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Effects of carbohydrate and nitrogen supplementation on fermentation of cheatgrass (*Bromus tectorum*) in a dual-flow continuous culture system[1]

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ABSTRACT: Cheatgrass (CG; *Bromus tectorum*), an introduced winter annual grass, is an aggressive invader of the sagebrush community in the Western United States. Because of its greater flammability, mature CG constitutes a fire hazard leading to repeated wildfires. One fuel-reduction strategy is livestock grazing. The objective of this study was to evaluate the effects of urea, molasses, or a combination of urea and molasses supplementation of a CG-based diet on digestibility, microbial fermentation, bacterial protein synthesis, and nutrient flow using a dual-flow continuous culture system. Eight fermenters were used in a replicate 4 × 4 Latin square design with four 10-d experimental periods. Experimental treatments (DM basis) were 1) forage only (CON), 2) CG plus urea alone (URE; 1.36% urea), 3) CG plus molasses alone (MOL; 15.9% molasses), and 4) CG plus urea and molasses combined (URE+MOL; 1.28% urea plus 19.3% molasses). Each fermenter was fed 72 g/d of DM, and data were analyzed using the GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC). The true digestibilities of NDF and ADF were not affected by diets ($P > 0.05$). Molasses-containing diets had greater true digestibility of OM ($P = 0.02$). However, true digestibility of CP was increased when molasses was fed alone ($P < 0.01$). Molasses-containing diets had lower pH ($P < 0.01$) and greater VFA concentrations ($P < 0.01$) compared to those of the other diets. The URE+MOL diet resulted in a greater VFA concentration ($P < 0.01$). Propionate concentration increased ($P < 0.01$), whereas acetate concentration decreased ($P < 0.01$) when molasses alone or in combination with urea was added to the diets. Supplying molasses alone resulted in greater ($P = 0.03$) total branched-chain VFA compared to the other diets. The concentration of NH_3–N and total N flow increased ($P < 0.01$) in response to urea supplementation and was greater ($P < 0.01$) when urea alone was supplemented in the diet. On the other hand, molasses-supplemented diets yielded more non-ammonia N ($P < 0.01$) and bacterial N ($P = 0.04$). Supplementation had no effect ($P = 0.83$) on bacterial efficiency. Results from this study indicate that the addition of urea and molasses in a CG-based diet could improve nutrient supply to animals, notably VFA supply and microbial N supply; however, in the levels tested in this study, it did not improve CG utilization as assessed by NDF digestion.

Key words: ammonia nitrogen, in vitro system, liquid molasses, urea, wildfire

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INTRODUCTION

From 2011 to 2015, wildfires have burned more than 13 million ha in the United States (NIFC, 2015). In Nevada, the burned areas have increased 0.6 million ha per decade since the 1970s (Gruell and Swanson, 2012), and cheatgrass (**CG**; *Bromus tectorum L.*) dominated areas burned 4 times more frequently than native vegetation (Whisenant, 1990). Platt and Jackman (1946) reported that CG becomes

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flammable 4 to 6 wk earlier and remain susceptible to wildfires 4 to 8 wk later than native perennials plants.

Cheatgrass is an introduced annual grass and an aggressive invader (Schmelzer et al., 2014). It is a prolific seed producer (Hulbert, 1955), drought tolerant (Melgoza et al., 1990), and grows well up to 2,000 m (NRCS, 2008). The Bureau of Land Management (BLM, 1991) has estimated that CG-dominated landscapes could reach 16.2 million ha.

Consumption of CG by livestock is a strategy that can reduce the fire load (Schmelzer et al., 2008); however, alone, CG does not meet animal requirements, as its CP content can be as low as 3.5% in the winter (Cook and Harris, 1952) and below 7.5% in the summer and fall (Ganskopp and Bohnert, 2001), which are below most ruminant requirements (NRC, 2000). Therefore, N and energy supplementation may provide opportunities to improve CG management strategies.

Scientific literature reporting the effects of carbohydrate and N supplementation on CG-based diets regarding digestion and fermentation is scarce. Therefore, the objective of this study was to evaluate the effects of urea, molasses, or a combination of urea and molasses supplementation on a CG-based diet regarding digestibility, microbial fermentation, bacterial protein synthesis, and nutrient flow using a dual-flow continuous culture system. We hypothesized that supplying carbohydrate and N sources concurrently on a CG-based diet would improve fermentation, which could improve microbial digestion, fermentation, and bacterial protein synthesis.

MATERIALS AND METHODS

Animal care and handling were conducted under protocols approved by the University of Nevada, Reno Institutional Animal Care and Use Committee (IACUC protocol number 00588).

