Nutritional evaluation and ruminal fermentation patterns of kochia compared with alfalfa and orchardgrass hays and ephedra and cheatgrass compared with orchardgrass hay as alternative arid-land forages for beef cattle in two dual-flow continuous culture system experiments[1]

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ABSTRACT: The objective was to evaluate the ruminal fermentation patterns of forage kochia (**FK**) compared with alfalfa hay (**AH**) and orchardgrass hay (**OH**) (Exp. 1), and ephedra (**EPH**) and immature cheatgrass (**CG**) compared with OH (Exp. 2), using a dual-flow continuous culture system. Two in vitro experiments were conducted, and in each experiment, treatments were randomly assigned to six dual-flow fermenters (1,223 ± 21 mL) in a replicated 3 × 3 Latin square design, with three consecutive periods of 10 d each, consisting of 7 d for diet adaptation and 3 d for sample collection. Each fermenter was fed a total of 72 g/d (DM basis) and treatments were as follows: Exp. 1: 1) 100% OH, 2) 100% AH, and 3) 100% dried FK. Exp. 2: 1) 100% OH, 2) 100% dried CG, and 3) 100% dried EPH. On day 8, 9, and 10, samples of solid and liquid effluent from each fermenter were taken for digestibility analysis, and subsamples were collected for NH_3-N, VFA, and bacterial N determinations. Data were analyzed using the MIXED procedure of SAS. In Exp. 1, treatments did not affect DM, OM, and NDF digestibilities, total VFA and molar proportions of acetate, propionate, butyrate, and branched-chain VFA. True CP digestibility, ruminal NH_3-N concentration, and total N, NH_3-N, NAN, and dietary N flows (g/d) were greater ($P < 0.05$) for FK compared with the other forages. However, treatments did not affect bacterial efficiency. In Exp. 2, DM, OM, and CP digestibilities were greater ($P = 0.01$) for EPH, and NDF digestibility was greater ($P < 0.05$) for EPH and CG compared with OH. Ephedra had the highest ($P < 0.05$) pH and acetate:propionate ratio and the lowest ($P < 0.05$) total VFA concentration. Total VFA, ruminal NH_3-N concentration, and NH_3-N flow (g/d) were highest ($P < 0.05$) for CG. Total N flow and bacterial efficiency were highest ($P < 0.05$) for OH and CG, while the flows (g/d) of NAN, bacterial N, and dietary N were greater ($P < 0.05$) for OH compared with the other forages. Results indicate that when compared with AH and OH (Exp. 1), FK has similar ruminal fermentation patterns and may be an adequate alternative for beef cattle producers. Furthermore, when compared with OH (Exp. 2), immature CG may also be an adequate forage alternative. This is especially important for areas in which conventional forages may not grow well such as the U.S. arid-land. However, EPH should not be used as the sole forage due to its poor ruminal fermentation as evidenced by the lowest total VFA concentration and propionate molar proportion.

Key words: *Bromus tectorum*, digestibility, *Ephedra nevadensis*, in vitro, *Kochia prostrata L.*, volatile fatty acid

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INTRODUCTION

Arid and semiarid lands make up more than a third of western North America (Huntsinger and Starrs, 2006) with over 160 million hectares of grazing lands located in the western U.S. dry lands (Sobecki et al., 2001). Arid and semiarid lands rely on irrigation to produce biomass, and given that water availability is among the most critical resources in agriculture, alternative water-efficient crops should be evaluated.

Kochia (*Kochia prostrata* L.), ephedra (*Ephedra nevadensis*), and cheatgrass (*Bromus tectorum*) are forages well adapted to arid and semiarid lands. Kochia (FK) is a semievergreen, half-shrub, native to regions of Central Eurasia, is adapted to high saline sites, and is fire tolerant (Harrison et al., 2002). It is known as "alfalfa of the desert" (Waldron et al., 2005), in reference to its nutritive value. The FK crude protein concentration may decrease with maturity; however, it can reach up to 21% in the autumn (Shenkoru et al., 2015). Ephedra (**EPH**) is a xerophytic evergreen shrub, native to dry areas of western North America and can be found in desert grasslands (USDA, 2006). Some EPH genera may contain ephedrine, a toxic compound; however, the *Nevadensis* genus can be grazed by cattle without causing toxicity (Keeler, 1989). Cheatgrass (**CG**) is an annual grass, native to the Mediterranean region (USDA, 2008), is drought tolerant (Melgoza et al., 1990), and its CP concentration can range from 3.0% to 22% depending on the season (Ganskopp and Bohnert, 2001).

