Research Article

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Effects of *Euphorbia esula* L. (leafy spurge) on cattle and sheep *in vitro* fermentation and gas production†

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Abstract

BACKGROUND: *Euphorbia esula* L. (leafy spurge) is indigenous to Eurasia and has been known to cause grazing aversion in ruminant species. As a result, *E. esula* encroachment has negatively impacted rangelands in the Northern Great Plains and Intermountain West of the USA, as well as southern Canada. Our objectives were to evaluate the effect of increasing concentrations of *E. esula* on *in vitro* dry matter digestibility (DMD) and gas production. Two ruminally-cannulated cows and ewes were used as rumen inoculum donors. To accomplish objectives, two studies were conducted. In study 1, animals were fed exclusively a barley hay (12% crude protein (CP), 55.4% neutral detergent fiber (NDF), DM basis) diet; whereas in study 2, animals were fed a diet of 15% *E. esula* (21.9% CP, 48% NDF, DM basis) and 85% barley hay based on previous day intake.

RESULTS: The 24 and 48 h *in vitro* and 96 h gas production indicate that, regardless of inoculum source or substrate fermented, DMD was not influenced. Differences, however, were consistently observed across studies for NDF disappearance.

CONCLUSION: Regardless of inoculum source NDF disappearance was greater when substrate being fermented contained 0%, 80%, or 100% *E. esula*.

Keywords: cattle; *Euphorbia esula* L.; gas production; *in vitro* fermentation; sheep

INTRODUCTION

Euphorbia spp. are predominant noxious weed species impacting rangelands throughout Europe and North America.[1] *Euphorbia esula* L. (leafy spurge) has infested the Northern Great Plains of North America since the early 1900s.[2] *Euphorbia* spp., in general, have high nutritive value compared to most phonologically mature grasses and are comparable to alfalfa and other regional pre-bud cool season grasses;[1,3] however, domesticated livestock and wildlife tend to avoid *Euphorbia* spp., especially larger herbivores such as cattle. Research has shown that cattle foraging behavior is altered and utilization of non-infested rangeland increased due to under-utilization of adjacent moderate to high infested *E. esula* sites.[4] This change in rangeland utilization by herbivores creates additional opportunities for further encroachment of *E. esula*. Avoidance of *E. esula* by cattle is considered to be related to aversion-eliciting condensed tannins and diterpenoids (i.e. secondary compounds) that create ruminal or post-ruminal digestive disturbance.[5–7] Gradual adaptation to plant secondary compounds has been suggested as a viable approach to improve tolerance.[8,9] Researchers observed equal *in vitro* dry matter (DM) and neutral detergent fiber (NDF) disappearance of a standard substrate when ewes received a diet containing either 50% *E. esula* and 50% grass hay or 100% grass hay.[10] In the Northern Great Plains, ovine production has diminished over the past 50 years as livestock producers have focused their operations more towards beef production. The fundamental basis of this research is to ascertain differences that may exist between ovine and bovine fermentation characteristics which may lead to manipulations of the bovine rumen to better utilize noxious weeds. Therefore, objectives for the present studies were to evaluate fermentation characteristics of *E. esula* using *in vitro* dry matter disappearance (DMD) and gas production. Fermentable substrates for DMD and gas production included incremental concentrations of *E. esula* added to typical production diet ingredients. Rumen liquor used in the *in vitro* incubations was donated by either ruminally cannulated cows or ewes consuming or not consuming *E. esula* in their basal diet. Our hypotheses were twofold: first, that as *E. esula* increased in the fermentation substrate, *in vitro* DMD and gas production would decline; secondly, using ruminal inoculum from donor animals consuming *E. esula* in their diet would result in increasing *in vitro* DMD and gas production with increasing proportions of *E. esula* in the fermentation substrate for both sources of ruminal fluid (cows and ewes).

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MATERIAL AND METHODS

The Fort Keogh Livestock and Range Research Laboratory (LARRL) Institutional Animal Care and Use Committee approved all animal handling and experimental procedures used in this study.

Euphorbia esula L. collection and preparation

Collection of *E. esula* occurred on US Department of Interiors, Bureau of Land Management property near Terry, Montana, USA (latitude 46° 8′ N, longitude 105° 3′ W) at an average elevation of 687 m and annual precipitation of 293 mm, with the majority of precipitation occurring from early spring to mid-summer.[11]

Initial collection for *E. esula* occurred on 24 June 2005 when plants were hand clipped at soil surface in the early shoot, pre-bloom stage (approximately 8 cm in height). Hand clippings were placed in paper bags, sealed, and stored on ice (32 °C) to protect the integrity of the *E. esula* during transport back to the LARRL. Once at the LARRL, leaves were manually stripped from harvested *E. esula* and stored in plastic bags at −20 °C. Leaves were lyophilized and an aliquot was analyzed for nutrient composition by an independent laboratory. Leaves of *E. esula* were employed as substrate for *in vitro* fermentations. Two subsequent collections of *E. esula* occurred on 8 and 13 July 2005, respectively, from a property immediately adjacent to the initial collection site. During these collections, a hand-held, gas-powered, sickle mower (Jari Monarch, 91.44 cm sickle; Year-A-Round Cab Corp. Mankato, MN, USA) was utilized in harvesting of *E. esula*, with no discrimination made for growth stage. Harvested *E. esula* was then hand raked, placed onto a plastic sheet lining the bed of a pickup truck and covered for prompt transportation back to the LARRL. Upon arrival at LARRL, *E. esula* was transferred into a temperature-controlled room (20 °C) and was subsequently separated from other plant species collected during harvest. Separated *E. esula* was then stored at −4 °C until inclusion in experimental diets (fed within 30 days). A random sample representing each *E. esula* collection was reserved and analyzed for nutrient content by an independent laboratory.