Experimental Design and Diets

For this study, an 8-unit dual-flow continuous culture system (1,223 ± 21 mL; Omni-Culture Plus; Virtis Co. Inc., Gardiner, NY) was used similarly as in Benedeti et al. (2015) and Silva et al. (2016) and described in details below. Each fermenter unit was randomly assigned to receive each diet once over the 4 periods in a replicate 4 × 4 Latin square design. Each 10-d period consisted of a 7-d diet adaptation period followed by a 3-d sampling period. Experimental treatments given on a DM basis were 1) forage only (**CON**; 98% CG), 2) CG plus urea alone (**URE**; 1.36% urea plus 96.5% CG), 3) CG plus molasses alone (**MOL**; 15.9% molasses plus 82.1% CG), and 4) CG plus urea and molasses combined (**URE + MOL**; 1.28% DM urea plus 19.3% molasses plus 77.3% CG). Chemical composition of the ingredients, individual ingredients, and dietary chemical composition are presented in Tables 1 and 2.

Table 1. Chemical composition of the ingredients

	Ingredient	
Item	Cheatgrass	Molasses[1]
DM, %	94.6	70.9
OM, % DM	95.2	90.4
CP, % DM	4.09	5.40
NDF, % DM	69.6	
ADF, % DM	53.2	
NFC[2], % DM	20.3	83.1
EE[3], % DM	1.15	1.86
Ash, % DM	4.80	9.63

[1]Liquid molasses obtained from Cerri Feed Co. (Stockton, CA). Sugar content: minimum 43% guaranteed analysis.

[2]NFC = nonfiber carbohydrates; NFC = 100 − (% NDF + % CP + % EE + % ash), according to NRC (2001).

[3]EE = ether extract.

Ammonium sulfate at a 9:1 ratio was added to urea-containing diets. The CG used was pelleted, molasses was liquid, and forage and supplements were combined at each feeding time. Matured CG was harvested in Reno, NV in August 2014 at 10 cm above soil level. Samples of CG were ground through a 2-mm screen in a Wiley Mill (model 2; Arthur H. Thomas Co., Philadelphia, PA) and pelleted (ECO-3 Pellet Mill and CME ECO-3; Colorado Mill Equipment, LLC, Cañon City, CO).

Dual-Flow Continuous Culture System

Fermentation conditions were maintained constant with temperature set at 39°C and N_2 gas continuously infused at a rate of 40 mL/min. Individual pH meters (model 5997-20; Cole-Parmer, Vernon Hills, IL) were used to monitor the pH of each fermenter. A central propeller apparatus driven by magnets was used to continuously agitate the fermenters contents at a rate of 155 rpm. Artificial saliva (Weller and Pilgrim, 1974) containing 0.4 g/L of urea to stimulate recycled N was continuously infused into fermenters at rate of 2 mL/min, and artificial saliva flow was measured twice daily to monitor the flow rate. The solid mean retention time, solid dilution rate, and liquid dilution rate of the fermenters were 24 h, 5%/h, and 10%/h, respectively, and were maintained by adjusting the buffer input and solid and liquid output from fermenters. Each fermenter was manually fed a total of 72 g DM/d divided in 4 equal portions at 0230, 0830, 1430, and 2030 h.

Experimental Procedures and Sample Collections

Rumen fluid was collected approximately 2 h after morning feeding from 2 ruminally cannulated Aberdeen Angus steers (average BW of 770 kg). For 1 wk before

Table 2. Ingredient and chemical composition of experimental diets

Item	Treatment[1]			
	CON	URE	MOL	URE+MOL
Ingredient, % DM				
Pelleted cheatgrass	98.0	96.5	82.1	77.3
Liquid molasses[2]	0.00	0.00	15.9	19.3
Urea	0.00	1.36	0.00	1.28
Ammonium sulfate	0.00	0.15	0.00	0.14
Mineralized salt[3]	2.00	2.00	2.00	2.00
Chemical composition, % DM				
DM, %	95.3	95.3	91.4	90.6
OM	93.7	92.2	92.8	91.3
CP	4.01	8.24	4.21	8.23
NDF	68.2	67.2	57.1	53.8
ADF	52.2	51.4	43.7	41.1
NFC[4]	19.9	18.1	29.9	30.3
EE[5]	1.13	1.11	1.24	1.25
Ash	6.32	7.77	7.19	8.74

[1]CON = control diet (98% DM of cheatgrass [CG]); URE = CG plus urea alone (1.36% DM of urea plus 96.5% DM of CG); MOL = CG plus molasses alone (15.9% DM of molasses plus 82.1% DM of CG); URE+MOL = CG plus urea and molasses combined (1.28% DM of urea plus 19.3% DM of molasses plus 77.3% DM of CG).

