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Can Sheep and Cattle Rumen Microorganisms be Conditioned to Invasive Weeds?[1]

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Summary

The invasive plant species, Spotted knapweed *(Centaurea maculosa* Lam.*)* and common tansy *(Tanacetum vulgare* L.*)*, are altering native rangeland communities in western North America (Tyser and Key, 1988; Jacobs, 2008). To increase our understanding of why sheep consume these species to a certain extent and cattle avoid them, *in vitro* dry matter digestibility (IVDMD), microbial gas production (MGP), and microbial purine concentrations (MPC) of *C. maculosa* or *T. vulgare* leaves or stems incubated in sheep or cattle rumen fluid were measured. Rumen microbes were not conditioned to these plants in Trials 1a and 1b, but were conditioned in Trials 2a and 2b. Total MGP of *C. maculosa* leaves or stems (Trial 1a; $P < 0.004$) and *T. vulgare* leaves (Trial 1b; $P < 0.07$) was less, but IVDMD of these plant parts were greater ($P < 0.05$) with cattle than sheep-rumen fluid. Conditioning ewe or cow rumen microbes to *C. maculosa* did not enhance 24-h MGP, IVDMD, or MPC of *A. arundinaceus* grass hay or *C. maculosa* leaves or stems (Trial 2a; $P > 0.10$). Conversely, conditioning ewe rumen microbes to *T. vulgare* increased (Trial 2b; $P < 0.04$) IVDMD of *A. arundinaceus* hay and *T. vulgare* leaves or stems. *Centaurea maculosa* leaves and stems and *T. vulgare* leaves were used by rumen microbes as a nutritious feedstuff and nutrient characteristics and overall low IVDMD and MPC suggest that *T. vulgare* stems represent a poor quality forage. To increase consumption, further research is warranted to determine species composition and physiological differences between sheep- and cattle-adapted rumen microbes.

Key Words: *Centaurea Maculosa*, Conditioning, Nutrient-Toxin Interactions, Rumen Microorganisms, *Tanacetum Vulgare*

Introduction

Although considered grazers, sheep and cattle select different proportions and species of grasses and forbs. For example, at times, sheep consume the invasive, perennial forbs common tansy (*Tanacetum vulgare* L.) and spotted knapweed (*Centaurea maculosa* Lam.), whereas cattle generally avoid them (Hein and Miller, 1992; Kelsey and Mihalovich, 1987; Lym and Kirby, 1987; Jacobs, 2008). An animal's ability to tolerate and continue to consume certain aversive plants may depend on its ability to digest its fiber and neutralize toxic compounds before body tissues are affected (Smith, 1992).

The complex rumen ecosystem contains general and specialized microorganisms, which allow ruminants to detoxify certain plant compounds (Weimer, 1998; Asanuma et al., 2002). Further, specialized bacteria present in some herbivores are not present in others, or at least not in significant quantities. These specialized bacteria may adapt to allow the host to consume plant materials that are toxic to non-adapted animals (Jones and Megarrity, 1986). Ruminants can tolerate higher quantities of toxins if the toxic plant is introduced gradually into their diet (Allison and Cook, 1981). In addition, extent and rate of digestion may also affect the ability of an animal to tolerate certain toxins (Kronberg and Walker, 1993), and cattle digest more fiber than sheep (Playne, 1978). Thus, greater microbial digestibility of toxic plant fiber in cattle may increase the release of chemicals associated with structural plant tissues (Hagerman and Butler, 1991). The objectives were to determine IVDMD, rumen microbial gas production (MGP), and microbial purine concentrations (MPC; microbial biomass indicator) from invasive plants incubated with non-conditioned or conditioned rumen fluid collected from sheep or cattle.