Animals and management

Rumen liquor for the *in vitro* experiments were collected from ruminally-cannulated cows (two beef cows weighing approximately 770 kg) and ewes (two ewes weighing approximately 90 kg), neither of which had previous exposure to *E. esula*. Animals were adapted to individual research pens, allowed *ad libitum* access to water, and received a diet of 100% barley hay daily at 0800 for 14 days prior to the start of study 1. Barley hay and *E. esula* fed to cannulated experimental animals was the same substrate material incubated in the *in vitro* evaluations (Table 1).

Treatments

Inoculum source treatments included either cows or sheep. Inoculum source was factorially arranged with six *in vitro* fermentation substrate mixtures of barley hay and *E. esula*. The combinations were 100 : 0, 80 : 20, 60 : 40, 40 : 60, 20 : 80 and 0 : 100 for barley hay: *E. esula* respectively.

Study 1

Rumen liquor donor animals (cows and ewes), were fed a diet containing 100% barley hay for a 7-day adaptation maintaining intake proportional to 2.0% of body weight (DM basis). Rumen liquor collections occurred on day 7, after adaption, for *in vitro* DMD determination and day 12 for the *in vitro* gas production.

Table 1. Nutrient composition for barley hay and *E. esula* L. (leaves for *in vitro* fermentation substrate (FS) combinations and whole plant for animal diets)

Item	Barley hay	*E. esula* (leaves)	*E. esula* (whole plant)
		%	
DM	91.05	89.87	90.97
OM	91.53	92.18	93.04
		% of DM	
CP	12.0	21.9	14.1
TDN	64.1	86.1	69.7
NDF	55.4	26.4	36.3
ADF	33.7	14.4	28.8
S	0.29	0.56	0.41
P	0.18	0.51	0.34
K	2.21	2.23	1.70
Mg	0.17	0.35	0.23
Ca	0.41	1.27	1.02
Na	0.95	0.02	0.04
		ppm	
Fe	112	99	87
Mg	36	24	22
Cu	6	8	7
Zn	32	67	43

Study 2

Ruminally cannulated cows and ewes were allowed an initial 7-day adaptation to the experimental diet. The experimental diet consisted of 85% barley hay and 15% *E. esula* fed at a constant proportion of body weight (2.0% body weight on a DM-fed basis). To avoid *E. esula* refusals, *E. esula* was hand cut daily to a length of <8.0 cm and equally divided by weight into two portions for placement into the rumen via the rumen cannula at 0800 h and 1800 h. Gavage of *E. esula* occurred simultaneously with daily offering of barley hay at 0800 h. Rumen liquor collections occurred on day 7, after concluding the adaptation period for *in vitro* DMD measurements and day 12 for the *in vitro* gas production.

Digesta collection and preparation of fermentation samples

Samples of barley hay and *E. esula* leaves (at time of collection) were individually packaged in airtight plastic bags, frozen at −20 °C, and subsequently lyophilized. Lyophilized samples of both barley hay and *E. esula* leaves were ground (Thomas-Wiley Laboratory Mill, Model 4, Arthur H Thomas Company, Philadelphia, PA, USA) to pass a 1 mm screen.[12] Ground fermentation substrate combinations of barley hay–*E. esula* were placed in square roller bottles (Pyrex square bottles, Corning Inc., Corning, NY, USA). Metal rod inserts were placed in the bottles, capped with lids, and placed on a roller grinder for 24 h to ensure complete mixing of barley hay–*E. esula* substrate combinations.[13] Substrate combinations of barley hay–*E. esula* were then weighed (0.5 g) and placed in 30 mL *in vitro* digestion tubes (Pyrex 9825, Corning Inc.).

In vitro substrate fermentation and gas production

In vitro digestion was conducted in the same manner for both studies following procedures previously reported.[14] Rumen liquor was collected at 0600 h on the day of collection. In brief, rumen extrusa was collected at the mat layer and liquid interface and

strained through four layers of cheesecloth into a collection Dewar (Nalgene 4150-200- StevenJo & Steph, Rochester, NY, USA) that had been warmed to 39 °C for 24 h. Each donor animal provided approximately 350 mL of rumen liquor at each collection. Rumen liquor samples were transported to LARRL immediately after collection. A 250 mL sample of rumen liquor from each animal and was then combined to make a 500 mL sample for each animal species. The 500 mL of rumen liquor was then combined with 500 mL of a phosphate buffer (70.8% Na_2HPO_4 and 29.2% KH_2PO_4)[15] and 1000 mL of McDougal's buffer,[14] and placed in a pre-warmed 39 °C water bath under continuous CO_2 bubbling. Previously weighed *in vitro* tubes containing 0.5 g substrate (six tubes per substrate combination) were then filled with 15 mL buffer plus rumen liquor using a Brinkman dispenser (5–25 mL bottle top dispenser; Brinkman Instruments, Westbury, NY, USA), flushed with CO_2, and sealed with plastic screw caps. Tubes were randomly placed in one of four metal racks and manually agitated 10 times and inserted into an incubator (39 °C). *In vitro* racks were agitated every 2 h for the first 12 h and then every 4 h throughout 48 h.

Duplicate tubes were promptly removed from the incubator at 24 or 48 h. Upon removal, two 10 mL aliquots were collected from each duplicate: one for VFA and another for ammonia (NH_3; fixed with 2 mL of 6 mol L^{-1} HCL) concentrations and stored at −20 °C until analysis. Concentrations of VFA were measured using gas chromatography (Hewlett-Packard 5890 series II gas chromatograph, 2 m × 2 mm column, Supelco 10% SP-1200/1% H_3PO_4 on 80/100 Chromosorb WAW, N_2 carrier at 20 mL min^{-1}, flame ionization detector at 195 °C) as previously reported.[16] Ammonia concentrations were determined using the phenol–hypochlorite method.[17]

For assessment, *in vitro* gas production rumen liquor was collected at 0600 on the day of collection as described above.[18] *In vitro* gas production syringes (100 mL) were filled with 20 mL of inoculum using a Brinkman dispenser (5–25 mL bottle top dispenser; Brinkman Instruments) which contained 0.5 g substrate (five syringes per substrate combination). Upon sealing, excess air was released and placed into the 120-syringe water bath maintained at 39 °C. Gas measurements were recorded at 0, 2, 4, 6, 8, 10, 12, 14, 16, 24, 30, 36, 48, 54, 60, 72 and 96 h by measuring gas displacement of syringes. Gas was released from syringes when gas displacement exceeded 80 mL.