Although these forages have potential to grow in arid regions, their ruminal fermentation patterns are not well known. Therefore, the objective was to conduct two experiments to evaluate the ruminal fermentation patterns of FK compared with AH and OH (*Exp. 1*), and EPH and CG compared with OH (Exp. 2), using a dual-flow continuous culture system. We hypothesized that FK compared with AH or OH and EPH and CG compared with OH may have similar ruminal fermentation patterns and therefore may be alternative forages for beef cattle producers in arid-land.

MATERIAL 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 # 00588).

Two in vitro experiments (Exp. 1 and 2) were conducted in which experimental procedures were similar unless otherwise stated.

Experimental Design and Diets

For each experiment, 6-unit dual-flow continuous culture system (1,223 ± 21 mL; Omni-Culture Plus; Virtis Co. Inc, Gardiner, NY) was used similar to that described by Hoover et al. (1989) and recently modified by Benedeti et al. (2015), Silva et al. (2016), and Paula et al. (2017). Modifications included smaller fermentation jars, different saliva solution and flow, N_2 gas infusion rate, and microbial markers; specifics are detailed below. Fermenter units were randomly assigned to receive each diet once over each period. Experiments were designed as a replicated 3 × 3 Latin square, with three consecutive periods of 10 d each, consisting of 7 d for diet adaptation and 3 d for sample collection.

In Exp. 1, treatments given on a DM basis were 1) 100% orchardgrass hay (**OH**), 2) 100% alfalfa hay (**AH**), and 3) 100% dried forage kochia (**FK**). The AH was prepared from the second cutting at 10% bloom and was harvested in June. The FK samples were harvested in November (late fall) from different locations in the Gund Ranch Research and Training Facility, Austin, Nevada. The OH was purchased from a local animal feed store. All forages were dried at 55 °C for 48 h and ground through a 2 mm screen in a Wiley Mill (Model #2, Arthur H. Thomas Co., Philadelphia, PA).

In Exp. 2, treatments given on a DM basis were 1) 100% OH, 2) 100% immature dried CG, and 3) 100% dried forage ephedra (EPH). Immature CG used was in the vegetative stage of growth and was harvested in April from different locations in an area near the Rancho San Rafael Regional Park, Reno, Nevada. The EPH was harvested in December from different locations at Lincoln County, Nevada. The OH was purchased from a local animal feed store. All forages were dried at 55 °C for 48 h and ground through a 2 mm screen and pelleted. Forages chemical compositions are presented in Table 1 and 2.

Dual-flow Continuous Culture System

Fermentation conditions were maintained constant with temperature set at 39 °C, and N_2

Table 1. Feed chemical composition (% DM, unless otherwise stated; Exp. 1)

	Forages		
Item[a]	OH	AH	FK
DM, %	95.2	93.8	94.2
OM	91.8	91.9	91.3
CP	10.3	15.7	20.9
NDF	59.5	44.3	30.9
ADF	39.9	32.5	19.3
NFC[b]	14.7	29.4	33.6
Ether extract	3.04	2.54	5.93
Ash	8.20	8.10	8.70

OH, 100% orchardgrass hay; AH, 100% alfalfa hay; FK, 100% dried forage kochia.

[a]Each fermenter was fed 2 g/d of mineralized salt (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).

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

Table 2. Feed chemical composition (%DM, unless otherwise stated; Exp. 2)

	Forages		
Item[a]	OH	CG	EPH
DM, %	94.3	92.1	93.6
OM	93.6	93.5	93.3
CP	9.94	16.1	10.4
NDF	48.7	52.2	46.8
ADF	46.4	30.7	27.1
NFC[b]	31.9	22.7	34.7
Ether extract	2.97	2.46	1.35
Ash	6.36	6.42	6.63

OH, 100% orchardgrass hay; CG, 100% immature dried cheatgrass; EPH, 100% dried forage ephedra.

[a]Each fermenter was fed 2 g/d of mineralized salt (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).

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

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. In Exp. 1, the culture pH was maintained under constant control at 6.8 ± 0.05 using an automatic addition of 5 N NaOH or 3 N HCl when necessary.