Materials and Methods

Experimental Design

The experimental protocol was approved by Montana State University's Institute for Animal Care and Use Committee. Two fistulated crossbred beef cows (mean BW = 816 kg) and two fistulated white-faced yearling ewes (mean BW, 63 kg) were housed in individual pens and used as donors of fresh rumen fluid. Trial 1 was used to determine inherent differences in MGP, MPC, and IVDMD of Garrison creeping foxtail (*Alopecurus arundinaceus* Poir) hay, *C. maculosa*, or *T. vulgare* between sheep and cows fed only a grass hay diet (2 percent of BW, DM-basis) at 1100 h mainly consisting of *A. arundinaceus* hay.; (Table 1). During Trial 1, fresh rumen fluid was collected from each sheep and cow, kept separate for each animal, and immediately incubated in flasks containing a buffer and either dried *A. arundinaceus* hay, or leaves or stems of *C. maculosa* (Trial 1a) or *T. vulgare* (Trial 1b).

Olson and Kelsey (1997) describe the *in vitro* system used to measure MGP, MPC, and IVDMD. Each plant species within each trial included three runs, with duplicates (n = 2 baths) in each run. Gas production was measured by water displacement in inverted burettes at 2 h, 3 h, 4 h, 6 h, 8 h, 12 h, 18 h, and

Table 1. Trials 1a, 2a (*Centaurea maculosa* Lam.) and 1b, 2b (*Tanacetum vulgare* L.): nutritive value of *A. arundinaceus* hay, *Centaurea maculosa,* and *Tanacetum vulgare*[1]

Item, %	CP	NDF	ADF	ASH
Ground forage material[2]				
A. arundinaceus hay	8.1	57.7	36.6	9.4
C. maculosa leaves	14.4	23.0	16.5	10.0
C. maculosa stems	7.5	53.0	44.6	6.1
T. vulgare leaves	17.6	25.2	17.6	12.2
T. vulgare stems	3.4	75.1	65.9	4.6
Chopped forage material fed to the animals				
Trial 1a: *C. maculosa*				
Sept. 27				
A. arundinaceus hay	6.8	53.9	35.5	9.5
Oct. 9				
A. arundinaceus hay	6.7	58.6	34.3	9.5
Trial 1b: *T. vulgare*				
Oct. 21				
A. arundinaceus hay	6.5	59.5	36.6	11.4
Trial 2a: *C. maculosa*				
Oct. 26				
A. arundinaceus hay, chopped	8.6	63.0	39.1	9.8
C. maculosa, chopped	10.6	40.2	33.7	7.5
Oct. 29				
A. arundinaceus hay, chopped	9.4	59.0	37.1	10.4
C. maculosa, chopped	10.7	38.9	32.3	8.0
Nov. 4				
A. arundinaceus hay, chopped	9.6	61.3	39.4	10.4
C. maculosa, chopped	12.1	31.3	25.4	9.2
Trial 2b: *T. vulgare*				
Nov. 10				
A. arundinaceus hay, chopped	9.3	59.5	37.5	11.4
Nov. 13				
T. vulgare, chopped	6.3	56.2	48.1	6.6
Nov. 16				
A. arundinaceus hay, chopped	9.2	58.7	37.9	11.4
T. vulgare, chopped	6.3	53.3	44.9	7.2

[1] Trial 1a: Sept. 27 to Oct. 15, 2004; Trial 1b: Oct. 16 to Oct. 22, 2004; Trial 2a: Oct. 26 to Nov. 5, 2004; Trial 2b: Nov. 6 to 21, 2004.
[2] Forage was ground to pass a 1-mm screen and placed into the flask during the *in vitro* trials.

24 h according to procedures of Roberts and Olson (1999) to determine MGP. After the 24-hr reading, flask contents were filtered to separate residue from the fluid fraction. Residues were dried at 60 ºC for 48 hr and weighed to determine IVDMD, and then analyzed for MPC (Zinn and Owens, 1986) as an indicator of microbial biomass.

For Trial 2a, one ewe and one cow were randomly selected to be conditioned over 10 d to *C. maculosa*, whereas the other ewe and cow were only fed *A. arundinaceus* hay at 2 percent BW (DM-basis). For the conditioned ewe and cow, quantity of *C. maculosa* in the diet was increased every other day in 5 percent increments until it comprised 25 percent of the diet. After Trial 2a, all ewes and cows were only fed *A. arundinaceus* hay for 7 d before Trial 2b. Protocol for Trials 2a and 2b was the same, except that *T. vulgare* was fed and the ewe and cow to be conditioned were switched.