Calculations and statistical analysis

Rates and cumulative gas volume produced were fitted to an exponential model and analyzed using GraphPad Prism.[19] The model included terms for asymptotic gas production (AGP), extent of degradation (ED), and average fermentation rate (AFR).[1,20]

In vitro dry matter disappearance (DMD) was calculated at 24 and 48 h of incubation and at 96 h of gas production. The residues after incubation were analyzed for DM and NDF disappearance (NDFD).

Data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA).[21] The model included the effect of fermentation substrate, inoculum source (cow or sheep), and incubation duration (for *in vitro* DMD) along with pre-planned orthogonal contrasts to evaluate the inclusion rate of *E. esula* in the substrate fermented. When significant ($P < 0.05$), main effect means were separated using LSMEANS. The Tukey–Kramer method was used to adjust *P*-values to correct for multiple comparisons in the current studies.[21] Means separations were conducted using PDMIX800.[22]

RESULTS

Study 1

In vitro DMD utilizing inoculum from animals fed barley hay only did not differ between any barley hay:*E. esula* substrate ratios ($P = 0.37$; Table 2). However, *in vitro* DMD were influenced by an inoculum source × time interaction ($P = 0.01$). Inoculum from cows provided for 341 g kg^{-1} and 497 ± 9.28 g kg^{-1} at 24–48 h of incubation, whereas inoculum from ewes allowed for a disappearance of 354 and 458 ± 9.28 g kg^{-1} from 24 to 48 h of incubation, which was less of an increase than when inoculum from cows was used. *In vitro* NDFD (g kg^{-1}) was not different for any of the barley hay:*E. esula* substrate ratios ($P = 0.20$) or inoculum sources ($P = 0.26$); however, NDFD was greatest ($P < 0.0001$) at 48 h (Table 2).

Ammonia (mg 100 mL^{-1}) concentrations were influenced by a fermentation substrate × inoculum source × time interaction ($P = 0.02$; Fig. 1). These data indicate that NH_4 concentrations at 24 h with 20% *E. esula* substrate had lower NH_4 concentrations from ewe donors compared to 100% barley hay fermentation substrate. No differences were found for NH_4 concentrations when using cow inoculum. Ammonia concentrations were greatest, regardless of inoculum source, after 48 h of incubation, while concentrations were lowest with higher ratios of *E. esula* in the substrate. *In vitro* pH responded linearly ($P = 0.002$) with a decline in pH as *E. esula* increased in the fermentation substrate. Additionally, *in vitro* pH was greater ($P = 0.03$) when cow inoculum was used, and time of incubation had no influence on pH ($P = 0.13$; Table 2).

Acetate concentrations were not influenced by substrate ratios ($P = 0.08$), but were greater if cow inoculum was used ($P = 0.001$) and incubated for 48 h ($P = 0.04$; Table 2). A fermentation substrate × inoculum source interaction was measured for propionate concentrations ($P = 0.0006$). Concentrations of propionate were lower when cow inoculum was used and were lowest with the 80 : 20 substrate combination, whereas propionate concentrations were greater and not influenced by substrate ratio when ewe inoculum was incubated (data not presented). Acetate : propionate ratio was affected by a fermentation substrate × inoculum source × time interaction ($P = 0.05$; Fig. 1). The acetate : propionate ratio was greatest at both 24 and 48 h when cow inoculum was used, although the ratio was variable and appeared to reflect no obvious biological influences due to barley hay:*E. esula* ratios. Butyrate concentrations were highest when substrate ratio was 80 : 20 and declined as barley hay:*E. esula* ratio decreased ($P = 0.0006$). Butyrate concentrations were greater when cow inoculum was used ($P = 0.01$; Table 2).

Asymptotic gas production after 96 h showed a fermentation substrate × inoculum source interaction ($P = 0.03$; Fig. 2). The interaction was the result of a change in rank due to inoculum source from 60 : 40 to 40 : 60 (barley hay:*E. esula*). This change resulted from ewe inoculum having a greater asymptotic gas production. Similarly, a fermentation substrate × inoculum source interaction was measured for fractional rate of gas production (h^{-1}; $P < 0.0001$) and average fermentation rate (mL h^{-1}; $P = 0.02$, Fig. 2). Lag time responded quadratically ($P < 0.0001$) and decreased as proportions of *E. esula* increased. Lag time was also greater when inoculum from cows was used ($P = 0.05$; Table 3).

Based on 96 h gas production kinetics DMD or ED was not influenced by substrate ratio ($P = 0.19$) or source of inoculum ($P = 0.48$; Table 3). However, NDFD responded quadratically to substrate ratio ($P < 0.0001$; Table 3), the lowest NDFD being found with 80 : 20 ratio, whereas each additional increase in *E. esula* provided greater NDFD. Also, NDFD was greatest ($P = 0.006$) with

Table 2. Least square means ± SEM for *in vitro* dry matter disappearance (DMD; g kg^{-1}), NDF disappearance (NDFD; g kg^{-1}), ammonia (NH_4; mg 100 mL^{-1}), pH, acetate (Ac; mol 100 mol^{-1}), propionate (Pr; mol 100 mol^{-1}), Ac : Pr ratio, butyrate (But; mol 100 mol^{-1}), and other VFA (calculated as the sum of isobutyrate, isovalerate, and valerate; mol 100 mol^{-1}) for study 1 when inoculum was collected from cows (C) and ewes (E) consuming a barley hay diet

