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C. P. Murphy
Oklahoma State University, david.lalman@okstate.edu

A. J. Sexten
Oklahoma State University

G. L. Mourer
Oklahoma State University

E. D. Sharman
Oklahoma State University

S. J. Trojan
Oklahoma State University

See next page for additional authors

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C.P. McMurphy[a], A.J. Sexten[a], G.L. Mourer[a], E.D. Sharman[a], S.J. Trojan[a], M.J. Rincker[b], W.K. Coblentz[c], D.L. Lalman[a,*]

[a] Department of Animal Science, Oklahoma State University, Stillwater, OK 74078, United States
[b] DPI Global, Porterville, CA 93257, United States
[c] USDA-ARS, US Dairy Forage Research Center, Marshfield, WI 54449, United States

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ABSTRACT

Sixteen ruminally cannulated crossbred steers (529 ± 45 kg initial body weight, BW) were used to evaluate *in situ* dry matter (DM), neutral detergent fiber (aNDF), and N degradation characteristics of low-quality prairie hay, blood urea-N (BUN) and rumen fermentation parameters in steers provided a protein supplement with or without Micro-Aid® (MA; plant derived saponin). Steers were allowed ad libitum access to chopped prairie hay (49 g crude protein (CP)/kg DM and 738 g aNDF/kg DM) and randomly assigned to one of four treatments: (1) no supplement (C), (2) cottonseed meal and wheat middlings: 920 g DM/d (PC; positive control), (3) MA added to PC to supply 1 g MA/d (MA1), and (4) MA added to PC to supply 2 g MA/d (MA2). Steers were individually supplemented 920 g DM once daily at 08:00 along with a vitamin and mineral mix to ensure requirements were met. Orthogonal contrasts were used to determine the effects of protein supplementation, addition of MA and level of MA inclusion. During *in situ* phase, forage samples were incubated for a 96 h period. Protein supplementation increased DM intake (DMI), particulate passage rate (Kp), and rumen digestibility of DM and NDF ($P < 0.001$), but there was no effect on rumen N degradability. The inclusion of MA did not impact DMI in either phase. Compared to PC, MA decreased K_p (27.8 and 22.7 g DM/kg/h, respectively; $P = 0.02$), resulting in an increase in rumen aNDF and DM digestibility ($P < 0.001$). However, there was no influence of MA on apparent total tract digestibility in the metabolism phase. Rumen protozoa concentrations were suppressed ($P = 0.01$) with MA inclusion while lactate concentrations and microbial crude protein (MCP) flow to the small intestine were increased ($P = 0.05$). There was no impact on BUN, rumen ammonia, pH, volatile fatty acid (VFA) concentrations or N balance for MA compared to PC diets. Supplementation improved N balance, MCP synthesis and increased total concentrations of VFA and independent acetate and propionate concentrations. In conclusion, including MA in protein supplements increased rumen DM and a NDF digestibility of forage, reduced protozoa concentrations and increased daily outflow of MCP. This is indicative of increased rumen fermentation rate and may ultimately impact animal performance via increased energy and amino acid supply to the small intestine. However, more research is needed to validate this potential impact on animal performance.

Abbreviations: MA, Micro-Aid®; CP, crude protein; VFA, volatile fatty acid; BW, body weight; DM, dry matter; C, Control; PC, Positive Control; RDP, rumen degradable protein; NEm, net energy maintenance; NEg, net energy gain; K_d, fractional rate constant; aNDF, neutral detergent fiber inclusive of residual ash; ADF, acid detergent fiber inclusive of ash; ADIA, acid detergent insoluble ash; OM, organic matter; RD, rumen degradability; K_p, rate of particulate passage from the rumen; BUN, blood urea-N; RAN, rumen ammonia-N; MCP, microbial crude protein.

* Corresponding author. Tel.: +1 405 744 606; fax: +1 405 744 7390.
E-mail address: david.lalman@okstate.edu (D.L. Lalman).

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1. Introduction

It is a common practice to supply additional protein to cattle consuming low-quality forage because of its positive impact on performance from increased intake and digestibility (McCollum and Horn, 1990). This results from meeting a deficiency in rumen ammonia-N (RAN) and is thought to be effective when the crude protein (CP) of forage is less than 70 g CP/kg dry matter (DM; McCollum and Horn, 1990). In the Southern Great Plains, it has been reported that standing native range pastures reach this minimum CP content in late July and continue to decline thereafter (McMurphy et al., 2011). This makes it imperative to provide supplemental protein to meet the CP demand and subsequent energy demand in grazing cattle when consuming low-quality native range. However, the cost of supplying purchased and harvested hay and concentrate feeds account for the majority of the nutrition costs associated with a cow–calf enterprise in the Southern Great Plains, which on average is nearly 40% of total operating costs (Lalman, 2008). Therefore, practices to improve efficiency of use of low-quality forage have been the recent focus of research in cattle consuming these diets.

Products such as ionophores can be added to these supplements to improve supplementation efficiency by shifting microbial populations toward the production of propionate and reducing the precursors for methanogenesis. There are limitations to supplying some ionophores to grazing cattle and producers are continually looking for options to improve efficiency while still having the option to market their cattle for use in natural finishing programs. Micro-Aid® (MA; DPI Global, Porterville, CA, USA) is a plant derived, dry or liquid feed additive for use in animal feeds. It is manufactured from a purified extract of the *Yucca schidigera* plant that grows in the southwest United States and Mexico and contains saponins. Saponins are either triterpenoids or steroids in nature and have a hydrophobic aglycone, more commonly named sarsapogenin, attached to a sugar (Wina et al., 2005). The interest in steroidal saponin technology, like those in MA, can be attributed to their known lytic action on rumen protozoa (Wallace et al., 1994). This action is believed to be due to their affinity to membrane sterols, particularly cholesterol (Glauert et al., 1962). The results of defaunating the rumen include, but are not limited to decreased bacterial proteolysis, improved N conservation, decreased methanogenesis, and a shift in VFA production toward propionate, which all improve animal efficiency. These benefits to animal efficiency may be a direct effect of reduced protozoa concentrations or mere functions of the yucca extract itself.