Plant Material and Forage Analysis

During the summer of 2004, *C. maculosa* (early-maturity stage) and *T. vulgare* (late-maturity growth stage) were cut separately about 4 cm above the ground. Plants were separated into leaves and stems, dried at 45°C for 48 h, and ground in a Wiley Mill to pass a 1-mm screen; this material was placed into the flasks during the *in vitro* trials. To condition rumen microbes, *C. maculosa* (Trial 2a) and *T. vulgare* (Trial 2b) plants were chopped (less than 5 cm), air-dried, and then fed. Subsamples of *A. arundinaceus* hay, *C. maculosa*, and *T. vulgare* were ground and analyzed separately for ash and N by standard methods (AOAC, 1990); CP was calculated as 6.25 × N. The NDF and ADF were analyzed using Van Soest et al. (1991) procedures modified for an Ankom 2000 Fiber Analyzer (Ankom Technol. Corp., Fairport, N.Y.).

Statistical Analyses

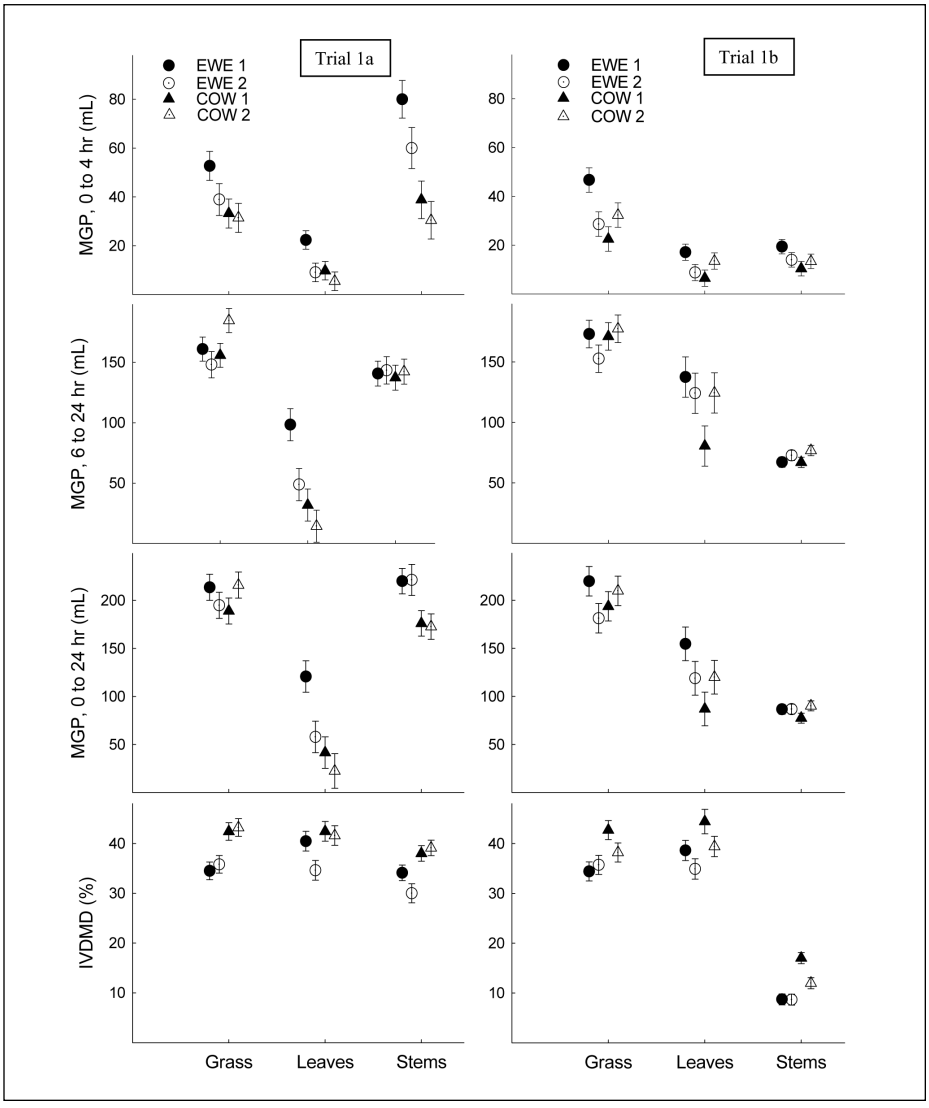
In both trials, the dependent variables MGP, MPC, and IVDMD of *A. arundinaceus* hay, and leaves or stems of the invasive species were analyzed using the Mixed procedure of SAS (SAS Inst. Inc., Cary, N.C.) and a model that included animal (treatment) as the fixed effect and flask as the experimental unit. Each trial was repeated three times (3 d per trial) and flasks were replicated in two water baths within each day; thus, six replications (flasks) per animal/trial were evaluated for each forage type (hay, leaves, or stems). A compound symmetry covariance structure was used for all variables in each model. For Trials 1a and 1b, orthogonal contrasts included: 1) ewe1 vs. ewe2, 2) cow1 vs. cow2, and 3) average of both ewes vs. average of both cows. For Trials 2a and 2b, contrasts included: 1) conditioned ewe vs. non-conditioned ewe, 2) conditioned cow vs. non-conditioned cow, and 3) conditioned ewe vs. conditioned cow. Spearman correlation coefficients were used to determine correlations of MGP, MPC, and IVDMD among ewes and cows (Trials 1a, b) and among the conditioned ewe and cow (Trials 2a, b).