A central propeller apparatus driven by magnets was used to continuously agitate the fermenter content at the rate of 155 rpm. Artificial saliva was continuously infused into fermenters at rate of 2 mL/min and was prepared as described by Weller and Pilgrim (1974); however, urea was not added in Exp. 1 given the high CP concentration of FK. Solid mean retention time, solid dilution rate, and liquid dilution rate of fermenters were 24%/h, 5%/h, and 10%/h, respectively and were maintained by adjusting artificial saliva input and solid and liquid output from fermenters. Each fermenter was manually fed a total of 72 g DM/d divided into 12 portions (at 0800, 1000, 1200, 1400, 1600, 1800, 2000, 2200, 2400, 0200, 0400, and 0600 h, Exp. 1) to reduce the need for external acid/base infusion for pH controlling or 2 equal portions (at 0800 and 2000 h, Exp. 2) to optimize labor and better represent animal conditions.

Experimental Procedures and Sample Collections

Ruminal fluid was collected approximately 2 h after morning feeding from two ruminally cannulated steers (average BW of 650 and 800 kg, Exp. 1 and 2, respectively). During 2 wk before collection, animals were fed an adaptation diet composed of 100% AH (Exp. 1) or a commercial mixture of AH and grass hay (Exp. 2). Ruminal content was collected manually from the ventral, central, and dorsal areas of the rumen and was strained through four layers of cheesecloth, and approximately 10 L (5 L/animal) of ruminal fluid was poured into prewarmed insulated containers. Equal amounts of ruminal content from each animal were homogenized thoroughly by agitation, infused with N_2 gas to maintain an 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 ruminal 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, during the first 7 d adaptation period the weights of digesta effluents were recorded daily and discarded. On day 8, 9, and 10 digesta effluents containers were submerged approximately two-thirds of the way in a chilled (2 °C) water bath, to prevent further microbial fermentation. During the last 3 d of each period, digesta effluents from each fermenter were weighed, combined, and homogenized using a mixer (T25 basics, IKA Works, Inc, Wilmington, NC) for 1 min at 13,400 rpm. After mixing, a 500 mL sample was collected via vacuum system and stored at −20 °C for analysis.

An additional two, 10 mL digesta effluents subsamples were collected and filtered through four layers of cheesecloth. Both 10 mL aliquots of fluid were preserved with 0.2 mL of 0.2 N sulfuric acid (Exp. 1) or one 10 mL aliquot was preserved with 0.2 mL of 0.2 N sulfuric acid (NH_3-N) and

another 10 mL aliquot was preserved with 2 mL of 25 N metaphosphoric acid (VFA; Exp. 2) due to the gas chromatography columns used, which are described below. The samples were then centrifuged for 10 min at 10,000 × *g* at 4 °C, and the supernatant was stored at −20 °C for later VFA and NH_3-N analyses.

The 500 mL digesta effluents samples collected on each of the three collection days per period were composited by fermenter by period. Digesta effluent composite (approximately 1,500 mL/fermenter per period) was thawed, placed in a container, and homogenized by hand using a glass stirring rod. Then 300 mL subsample was collected, freeze-dried, and ground using mortar and pestle method. Samples were placed in a plastic container for further analyses of DM, OM, ash, NDF, CP, and ether extract (**EE**).

In Exp. 2, on day 5, digesta effluents (liquid and solid) were homogenized, and samples were collected to determine the 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 pool in the fermenters. 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 the fermenters. On days 7, 8 and 9 of Exp. 2, pH of each fermenter was measured 0, 2, 6, and 10 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 for 30 s at 8,000 rpm (Conair Waring Seven-Speed Blenders) and straining through four layers of cheesecloth. Strained contents were centrifuged at 1,000 × *g* at 5 °C for 10 min (Sorvall RC-5B Refrigerated Super Speed Centrifuge, DuPont Instruments, Wilmington, DE) to remove feed particles (Bach et al., 2008). In Exp. 1, supernatant was centrifuged at 10,000 × *g* at 5 °C for 20 min to isolate bacterial pellets (Bach et al., 2008). In order to yield greater bacterial pellets for ^{15}N analysis, in Exp. 2, supernatant was centrifuged at 11,250 × *g* at 5 °C for 20 min (Beckman Coulter Avanti® JXN- 30 refrigerated high-speed centrifuge), then supernatant was discarded and pellets were resuspended in 200 mL of McDougall solution (McDougall, 1948) and were centrifuged at 16,250 x *g* at 5 °C for 20 min to isolate microbial biomass pellets. Bacterial pellets were obtained following the procedure described by Krizsan et al. (2010). In both experiments, bacterial pellets were freeze-dried, ground using mortar and pestle, and stored for further analyses of total N, DM, ash, and total purines (Exp. 1), and ^{15}N enrichment (Exp. 2).