There are numerous products on the market utilizing saponin technology. However, not all manufacturers use the same procedures to harvest the phytogenic extract and incorporate it into an animal feed product. Most manufacturers mechanically macerate, grind and dry the trunk and root of the plant to produce a yucca powder; others squeeze these plant parts in a press to produce a yucca juice (Cheeke, 2000; Oleszek et al., 2001). These processes can yield differing concentrations of saponins in these products. Singer et al. (2008) assayed four commercial products, including MA, for their saponin concentrations and MA was reported to have 181.6 g sarsaponins/kg of DM compared to 189.1, 170.6 and 95.4 g sarsaponin/kg of DM for DK sarsaponin 30®, Alltech De-Odorase®, and Monterey Sarsaponin 15®, respectively. This suggests that MA is one of the more highly saponin concentrated products on the market and research is needed to determine if MA has an impact on fiber digestion in cattle consuming low-quality forage and supplemented with additional protein.

The objectives were to investigate the effects of two different MA inclusion rates, in a protein supplement, on *in situ* rumen degradation of low-quality forage and its components, rumen fermentation parameters, N metabolism, and total tract digestibility of low-quality forage and its components to determine if production efficiency could be improved with the use of MA. The hypotheses was that the inclusion of MA in supplements offered to forage-fed cattle would reduce protozoa populations and subsequently increase rumen digestibility of low-quality forage, improve N metabolism, and total tract digestibility of low-quality forage, ultimately improving production efficiency.

2. Materials and methods

This experiment was conducted at the Nutrition and Physiology Barn located on campus at Oklahoma State University in accordance with an approved Oklahoma State University Animal Care and Use Committee protocol.

2.1. Animals and diet

Sixteen ruminally cannulated, crossbred steers (529 ± 45 kg initial body weight; BW) were housed individually in slatted floor pens (2.4 m × 4.6 m) during *in situ* digestibility determination and individual stanchions for the metabolism phase of the trial. During both periods, steers were allowed ad libitum access to chopped prairie hay (5 cm; 930 g/kg DM, 46 g CP/kg DM, 8.67 MJ net energy maintenance (NEm)/kg DM, 3.69 MJ net energy gain (NEg)/kg DM, 757 g aNDF/kg DM, 42 g acid detergent insoluble ash (ADIA)/kg DM. Hay was harvested in late July 2008 from an old world bluestem (*Bothriochloa ischaemum*) meadow. Steers were randomly assigned to one of four supplement treatments in a completely randomized design. Supplement treatments (Table 1) included, (1) no supplement (C), (2) cottonseed meal and wheat middlings: 920 g DM/d (PC; positive control), (3) MA added to PC to supply 1 g MA/d (MA1), and (4) MA added to PC to supply 2 g MA/d (MA2). Treatment levels for MA were recommended by manufacturer based on previous proprietary research. Steers were provided 920 g DM of supplement once daily in order to meet rumen degradable protein requirements (RDP; NRC, 1996). A vitamin and mineral mix was provided in feed pans with supplement daily to all steers. Steers had continuous access to fresh water and diets were fed at 08:00 for 10 days prior to initiation of the study to allow for ruminal adaptation.

Table 1
Supplement composition and amount of nutrients supplied daily.

Item (DM basis)	Supplement[a] (g/kg of DM)			
	C	PC	MA1	MA2
Cottonseed meal	–	755.0	755.0	755.0
Wheat middlings	–	195.0	195.0	195.0
Cane Molasses	–	50.0	48.9	47.8
Micro-Aid®	–	–	1.1	2.2
Nutrient supplied				
DM (g/day)	–	920	920	920
CP (g/day)	–	368	368	368
Crude fat (g/day)	–	17.2	17.2	17.2
NE_m (MJ/day)	–	6.9	6.9	6.9
NE_g (MJ/day)	–	4.4	4.4	4.4

Abbreviations: DM: dry matter, CP: crude protein (N × 6.25), NEm: net energy maintenance, NEg: net energy gain.

[a] Supplements included (DM basis) (1) no supplement (C), (2) cottonseed meal and wheat middlings: 920 g DM/d (PC; positive control), (3) MA added to PC to supply 1 g MA/d (MA1), and (4) MA added to PC to supply 2 g MA/d (MA2).

2.2. In situ degradation

Characteristics of forage *in situ* degradation were evaluated in steers consuming the experimental diet (four steers per treatment) and using standardization techniques presented by Vanzant et al. (1998). Dacron bags (Ankom Technology, Macedon, NY; 10 cm × 20 cm, 53 ± 15 μm pore size) were labeled with waterproof permanent marker and bag weight was recorded. All forage samples were ground in a Wiley mill (Model-4, Thomas Scientific, Swedesboro, NJ) to pass a 2 mm screen prior to incubation. Forage samples were weighed into duplicate Dacron bags (5 g; as-fed; AF) and heat sealed for each incubation time point. Prior to ruminal insertion, bags were soaked in tepid water (39 °C) for 20 min to remove water soluble fractions and reduce lag time associated with wetting. All bags (except 0 h) were inserted into the ventral rumen, under the ruminal mat, in a mesh laundry bag in reverse order for incubation times of 2, 4, 6, 8, 12, 16, 24, 36, 48, 72 and 96 h. At 07:30 on day 4, all bags were removed from the rumen and 0 h bags were soaked in tepid water for 20 min. All bags were rinsed with 39 °C water to remove particles adhering to the outside of the bags and then washed, by steer, in a washing machine (Model LSR7233EQO, Whirlpool, Benton Harbor, MI) on delicate setting 10 times for 1 min rinse and 2 min spin cycles. Following rinsing, bags were oven dried at 50 °C for 72 h. Dry sample bags were allowed to equilibrate with atmospheric conditions at room temperature for 1 h before being weighed. Samples from each incubation time were composited and subsamples from each composite were analyzed for N and aNDF.

Total N, aNDF and DM were segmented into three fractions (A, B and C) based on susceptibility to ruminal degradation. The A fraction was considered to be immediately soluble while the C fraction was deemed unavailable to rumen degradation and the B fraction was the portion that was degraded at a measurable rate (Coblentz et al., 2002). Nonlinear regression was used to determine degradation kinetics of the percentage of DM and aNDF remaining on incubation time, using the PROC NLIN procedure of SAS (SAS Inst. Inc., Cary, NC). Data were fitted to the nonlinear regression model described by Mertens and Loften (1980). The A and B fractions, lag time and the fractional rate constant (k_d) were determined directly from the nonlinear model. The C fraction was determined experimentally, and is defined as the residual DM, aNDF, or N remaining after 96 h. The effective rumen degradability (RD) was calculated according to Orskov and McDonald (1979) using the equation:

$$RD = A + \left[\frac{B \times k_d}{k_d + k_p}\right]$$

where k_d is rate of degradation of *B* fraction and k_p is rate of particulate passage from the rumen as described below.