	Fermentation substrate									Inoculum				Time			
Item	100 : 0	80 : 20	60 : 40	40 : 60	20 : 80	0 : 100	SEM	*P*-value	Effect[a]	C	E	SEM	*P*-value	24	48	SEM	*P*-value
DMD[b]	417	414	399	404	433	407	11.4	0.37	–	419	406	6.6	0.16	348	477	6.6	<0.0001
NDFD	449	470	409	379	447	517	42.2	0.20	Q	432	465	53.3	0.26	358	532	22.7	<0.0001
NH_4[c]	4.9	4.3	4.1	3.5	2.7	3.4	0.25	<0.0001	L, Q	3.9	3.7	0.15	0.27	1.6	5.9	0.15	0.004
pH	6.89	6.87	6.78	6.78	6.73	6.75	0.03	0.006	L	6.83	6.78	0.02	0.03	6.82	6.78	0.02	0.13
Ac	68.4	68.8	69.9	70.5	69.8	70.2	0.56	0.08	L	70.4	68.7	0.32	0.001	70.1	69.1	0.32	0.04
Pr[d]	20.0	19.1	19.6	19.7	20.2	20.1	0.19	0.005	L, Q, C	18.1	21.4	0.11	<0.0001	19.4	20.2	0.11	<0.0001
Ac : Pr[c]	3.4	3.7	3.6	3.6	3.5	3.5	0.04	0.003	Q,C	3.9	3.2	0.02	<0.0001	3.7	3.4	0.02	<0.0001
But	8.6	9.3	7.6	7.0	6.7	6.7	0.53	0.009	L	8.2	7.1	0.31	0.01	7.8	7.5	0.31	0.64
Others[e]	3.0	2.8	2.9	2.8	3.3	2.9	0.22	0.57	–	3.2	2.8	0.13	0.02	2.8	3.2	0.13	0.05

[a] Significant ($P \leq 0.05$) orthogonal estimates for substrate concentrations; linear (L), quadratic (Q), and cubic (C).
[b] Inoculum × time interaction ($P = 0.01$).
[c] Substrate concentrations × inoculum × time interaction ($P \leq 0.05$).
[d] Substrate concentrations × inoculum ($P = 0.0006$).
[e] Calculated as the sum of isobutyrate, isovalerate and valerate.

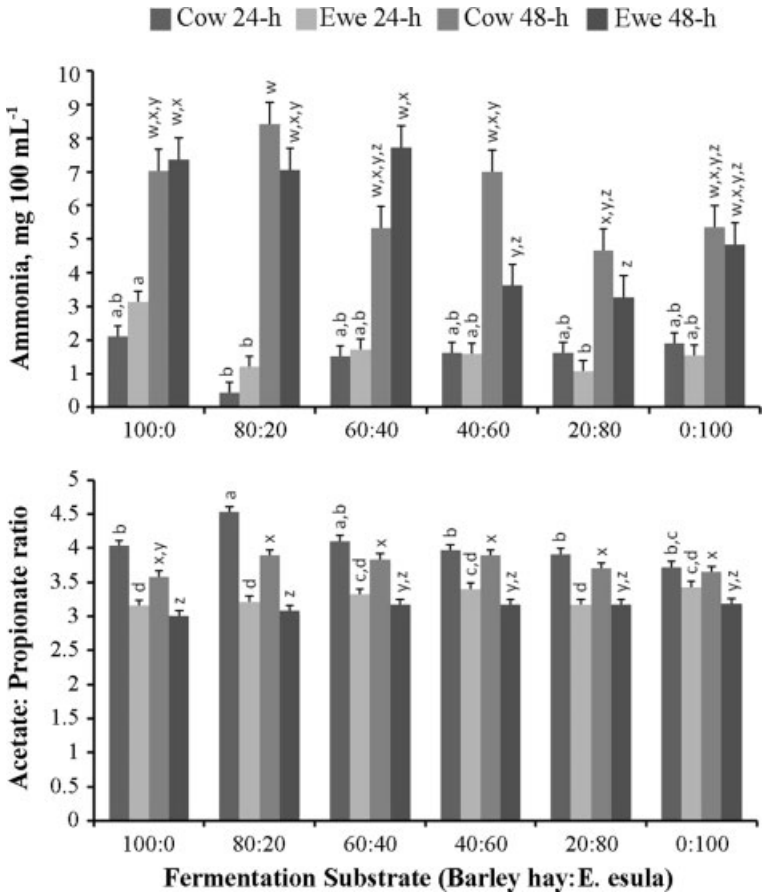

Figure 1. Least square means ± SEM for fermentation substrate (combinations of barley hay and *E. esula* L. × inoculum source (cow and ewe on a 100% barley hay diet) × time (24 and 48 h) for *in vitro* ammonia concentrations ($P = 0.02$; mg 100 mL^{-1}) and acetate : propionate ratio ($P = 0.05$). Superscripts within time indicate significant difference ($P < 0.05$).

