. (1974) glycosylation of a saponin must occur in order for saponins to become biologically active. In a study by Wang et al. (1999), where an *in vitro* mixed rumen microbial population was exposed to steroidal saponins, although overall saponin content was not affected, the soluble steroidal saponin content decreased suggesting that deglycosylation had occurred and rendered the steroidal saponin inactive. Results from Wang et al. (1999) also confirm the possibility that microbial adaptation can occur and may therefore necessitate higher levels of YSE feeding in order to induce an effect on gas production at longer incubation times. Deglycosylation of saponins from YSE at 4 h of *in vitro* incubation was reported (Wang et al., 2005) and is probably a major reason for differences in gas production at 4 h of *in vitro* incubation. In *in vitro* studies, including the current one, gas syringes are supplied with organic ammonia to prevent lack of N from impacting gas production due to reduced bacterial growth. Effects of ammonia on deglycosylation of saponins are not known, although it is possible that ammonia may increase the rate of saponin deglycosylation.

Table 6
Nutrient composition (g/kg DM) of the feedstuffs evaluated

Feed	Ash	Fat	CP	dNDF30	iNDF[a]	Starch
Alfalfa hay (low NDF)	110	25	250	160	160	40
Alfalfa hay (high NDF)	110	25	200	175	205	40
Almond hulls	60	30	60	110	440	20
Barley grain (ground)	30	20	130	83	68	580
Beet pulp (dehy)	60	5	110	360	90	10
Corn grain (ground)	20	45	101	50	50	720
Corn silage	50	20	89	303	248	320
Cottonseed (with lint)	40	210	230	180	220	20
Distillers dried grains	50	100	285	384	96	30
Soybean meal (480 g/kg CP)	50	15	540	18	42	120
Wheat silage	80	20	115	270	330	280

[a] iNDF was calculated as: aNDFom − dNDF30.

4.3. Impacts of YSE supplementation on animal performance and implications

Despite changes in the ruminal bacterial populations suggested from increases in *in vitro* gas production in starch-rich feedstuffs, and reductions in rumen ammonia concentrations, no differences in production characteristics of the cows occurred.

In the current study, 4 and 24 h time values for *in vitro* fermentation were chosen to be key points of evaluation. This was based, in the case of 4 h, on Hungate (1966) who suggested that it was an important point for evaluation of impacts of treatments that may modify rumen fermentation because this time provided a more reliable estimation of fermentation parameters based upon the bacterial population of the rumen inoculum than longer times when the bacterial population may have changed (Hungate, 1966). Indeed, Hungate (1966) suggested that short term *in vitro* methods provide very reliable estimates of rates of gas production, as well as substrate wetting on lag time before fermentation begins, and offers advantages of repeatability and practicality for evaluating microbial activity in the rumen before accumulation of fermentation products occurs. The 24 h fermentation time period is also important, as it is used as the time to estimate feed ME values with gas production values (Menke and Steingass, 1988).

To determine if effects of increasing feeding levels of YSE on proportional increases in 4 and 24 h gas production among feeds could be predicted from their nutrient profiles and the YSE dose level, multivariate analysis was used to create equations to predict impacts of nutrients in the 11 feeds (Table 6) on predicted *in vitro* gas production increases. These equations (Table 7) describe the degree to which each nutrient and YSE dose level is responsible for proportional increases in gas production at each of the 4 and 24 h time periods, and provide a way to predict proportional response of any feed based on the nutrients that comprise it, and the YSE dose level.

The equations in Table 7 were then used to estimate predicted 4 and 24 h proportional gas increases for the ration fed to our cows and these were in turn related to the key energetic output parameter of the cows (*i.e.*, NE output; Fig. 2). This figure suggests a correlation between the proportional 4 h gas value increases and the total NE output, and no correlation between NE output and proportional increases in 24 h gas production. This may not support

Table 7
Compositional characteristics utilized to predict sarsaponin impacts on 4 and 24 h gas production *in vitro* based on YSE dose level and composition of the diet

	4 h		24 h	
	Value	P	Value	P
Intercept	0.9933	<0.001	0.9876	<0.001
Dose level	0.0252	<0.001	–	–
(Dose level)^2	−0.0011	<0.001	0.0000016	0.006
Ash	–	–	0.0036	0.060
Fat	–	–	0.0038	<0.001
CP	–	–	−0.0013	0.002
iNDF	−0.000875	0.151	–	–
dNDF30	0.000724	0.254	–	–
Starch	0.000591	0.090	–	–
S.E.M.	0.019596		0.01628	
r^2	0.685		0.458	

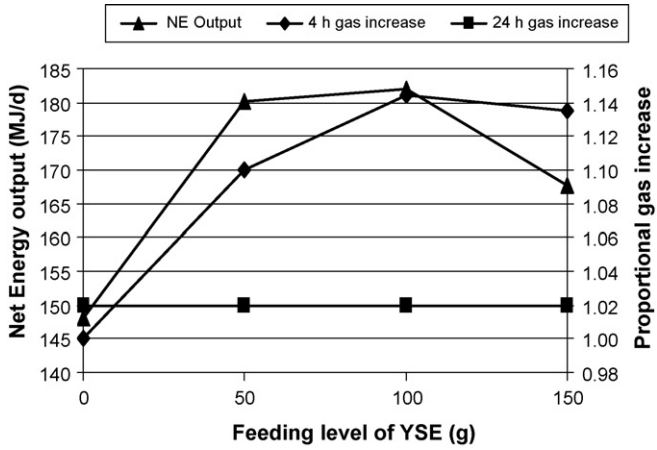

Fig. 2. Relationship between net energy output of the cows and 4 and 24 h predicted proportional gas increase *vs.* YSE feeding level.

Menke and Steingass (1988), who showed that 24 h *in vitro* gas production values were correlated to ME output, but it appears to support Hungate (1966) who stated that short-term *in vitro* incubations are better to evaluate activity of the actual microbial population in the rumen. That the proportional 4 h gas production increase was correlated to the NE output of the cows in the current study suggests that it may be possible to utilize it to predict the response of mixed rations to YSE supplementation on animal NE output.

5. Conclusions

YSE supplementation to rations of lactating dairy cattle has been a renewed subject of research by ruminant nutritionists in the search for ionophore mimicking 'natural' products

that can be safely fed to ruminants. Our results show that YSE is an effective way of reducing rumen ammonia levels, and YSE-modified rumen fluid affected gas production of almond hulls, barley grain, beet pulp, corn silage, soybean meal and wheat silage, sharply increasing 4 h gas production of starch-containing feedstuffs. This decrease in rumen ammonia N levels may have caused a shift in rumen bacterial populations with increased amylolytic bacterial numbers at the expense of fibrolytics. Despite YSE feeding having had no effect on milk production, milk components or energy balance, a correlation between NE output and proportional increases in 4 h gas production with increasing levels of YSE in the diet, suggests that this measure may be important in predicting responses to YSE supplementation. Multivariate analysis suggested that the increase in 4 h gas production of any feed, and perhaps rations, can be predicted from its nutrient profile, thereby offering the potential to optimize the YSE feeding level based on nutrients in the ration.

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