After *in situ* procedures were completed steers were allowed an additional 10 day adaptation period in which hay intake was measured during the final 5 days. Following measurement of intake, four consecutive days were used to ascertain passage rate by procedures described by Coblentz et al. (2002). Briefly, manual evacuation of ruminal contents of each of four steers (one treatment replication/day) was conducted before feeding (0 h) and at 4 h post-feeding. Total ruminal contents were weighed, mixed, subsampled in triplicate, and returned to the rumen. Ruminal subsamples were dried at 50 °C in a forced air oven for 96 h. Hay and ort samples were collected throughout the study, composited by steer and dried at 50 °C in a forced air oven for 48 h. All dried samples were ground with a Wiley mill (Model-4, Thomas Scientific, Swedesboro, NJ) to pass through a 2 mm screen. Fractional passage rate of ADIA was determined by dividing the mean ADIA intake (g/h) by the mean (from the 0 and 4 h ruminal evacuation) ruminal mass of ADIA (Waldo et al., 1972). The hourly intake of ADIA for each steer was calculated by dividing total daily intake of ADIA by 24 h.

2.3. Metabolism measurements

The same sixteen ruminally cannulated, crossbred steers remained on their treatments and were moved to individual metabolism stanchions to evaluate rumen fermentation, blood urea-N (BUN) and urine and fecal excretion. Once in place, steers were adapted to the metabolism stanchions for 7 days before 5 days of forage intake measurement and sample collection (days 8–13). Steers had unlimited access to fresh water and were offered the same chopped prairie hay used for *in situ* degradability (5 cm; 46 g CP/kg DM, 8.67 MJ NEm/kg DM, 3.69 MJ NEg/kg DM, 757 g aNDF/kg DM, 42 g ADIA/kg DM) at 130% of each steer's average voluntary intake measured during the 7 day adaptation. Supplement and mineral was provided using the same methods as described previously.

Forage and supplement samples were collected from day 8 through 12, while orts, urine and fecal samples were collected from day 9 through 13. Urine was kept in an environment with a pH < 3 between sampling periods by using 6N HCl in the urine containers (Farmer et al., 2004). Urine was weighed and sampled every 24 h unless the sample collection container was more than half full at 12 h; when that situation occurred, subsamples were collected and weights were determined at that time. Specific gravity was determined using a hydrometer and subsamples were collected and frozen (−20 °C) for later analysis of purine derivatives and urinary-N (2400 Kjeltec, FOSS Analytical, Slangerupgade, Denmark). Fecal output was weighed every 24 h and a subsample was immediately placed in the forced air drying oven (50 °C) for DM determination.

Before conducting laboratory analysis, supplement, hay, orts and fecal samples were dried at 50 °C in a forced air oven and ground in a Wiley mill (Model-4, Thomas Scientific, Swedesboro, NJ) to pass through a 2 mm screen. At 08:00 on day 16, 0-h blood and rumen samples were collected. Blood samples were collected with a BD vacutainer (BD, Franklin Lakes, NJ), immediately placed on ice and allowed to coagulate before serum harvest. Serum was harvested via centrifugation at 1500 × *g* at room temperature for 20 min and stored (−20 °C) for later evaluation of BUN. After blood collection, 0-h rumen fluid was hand collected from the ventral rumen. Following 0 h, rumen and blood samples were collected at 3, 6, 9, 12, 15, 18, 21, and 24 h post-feeding. Rumen fluid pH was immediately determined using a portable pH meter. Two whole rumen content samples were collected. One sample was strained through four layers of cheesecloth and 100 mL of strained rumen fluid was acidified with 10 mL of 0.1 N HCl for subsequent determination of volatile fatty acid analysis (VFA; mM), RAN (mg/dL) and lactate (mM). A second sample (50 mL) was mixed with 50 mL of 50% (v/v) formalin (1:2 dilution) for determination of protozoa concentrations.

2.4. Lab analyses

Supplement, hay, orts and fecal samples were composited within steer across all days for the experiment. All composite samples were analyzed for laboratory DM (100 °C), concentration of aNDF (Van Soest et al., 1991) and ADF (AOAC, 1990, #973.18 using an ANKOM200 Fiber Analyzer Unit, Ankom Tech Corp, Fairport, NY, USA), ash (combusted 6 h in a muffle furnace at 500 °C), and N (TruSpec CN, LECO Corporation, St. Joseph, MI, USA). All samples analyzed for aNDF were assayed with alpha amylase and sodium sulfite and then expressed inclusive of residual ash and N. *In situ* residues were analyzed for aNDF, and prairie hay, orts, supplements and ruminal contents were analyzed for ADIA by ashing ADF residues in a muffle furnace at 500 °C for 8 h.

Blood urea–N concentrations were determined according to manufacturers guidelines (TECO Diagnostics, Anaheim, CA, USA) using 96 well plates and a spectrophotometer (Multiskan Ascent, MTX Labsystems Inc., Vienna, VA, USA; filter 595).

Rumen samples stored for RAN analysis were thawed and analyzed using a phenol–hypochlorite assay adapted from Broderick and Kang (1980), and modified by Galyean (1997), using a spectrophotometer (Multiskan Ascent, MTX Labsystems Inc., Vienna, VA, USA). Samples for VFA analysis were centrifuged at 3800 × *g* for 10 min. Rumen fluid was then removed from the centrifuge and 1 mL of supernatant was filtered through a 0.45 μm filter into 1.5 mL microcentrifuge tubes. At this time 250 μL of 25% (w/v) metaphosphoric acid solution was added to the supernatant. Tubes were vortexed, allowed to stand in ice water for 30 min, and then centrifuged at 15,000 × *g* for 15 min. Supernatant was loaded into gas chromatography (GC) vials at 900 μL with 100 μL 2-ethyl butyric acid as the internal standard and concentrations were determined by GC and injected onto a ZB-FFAP capillary column (30 mm × 0.53 mm × 1 μm; no. 7HK-G009-22, Phenomenex Inc., Torrance, CA) with helium carrier gas at 620 kPa and a flow rate of 8.0 mL/min. Injector and detector temperatures were 250 and 280 °C. Lactate was determined using a spectrophotometer (Multiskan Spectrum, Thermo Scientific, Waltham, MA; filter 560) from a protocol adapted from Barker and Summerson (1941) and Pennington and Sutherland (1956).