[2]Liquid molasses obtained from Cerri Feed Co (Stockton, CA).

[3]Provided per kilogram of DM: Zn, 56 mg; Mn, 46 mg; Fe, 22 mg; Cu, 12 mg; I, 0.9 mg; Co, 0.4 mg; Se, 0.3 mg; vitamin A, 6,440 IU; vitamin D, 2,000 IU; vitamin E, 16 IU.

[4] NFC = nonfiber carbohydrates; NFC = 100 − (% NDF + % CP + % EE + % ash), calculated according to NRC (2001) , except for diets containing urea and ammonium sulfate, where NFC was calculated according to Hall (2000): NFC = 100 − [% CP − (% CP from urea + % CP from ammonium sulfate) + (% of urea + % of ammonium sulfate) + % NDF + % EE + % ash].

[5]EE = ether extract.

collection, animals were fed an adaptation diet composed of 60% straw, 38% orchard hay, and 2% mineralized salt (DM basis). The rumen content was collected manually from the ventral, central, and dorsal areas of the rumen and was strained through 4 layers of cheesecloth, and approximately 10 L (5 L/animal) of rumen fluid was poured into prewarmed insulated containers. Equal amounts of rumen content from each animal were homogenized thoroughly by agitation, infused with N_2 to maintain the anaerobic environment, and adjusted to 39°C by submerging a 5,000-mL Erlenmeyer flask in a preheated water bath. About 1,250 mL of rumen fluid was then poured into each of the fermentation jars until it cleared the overflow spout.

During the experiment, digesta effluents (liquid and solid) were collected in 4-L plastic containers. To monitor the flow rates, the weights of digesta effluents were recorded daily at 0800 h and discarded during the first 7-d adaptation period. After being emptied on d 7 and during d 8, 9, and 10, digesta effluent containers were submerged approximately two-thirds of the way in a chilled (2°C) water bath, and 25 mL of 50% sulfuric acid was added in each effluent container to prevent further microbial fermentation. During the last 3 d of each period, liquid and solid digesta effluents from each fermenter were combined and homogenized using a mixer (T25 basics; IKA Works, Inc., Wilmington, NC) for 1 min. After mixing, a 500-mL sample was collected via vacuum system and stored at −20°C. An additional 20-mL overflow sample was squeezed through 2 layers of cheesecloth, and two 10-mL aliquots of fluid were preserved with 0.2 mL of 0.2 *N* sulfuric acid and swirled. Then, the samples were centrifuged for 10 min at 10,000 × *g* at 4°C, and the supernatant was frozen at −20°C for later analyzes of NH_3–N and VFA. The 500-mL digesta effluent samples collected on each of the 3 collection days were composited by fermenter by period. The digesta effluent composite (approximately 1,500 mL/fermenter per period) was thawed, placed in a container, and with the aid of a stick was manually homogenized. Then a 300-mL subsample was collected, freeze-dried, and ground using pestle and mortar. The samples were placed in a plastic container for further analyses of DM, OM, ash, NDF, ADF, CP, and ether extract (**EE**).

On d 5, digesta effluents (liquid and solid) were homogenized by vigorous manual shaking, and samples were collected to determine the liquid and solid effluents' background ^{15}N abundance (Calsamiglia et al., 1996). Then, 0.077 g of 10.2% excess $^{15}(NH_4)_2SO_4$ (Sigma-Aldrich Co., St. Louis, MO) was infused into each fermenter to instantaneously label the NH_3–N pool. Artificial saliva was reformulated, and 0.077 g/L of enriched $^{15}(NH_4)_2SO_4$ (Sigma-Aldrich Co.) was added in replacement of isonitrogenous amounts of urea to maintain a steady state concentration of ^{15}N enrichment in fermenters.

On d 7, 8, and 9, the pH of each fermenter was measured 0, 1, 2, 3, 4, 5, and 6 h after feeding using an Accumet portable AP61 pH meter (Fisher Scientific, Atlanta, GA).

On the last day of each period, the entire fermenter content was used to harvest bacteria by mixing in a blender and straining through 2 layers of cheesecloth. Strained contents were centrifuged at 1,000 × *g* at 5°C for 10 min (Sorvall RC-5B Refrigerated Superspeed centrifuge; DuPont Instruments, Wilmington, DE) to remove undigested substrate and possibly some attached microbial biomass. Then, the supernatant was centrifuged at 10,000 × *g* at 5°C for 20 min to isolate microbial biomass pellets (Bach et al., 2008). Supernatant was discarded, and bacterial pellets were prepared by freeze-drying and grinding using mortar and pestle for analyses of ^{15}N enrichment, total N, DM, and ash.