Chemical Analyses

According to AOAC (2016), feed and digesta effluents samples were analyzed for DM (method 930.15), ash (method 942.05), and EE (method 2003.05), with OM calculated as the difference between DM and ash concentrations. Total N (method 990.03) was determined by Tecator Kjeltec auto 2400 analyzer (FOSS Tecator, Sweden; Exp. 1) and by Leco combustion N analyzer (Leco CN628 Carbon/Nitrogen Analyzer, Leco Instruments Inc, St. Joseph, MI; Exp. 2). For NDF and ADF, samples were sequentially analyzed and treated with alpha 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); solutions as in method 973.18 (AOAC, 2016).

Nonfiber carbohydrate (% of DM) was calculated according to NRC (2001):

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

Bacteria samples were analyzed for DM, CP, and ash as described previously for feed samples. Digestibilities of DM, OM, CP, and NDF were calculated as described by Soder et al. (2013) and Benedeti et al. (2015).

Digesta effluent samples were analyzed for NH_3-N concentration (Chaney and Marbach, 1962). In Exp. 1, VFA concentrations were determined using gas chromatography (Varian Model 3800; Varian, Inc., Walnut Creek, CA; equipped with a glass column [180 cm × 4 mm i.d.] packed with GP 10% SP-1200/1% H_3PO_4 on 80/100 Chromosorb WAW [Supelco, Bellefonte, PA]), with N_2 used as a carrier gas at a flow rate of 85 mL/min. The oven, injection port, and detector port temperatures were 125, 175, and 180 °C, respectively. In Exp. 2, VFA concentrations were analyzed by capillary gas chromatography (Agilent 6890N, Agilent Technologies, Santa Clara, CA, USA) equipped with a capillary column (HP-FFAP, 10 m by 530 um by 1.00 um film thickness (Agilent Technologies). Helium was used as the carrier gas at a flow rate of 27 cm/s. The oven temperature was programmed as 90 °C for 2 min, increasing from 90 °C to 190 °C at 15 °C/min and holding 190 °C for 2 min.

In *Exp.1*, concentrations of total purines (Zinn and Owens, 1986) in digesta effluents and bacteria samples were used to partition N flow into bacterial and nonbacterial fractions and to calculate DM and OM digestibilities. In Exp. 2, samples of background, digesta effluents, and bacteria were analyzed for ^{15}N enrichment according to Werner et al. (1999). Isotope analyses were performed using a Eurovector elemental analyzer (Euro EA 3000, Eurovector S.P.A., Milano, Italy) interfaced to a Micromass Isoprime stable isotope ratio mass spectrometer (IsoPrime, Micromass UK Ltd., Manchester, UK). Bacterial nitrogen flow and bacterial efficiency were calculated as follows: Bacterial nitrogen flow (g/d) = (nonammonia nitrogen (NAN) flow × percentage of ^{15}N atom excess of digesta effluent)/(percentage of ^{15}N atom of bacterial pellet), with ^{15}N digesta effluents background subtracted from ^{15}N enrichment. Bacterial efficiency = Bacterial nitrogen flow (g)/OM truly digested (kg) (Calsamiglia et al., 1996).

Statistical Analysis

For both experiments, data were analyzed as a replicated 3 × 3 Latin square arrangement, using MIXED procedure of SAS (SAS, 2002). The following model was used:

$$Y_{ijkl} = \mu + S_i + F_j + P_k + T_l + \varepsilon_{ijkl}$$

where μ is overall mean; S_i is the square, F_j is the fermenter, P_k is the period, T_l is the treatment, and ε_{ijk} is the residual error associated with ijkl observation. Fermenters and period were random effects; whereas, all other factors were fixed effects. Least squares means and SEM were reported for all data with significance declared at $P < 0.05$.