To determine protozoa concentrations, two drops of Brilliant Green dye were added to 1 mL of the mixed rumen sample and then allowed to stand overnight. Nine millilitres of a 30:70 (v/v) glycerol solution was added to each sample, giving it a final dilution of 1:20. One millilitre of the 1:20 dilution was pipetted into a Sedgewick-Rafter chamber and protozoa were counted at 10× magnification as described by Dehority (1984).

To determine rumen microbial crude protein (MCP) flow to the small intestine total purine derivatives were determined in excreted urine. Prior to analysis, urine samples were composited by period, and were diluted with 39 parts urine diluent to 1 part urine. Urine samples were analyzed for allantoin, uric acid, xanthine, hypoxanthine, and creatinine by HPLC (Waters Corp., Milford, MA) at the University of Nebraska following the procedures of Shingfield and Offer (1999). Total purine derivatives excreted (Y mmol/d) were included in the model for cattle as suggested by Chen and Gomes (1992):

$$Y = 0.85X + (0.385\mathrm{BW}^{0.75})$$

Table 2
Effect of supplement on feed intake and passage rate in steers consuming low-quality prairie hay during *in situ* phase.

Item	Treatments[a]				SEM[b]	P-Value[c]		
	C	PC	MA1	MA2		Pro_1[d]	MA[d]	MALev[d]
No.	4	4	4	4				
Initial BW (kg)	523	510	526	556	23	0.78	0.29	0.38
Intake								
Hay DMI (g/kg BW)	7.8	15.0	14.4	12.7	1.5	<0.001	0.40	0.38
Total DMI (g/kg BW)	7.8	17.0	16.3	14.6	1.5	<0.001	0.36	0.35
Rumen Contents								
Fill (kg of DM)	7.9	9.4	10.6	10.7	0.8	0.02	0.15	0.88
ADIA (g/kg)	46.7	50.1	51.9	52.4	0.0	<0.001	<0.001	<0.001
Passage rate (g DM/kg/h)	18.2	27.8	23.8	21.5	1.8	0.001	0.02	0.31

Abbreviations: No.: Number of animals, SEM: standard error of the mean, BW: body weight, DMI: dry matter intake, ADIA: acid detergent insoluble ash.
[a] Supplements included (DM basis) (1) no supplement (C), (2) cottonseed meal and wheat middlings: 920 g DM/d (PC; positive control), (3) MA added to PC to supply 1 g MA/d (MA1), and (4) MA added to PC to supply 2 g MA/d (MA2).
[b] Most conservative SEM, $n = 4$.
[c] Probability of a greater F-statistic.
[d] Pro: C vs. others; MA: PC vs. MA1 + MA2; MALev: MA1 vs. MA2.

where X is purine absorption (mmol/d), and $BW^{0.75}$ is metabolic BW (kg). Then the amount of microbial purines absorbed can be used to calculate MCP yield using the equation of Chen and Gomes (1992):

$$\text{MCP}\ \ (\text{gN/d}) = \frac{X(\text{mmol/d}) \times 70}{0.116 \times 0.83 \times 1000}$$

where 70 is the N content of purines (70 mg N/mmol), 0.116 the ratio of purine N:total N in mixed rumen microbes, and 0.83 is the estimated digestibility of microbial purines.

2.5. Statistical analyses

For all measurements, animal was the experimental unit because supplements were individually fed. *In situ* degradation characteristics were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) and the Satterthwaite approximation for degrees of freedom. The model included supplement treatment as a fixed effect. Orthogonal contrasts were tested for: (1) C vs. supplemented, (2) PC vs. MA1 + MA2, (3) MA1 vs. MA2. Intake, digestibility, MCP synthesis and N retention data were analyzed using PROC MIXED procedures of SAS (SAS Inst. Inc., Cary, NC) and included in the Satterthwaite approximation for degrees of freedom. Model terms included supplement treatment, time and treatment × time as fixed effects. Blood urea-N, and rumen fermentation parameters were analyzed using time as a repeated measure factor, and an autoregressive (period 1) covariance structure was used to model within steer variability. Orthogonal contrasts were tested for: (1) C vs. supplemented, (2) PC vs. MA1 + MA2, (3) MA1 vs. MA2.

3. Results

3.1. Intake and passage rate during in situ measurements

Protein supplementation increased hay DMI by 80% ($P < 0.001$). However, MA inclusion did not impact hay DMI as compared to PC ($P = 0.40$; Table 2). As expected, increased DMI for protein-supplemented cattle resulted in greater rumen fill as compared to C cattle ($P = 0.02$).

Particulate passage rate calculated from ADIA concentrations was greater for supplemented steers than for C steers ($P = 0.01$). The inclusion of MA reduced particulate passage rate by 19% compared to PC ($P = 0.02$), with no detectable difference between MA1 and MA2 ($P = 0.31$).

3.2. In situ digestibility

Protein supplementation increased the *B* fraction, k_d, and rumen digestibility of DM ($P < 0.001$; Table 3), with a tendency to increase the immediately soluble fraction (A; $P = 0.08$). Protein supplementation did not affect time to onset of DM fermentation ($P = 0.26$). The inclusion of MA increased the proportion of the A fraction, k_d and rumen degradability; a numerical trend ($P = 0.14$) for decreased lag time was observed for MA diets compared to PC. Micro-Aid increased *in situ* rumen DM digestibility ($P < 0.001$) by seven units compared to PC.

The kinetics for aNDF portion were similar to DM, with the following exceptions. There were no differences among treatment comparisons for the A fraction ($P > 0.10$), with a tendency for decreased lag time ($P = 0.09$) for supplemented

Table 3
Effect of supplement on *in situ* digestibility kinetics of low-quality prairie hay during *in situ* phase.