Chemical Analyses

Feed and digesta effluent samples were analyzed for DM (method 934.01), ash (method 938.08), CP (meth-

od 984.13), and EE (method 920.85) according to the AOAC (1990). The OM was calculated as the difference between DM and ash contents. For NDF and ADF, samples were sequentially analyzed and treated with α thermo-stable amylase without sodium sulfite according to Van Soest et al. (1991) and adapted for the Ankom^{200} Fiber Analyzer (Ankom Technology, Macedon, NY).

Nonfiber carbohydrate (**NFC**; % of DM) was calculated as follows according to NRC (2001):

$$\text{NFC} = 100 - (\%\,\text{NDF} + \%\,\text{CP} + \%\,\text{fat} + \%\,\text{ash})$$

For diets containing urea and ammonium sulfate, NFC was calculated as follows according to Hall (2000):

$$\text{NFC} = 100 - [\%\,\text{CP} - (\%\,\text{CP from urea} + \%\,\text{CP from ammonium sulfate}) + (\%\,\text{of urea} + \%\,\text{of ammonium sulfate}) + \%\,\text{NDF} + \%\,\text{EE} + \%\,\text{ash}]$$

The digesta effluent samples were analyzed for NH_3–N content (Chaney and Marbach, 1962), and VFA concentrations were determined using gas chromatography (Varian, Inc., Walnut Creek, CA; Varian Model 3800 equipped with a glass column [180 cm by 4 mm i.d.] packed with GP 10% SP1200/1% H3PO4 on 80/100 Chromosorb WAW [Supelco, Bellefonte, PA]) with N2 used as a carrier gas at a flow rate of 85 mL/min. The oven, injection port, and detector port temperatures were 125°C, 175°C, and 180°C, respectively.

Samples of background, digesta effluents, and bacterial pellets were analyzed for DM, CP, and ash as described previously for feed samples and were analyzed for ^{15}N enrichment according to Werner et al. (1999). Isotope analyses were performed using a EuroVector model 3028 elemental analyzer interfaced to a Micromass IsoPrime stable isotope ratio mass spectrometer. Bacterial N flow and bacterial efficiency were calculated as follows: bacterial N flow (expressed in g/d) = [non-ammonia N (**NAN**) flow × percentage of ^{15}N atom excess of digesta effluent]/(percentage of ^{15}N atom of bacterial pellet), with the ^{15}N digesta effluent background subtracted from ^{15}N enrichment. Bacterial efficiency = bacterial N flow (g)/OM truly digestible (kg; Calsamiglia et al., 1996). Apparent and true digestibilities were calculated as described by Soder et al. (2013) and Benedeti et al. (2015).

Statistical Analysis

Data were analyzed as a replicated 4 × 4 Latin square arrangement using the GLIMMIX procedure of SAS (release 9.4; SAS Inst. Inc., Cary, NC). The following model was used:

$$Y_{ijkl} = \mu + S_i + F(S)_{ij} + P_k + T_l + e_{ijkl}$$

where μ is overall mean, S_i is the square, $F(S)_{ij}$ is the fermenter (F) within the square, P_k is the period, T_l is the treatment, and e_{ijkl} is the residual error associated with ijkl observation. Fermenters within square were random effects, whereas all other factors were fixed. LSMEANS/DIFF LINES was used as the mean method of separation. Least squares means and SEM are reported for all data with a significance declared at $P \leq 0.05$, and trends were discussed at $0.05 < P \leq 0.10$.

The pH data were analyzed using the GLIMMIX procedure of SAS (release 9.4; SAS Inst. Inc.) as a repeated measurement according to the following model:

$$Y_{ijklm} = \mu + S_i + F(S)_{ij} + P_k + T_l + Z_m + ZT_{ml} + e_{ijklm}$$

where μ is the overall mean, S_i is the square, $F(S)_{ij}$ is the fermenter (F) within the square, P_k is the period, T_l is the treatment, Z_m is the time, ZT_{ml} is the interaction between time and treatment, and $e$ijklm is the residual error associated with ijklm observation.

