Comparison of warm season and cool season forages for dairy grazing systems in continuous culture[1]

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ABSTRACT: The objective of this study was to compare warm-season annual grasses to cool-season perennial (**CSP**) grasses for ruminal nutrient digestibility and N metabolism in a dual-flow continuous culture fermentation system. Dietary treatments were 1) fresh alfalfa, 2) **CSP** grasses and legumes, 3) brown-midrib sorghum-sudangrass (**BMRSS**), and 4) teff grass from an organic dairy production system. Eight dual-flow continuous culture fermenters were used during two consecutive 10-d periods consisting of 7 d for stabilization followed by 3 d of sampling. Fermenter samples were collected on days 8, 9, and 10 for analysis of pH, NH3-N, and VFA. Apparent DM, OM, NDF, and ADF digestibility were on average lesser ($P < 0.05$) in CSP grasses and legumes and warm-season annual grasses compared with alfalfa. True DM and OM digestibility were lesser ($P < 0.05$) for CSP grasses and legumes and warm-season annual grasses compared with fresh alfalfa. Total VFA were not affected ($P > 0.05$) by forage. The NH3-N concentrations were highest ($P < 0.05$) with alfalfa compared with the other CSP grasses and legumes and warm-season annual grasses. CP digestibility was not affected ($P > 0.05$) by forage treatment. Flow of NH3-N was greatest ($P < 0.05$) for alfalfa, reflecting the greatest NH3-N concentration. Flow of total N was greatest ($P < 0.05$) for alfalfa, intermediate for teff, and lowest for CSP grasses and legumes and BMRSS. Flows of bacterial N, efficiency of bacterial N, non-NH3-N, and dietary N were not affected ($P > 0.05$) by forage source. Overall, fermentation of warm-season grasses was similar to the cool-season grasses and legumes which indicate dairy producers may use warm-season grasses without concerns about negative impact on rumen health.

Key words: continuous culture fermentation, grazing, sorghum-sudangrass, teff

Transl. Anim. Sci. 2018.2:125–134
doi: 10.1093/tas/txy014

[1]The authors express gratitude to the interns and graduate students at the University of Minnesota West Central Research and Outreach Center, Morris, for their assistance in data collection. This work is supported by Organic Agriculture Research and Extension Initiative (grant no. 2012-51300-20015/project accession no. 0230589) from the USDA National Institute of Food and Agriculture. Financial support was also provided for this project by the Ceres Trust (Chicago, IL). Authors confirm there is no conflict of interest with this study.

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Received March 27, 2018.
Accepted April 3, 2018

INTRODUCTION

Profitability of grazing dairy farms relies on pastures that produce a large quantity of high-quality forage for cattle to graze. In the upper Midwest, cool-season grass and clover species are traditional pasture forages for many dairy grazing producers. However, cool-season perennial (**CSP**) grasses and legumes experiences a decrease in growth rate during periods of high temperatures and low precipitation, as observed in July and August in the upper Midwest (Moore et al., 2004; Hudson et al., 2010). Warm-season annual grasses, such as sudangrass (*Sorghum bicolor* × *drummondii*), sorghum × sudangrass (*S. bicolor L. Moench* × *S. bicolor var. sudanense)*, and Japanese millet (*Echinochloa esculenta*), have been suggested as a potential solution to maintain pasture production and to overlap a decrease in cool-season forage biomass production during the warmest parts of summer (Najda, 2003). Incorporating warm-season annual grass into a grazing system provides an opportunity to rest CSP when decreased forage quality growth conditions are limiting and to add flexibility to the grazing system (Moore et al., 2004).

There has been interest by grazing producers in the Upper Midwest and Northeast United States to utilize warm-season annual grasses, such as sorghum (*S. bicolor*), sudangrass, and their hybrids as feed for dairy cattle. Use of sorghum and sudangrass and their hybrids are desired for their persistent yield in drought conditions in mid to late summer when CSP grasses may become dormant (White et al., 2002; McCartney et al., 2009; Tracy et al., 2010). Teff (*Eragrostis tef*) has also shown interest from dairy producers during drought stress periods. Saylor et al. (2017) reported that teff hay may be utilized to replace alfalfa in dairy cow diets without any loss of production.