RESULTS AND DISCUSSION

Experiment 1

Nutritional composition of tested forages. Producers from western United States and ranchers in Central Asia have been using FK due to its agronomic characteristics (drought-tolerance and low soil fertility requirements) and CP concentration for grazing beef cows during late fall and early winter as an alternative to traditional harvested hay winter feeding program such as AH and OH (USDA, 2012; Waldron et al., 2006, 2010).

Composition data on the forages used in the trial is shown in Table 1. Concentrations of DM, CP, NDF, and ADF in AH and OH suggest that these forages were of typical composition as previously reported by the NRC (2016). Dietary DM concentration was comparable among forages and CP (20.9%) was numerically greater, and NDF and ADF (30.9% and 19.3%, respectively) concentrations were numerically lower in FK compared with the other forages. These values are within the range described by NRC (2016) tabular values for AH, indicating that FK is a high CP source that can be used as alternative forage for beef cattle in arid-lands.

Nutrient digestibility. Treatments did not affect DM, OM, and NDF digestibilities (Table 3). However, true CP digestibility was highest for FK and lowest for OH ($P = 0.02$). A possible explanation for the greater true CP digestibility observed in the FK compared with the other treatments could have been due to a better AA profile in FK, which would impact CP digestion; however, we did not measure AA composition in the present study.

Nutrient digestibility is directly related with ruminal pH, according to Mould and Ørskov (1983), ruminal pH below 6.1 can depress fiber degradation. In a continuous culture study, Shriver et al. (1986) reported a reduction in OM digestibility with a 6.2 ruminal pH. In addition, de Veth and Kolver (2001) reported that ruminal digestibility can decrease when pH is less than 5.8; the authors also found that increases in forage digestion can be achieved within the pH range of 5.8 to 6.6. Yet, Waldo et al. (1972) observed that ruminal retention time has been associated with changes in digestibility. In Exp. 1, pH was automatically controlled and maintained at 6.8 ± 0.05; additionally, fermenters were fed equal amounts (72 g/d DM basis), forages had the same particle size, and liquid and solid were set to have the same passage rate, and these likely explain the lack of significant differences in nutrient digestibility among treatments.

Digestibility of DM observed in the FK treatment (41.3%) is in accordance with the reports of Bach et al. (1999) that in a continuous culture study observed a DM digestibility of 41.2% in high quality pasture (18.2% CP and 47.4% NDF). In Exp. 1, the average OM digestibility across treatments was 56%; this finding was consistent with other dual-flow in vitro studies, where Cerrato-Sanchez et al. (2008) found 52.4% of OM digestibility when fermenters were maintained in a constant 6.4 pH and were fed a diet with 55:45 (DM basis) forage to concentrate ratio, and Kokko et al. (2013) reported an average OM digestibility of 56.4% when evaluating

Table 3. Effects of different forage sources on dietary nutrient digestibility (%), volatile fatty acids concentration, and profile in dual-flow continuous culture (Exp. 1)

	Forages				
Item	OH	AH	FK	SEM	*P*-value
DM	35.7	37.4	41.3	4.76	0.54
OM	57.6	53.1	57.2	5.08	0.74
NDF	52.3	67.3	64.1	4.09	0.25
CP	38.7[a]	40.9[a]	56.8[b]	2.9	0.02
Total VFA, mM	85.6	90.6	91.0	9.39	0.89
VFA, mol/100 mol					
Acetate	72	74.8	73.9	2.64	0.68
Propionate	18.3	17.1	16.8	1.9	0.81
Butyrate	7.6	6.1	6.7	1.23	0.47
Isobutyrate	0.18	0.23	0.3	0.06	0.05
Valerate	1.47	1.37	1.44	0.27	0.91
Isovalerate	0.36[a]	0.34[a]	0.56[b]	0.1	0.02
Acetate:propionate	4.43	4.56	4.58	0.6	0.98
Total BCVFA, mM	1.8	2.2	2.4	0.4	0.55

Least squares means within the same row with different superscripts differ ($P < 0.05$).

OH, 100% orchardgrass hay; AH, 100% alfalfa hay; FK, 100% dried forage kochia; BCVFA, branched-chain VFA computed as the sum of isobutyrate and isovalerate.

the effects of time of cutting and mechanical maceration on birdsfoot trefoil-timothy hay.