RESULTS AND DISCUSSION

Nutrient Digestibility

To our knowledge, this is the first study to report the effects of CG-based diet supplementation on fermentation. Results for apparent and true digestibilities of dietary nutrients are provided in Table 3. Adding urea alone had a tendency to decrease the apparent digestibilities of DM ($P = 0.10$) and OM ($P = 0.06$). According to Hersom (2008), supplemental feeds provide feed fractions that often have solubility, degradation, and chemical characteristics that are different from the base forage utilized, and these properties of the supplement may exacerbate an asynchronous dietary nutrient supply. It is important to point out that CG and urea have different rates of degradation, and this may be the reason why the URE diet had a tendency to decrease the apparent digestibilities of DM and OM.

The true digestibilities of NDF and ADF (Table 3) were not affected by diets ($P > 0.05$). Although sugars added to forage-based diets have been found to decrease fiber digestion (Khalili and Huhtanen, 1991b; Heldt et al., 1999), this was not the case in the current study. Brown (1990) reported that NDF digestibility was decreased by 5% units in feeding trials with ammoniated tropical grasses supplemented with urea or cottonseed meal and molasses at 25% of ration DM. In the present study, CON and MOL had lower dietary CP compared to that of the other diets; nevertheless, it did not compromise the fiber digestibility of these diets. According to Jones et al. (1998), the depression in NDF digestibility with sugar feeding may be a function of changing ruminal pH and/or

Table 3. Effects of cheatgrass (CG)-based diets supplemented with urea and molasses alone or in combination on digestibility in continuous culture

	Diet[1]					
Item	CON	URE	MOL	URE+MOL	SEM	*P*-value
Apparent digestibility, %						
DM	18.4	13.0	17.7	19.1	1.93	0.10
OM	29.5	23.0	30.2	29.4	2.09	0.06
True digestibility, %						
DM	31.7	28.8	34.6	37.0	2.80	0.18
OM	39.0^{ab}	33.8^{b}	42.2^{a}	42.7^{a}	2.12	0.02
CP	74.6^{b}	52.7^{c}	87.2^{a}	67.4^{b}	5.71	<0.01
NDF	69.4	66.0	67.7	68.2	2.13	0.71
ADF	69.6	65.9	69.1	71.5	1.94	0.20

$^{a–c}$Least squares means within the same row with different superscripts differ ($P \leq 0.05$).

[1]CON = control diet (98% DM of CG); URE = CG plus urea alone (1.36% DM of urea plus 96.5% DM of CG); MOL = CG plus molasses alone (15.9% DM of molasses plus 82.1% DM of CG); URE+MOL = CG plus urea and molasses combined (1.28% DM of urea plus 19.3% DM of molasses plus 77.3% DM of CG).

a competition between cellulolytic and amylolytic bacteria for N and other nutrients. In general, discrepancies in the literature regarding the response in fiber digestibility to molasses supplementation may be explained by the wide variability in molasses sources, supplementation levels, and forage quality (Soder et al., 2010).

Molasses-containing diets (MOL and URE+MOL) had greater true OM digestibility ($P = 0.02$; Table 3). However, true CP digestibility was increased when molasses was fed alone ($P < 0.01$). This may indicate that the energy in molasses was used to release the protein in the forage content, resulting in a greater CP true digestibility. Moore et al. (1999) stated that the inclusion of supplemental feeds creates a complexity in the feeding scenario that may result in an improved or detrimental animal response. Thus, this may explain why CG supplemented with urea and molasses had lower ($P < 0.01$) CP true digestibility compared with feeding CG alone.

pH and Volatile Fatty Acid Concentration

Not surprisingly, the pH and VFA concentration of rumen contents showed an inverse relationship. Molasses-containing diets had lower pH ($P < 0.01$) and greater VFA concentrations ($P < 0.01$) compared to the other diets (Table 4). However, the URE+MOL diet resulted in a greater ($P < 0.01$) VFA concentration. This result indicated that VFA concentration is improved to a greater extent when molasses is supplied in addition to urea rather than when they are fed separately. The greater VFA concentration in this diet may be the result of a more complementary utilization of energy and N by microorganisms, supporting our hypothesis that carbohydrate and N supplementation may improve fermentation. According to Mould et al. (1983), nutrient supplementation may enhance rumen microbial population and VFA concentration. Moreover, many studies have found that supplemental rumen-degraded carbohydrates can result in increased VFA concentration and elicit ruminal pH depression (Kennedy and Bunting, 1992; Bargo et al., 2002).