Item	Treatments[a]				SEM[b]	P-Value[c]		
	C	PC	MA1	MA2		Pro[d]	MA[d]	MALev[d]
No.	4	4	4	4				
DM								
A (g/kg)	129	130	144	142	4.3	0.08	0.02	0.66
B (g/kg)	439	553	565	545	12.0	<0.001	0.87	0.21
C (g/kg)	432	317	291	313	12.7	<0.001	0.30	0.18
Lag (h)	9.9	7.0	9.4	8.6	1.2	0.26	0.14	0.60
k_d (g/kg/h)	20	32	39	40	2.8	<0.001	0.02	0.84
RD (g/kg)	357	424	494	494	15.9	<0.001	<0.001	0.99
aNDF[e]								
A (g/kg)	21	24	34	39	7.3	0.20	0.15	0.58
B (g/kg)	526	654	675	644	12.5	<0.001	0.69	0.07
C (g/kg)	453	322	292	317	15.8	<0.001	0.32	0.21
Lag (h)	11.5	6.9	7.9	8.4	1.9	0.09	0.54	0.83
k_d (g/kg/h)	21	33	42	41	2.9	<0.001	0.02	0.84
RD (g/kg)	296	379	462	461	21.9	<0.001	<0.001	0.96
N[f]								
A (g/kg)	210	199	177	196	17.0	0.33	0.50	0.40
B (g/kg)	278	331	392	320	23.8	0.02	0.34	0.03
C (g/kg)	512	470	430	485	23.5	0.08	0.62	0.09
Lag (h)	38.6	34.1	25.4	23.6	3.9	0.03	0.04	0.71
k_d (g/kg/h)	32	39	40	34	3.2	0.15	0.55	0.17
RD (g/kg)	383	389	421	391	20.8	0.48	0.46	0.26

Abreviations: No.: Number of animals, SEM: standard error of the mean, DM: dry matter, aNDF: neutral detergent fiber inclusive of ash, *A*: Immediately soluble fraction, *B*: Fraction degraded at a measurable rate, *C*: Fraction unavailable to rumen degradation, Lag: Time lapse until fermentation begins, k_d: Rate of degradation, RD: Rumen degradability, h: hour.

[a] Supplements included (DM basis) (1) no supplement (C), (2) cottonseed meal and wheat middlings: 920 g DM/d (PC; positive control), (3) MA added to PC to supply 1 g MA/d (MA1), and (4) MA added to PC to supply 2 g MA/d (MA2).

[b] Most conservative SEM, $n = 4$.

[c] Probability of a greater F-statistic.

[d] Pro: C vs. others; MA: PC vs. MA1 + MA2; MALev: MA1 vs. MA2.

[e] aNDF presented as g/kg of aNDF.

[f] N presented as g/kg of N.

compared to non-supplemented steers. Micro-Aid increased rate of degradation compared to PC (42 g/kg/h vs. 33 g/kg/h; P = 0.02).

Protein supplementation increased fraction B of N, resulting in a trend for a reduction in the insoluble portion when expressed as a percent of total N (P = 0.08). The discrete lag time for N was reduced when steers were supplemented with protein (P = 0.03), and further reduced for steers consuming MA (P = 0.04).

3.3. Intake and digestibility during metabolism measurements

There was no significant difference (P = 0.33) in hay DMI for supplemented steers as compared to C (Table 4), nor did the inclusion of MA affect DMI (P = 0.83).

Total tract apparent digestibility of DM, aNDF, ADF and CP was increased due to protein supplementation (P < 0.001) with no change due to dietary MA inclusion (P ≥ 0.39) or MA inclusion level (P ≥ 0.33).

3.4. Nitrogen balance and microbial protein flow

Protein supplementation improved N balance in steers consuming low-quality prairie hay. Even though N intake and fecal excretion of N were increased (P < 0.02) and urinary N tended to increase (P = 0.07) for supplemented steers, there was still a substantial improvement in retained N. Nitrogen excretion or retention was not different for MA-fed steers as compared to PC-fed steers (P ≥ 0.42).

Protein supplementation resulted in an increase in MCP flow to the small intestine (P < 0.001). The inclusion of dietary MA enhanced (P = 0.05) the flow of MCP to the small intestine by 45% when compared to PC-fed steers.

3.5. Rumen fermentation and blood urea nitrogen

There were no treatment by time interactions observed for BUN (P = 0.36) or any of the rumen fermentation parameters; therefore, main effect means for treatment over time are presented in Table 5.

Table 4
Effect of supplement on DMI, total tract apparent digestibility, and nitrogen balance in steers consuming low-quality prairie hay during metabolism phase.

Item	Treatments[a]				SEM[b]	P-Value[c]		
	C	PC	MA1	MA2		Pro[d]	MA[d]	MALev[d]
No.	3	3	4	4				
Initial BW (kg)	423	538	550	577	27	0.32	0.45	0.48
Hay intake (g/kg BW)	8.8	12.2	12.5	10.8	2.7	0.33	0.85	0.60
DMI (g/kg BW)	8.8	13.9	14.2	12.4	2.7	0.15	0.83	0.59
Apparent Digestibility (g/kg)								
DM	436	595	593	607	42.3	<0.001	0.90	0.80
NDF	475	625	647	644	43.4	<0.001	0.66	0.97
ADF	441	599	616	621	46.9	<0.001	0.70	0.93
CP	417	612	546	598	41.8	<0.001	0.39	0.33
N Balance								
Intake (g/d	39	114	118	114	12	<0.001	0.90	0.78
Fecal (g/d)[e]	23	46	55	47	9	0.02	0.61	0.48
Urine (g/d)	15	24	25	32	5	0.07	0.45	0.31
Retained (g/d)	1	45	39	35.4	8	<0.001	0.42	0.75
N retained/N intake (g/kg)	20	391	324	302	63	<0.001	0.27	0.77
Microbial N flow (g/d)[f]	117	196	239	330	38	<0.001	0.05	0.08

Abbreviations: No.: Number of animals, SEM: standard error of the mean, DMI: dry matter intake, DM: dry matter, BW: body weight, DMI: dry matter intake, aNDF: neutral detergent fiber inclusive of ash, ADF: acid detergent fiber inclusive of ash, CP: crude protein.

[a] Supplements included (DM basis) (1) no supplement (C), (2) cottonseed meal and wheat middlings: 920 g DM/d (PC; positive control), (3) MA added to PC to supply 1 g MA/d (MA1), and (4) MA added to PC to supply 2 g MA/d (MA2).

[b] Most conservative SEM, $n = 3$.

[c] Probability of a greater F-statistic.

[d] Pro: C vs. others; MA: PC vs. MA1 + MA2; MALev: MA1 vs. MA2.

[e] Fecal N was determined using fecal samples that were previously dried at 50 °C.