Results and Discussion

Chemical Composition of *A. arundinaceus* Hay, *C. maculosa*, and *T. vulgare*

Chemical composition of the forages (Table 1) was not statistically analyzed, but differences were observed, which was probably due to sampling collection date, slight differences in soil and microclimate, or both. Quality of *C. maculosa* leaves and stems was comparable to previous reports (Table 1; Kelsey and Mihalovich, 1987; Olson and

Fig. 1. *In vitro* microbial gas production (MGP: 0 to 4 hr, 6 to 24 hr, and 0 to 24 hr) and dry matter digestibility (IVDMD) of grass hay, *C. maculosa* (Trial 1a) and *T. vulgare* (Trial 1b) leaves or stems from ambient rumen fluid. Data are presented as least squares means ± SE.



Kelsey, 1997). Ground *C. maculosa* and *T. vulgare* leaves, which were used in the flasks, had greater CP and less NDF and ADF than *A. arundinaceus* hay. The *T. vulgare* stems were considered a poor quality forage due to low CP and high fiber (NDF and ADF) concentrations. Chopped *C. maculosa* and *T. vulgare* plants, which were fed to the animals, contained a mixture of leaves and stems; thus, quality of the mixture was between leaf and stem qualities.

Centaurea maculosa

The IVDMD of *A. arundinaceus* hay and *C. maculosa* leaves and stems was greater with cow than with sheep rumen fluid ($P < 0.04$; Figure 1, Trial 1a). Cattle digest certain plant materials more than sheep (Playne, 1978), but cattle avoid *C. maculosa* in the field whereas sheep graze it. Our *in vitro* approach bypassed the animals' sensory capacity; thus, further research is warranted to determine if the bitter-tasting sesquiterpene lactone (cnicin) located in glandular trichomes on leaves of *C. maculosa* is more taste-aversive to cattle than sheep.