Total VFA concentrations observed in the present study (mean, 68.15 m*M*; Table 4) for molasses-supplemented diets are slightly lower than those in previous continuous culture experiments in which molasses was supplemented at 5% (DM basis) in an orchardgrass-based diet (mean, 74.30 m*M*; Soder et al., 2010). A possible reason for the different outcome of this study compared with that of Soder et al. (2010) may be due in part to the type of forage and molasses used in this trial. Just as it did for true CP digestibility, supplying the diet with only urea resulted in a decreased VFA concentration ($P < 0.01$). The mean VFA concentration for the CON diet was 60.5 m*M*. Results in the present trial are slightly lower than those in the work of Lourenco et al. (2008), which found a 67.0 m*M* ruminal VFA concentration when 60 g of dried perennial ryegrass was fed in a dual-flow continuous culture system. This shows that CG has the potential to be used as feed for ruminant animals and that the difference in the VFA concentration may be attributed to the different forage quality in these 2 studies.

Propionate concentration increased ($P < 0.01$) whereas acetate concentration decreased ($P < 0.01$) when molasses alone or in combination with urea was added to the diets (Table 4). Changes in fermentation patterns likely reflect shifts in bacterial population that lead to changes in fermentable substrates (Ribeiro et al., 2005). Soluble carbohydrates have been found to increase the molar proportion of propionate in the rumen (Kellogg and Owen, 1969). The greater propionate concentrations in the diets supplemented with molasses may be beneficial to the animal since propionate is the main gluconeogenic precursor in ruminants (Bergman et al., 1968). Butyrate concentration had a tendency to

Table 4. Effects of cheatgrass (CG)-based diets supplemented with urea and molasses alone or in combination on pH and total VFA concentration and profile in continuous culture

	Diet[1]					
Item[2]	CON	URE	MOL	URE+MOL	SEM	*P*-value
pH	6.99[b]	7.50[a]	6.45[d]	6.59[c]	0.05	<0.01
Total VFA, m*M*	60.5[b]	41.5[c]	67.1[ab]	69.2[a]	2.71	<0.01
Individual VFA, mol/100 mol						
Acetate	70.7[a]	70.0[a]	66.3[b]	66.2[b]	0.64	<0.01
Propionate	21.6[b]	21.8[b]	24.1[a]	25.6[a]	0.61	<0.01
Butyrate	7.90	8.26	9.32	8.51	0.38	0.07
Isobutyrate	0.15[ab]	0.34[a]	0.08[b]	0.03[b]	0.08	0.02
Valerate	0.05	0.03	0.22	0.04	0.06	0.07
Isovalerate	0.05	0.03	0.21	0.05	0.06	0.08
A:P ratio	3.30[a]	3.24[a]	2.80[b]	2.60[b]	0.10	<0.01
Total BCVFA, m*M*	0.07[b]	0.06[b]	0.14[a]	0.04[b]	0.03	0.03

[a–d]Least squares means within the same row with different superscripts differ ($P \leq 0.05$).

[1]CON = control diet (98% DM of CG); URE = CG plus urea alone (1.36% DM of urea plus 96.5% DM of CG); MOL = CG plus molasses alone (15.9% DM of molasses plus 82.1% DM of CG); URE+MOL = CG plus urea and molasses combined (1.28% DM of urea plus 19.3% DM of molasses plus 77.3% DM of CG).

[2]A:P = acetate to propionate ratio; BCVFA = branched-chain VFA computed as the sum of isobutyrate and isovalerate.

be greater ($P = 0.07$) when molasses was supplemented alone or in combination with urea. These results are in agreement with the findings of Piwonka and Firkins (1996), which reported an increase in butyrate concentration with the addition of soluble sugars. Furthermore, according to these authors, the increase in butyrate concentration could be attributed to a change in fermentation pathways to accommodate the greater flux of hydrogen from rapidly fermented sugar sources. In agreement, other studies observed that soluble carbohydrates increased the molar proportion of propionate and/or butyrate at the expense of acetate (Chamberlain et al., 1985; Khalili and Huhtanen, 1991a). The inverse relationship observed between acetate and butyrate is reasonable given that both are derived from acetyl-CoA. Additionally, acetate can be converted to butyrate in the rumen as a mechanism to incorporate metabolic hydrogen in accumulated H_2 into VFA (Ungerfeld et al., 2006).

Molasses-containing diets resulted in a decreased isobutyrate concentration ($P = 0.02$) and acetate to propionate ratio ($P < 0.01$; Table 4). This result is similar to those of Bond and Rumsey (1973), who reported lower concentrations of isobutyrate when cattle were provided ad libitum access to a liquid molasses-urea supplement. The reduction in the acetate to propionate ratio was expected considering that molasses-containing diets had a greater ($P < 0.01$) molar proportion of propionate. Adding molasses alone to the diet resulted in a tendency to increase the concentration of valerate ($P = 0.07$) and isovalerate ($P = 0.08$). Yet, the MOL diet had greater ($P = 0.03$) total branched-chain VFA compared to the other diets. The branched-chain VFA are products of protein degradation as well as substrates for synthesis of leucine, isoleucine, and valine in certain ruminal bacteria (Allison, 1970). The change in branched-chain VFA may be related to the difference in CP digestibility observed in the present study.