Volatile fatty acids. Consistent with our digestibility findings, treatments did not affect total VFA concentration and molar proportions of acetate, propionate, butyrate, and branched-chain VFA (Table 3). Isovalerate is a branched-chain VFA derived from branched-chain AA oxidative deamination and decarboxylation (Allison and Bryant, 1963). The highest ($P = 0.02$) molar proportion of isovalerate in FK suggests an increase of branched-chain AA degradation in this treatment. The average total VFA concentration across treatments was 89 mM, which is close to the average (93.8 mM) reported in a meta-analysis by Hristov et al. (2012) for in vitro studies. In agreement with our results, Kolver et al. (1998) using a dual-flow continuous culture system found similar ruminal total VFA concentration (82.6 mM) for an orchardgrass diet.

Hoover (1986) reported that when the range of ruminal pH is from 5.0 to 5.5, it can negatively affect the activity of fibrolytic microorganisms resulting in a decrease of the fermentation end products, such as VFA; therefore, according to this author, controlling pH would be necessary to assure that pH was not limiting fiber digestion. In Exp. 1, pH was automatically controlled; therefore, it is possible that controlling ruminal pH affects VFA concentration, especially when diets vary in NFC; however, it is unlikely that the tested diets (100% forages) would play a major role in pH fluctuation. Calsamiglia et al. (2002) evaluated the effect of pH when pH values fluctuated between 5.7 and 6.4 and concluded that within that range, pH was not a major player on VFA concentration.

Nitrogen metabolism. Ruminal NH_3-N concentration was greater ($P < 0.01$) for FK compared with the other forages (Table 4), possibly as a result of highest ($P < 0.05$) true CP digestibility (Table 3). In agreement with the current study, Dillard et al. (2017) also found a positive relationship between true CP digestibility and effluent NH_3-N concentration when dual-flow continuous culture fermenters were fed 100% orchardgrass. Nevertheless, the accumulation of NH_3-N in the fermenter fluid not only depends upon the extent of CP digestibility but also on the rate of N utilization by ruminal bacteria (Bach et al., 1999). Although FK treatment had increased ($P < 0.05$) true CP digestibility, which led to an increase ($P < 0.01$) in ruminal NH_3-N concentration, it was not followed by improvements in N utilization since bacterial efficiency showed no concurrent increase. Bacterial efficiency averaged 7.84 g of bacterial N per kilogram of OM truly digested, which agrees with data reported by Dillard et al. (2017), who found a bacterial efficiency average of 7.32 g in dual-flow continuous culture when fermenters were fed diets containing 100% orchardgrass or

Table 4. Effects of different forage sources on nitrogen metabolism in dual-flow continuous culture (Exp. 1)

	Forages				
Item	OH	AH	FK	SEM	*P*-value
NH_3-N, mg/100ml	2.58[b]	4.34[c]	6.13[d]	0.76	<0.01
N flow, g/d					
Total N	0.52[b]	1.12[c]	1.48[d]	0.06	<0.01
NH_3-N	0.06[b]	0.11[c]	0.15[d]	0.02	<0.01
NAN	0.60[b]	1.02[c]	1.34[d]	0.05	<0.01
Bacterial N	0.40[d]	0.25[c]	0.22[c]	0.04	0.03
Dietary N	0.20[b]	0.80[c]	1.11[d]	0.07	<0.01
Bacterial efficiency	8.50	6.84	8.26	0.87	0.38

Least squares means within the same row with different superscripts differ ($P < 0.05$).

OH = 100% orchardgrass hay; AH = 100% alfalfa hay; and FK = 100% dried forage kochia; NAN = nonammonia N; Bacterial efficiency = grams of bacterial N per kilogram of OM truly digested.

a combination of orchardgrass and warm-season annual forages.

The NH_3-N concentration in the FK treatment was 6.13 mg/100 mL, which is close to the 5 mg/dL reported by Satter and Slyter (1974) as the minimum NH_3-N concentration required in the rumen to ensure maximum microbial growth. In agreement with our findings, in a dual-flow continuous culture study, Soder et al. (2010) used orchardgrass with similar CP concentration (21.3% of DM) than FK (20.9 % of DM) and reported a similar ruminal NH_3-N concentration value (6.07 mg/100 mL). The lowest ($P < 0.01$) NH_3-N concentration found in the OH treatment (Table 4) can be linked to its decrease ($P < 0.05$) in ruminal true CP digestibility (Table 3). However, despite the lowest NH_3-N concentration observed in the OH, bacterial N flow (g/d) was highest ($P = 0.03$) in this treatment. One possible explanation for that may be that most bacteria are capable of scavenging NH_3-N from very dilute solutions (Satter and Slyter, 1974) and convert it into bacterial N, suggesting better utilization of N by ruminal microorganisms.