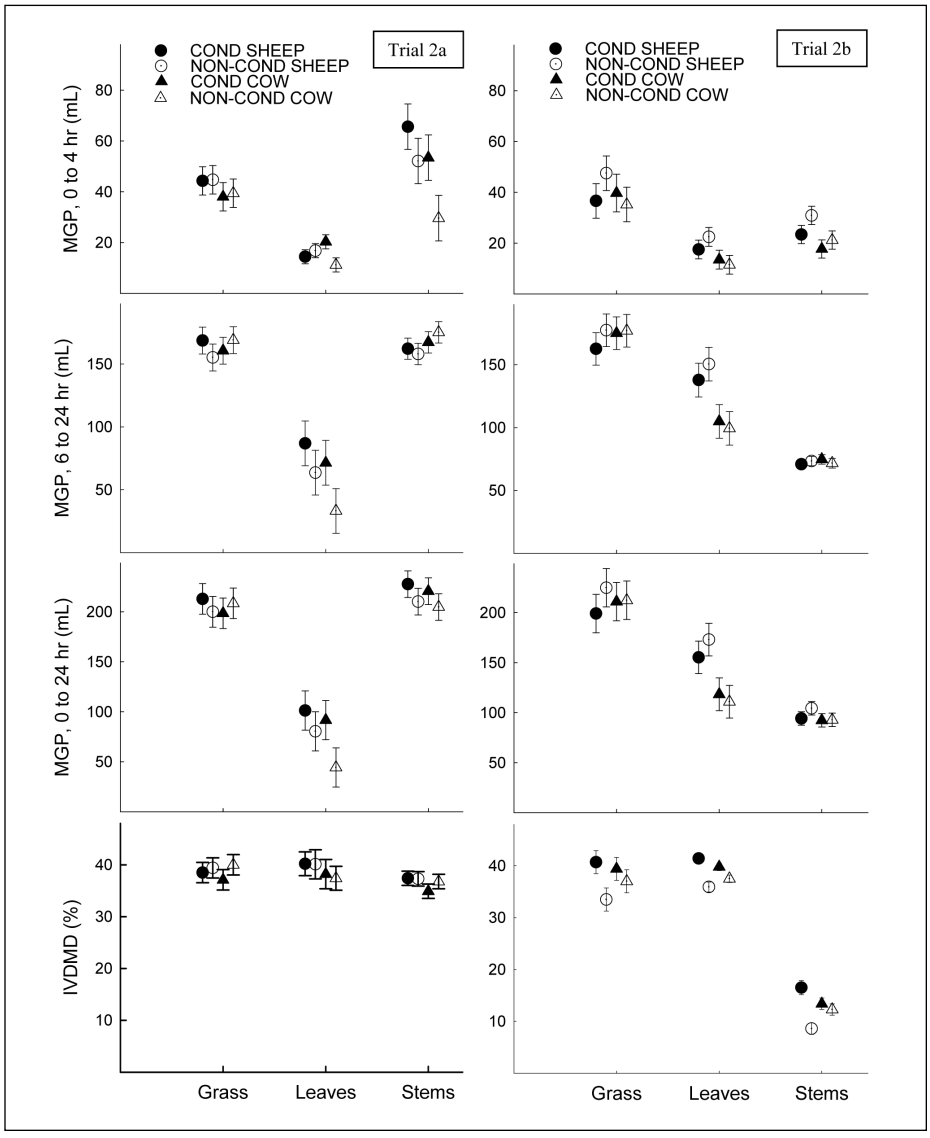
Total 24-h MGP from *C. maculosa* leaves and stems was low, but greater with sheep than cow rumen fluid ($P < 0.004$; Figure 1), even though MPC were similar ($P > 0.25$; 1.88 µg/mL to 3.57 µg/mL for leaves, 3.06 µg/mL to 4.73 µg/mL stems. Furthermore, MGP from *C. maculosa* leaves with sheep and cow rumen fluid was correlated ($r > 0.77$; $P < 0.05$) with MPC. Gas production from *C. maculosa* stems in sheep rumen fluid was not correlated with MPC, but was correlated ($r > 0.71$; $P < 0.05$) with MPC in cow rumen fluid.