Nitrogen Metabolism

As expected, NH_3–N concentration increased ($P < 0.01$) in response to urea supplementation and was greater ($P < 0.01$) when urea alone was supplemented in the diet (Table 5). The NH_3–N concentration observed in the present study (mean, 22.1 mg/100 ml) for the URE diet was similar to that of previous continuous culture experiments that replaced highly degraded protein (soybean meal) with less degraded sources (fish meal) in a grass hay and wheat straw-based diet (mean, 19.51 mg/100 ml; Hussein et al., 1991). Ammonia can serve as a N source for microbial growth when carbohydrate is available as an energy source (Allison, 1969). Given that, the optimal NH_3–N concentration for the maximal rate of fermentation of high-forage diets is related to the availability of an energy-yielding substrate (Mehrez et al., 1977). Thus, the excessive NH_3–N accumulation in the rumen when urea was supplemented may indicate that the rate of urea degradation exceeded that of forage carbohydrate fermentation and the rate of ammonia uptake by the ruminal microorganisms.

Compared to the URE diet, the NH_3–N concentration was decreased ($P < 0.01$) when urea was supplemented in combination with molasses (Table 5). It has been suggested that the ruminal NH_3–N concentration decreases in response to factors that promote NH_3–N utilization, such as synchronization of fermentable energy and N (Petit and Veira, 1994; Kolver et al., 1998; Olson et al., 1999). Hersom (2008) reported that the efficiency

Table 5. Effects of cheatgrass (CG)-based diets supplemented with urea and molasses alone or in combination on N metabolism in continuous culture

	Diet[1]					
Item	CON	URE	MOL	URE+MOL	SEM	*P*-value
NH_3–N, mg/100 ml	4.49[c]	22.1[a]	0.85[d]	13.7[b]	1.25	<0.01
CP digestibility, %	74.6[b]	52.7[c]	87.2[a]	67.4[b]	5.71	<0.01
N flow, g/d						
Total N	0.90[b]	1.26[a]	0.96[b]	1.28[a]	0.04	<0.01
NH_3–N	0.13[c]	0.65[a]	0.03[d]	0.41[b]	0.04	<0.01
NAN[2]	0.77[b]	0.60[c]	0.94[a]	0.87[ab]	0.06	<0.01
Bacterial N	0.53[b]	0.53[b]	0.65[ab]	0.69[a]	0.06	0.04
Dietary N	0.24[ab]	0.07[c]	0.28[a]	0.18[b]	0.03	<0.01
Bacterial efficiency[3]	22.1	24.1	23.6	24.6	2.20	0.83

[a–d]Least squares means within the same row with different superscripts differ ($P \leq 0.05$).

[1]CON = control diet (98% DM of CG); URE = CG plus urea alone (1.36% DM of urea plus 96.5% DM of CG); MOL = CG plus molasses alone (15.9% DM of molasses plus 82.1% DM of CG); URE+MOL = CG plus urea and molasses combined (1.28% DM of urea plus 19.3% DM of molasses plus 77.3% DM of CG).

[2]NAN = non-ammonia N.

[3]Bacterial efficiency = grams of bacterial N per kilogram of OM truly digested.

of nutrient utilization dictates whether nutrient synchrony may be a successful strategy. Moreover, according to this author, ruminal variables such as pH, total VFA, individual VFA concentrations, and NH_3–N concentration are measurements associated with nutrient synchrony. In the current study, the URE diet may not have provided the rumen microorganisms with the environment necessary to use the NH_3–N produced through urea degradation.

Molasses supplementation decreased ($P < 0.01$) the NH_3–N concentration compared with the concentrations of the CON and URE diets (Table 5). In alignment with the present study, Petit and Veira (1994) also observed a decrease in the NH_3–N concentration of timothy silage-based diets when molasses was incorporated at 7.5 and 15% of the total feed DM, and some values were lower than 5 mg/100 mL, which is considered the minimum value needed to support maximum net microbial protein production (Satter and Slyter, 1974). In addition, several in vitro (Stern et al., 1978; Henning et al., 1991) and in vivo (Casper and Schingoethe, 1989; Cameron et al., 1991) studies demonstrated that infusions of increasing amounts of readily fermentable carbohydrates may decrease NH_3–N concentrations due to improved N uptake by ruminal microorganisms.