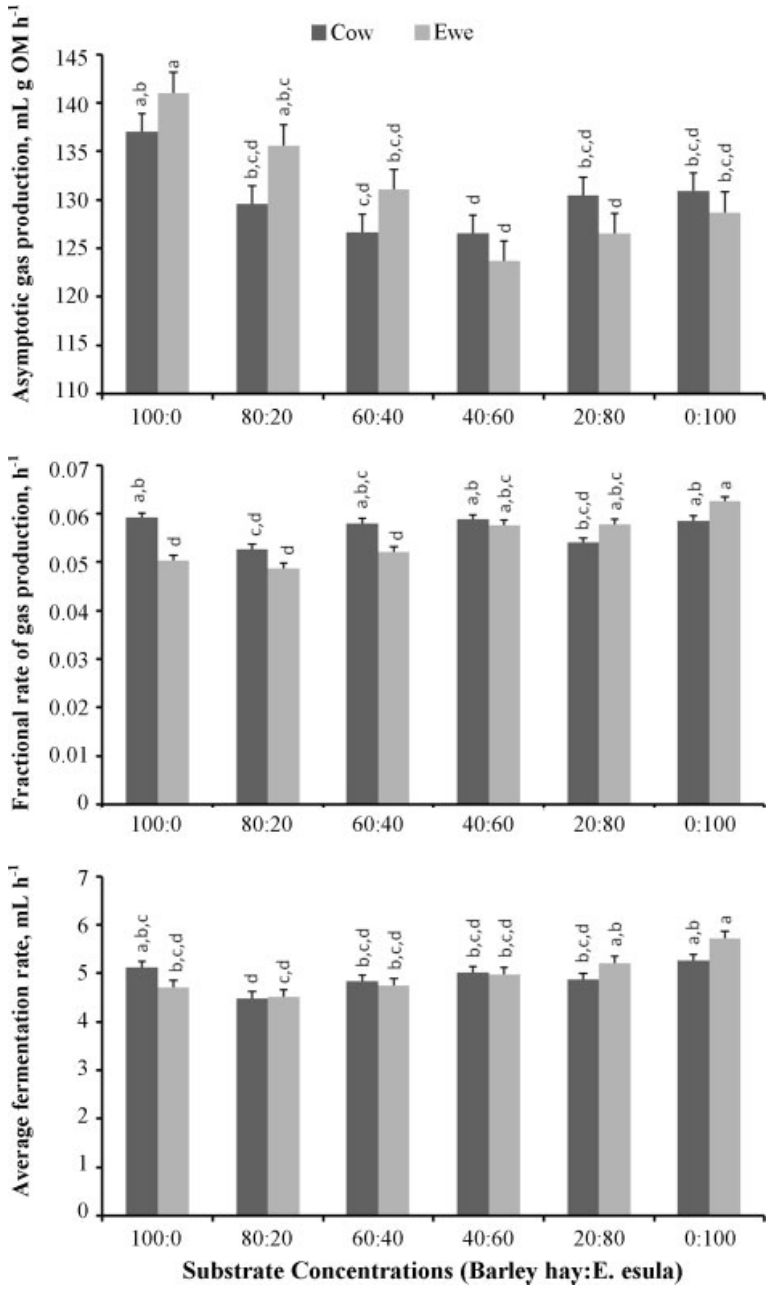

Figure 2. Least square means ± SEM for fermentation substrate (combinations of barley hay and *E. esula* L. × inoculum source (cow and ewe on a 100% barley hay diet) for asymptotic gas production ($P = 0.03$; mL g^{-1} OM), fractional rate of gas production ($P < 0.0001$; h^{-1}), and average fermentation rate ($P = 0.02$; mL h^{-1}). Superscripts within time indicate significant difference ($P < 0.05$).

cow inoculum. Lastly, pH linearly ($P = 0.0003$) decreased as *E. esula* increased in the fermentation substrate and was greater when inoculum from cow was used ($P = 0.0001$; Table 3).

Study 2

When inoculum donor animals consumed *E. esula*, DMD was not affected by substrate combinations ($P = 0.13$; Table 4). However, *in vitro* DMD (g kg^{-1}) was influenced by ruminant inoculum source × time ($P < 0.0001$). Inoculum from cows consuming *E. esula* in their diet had a disappearance of 335 g kg^{-1} at 24 h, which increased to 770 ± 14.9 g kg^{-1} at 48 h of incubation, whereas, when inoculum from ewes consuming *E. esula* in their diet was used, disappearance at 24 h was 335 g kg^{-1} and only 410 ± 16.1 g kg^{-1} from 24 to after 48 h, which was much less of an increase from 24 to 48 h in comparison to bovine inoculum. *In vitro* NDFD (g kg^{-1}) resulted in a quadratic response to the addition of *E. esula* in the substrate ($P = 0.01$), where DMD decreased when the proportion of *E. esula* was greater than 60%, and then DMD was higher as *E. esula* increased to 80% and 100% of the fermentation substrate. This would suggest that some type of interaction occurs in relation to the proportion of *E. esula* in the substrate and DMD.

The *in vitro* NH_4 (mg 100 mL^{-1}) concentrations as influenced by incremental proportions of *E. esula* did not differ ($P = 0.06$; Table 4). However, *in vitro* NH_4 concentrations were influenced by inoculum source × time ($P < 0.0001$). When sheep inoculum

Table 3. Least square means ± SE for asymptotic gas production (AGP; mL g^{-1} OM), fractional rate of gas production (K; h^{-1}), lag time (Lag; h), average fermentation rate (AFR; mL h^{-1}), dry matter disappearance (DMD; g kg^{-1}), estimated extent of degradation (ED; g kg^{-1}), NDF disappearance (NDFD; g kg^{-1}) and pH for study 1 when inoculum was collected from cows (C) and ewes (E) consuming a barley hay diet

	Fermentation substrate (FS)									Inoculum (I)				
Item	100:0	80:20	60:40	40:60	20:80	0:100	SEM	*P*-value	Effect[a]	C	E	SEM	*P*-value	FS × I
AGP	139	133	129	125	128	130	1.31	<0.0001	L, Q	130	131	0.77	0.40	0.03
K	0.05	0.05	0.06	0.06	0.06	0.06	0.0008	<0.0001	L, Q	0.06	0.05	0.0005	0.004	<0.0001
Lag	1.44	1.05	0.83	0.62	0.36	0.38	0.05	<0.0001	L, Q	1.02	0.54	0.03	<0.0001	0.43
AFR	4.92	4.50	4.79	4.99	5.04	5.50	0.09	<0.0001	L, Q	4.93	4.98	0.05	0.52	0.02
DMD	414	536	525	516	454	413	47.4	0.19	Q	463	489	26.2	0.48	0.41
ED	273	350	354	356	311	289	30.1	0.24	Q	313	331	17.7	0.48	0.36
NDFD	559	542	546	534	618	693	10.5	<0.0001	L, Q	594	570	6.0	0.006	0.37
pH	6.59	6.59	6.56	6.57	6.56	6.52	0.01	0.003	L	6.59	6.54	0.007	0.0001	0.76

[a] Significant ($P \leq 0.05$) orthogonal estimates for substrate concentrations; linear (L), quadratic (Q), and cubic (C).