[f] Calculated using urinary purine derivatives and the equations: $Y = 0.85X + (0.385BW^{0.75})$ where Y is the excretion of purine derivatives, X the purine absorption, 0.385 $BW^{0.75}$ the correction for endogenous purine contribution, and 0.85 is recovery coefficient. After X is determined then microbial N yield is calculated: Microbial N (gN/d) = (X (mmol/d) × 70)/(0.116 × 0.83 × 1000) where 0.83 = microbial purine digestibility, 70 = [N] of purines (mg N/mmol) (Verbic et al., 1990).

Table 5
Effect of supplement on rumen fermentation, blood urea nitrogen and protozoa counts in steers consuming low-quality prairie hay during metabolism phase.

Item	Treatments[a]				SEM[b]	P-Value[c]			
	C	PC	MA1	MA2		Trt[d]	Pro[e]	MA[e]	MALev[e]
No.	3	3	4	4					
BUN (mg/dL)	1.8	2.7	3.0	2.9	0.21	<0.001	<0.001	0.26	0.72
Rumen									
pH	7.1	6.5	6.5	6.3	0.16	0.03	<0.001	0.55	0.47
RAN (mM)	0.1	0.6	0.6	0.8	0.18	0.11	0.02	0.57	0.49
Lactate (mM)	0.1	0.1	0.2	0.1	0.02	0.07	0.09	0.05	0.69
Protozoa (10^3/mL)	5.5	12.5	10.5	10.2	0.73	<0.001	<0.001	0.01	0.69
VFA (mM)									
Total	56.0	75.8	83.5	73.7	3.79	<0.001	<0.001	0.49	0.04
Acetate	37.6	54.2	60.5	51.8	3.01	<0.001	<0.001	0.54	0.03
Propionate	8.6	10.6	11.5	10.2	0.52	<0.001	<0.001	0.66	0.05
Butyrate	5.4	6.4	6.8	6.6	0.34	0.03	<0.001	0.43	0.65
Valerate	1.5	1.6	1.7	1.7	0.04	<0.001	<0.001	0.47	0.84
Isobutyrate	1.6	1.7	1.7	1.8	0.12	0.57	0.51	0.47	0.31
Isovalerate	1.3	1.4	1.4	1.6	0.12	0.33	0.21	0.30	0.36
Acetate:Propionate	4.3	5.1	5.2	5.1	0.10	<0.001	<0.001	0.64	0.16

Abreviations: No.: Number of animals, SEM: standard error of the mean, BUN: blood urea-N, RAN: rumen ammonia-N, VFA: volatile fatty acid.

[a] Supplements included (DM basis) (1) no supplement (C), (2) cottonseed meal and wheat middlings: 920 g DM/d (PC; positive control), (3) MA added to PC to supply 1 g MA/d (MA1), and (4) MA added to PC to supply 2 g MA/d (MA2).

[b] Most conservative SEM, n = 3.

[c] Probability of a greater F-statistic.

[d] Trt: Treatment.

[e] Pro: C vs. others; MA: PC vs. MA1 + MA2; MALev: MA1 vs. MA2.

Protein supplementation increased BUN in steers by 59% ($P < 0.001$), with no additional effect from MA ($P = 0.26$). As compared to C steers, supplemental protein decreased ruminal pH ($P < 0.001$). Nevertheless, there was no difference ($P = 0.55$) in pH between PC and MA supplemented steers. Rumen ammonia-N concentrations were greater for supplemented steers ($P = 0.02$), but MA inclusion did not impact RAN ($P = 0.57$).

Protozoa concentrations were the lowest ($P < 0.001$) for the C steers. Positive control steers had the greatest concentration of protozoa with MA supplemented steers being intermediate and less than PC steers ($P = 0.01$).

Rumen lactate concentrations were minimal, as expected by diet, but there was a 50% increase in lactate concentrations for MA steers as compared to PC ($P = 0.05$), and a trend ($P = 0.09$) for increased lactate due to protein supplementation.

Protein supplementation increased ($P < 0.001$) total VFA concentrations, as well as concentrations of acetate, propionate, butyrate, and valerate, but no effect ($P \geq 0.21$) on isobutyrate and isovalerate were observed. However, the acetate to propionate ratio was the lowest ($P < 0.001$) for C steers, which is a consequence of having the lowest total VFA concentrations. There were no differences in total and individual VFA concentrations or acetate to propionate ratios for PC as compared to MA ($P \geq 0.30$). Still, there was a decrease ($P \leq 0.05$) in acetate, propionate and total VFA for MA2 versus MA1 steers.

4. Discussion

4.1. Intake and passage rate during in situ measurements

Hay intakes were similar to those observed with cannulated steers in a similar confinement setting fed similar low-quality hay in Oklahoma (Guthrie and Wagner, 1988). Protein supplementation of steers consuming low-quality prairie hay has consistently resulted in greater forage DMI (Guthrie and Wagner, 1988; Mathis et al., 1999; Bodine et al., 2001). This is a derived benefit of increasing RAN concentrations, fulfilling a deficiency, with a supplement high in RDP (McCollum and Horn, 1990). The lack of response to DMI with the inclusion of MA is consistent with other reported data when saponins were included in the diet (Valdez et al., 1986; Wilson et al., 1998; Hristov et al., 1999).

Particulate passage rate has been consistently increased when supplemental protein is provided. Other research evaluating passage rate in cattle consuming low-quality forage reported similar rates of passage using ADIA as a marker. Olson et al. (1999) reported an ADIA passage rate of 18.8 g/kg/h for non-supplemented steers and a rate of 24.6 g/kg/h for steers provided similar amounts of RDP. This measured rate of passage for supplemented steers is further supported by Winterholler et al. (2009) using similar feedstuffs and facilities as the current study. The simultaneous increase in DMI and particulate passage rate has been described in previous research from protein supplemented cattle consuming low-quality prairie hay (McCollum and Galyean, 1985; Guthrie and Wagner, 1988; Olson et al., 1999). The caveat in this study is that MA supplemented steers had similar DMI, but slower particulate passage rates than PC. This can be possible if the average retention time in one part of the gut is balanced by a decrease in another part (Grovum, 1988) and may be a possibility since there was no detectable difference in apparent total tract digestibility during the metabolism phase, but needs further investigation to validate.