Molasses supplemented alone also decreased ($P < 0.01$) pH compared to the other diets. The mean pH for molasses-containing diets was 6.52 (Table 4). This result is similar to the findings of Hoover (1986), which identified 6.4 as the optimum ruminal pH for cellulose digestion. Greater ruminal pH could be associated with a greater ruminal NH_3–N concentration derived from greater dietary protein or urea concentrations (Haaland et al., 1982). According to Owens et al. (1998), ammonia is the primary ruminal base, which may explain the reason that the URE diet had greater pH ($P < 0.01$).

Urea-containing (URE and URE+MOL) diets had a greater flow of total N and NH_3–N ($P < 0.01$), which is consistent with the urea supplementation that provided additional N compared with the other diets (Table 5). On the other hand, molasses-supplemented diets yielded more NAN ($P = 0.04$) and bacterial N ($P < 0.01$) in the effluent than CON and URE. Results from the current study and others (Hoover and Stokes, 1991) suggest that the rate of digestion of carbohydrates is the major factor controlling the energy available for microbial growth. Urea is rapidly hydrolyzed to ammonia by the ruminal bacteria (Bunting et al., 1989a), with amounts ranging from 0 to over 80% of ammonia from urea degradation, which may be incorporated into bacterial N (Salter et al., 1979; Bunting et al., 1989b), and the availability of energy is the major determinant of that percentage. The ^{15}N studies of Nolan (1975) indicated that 50% or more of microbial N is derived from NH_3 and the rest from peptides and AA. This may explain why the MOL diet, which had the lowest ($P < 0.01$) NH_3–N concentration, had greater ($P < 0.01$) bacterial N flow compared to the URE diet.

According to Rooke et al. (1987), a reduction in ruminal NH_3–N concentration may reflect an improvement in N use and subsequent increase on bacterial CP synthesis in the rumen. However, this indicator suggesting improved N use was not accompanied by a general improvement in bacterial efficiency in the present study since supplementation had no effect ($P = 0.83$) on bacterial efficiency (Table 5). In the current study, bacterial efficiency was calculated using bacterial growth divided by OM truly digested in the rumen; therefore, possible explanations for the lack of response in bacterial efficiency when molasses and urea were supplemented may be bacterial washout or greater OM digested in the molasses- and urea-supplemented diets.

Supplying N and energy to rumen microorganisms has been shown to enhance the capture of rumen-degraded N and increase microbial growth and efficiency (Sniffen et al., 1983). However, experimental evidence showing that N and energy synchronization in fact increase microbial protein syntheses are somewhat controversial. Witt et al. (1999a) reported that synchronous treatments containing rapidly degraded OM sources yielded more purine derivatives and increased the efficiency of microbial protein synthesis. However, another study conducted by the same group (Witt et al., 1999b) did not show any improvement in microbial protein synthesis with synchronized diets. Similarly to the current study, Salter et al. (1983) reported no significant effects of synchronizing glucose and urea supply in the rumen of straw-fed steers on microbial efficiency. Although not significantly affected by diet, bacterial efficiency was slightly greater in the URE + MOL diet (Table 5). The synchrony index (**SI**) has been used to assess energy and N synchronization in the rumen. Bacterial efficiency found in the present study for the URE+MOL diet was 24.6 g of N/kg of OM truly digested in the rumen, which is close to the optimal ratio of 25 g of N/kg of OM truly digested in the rumen proposed by Czerkawski (1986). This is very close to the mean bacterial efficiency found in the present study for the URE + MOL diet (24.6 g of N/kg truly rumen-digested OM).

When taken together, the results of true OM digestibility, total VFA concentration, pH, NH_3–N concentration, and bacterial N flow, which were all greater in the diet where molasses was supplemented in combination with urea, support our hypothesis that carbohydrate and N supplementation in CG-based diets may positively affect fermentation.

Conclusion

Results from this study indicate that the addition of urea and molasses in a CG-based diet may improve nutrient supply to animals, notably VFA supply and microbial N supply; however, the levels tested in this study did not improve CG utilization as assessed by NDF digestion. Ruminal VFA and microbial N are indicators of rumen function and may indicate a better ruminal environment; however, they did not translate to greater digestibility.

Future studies should focus on CG supplementation strategies that would allow improvements in digestibility and possibly greater animal intake to reduce CG accumulation and therefore alleviating large-scale wildfires in the United States.

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