Table 4. Least square means ± SEM for *in vitro* dry matter disappearance (DMD; g kg^{-1}), NDF disappearance (NDFD; g kg^{-1}), ammonia (NH_4; mg 100 mL^{-1}), pH, acetate (Ac; mol 100 mol^{-1}), propionate (Pr; mol 100 mol^{-1}), Ac:Pr ratio, butyrate (But; mol 100 mol^{-1}), and other VFA (calculated as the sum of isobutyrate, isovalerate, and valerate; mol 100 mol^{-1}) for study 2 when inoculum was collected from cows (C) and ewes (E) consuming barley hay and *E. esula* diet

	Fermentation substrate							*P*-		Inoculum			*P*-	Time			*P*-
Item	100:0	80:20	60:40	40:60	20:80	0:100	SEM	value	Effect[a]	C	E	SEM	value	24	48	SEM	value
DMD[b]	465	428	445	467	467	503	20.4	0.13	L	552	372	11.0	<0.0001	335	590	11.0	<0.0001
NDFD	361	321	291	210	271	309	32.0	0.04	Q	269	319	17.2	0.05	206	382	17.2	<0.0001
NH_4[b]	2.24	1.50	1.67	1.36	0.88	1.47	0.31	0.06	L	1.04	2.00	0.17	0.0004	0.43	2.61	0.17	<0.0001
pH	7.1	7.1	7.2	6.9	7.1	7.1	0.07	0.30	–	7.1	7.1	0.04	0.71	7.2	7.0	0.04	0.0011
Ac	68.7	71.6	72.1	73.9	73.9	73.2	0.67	<0.0001	L, Q	71.1	73.4	0.36	0.0001	72.6	71.9	0.36	0.17
Pr	16.4	15.5	15.0	15.2	15.9	16.4	0.73	0.56	–	15.3	16.2	0.39	0.09	15.6	15.9	0.38	0.57
Ac:Pr	4.2	4.6	4.9	5.2	4.6	4.5	0.35	0.43	Q	4.7	4.7	0.19	0.81	4.7	4.7	0.19	0.90
But	11.6	9.3	10.2	8.3	7.4	7.6	1.05	0.03	L	10.5	7.7	0.56	0.0013	9.2	9.0	0.56	0.77
Others[c]	3.2	3.5	2.8	2.5	2.7	2.8	0.37	0.33	–	3.1	2.7	0.20	0.15	2.6	3.2	0.20	0.03

[a] Significant ($P \leq 0.05$) orthogonal estimates for substrate concentrations; linear (L), quadratic (Q), and cubic (C).
[b] Inoculum × time interaction ($P < 0.001$).
[c] Calculated as the sum of isobutyrate, isovalerate and valerate.

was used the concentration of ammonia increased more at 48 h compared to the concentration at 24 h. *In vitro* pH was not affected by proportion of fermentation substrate ($P = 0.30$) or inoculum source ($P = 0.71$); however, *in vitro* pH declined slightly from 24 to 48 h ($P = 0.001$; Table 4).

Concentration of acetate (mol 100 mol^{-1}) in buffer and ruminal fluid solution fitted a quadratic response ($P = 0.002$) where acetate increased as the proportion of *E. esula* increased to a ratio of 40:60 (barley hay:*E. esula*), then remained unchanged with the higher *E. esula* substrate combinations. Additionally, the concentration of acetate was greater when ewe inoculum was donated compared to the cow inoculum ($P = 0.001$), with no differences between 24 and 48 h ($P = 0.17$; Table 2). Propionate concentration and acetate:propionate ratio were not influenced by barley hay:*E. esula* ratios, inoculum source, or incubation time ($P > 0.05$; Table 4).

Fermentation kinetics based on 96 h gas production showed rumen inoculum from cows or sheep consuming *E. esula* in their diet did not differ for fractional rate of gas production (K (h^{-1}); $P = 0.60$), DMD (g kg^{-1}; $P = 0.13$), ED (g kg^{-1}; $P = 0.21$), or pH ($P = 0.32$) at any of the barley hay:*E. esula* substrate ratios (Table 5). Asymptotic gas production (mL g^{-1} organic matter (OM)) followed a cubic response ($P = 0.0008$) (Table 5). Lag time (h) decreased linearly ($P < 0.0001$) as *E. esula* increased in the fermentation substrate, whereas AFR (mL h^{-1}) increased linearly ($P = 0.002$) with incremental inclusion of *E. esula* in fermentation substrate (Table 5). The NDFD resulted in a quadratic response ($P < 0.0001$) with a general trend to increase as *E. esula* increased in the substrate fermented. A similar trend was observed in the *in vitro* experiment previously mentioned, especially for substrates containing 80% and 100% *E. esula*.

Cow or sheep inoculum did not influence AGP ($P = 0.12$), K ($P = 0.09$), lag time ($P = 0.60$), NDFD ($P = 0.68$), or pH ($P = 0.34$; Table 5) estimated from the gas production results. However, inoculum source was found to influence AFR ($P = 0.04$), DMD ($P = 0.0007$), and ED ($P = 0.0009$), which were greater when using inoculum from ewes consuming *E. esula* in their basal diet (Table 5).