Most reports suggest that saponins have no effect on particulate passage rate (Goetsch and Owens, 1985; Hristov et al., 2004). However, the reduced rate in particulate passage observed in this study coincides with findings from Goodall and Matsushima (1980). Because DMI for MA steers was not statistically different from PC steers, it cannot be assumed that slower particulate passage rates were a derived result of a reduction in DMI. A mechanism for slowing particulate passage rate is unclear, although it could be attributed to animal or microbial factors (Fahey and Berger, 1988). Another possibility is a potential change in rumen fluid viscosity from the foam-forming characteristics of saponins found in yucca extracts (Cheeke, 2000). It has been shown that foam formation is negatively correlated with particulate passage rate (Okine et al., 1988). These suggestions were not evaluated in the current study and should be further evaluated to determine the mode of action of MA on particulate passage rate.

4.2. In situ digestibility

To date, publications evaluating *in situ* digestibility kinetics of low-quality forage with differing dietary supplements are limited. However, McCollum and Galyean (1985) did evaluate *in vitro* 72 h DM disappearance of low-quality forage (6.1 g/kg CP and 677.7 g/kg NDF) in rumen inoculum from protein supplemented and non-supplemented steers and observed a 6.7% improvement in DM digestibility of forage compared to a 19% improvement between C and PC in the current study. Calculated *in situ* rumen digestibility is highly dependent on passage rate and because it was significantly slower for MA steers and the total undigested DM was similar between treatments ($P = 0.30$), there was a 16.5% improvement in calculated *in situ* rumen DM digestibility for MA supplemented steers compared to PC steers.

The calculated increase in k_d for DM and NDF of protein supplemented steers in the current study was observed as a numerical increase for DM *in vitro* digestibility by McCollum and Galyean (1985) (45.0 g DM/kg/h compared to 39.0 g DM/kg/h for supplemented and non-supplemented, respectively, $P > 0.10$).

The inability to detect an improvement in the degradable N portion due to supplementation may be because of the relatively low availability of the N to the rumen microbes. The concentration of available degradable N measured in the

forage in this study is relatively low compared to other published values (Vanzant et al., 1996), but with warm season grasses the low degradability is thought to be due to the N association with bundle-sheath cells (Mullahey et al., 1992).

4.3. Intake and total tract digestibility during metabolism measurements

The inability to detect a statistical difference in DMI during this phase of the study not only contradicts the *in situ* observations, but also disagrees with other published data (Guthrie and Wagner, 1988; Mathis et al., 1999; Bodine et al., 2001). However, there was still a 37% numerical increase in DMI ($P = 0.15$) for PC steers compared to C steers. The lack of significance can be explained by the combination of reduced treatment means for DMI and the nearly two fold difference in standard errors compared to the *in situ* phase. These compressed and variable intakes are most likely due to high temperatures during this time period in July because of conducting this study in a non-climate controlled facility.

Total tract apparent digestibility of DM, aNDF, ADF and CP was increased due to protein supplementation as observed by others (Guthrie and Wagner, 1988; Beaty et al., 1994; Mathis et al., 1999). It was anticipated that MA supplemented steers would have a further increase in apparent total tract digestibility after observing an increase in *in situ* rumen digestibility of DM and aNDF. However, this was not observed and is consistent with Holtshausen et al. (2009) where *in vitro* digestibility contradicted *in vivo* total tract digestibility. It is suggested that this is due to compensatory hind gut digestibility for PC steers. Goetsch and Owens (1986) suggested that particles passing from the rumen the slowest must pass through the hindgut the fastest in high roughage diets. Sultan and Loerch (1992) observed an 8 unit improvement in apparent rumen aNDF digestion when lambs were supplied a high protein supplement, but actually observed a 1.3 unit decrease in apparent total tract digestibility. They also recorded a 3 unit increase in rumen DM digestibility when total tract apparent DM digestibility was again 1.3 units lower than the low-protein supplemented lambs. Brink and Steele (1985) also demonstrated that postruminal digestion was inversely related to ruminal digestion of OM and that post-ruminal digestion increases as OM supply increases. The slower passage rate observed in the *in situ* phase combined with similar intakes between PC and MA supplemented steers also suggests that passage through the hind-gut was faster for MA steers, allowing for increased hind-gut retention and digestion for PC cattle.

4.4. Nitrogen balance and microbial protein flow

The increase in N balance has been demonstrated in steers supplemented with protein while consuming subtropical forages (Hennessy and Nolan, 1988) and is a result of combined increases in amino acid supply, via MCP, to the small intestine and dietary energy available for tissue synthesis and maintenance. The hypothesis that MA would shift microbial populations to conserve supplemental N was not supported with N balance data in this study. Although basal diets in the current study are unique to most literature, the inability of MA to reduce N excretion when N intake was similar to cattle not supplemented with MA are consistent with those data reported by Doerr et al. (2012).

Providing RDP to cattle consuming low-quality forage provides RAN needed for the synthesis of MCP and ultimately increases the flow of MCP to the small intestine. This was confirmed in this study, but in agreement with the hypothesis MA supplemented cattle had an increase in flow of MCP to the small intestine. The further improvement in MCP flow for MA is consistent with in vitro data reported by Hales et al. (2007), when MA was dosed at 0.75 g MA/d or 1.0 g MA/d they saw a 34% increase ($P < 0.04$) in the quantity of MCP. The increased MCP flow in the current study may be a combined response to an increase in rumen retention time and a reduction in protozoa. Data summarized by Veira (1986) demonstrates a consistent improvement in rumen MCP synthesis in ciliate-free ruminants compared to faunated animals. Although not measured in this study, a reduction in protozoa from MA could potentially increase microbial efficiency as compared to PC due to a reduction in predation of bacteria. Alternatively, the increased particulate passage rate from PC-fed steers could increase microbial efficiency. This is important because MCP yield is the multiple of microbial efficiency and the amount of OM truly fermented in the rumen. When rate of digestion is low and rate of passage is high (i.e. PC-fed steers) total MCP yield can be decreased as particulate passage increases, regardless of a potential increase in microbial efficiency (Owens and Goetsch, 1988). The increase in MCP flow to the small intestine is important because it could more appropriately meet amino acid demands and be beneficial to ruminants with a greater demand for metabolizable protein, such as lactating cows.