Greater bacterial efficiency has been defined as less gas produced per unit of degraded organic matter (Blummel et al., 1997; Mlambo et al., 2008); thus, results indicated that rumen microbial populations from cattle rumen fluid were more efficient than from sheep rumen fluid. Greater efficiency may reflect that the secondary metabolites in *C. maculosa* differentially affect ambient sheep and cow rumen microbial populations. Soluble phenolics can be negatively correlated with MGP, but positively correlated with digestion (Mlambo et al., 2008). Cnicin concentrations in *C. maculosa* leaves may have been high enough to reduce MGP in cow rumen fluid only, without negatively affecting IVDMD. For example, a low saponin supply enhanced rumen microbial population growth, but reduced it when the dose was excessive (Sliwinski, et al., 2002). Leaves of *C. maculosa* collected in the same region at the same growth stage contained 3.6 percent cnicin concentrations (DM basis). Cnicin depresses MGP (Olson and Kelsey, 1997) and can inhibit rumen fermentation (Lowery and Kennedy, 1996).

Cnicin may have killed more cow than sheep rumen microbes, which would have reduced MGP, but would not have been detected in MPC; the technique used in the current study does not differentiate between alive and dead microbes. Even so, direct effects of toxicity may be more important than digestion inhibition when assessing deterrence by secondary metabolites (Bryant, et al., 1992). Thus, primary differences between non-conditioned sheep and cattle consuming *C. maculosa* may occur past the rumen, i.e., gut tissue or liver detoxification and excretion mechanisms (Foley et al., 1995; Provenza, 1995).

Conditioning rumen microbial population to *C. maculosa* (Figure 2, Trial 2a) was expected to increase IVDMD

Figure 2. *In vitro* microbial gas production (MGP: 0 to 4 hr, 6 to 24 hr, and 0 to 24 hr) and dry matter digestibility (IVDMD) of grass hay, *C. maculosa* (Trial 2a) and *T. vulgare* (Trial 2b) leaves or stems from rumen fluid conditioned with either *C. maculosa* (Trial 2a) or *T. vulgare* (Trial 2b). Data are presented as least squares means ± SE.



and MPC from leaves and stems with cow, and especially, sheep rumen fluid, because sheep graze the plant. However, conditioning did not enhance ($P > 0.10$) IVDMD, MGP, or MPC with rumen fluid from either animal species, which may have been due to the length of conditioning period not being long enough to allow microbial species to fully adapt. In the current study, nutrients were not limited, e.g., CP concentration in *C. maculosa* leaves was greater than 14 percent. Thus, conditioning with *C. maculosa* may not have been effective because anti-nutritional compounds in plants primarily affect microbial efficiency and production when nutrient availability is limiting (Lanyasunya et al., 2008). However, it should also be noted that some of the CP in *C. maculosa* leaves was from cnicin and not totally available to the animal.

With fluid from the conditioned cow, MPC and 24-h MGP from leaves were negatively correlated with IVDMD ($r = -0.97$; $P < 0.01$ and $r = -0.92$; $P < 0.05$, respectively), but positively correlated with IVDMD in the non-conditioned cow ($r = 0.61$ and $r = 0.70$; $P < 0.05$, respectively). A negative correlation between IVDMD, and MPC and 24-h MGP with conditioned cow rumen fluid indicates that microbial protein synthesis was favored over short-chain fatty acid production (Blummel and Orskov, 1993) and that the cow conditioned to *C. maculosa* was more efficient than the non-conditioned cow.

Unexpectedly, IVDMD, MGP, or MPC of the *A. arundinaceus* hay (control) did not differ between *C. maculosa*-conditioned and non-conditioned animals, indicating *in vitro* microbial populations were not negatively affected by cnicin. Adding *C. maculosa* to the basal diet could have increased bacterial utilization of the *A. arundinaceus* hay because of the added nutrients from *C. maculosa* leaves, or decreased bacterial utilization, because of secondary metabolites, e.g., cnicin, inhibiting MPC or MPG. Any negative effect associated with this secondary metabolite in *C. maculosa* may have been mitigated by its relatively high quality.