DISCUSSION

The use of *in vitro* techniques to elucidate potential functionality differences between cattle or sheep ruminal microbial activity

Table 5. Least square means ± SE for asymptotic gas production (AGP; mL g^{-1} OM), fractional rate of gas production (*K*; h^{-1}), lag time (Lag; h), average fermentation rate (AFR; mL h^{-1}), dry matter disappearance (DMD; g kg^{-1}), estimated extent of degradation (ED; g kg^{-1}), NDF disappearance (NDFD; g kg^{-1}) and pH for study 2 when inoculum was collected from cows (C) and ewes (E) consuming a barley hay and *E. esula* diet

	Fermentation substrate (FS)									Inoculum (I)				
Item	100 : 0	80 : 20	60 : 40	40 : 60	20 : 80	0 : 100	SEM	*P*-value	Effect[a]	C	E	SEM	*P*-value	FS × I
AGP	123.7	135.3	131.7	126.9	123.3	127.5	2.56	0.01	C	129.7	126.4	1.48	0.12	0.07
K	0.05	0.05	0.05	0.05	0.05	0.05	0.004	0.60	–	0.05	0.05	0.002	0.09	0.61
Lag	1.09	0.93	0.57	0.41	0.54	0.32	0.14	0.001	L	0.67	0.62	0.07	0.60	0.57
AFR	4.07	4.15	4.27	4.59	4.34	4.76	0.15	0.03	L	4.23	4.50	0.09	0.04	0.96
DMD	562	520	524	525	515	540	12.9	0.13	Q	512	550	7.4	0.0007	0.86
ED	361	326	336	350	340	365	12.5	0.21	–	328	365	7.2	0.0009	0.87
NDFD	597	579	600	578	654	728	10.3	<0.0001	L, Q	621	624	5.9	0.68	0.67
pH	6.7	6.7	6.6	5.9	6.6	6.5	0.27	0.32	–	6.4	6.6	0.16	0.34	0.45

[a] Significant ($P \leq 0.05$) orthogonal estimates for substrate concentrations; linear (L), quadratic (Q), and cubic (C).

can be an effective screening method. Grazing sheep have been studied extensively as a biological tool for controlling *E*. esula.[23] Research has implied that goat rumen microflora may contain specific microorganisms better adapted to minimize the effect of secondary compounds found in *E. esula* even when fermented in goat digesta and introduced into the sheep rumen.[24] A better understanding of ruminal capacity in both cattle and sheep may lead to advances that encourage the use of all herbivores to control *E. esula* and other noxious weeds.

In the present studies, it is evident that differences exist in regards to source of rumen inoculum (cow or ewe) and fermentation substrate ratio of barley hay:*E. esula* in fermentation substrate. For instance, *in vitro* DMD was not influenced by experimental treatments in study 1 or 2; however, when comparing the two studies it appears that donors receiving *E. esula* in their basal diet may have improved DMD (Tables 2 and 4). Unfortunately, the number of animals that were available for this experiment did not allow the design of the study to test that implication. Interpretation of the 96 h gas production results predicted a slightly greater DMD and ED in ewe inoculum, especially when ewe donor animals consumed *E. esula* in their diet.

Whether the *in vitro* or gas production method was used to measure NDFD, a similar quadratic pattern in response to substrate ratio was found. When lowest proportions of *E. esula* were added to the substrate NDFD was lowest and at ratios 40 : 60 (or higher ratios of *E. esula*) there was an increase in NDFD. This indicates a high probability of an associative affect between barley hay and *E. esula*. Other results common to both studies was that inoculum from ewes generally resulted in greater NDFD (with one exception when donor animals did consume *E. esula* then cow inoculum resulted in greater NDFD). Other studies have reported no differences when comparing *in situ* DMD or NDFD in ewes consuming a grass hay or a diet consisting grass hay and *E*. esula.[10]

Condensed tannins and terpenoids (e.g. secondary compounds) are present in *E. esula*[24–26] and may reduce microbial growth and plant cell wall digestion.[27–29] This would suggest that ruminal fibrolytic bacteria not accustomed to *E. esula* may be inhibited by exposure to *E. esula* and the secondary compounds it contains. However, in browsing ruminants (i.e. deer) no negative effects on cell wall digestion were associated with condensed tannins consumption.[30] Evaluating the 24 h, 48 h *in vitro* DMD and 96 h gas production the authors conclude that inoculum source or ratio of *E. esula* fermented had few if any negative effects. In regard to NDFD there was an improvement when the higher ratio of *E. esula* was added to the substrate. Under the conditions of this study it appears that the nutrient composition of *E. esula* (Table 1) overcomes any expected negative effects due to secondary compounds.

When donor animals were gavaged with *E. esula* (study 2), lower NH_4 concentrations were found. It is unknown whether *in vitro* microbial growth was impacted. Other researchers have reported greater NH_4 concentrations *in vitro* when substrates contain *E. esula*. This increase has been attributed to the higher CP concentrations of *E*. esula.[10] Furthermore, researchers have observed greater *in vitro* concentrations of NH_4 when sheep are used as donor animals than cattle with a tendency for greater NH_4 concentrations in animals previously exposed to *Euphorbia* spp.[1] These reports are contradictory to our results, where NH_4 concentrations were lower when inoculum was used from *E. esula* exposed animals.

Individual VFA concentrations such as acetate tended to increase with increasing *E. esula* whereas propionate remained unchanged. This could indicate that in a production setting animals consuming *E. esula* may not be able to sufficiently clear acetate and may have a higher capacity to convert acetate to ketones, which may result in a metabolic disturbance.

In vitro fermentation characteristics and gas production measurements were evaluated and differences were found to exist between cow and sheep inoculum and whether or not there had been previous exposure to *E. esula*. To what extent dietary *E. esula* consumption alters *in vitro* rumen ecology is not completely elucidated. The rumen is a complex ecosystem that relies not only on the consumption of dietary feeds but also on the synchrony of nutrient release and production fermentation end-products to be utilized by co-species of rumen microorganisms and the host animal. The presence of condensed tannins, terpenoids, and other plant-derived secondary compounds may influence the rumen ecosystem, and subsequently animal performance and animal preference to consume *Euphorbia* spp. Other results suggest advantages for either cattle or sheep inoculum when fermenting combinations of barley hay and *E. esula*. An important outcome of these experiments suggests that cattle may be a viable user of *E. esula* if adaptation is allowed to occur in the rumen prior to exposure to *E. esula*. A more difficult task may be changing the

behavioral nature of cattle that tend to avoid *E. esula* infested areas.