The flows (g/d) of total N, NH_3-N, NAN, and dietary N were greater ($P < 0.01$) for FK compared with the other forages (Table 4), possibly as a result of the highest true CP digestibility in this treatment (Table 3).

Experiment 2

Nutritional composition of tested forages. Forages CG and EPH are plants widely available in western United States, in particular northern Nevada, that are used by ranchers but with virtually little to no documentation about their potential as livestock feed (Cook and Harris, 1952; USDA, 2008; USDA, 2006).

Composition data on the forages used in the trial is shown in Table 2. Dietary DM, CP, and NDF concentration in OH indicate that this forage was of typical composition as previously reported by the NRC (2016). Dietary DM and OM concentrations were comparable among forages. Dietary CP and NDF concentrations in OH were 9.94% and 48.7%, respectively; EPH had comparable values (10.4% and 46.8%, respectively), and immature CG had the numerically highest values (16.1% and 52.2%, respectively). However, all these values are close to the range described by the NRC (2016) tabular values for OH, indicating that immature CG and EPH can be used as alternative forages for beef cattle in arid-lands, and furthermore, it indicates that immature CG can be a high CP source for ruminants.

Nutrient digestibility. To our knowledge, this is the first study to report immature CG and EPH ruminal fermentation patterns. Digestibilities of DM, OM, and CP were highest ($P = 0.01$) for EPH, and NDF digestibility was highest ($P < 0.05$) for CG and EPH (Table 5). Although EPH had the highest nutrient digestibility value, it was observed that fermenters fed EPH had some feed particle accumulation in the bottom of the fermenter jars, which might have slightly increased its digestibility so readers should be caution interpreting this particular value.

Numerically greatest dietary NDF concentration was observed on CG treatment (Table 2); however, it did not result in reduction of NDF digestibility (Table 5). The numerically greater CG NDF concentration may not limit digestibility presumably because of the low indigestible NDF, which is a common characteristic of forages in immature stage. On the other hand, compared with CG, OH had lower NDF digestibility ($P = 0.01$) possibly as

Table 5. Effects of different forage sources on dietary nutrient digestibility (%), volatile fatty acids concentration, and profile in dual-flow continuous culture (Exp. 2)

	Forages				
Item	OH	CG	EPH	SEM	*P*-value
DM	52.6^{b}	51.6^{b}	60.6^{c}	2.29	0.01
OM	59.0^{b}	58.6^{b}	68.1^{c}	2.64	0.01
NDF	52.6^{b}	77.4^{c}	70.8^{c}	2.42	0.01
CP	67.3^{b}	62.7^{b}	76.0^{c}	3.7	0.01
pH	6.83^{b}	6.78^{b}	7.73^{c}	0.16	<0.01
Total VFA, mmol	77.5^{b}	86.9^{c}	37.8^{d}	3.18	<0.01
VFA, mol/ 100 mol					
Acetate	58.9^{b}	69.7^{b}	74.3^{c}	1.5	<0.01
Propionate	24.5^{c}	20.8^{b}	17.1^{b}	1.3	<0.01
Butyrate	13.3^{c}	8.30^{b}	7.42^{b}	1.27	<0.01
Isobutyrate	ND	ND	ND		
Valerate	0.62	0.72	0.28	0.19	0.47
Isovalerate	ND	ND	ND		
Acetate:Propionate	2.25^{b}	3.48^{cb}	4.77^{c}	0.42	<0.01

Least squares means within the same row with different superscripts differ ($P < 0.05$).