4.5. Rumen fermentation and blood urea nitrogen

Similar to the BUN differences in the current study, Caton et al. (1988) observed an increase in BUN when providing a cottonseed-meal based supplement to forage fed lambs. As seen here, some reports show no effect on BUN when ruminants are supplemented with saponins (Wilson et al., 1998; Hristov et al., 1999), but more often it has been cited to reduce BUN (Hussain and Cheeke, 1995; Hussain et al., 1996; Killeen et al., 1998).

The reduction in pH from supplementing protein has also been seen by others and would be expected from the increase in rate of fermentation detected during the *in situ* phase. Olson et al. (1999) measured a linear reduction in pH when intraruminally infusing casein to steers consuming low-quality forage. This was also the case for Guthrie and Wagner (1988) when increasing the level of soybean meal provided to cattle consuming low-quality hay. Even though there was an increase in lactate production for MA steers, there was not a measurable decrease in pH. Hristov et al. (1999) observed no decrease in pH from the control diet when yucca extract was added at 20 and 60 g DM/d (6.28 vs. 6.18 and 6.19, respectively). Similarly,

others have not observed differences in pH with yucca extract supplementation (Valdez et al., 1986; Wilson et al., 1998) and more specifically MA (Sliwinski et al., 2002).

Because of the urea cycle in cattle, it can be expected that protein supplementation will increase RAN similar to BUN. This was true in the current study and as mentioned previously, providing protein to cattle consuming low-quality forage increases RAN. This was observed and the measured concentrations for supplemented and non-supplemented steers were similar to those reported by Olson et al. (1999). It has been shown that MA reduces RAN when saponins were dosed at 600 mg/kg (Sliwinski et al., 2002). This reduction may be due to the ability of yucca saponins to bind N and subsequently release it when concentrations are low (Hussain and Cheeke, 1995). This response was not expressed here where cattle were consuming ad libitum low-quality forage. However, there was a numerical increase in rumen fill ($P = 0.15$) during the *in situ* phase and therefore it warrants further investigation to determine if total RAN is influenced by MA as compared to concentration of RAN.

The increase in protozoa concentrations due to supplementation was expected as protozoa have an amino acid requirement for proliferation and their excretion of ammonia-N is utilized by rumen bacteria as a substrate for synthesis of bacterial protein (Dehority, 2003). Therefore, when rumen degradable protein is deficient, microbial populations will be reduced (Dehority, 2003). It appears that a reduction in protozoa from yucca extract products may be dependent on saponin level, source and diet type. There are data supporting the findings in this study that when saponins are included in ruminant diets there is a suppression of protozoa populations (Valdez et al., 1986; Wallace et al., 1994; Hristov et al., 1999), while there are published data that contradict this when MA was included in high roughage dairy cow diets at three different levels (Sliwinski et al., 2002). The protozoa counts for the positive control diet in the current study were higher than those reported by Sliwinski et al. (2002) and therefore there may have been more opportunity to change these populations in these low-quality roughage diets. The interaction between saponins and membrane lipids is complicated, but it is thought that yucca saponins are effective at suppressing rumen protozoa by reacting with cholesterol in the protozoal cell membrane, causing it to lyse (Cheeke, 2000).

There are limited data available evaluating rumen lactate concentrations in cattle consuming low-quality forage. This is because there are minimal concentrations of lactate in high roughage diets and there is no concern for ruminal acidosis in cattle consuming ad libitum low-quality hay. However, the current study measured rumen lactate production to further validate MA role in altering rumen fermentation through its impact on protozoa. There are no published data to validate the increase in lactate production due to MA supplementation. Nevertheless, protozoa have been known to be mediators in lactate production by engulfing some of the lactate producing bacteria (Veira, 1986). It is suggested that yucca extract is more potent to gram-positive bacteria (Wang et al., 2000), which could lead to a subsequent increase in gram-negative bacteria. This may help explain the increase in lactate and also the increase in propionate (Table 5). Gram-negative bacteria such as *M. elsdenii* and *B. ruminicola* have been identified as bacteria that convert pyruvate to lactate which is converted to acrylyl-CoA and then reduced to propionyl-CoA (Fahey and Berger, 1988). The increased activity of this pathway would aid in explaining the increase in both lactate and propionate, but the concentrations of the bacteria referenced were not determined during this study and should be measured to validate this hypothesis.

The impact of protein supplementation on VFA production has been inconsistent. Olson et al. (1999) observed a linear increase in total VFA concentrations and independent concentrations of isobutyrate, valerate and isovalerate with increased ruminal protein and no impact on acetate or propionate, contradictory to these data. Guthrie and Wagner (1988) did not detect a difference in total VFA concentrations at −3, 0, 1, 3, 6 or 9 h with a reduction in acetate at −3, 0, 1, 3, and 6 h. When including dietary saponins, others have reported a shift toward propionate production, resulting in a decrease in the acetate to propionate ratio (Hristov et al., 1999), which is consistent with a decrease in protozoa numbers (Williams and Coleman, 1997). When MA was evaluated *in vitro*, in high concentrate diet inoculums, there was also no impact on VFA proportions or concentrations (Hales et al., 2007), but Holtshausen et al. (2009) did find an increase in propionate concentrations *in vitro* with no improvement in performance *in vivo*. Even though a reduction in protozoa population was observed, there was not a detectable decrease in the acetate to propionate ratio due to MA supplementation. The C cattle actually had the lowest acetate to propionate ratio which can be attributed to low total VFA concentrations.

5. Conclusion

Additional plant protein supplied to cattle consuming low-quality prairie hay improved hay intake and digestibility as well as N balance. The inclusion of MA in the protein supplement improved rumen DM and aNDF degradability via a decrease in rumen particulate passage rate, but was not successful at improving apparent total tract digestibility. In addition, MA successfully suppressed protozoa after continuous administration during both phases of the trial, suggesting that complete adaptation to saponins by protozoa was not observed in this trial as suggested by Cheeke (2000). The reduction in rumen protozoa may have several positive associative effects including improved N metabolism efficiency, a reduction in methane emissions, a shift in bacterial and fungal populations and a potential increase in MCP flow to the lower gastrointestinal tract (Wallace et al., 1994). This study did observe a subsequent increase in MCP flow to the small intestine, which might prove to be beneficial to those ruminants with a greater metabolizable protein requirement. Micro-Aid® is effective at altering rumen fermentation, but more research is needed to determine the effects of MA on animal performance.

Conflict of interest

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information, and does not imply either recommendation or endorsement by the U.S. Department of Agriculture.

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