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REFERENCES

1 Mora MJ, Hervas G, Mantecon AR, Busque J and Frutos P, Ruminal degradation of mountain pastures infested by *Euphorbia polygalifolia* in cattle and sheep: effect of previous grazing in invaded areas. *J Sci Food Agric* **86**:2153–2160 (2006).
2 Bangsund DA, Nudell DJ, Sell RS and Leistritz FL, Economic analysis of using sheep to control leafy spurge. *J Range Manage* **54**:322–329 (2001).
3 Fox D, Kirby D, Lym R, Canton J and Krabbenhoft K, Chemical composition of leafy spurge and alfalfa. *North Dakota Farm Research Bi-monthly Bulletin, ND Agriculture Experiment Station* **48**:7–9 (1991).
4 Lym RG and Kirby DR, Cattle foraging behavior inleafy spurge (*Euphorbia esula* L.) infested rangeland. *Weed Technol* **1**:314–318 (1987).
5 Kronberg SL, Halaweish FT, Hubert MB and Weimer PJ, Interactions between *Euphorbia esula* toxins and bovine ruminal microbes. *J Chem Ecol* **32**:15–28 (2006).
6 Halaweish FT, Kronberg S, Hubert MB and Rice JA, Toxic and aversive diterpenes of *Euphorbia esula*. *J Chem Ecol* **28**:1599–1611 (2002).
7 Kronberg SL, Muntifering RB, Ayers EL and Marlow CB, Cattle avoidance of leafy spurge: a case of conditioned aversion. *J Range Manage* **46**:364–366 (1993).
8 Duncan AJ, Frutos P and Young SA, The effect of rumen adaptation to oxalic acid on selection of oxalic-acid-rich plants by goats. *Br J Nutr* **83**:59–65 (2000).
9 Dominguez-Bello MG, Detoxification in the rumen. *Ann Zootech* **45**:323–327 (1996).
10 Thomas VM, Clark CF, Kott RW and Olson B, Influence of leafy spurge on ruminal digestion and metabolism and blood metabolite profiles in sheep. *Sheep Goat Res* **10**:168–172 (1994).
11 WRCC, Western Regional Climate Center. *Western U.S. Climate Historical Summaries*. [Online]. Available: http://wwww rccdriedu/climsumhtml [1 March 2010].
12 AOAC, *Official Methods of Analysis* (15th edn). Association of Official Analytical Chemists, Arlington, VA (1990).
13 Mortenson MC, Effects of interseeded alfalfa (*Medicago sativa* ssp. *falcata*) on forage production, forage quality, and carbon sequestration on a mixed-grass rangeland. MS thesis, University of Wyoming, Laramie (2003).
14 Tilley JMA and Terry RA, A two stage technique for the *in vitro* digestion of forage crops. *J Br Grass Soc* **18**:104–111 (1963).
15 Menke KH, Raab L, Salewski A, Steingass H, Fritz D and Schneider W, The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor. *J Agric Sci* **93**:217–222 (1979).
16 Erwin ES, Marco GJ and Emery EM, Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. *J Dairy Sci* **44**:1768–1771 (1961).
17 Broderick GA and Kang JH, Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and *in vitro* media. *J Dairy Sci* **63**:64–75 (1980).
18 Blümmel M and Orskov ER, Comparison of *in vitro* gas production and nylon bag degradability of roughages in predicting food intake in cattle. *Anim Feed Sci and Tech* **40**:109–119 (1993).
19 GraphPad Prism Version 4.00, GraphPad Software Inc., San Diego, CA (2003).
20 France J, Dijkstra J, Dhanoa MS, Lopez S and Bannink A, Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed *in vitro*: derivation of models and other mathematical considerations. *Br J Nutr* **83**:143–150 (2000).
21 SAS Institute, *SAS/Stat 9.1 User's Guide*. SAS Institute, Cary, NC (2004).
22 Saxton AM, A macro for converting mean separation output to letter groupings in Proc Mixed, in *Proceedings of the 23rd SAS Users Group International*. SAS Institute, Cary, NC, pp. 1243–1246 (1998).
23 Bangsund DA, *Economic analysis of controlling leafy spurge with sheep*. Department of Agricultural Economics, North Dakota State University, Fargo, ND (2000).
24 Kronberg SL and Walker JW, Ruminal metabolism of leafy spurge in sheep and goats: a potential explanation for differential foraging on spurge by sheep, goats, and cattle. *J Chem Ecol* **19**:2007–2017 (1993).
25 Evans FJ and Kinghorn AD, A comparative phytochemical study of the diterpenes of some species of the genera *Euphorbia* and *Elaeophorbia* (Euphorbicaceae). *Bot J Linn Soc* **74**:23–27 (1991).
26 Roberts JL and Olson BE, Effect of *Euphorbia esula* on sheep rumen microbial activity and mass *in vitro*. *J Chem Ecol* **25**:297–314 (1999).
27 Barry TN and Manley TR, The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 2. Quantitative digestion of carbohydrates and proteins. *Br J Nutr* **51**:493–504 (1984).
28 Barry TN, Manley TR and Duncan SJ, The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 4. Sites of carbohydrate and protein digestion as influenced by dietary reactive tannin concentration. *Br J Nutr* **55**:123–137 (1986).
29 Lundberg P and Palo RT, Resource use, plant defenses, and optimal digestion in ruminants. *Oikos* **68**:224–228 (1993).
30 Robbins CT, Mole S, Hagerman AE and Hanley TA, Role of tannins in defending plants against ruminants: reduction in dry matter digestion? *Ecology* **68**:1606–1615 (1987).