Tanacetum vulgare

The IVDMD and MPC of *T. vulgare* leaves and stems were expected to be less with non-conditioned sheep and cow rumen fluid, because they do not readily consume the plant. *Tanacetum vulgare* contains tanecetin, a sesquiterpene lactone that has antimicrobial properties (Hein, 1952); however, IVDMD of leaves was high (Figure 1, Trial 1b). Livestock may also avoid mature *T. vulgare*, because of its hardened stems resembling thin, mature corn stalks with low CP, and high NDF and ADF (Table 1). The nutritive value of the stems indicates that they contain minor amounts of cell solubles with considerable non-digestible fiber, i.e. ADF (Table 1). Presumably, the tanecetin and high fiber concentrations resulted in the low IVDMD, 24-h MGP, and MPC (1.45 µg/mL to 1.73 µg/mL) of stems with sheep- and cow-rumen fluid. In contrast to *T. vulgare* stems, leaves could be classified as a high-quality forage, based on CP, NDF, and ADF concentrations, while ignoring palatability or toxin issues.

The IVDMD of the *A. arundinaceus* hay and *T. vulgare* leaves and stems was greater with cow- than sheep-rumen fluid ($P < 0.03$; Figure 1, Trial 1b). Total 24-h MGP and MPC were greater with sheep- than with cattle-rumen fluid ($P = 0.07$, $P = 0.003$, respectively). Further, MGP from *T. vulgare* leaves with sheep-rumen fluid was correlated with IVDMD ($r = 0.67$, respectively; $P < 0.04$), but not correlated ($P > 0.54$) with IVDMD with cow-rumen fluid. Similar to *C. maculosa*, cattle-rumen microbes seemed more efficient than sheep-rumen microbes digesting *T. vulgare* leaves, i.e., greater digestion with less 24-h MGP and MPC.

Conditioning rumen microbial populations to *T. vulgare* was expected to increase IVDMD and MPC from leaves and stems, with the increase being greater for sheep than cow rumen fluid. Conditioning ewe rumen microbes to *T. vulgare* resulted in 7.2 percent, 5.4 percent and 7.9 percent greater ($P > 0.04$; Figure 2, Trial 2b) IVDMD of *A. arundinaceus* hay and *T. vulgare* leaves and stems, respectively, compared with fluid from the non-conditioned ewe, but 24-h MGP was similar ($P > 0.30$). Conditioning cow rumen microbes to *T. vulgare* did not enhance or decrease ($P > 0.12$; Figure 2, Trial 2b) IVDMD or 24-h MGP of *A. arundinaceus* hay or *T. vulgare* leaves or stems relative to fluid from the non-conditioned cow. The MPC for both animal species and *A. arundinaceus* hay, *T. vulgare* leaves or stems were similar ($P > 0.14$). In addition, IVDMD of *T. vulgare* stems was greater ($P = 0.09$) with fluid from the conditioned ewe than the conditioned cow. Results indicated that conditioning sheep, but not the cow, to *T. vulgare* increased microbial efficiency and was effective. Sheep may have specialized bacteria, allowing them to adapt to certain plant chemicals, which are not tolerated by cow bacteria (Jones and Megarrity, 1983, 1986).

Conclusions

By traditional measures of forage quality, *C. maculosa* leaves and stems, and *T. vulgare* leaves used in this study seemed to be nutritious forages, at least to rumen microbial populations. Utilization of *T. vulgare* by sheep may be increased if they are conditioned to *T. vulgare* before grazing. In fact, depending on secondary metabolite concentrations and quantity and quality of other available forages, sheep grazing *T. vulgare* may actually have greater IVDMD of grasses, which may increase growth rate or milk production. Grazing "value" and definition of any weed can change if negative consequences of consuming that weed are countered by positive responses from nutrients it offers. Based on size, cattle could consume much more *T. vulgare* than sheep if their microbial populations could be conditioned as effectively. Therefore, additional research is needed to evaluate differences between conditioning sheep and cattle on microbial species concentrations and physiological functions. Furthermore, because IVDMD of *C. maculosa* and *T. vulgare* leaves were high but their intake in the field is generally low, effects of secondary metabolites on gut tissue and liver metabolism should be evaluated.

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