OH, 100% orchardgrass hay; CG, 100% immature dried cheatgrass; EPH, 100% dried forage ephedra; ND, not detected.

a result of the reduction in NH_3-N concentration ($P < 0.01$), which was below 5 mg/100 mL the recommended value to NDF degradation optimization (Satter and Slyter, 1974).

pH and volatile fatty acids. The pH was greater ($P < 0.05$), and total VFA concentration was lowest ($P < 0.05$) for EPH compared with the other forages (Table 5). Some *Ephedra* ssp. contain tannins (Keeler, 1989), which may affect ruminal pH. According to Bhatta et al. (2009), tannins could increase ruminal pH, which may decrease ruminal VFA concentration. In agreement with the present study, Dijkstra et al. (2012) and Peters et al. (1989) reported that VFA concentration is not only a direct result of dietary nutrient digestibility but is also affected by ruminal pH. The EPH pH value was out of the adequate range reported for ruminants fed forages (Russell, 2002).

Total VFA concentration was highest ($P < 0.05$) for CG, followed by OH and EPH (Table 5). Similar results of total VFA concentration and individual VFA proportions were found by Silva et al. (2017) using mature CG in an in vitro system. Total VFA concentration was lowest in EPH despite greatest digestibility. This might have happened as a result of denser EPH particles, leading to greater nutrient digestibility. Propionate and butyrate molar proportions were lowest ($P < 0.05$) and acetate:propionate ratio was highest ($P < 0.05$) for EPH.

Nitrogen metabolism. Ruminal NH_3-N concentration and NH_3-N flow (g/d) were highest ($P < 0.05$) for CG, and total N flow and bacterial efficiency were highest ($P < 0.05$) for OH and CG (Table 6). Dietary and bacterial N flows, and NAN were highest ($P < 0.05$) for OH, followed by CG and EPH. The energy availability is a factor that determines the amount of NH_3 that will be incorporated in bacterial N (Salter et al., 1979; Hoover and Stokes, 1991). However, in excess, degraded N will be absorbed from the rumen as NH_3 and excreted in the urine as urea and may represent a nutritional loss, a metabolic burden, and an environmental issue (Broderick and Albrecht, 1997). The highest ($P < 0.01$) NH_3-N concentration in CG treatment may be due to the decrease in NH_3-N incorporation into bacterial N, as evidenced by its lower ($P < 0.05$) bacterial N and bacterial efficiency compared with OH. Unlike CG, OH had the lowest ($P < 0.01$) NH_3-N concentration and highest bacterial N flow and bacterial efficiency ($P < 0.01$), indicating better microbial utilization of N. In the present study, we observed a greater ruminal NH_3-N concentration compared with Silva et al. (2017), who used mature CG (4.01% CP and 68.2% NDF) and that is likely due to the greater CP supply in the present study.

Overall, it can be concluded that CG may be used as alternative forage for grazing while in immature stage of growth. Its ruminal fermentation patterns are comparable with commonly used forages such as OH.

CONCLUSION

Results indicate that when compared with AH and OH (*Exp. 1*), FK has similar ruminal fermentation patterns and may be an adequate alternative

Table 6. Effects of different forage sources on nitrogen metabolism in dual-flow continuous culture (Exp. 2)

	Forages				
Item	OH	CG	EPH	SEM	*P*-value
NH_3-N, mg/100 mL	3.47[b]	18.09[c]	8.59[d]	0.92	<0.01
N flow, g/d					
Total N	1.20[c]	1.23[c]	0.67[d]	0.06	0.01
NH_3-N	0.09[b]	0.49[c]	0.23[d]	0.02	<0.01
NAN	1.15[c]	0.72[d]	0.44[d]	0.07	0.01
Bacterial N	0.60[c]	0.39[d]	0.17[b]	0.03	0.01
Dietary N	0.58[c]	0.32[c]	0.26[d]	0.04	0.01
Bacterial efficiency	14.1[c]	11.7[d]	3.39[b]	1.22	<0.01

Least squares means within the same row with different superscripts differ ($P < 0.05$).

OH, 100% orchardgrass hay; CG, 100% immature dried cheatgrass; EPH, 100% dried forage ephedra; NAN, nonammonia N; Bacterial efficiency, grams of bacterial N per kilogram of OM truly digested.

for beef cattle producers. Furthermore, when compared with OH (*Exp. 2*), immature CG may also be an adequate forage alternative for beef cattle producers. This is especially important for areas in which conventional forages may not grow well such as the U.S. arid-land. Based on the results of this research, forage *ephedra* should not be used as the sole forage in cattle diets due to its poor ruminal fermentation as evidenced by the lowest total VFA concentration and propionate molar proportion.

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