Continuous culture fermentation systems are in-vitro systems that provide estimates of ruminal fermentation for various dietary sources (Hannah et al., 1986). Previous continuous culture studies that used pasture-based diets generally compared pasture-based diets to diets with feed additives or non–pasture-based diets. Recently, Dillard et al. (2017) reported that warm-season grasses (sorghum-sudangrass and Japanese millet) in combination with orchardgrass (*Dactylis glomerata*) may provide an alternative to orchardgrass when summer productivity is lesser. A study by Soder et al. (2013a) compared a 100% orchardgrass diet to orchardgrass diets including 10% flaxseed, canola, or sunflower seed, and they reported that NH_3-N concentrations and flow of NH_3-N was lowest for a herbage only diet (Soder et al., 2013a). One study compared fresh alfalfa (*Medicago sativa*) to alfalfa hay in continuous culture to determine differences in ruminal fermentation when sucrose was added to the alfalfa diet (Ribeiro et al., 2005). There are few studies that compare digestibility and ruminal fermentation of pasture diets of different species composition in continuous culture, although one study was conducted that used fresh orchardgrass or red clover in combination with different inclusion of corn grain in continuous culture (Loor et al., 2003).

The brown-midrib sorghum-sudangrass (**BMRSS**) and teff grass were chosen as the warm-season grasses for the current study, because these grasses are starting to be utilized by farmers in their Midwest grazing systems. This study is one of the first to examine warm-season grasses (BMRSS and teff) as the only forage source grown in a pasture environment to be utilized in a continuous culture system. Grazing dairy producers that utilized warm-season grasses in the Midwest U.S. tend to grow the warm-season grasses in monoculture, and therefore, we chose to mimic current producer conditions to be able to provide valuable information back to producers. Fresh alfalfa was chosen as a treatment for the study, because grazing alfalfa is growing in the Upper Midwest of the United States, and dairy producers that are grazing cattle are adding alfalfa to grass pastures to maintain diversity. Additionally, by having fresh alfalfa as a treatment, we were able to compare to prior research that has utilized fresh alfalfa in continuous culture systems (Ribeiro et al., 2005).

The objectives of this study were to compare nutrient digestibility, ruminal fermentation, and microbial protein synthesis using different types of forages that may be used in grazing systems in the Midwest as substrate, and to compare cool-season grass species to warm-season grasses as the only forage source. Specifically, we will compare fermentation of warm-season grasses (BMRSS and teff grass) with two control diets (CSP and fresh alfalfa) in a dual-flow continuous culture fermentation system. Results from this study may provide insight into the digestibility and bacterial protein synthesis of warm-season grasses used in pasture systems for dairy cattle and how alternative forages may affect ruminal fermentation.

MATERIALS AND METHODS

Experimental Design and Treatments

This study was conducted at the University of Minnesota West Central Research and Outreach

Center organic dairy, Morris, MN, and the University of Minnesota Dairy Cattle Teaching and Research Center, St. Paul, MN. All animal procedures involving animal care and management were approved by the University of Minnesota Institutional Animal Care and Use Committee (#1508-32961A). A ruminally cannulated lactating crossbred (Swedish Red × Montbéliarde × Holstein) dairy cow was used as the rumen fluid donor. The diet fed to the donor cow was formulated to meet or exceed requirements of a Holstein cow producing 25 kg milk/d, with 3.8% fat and 3.7% protein (NRC, 2001). Diet ingredient composition was 39.8% alfalfa silage, 21.7% grass hay, 31.3% corn, and 3.6% each of soybean meal and vitamins and minerals on a DM basis.

A dual-flow continuous culture rumen fermentation system was used to evaluate digestibility and microbial fermentation response to four treatments: 1) fresh alfalfa (*M. sativa*), 2) CSP grasses and legumes, 3) BMRSS, and 4) teff grass. The CSP consisted of smooth bromegrass (*Bromus inermis Leyss*), orchardgrass (*D. glomerata*), meadow fescue (*Festuca pratensis*), and red (*Trifolium pratense*) and white clover (*Trifolium repens*). Botanical composition of the CSP was 30% smooth bromegrass, 20% orchardgrass, 10% meadow fescue, 20% red clover, and 20% white clover.

Four experimental forage treatments (fresh alfalfa, CSP, BMRSS, teff grass) were randomly allocated to an 8-unit, dual-flow continuous culture fermentation system designed to simulate ruminal fermentation and outflow to the small intestine. Four forage dietary treatments were compared during two experimental periods. Diet preparation resulted in four treatments arranged in a 2 × 2 completely randomized block design, because each treatment was assigned to two fermenters over two periods. The forage collection was conducted at the West Central Research and Outreach Center (Morris, MN) from May to October 2015. Perennial grasses and legumes were established in 2012 and rotational grazing began in 2013. Organic dairy cows were used to evaluate the effect of two pasture production systems (perennial vs. perennial/annual systems) over two grazing seasons with rotational grazing. Rotational grazing of lactating cows was initiated when forages were 20–30 cm tall and strip size was adjusted to leave 7–13 cm of refusals (Ruh et al., 2016). The current study was conducted with CSP and warm-season grasses during the 2015 grazing year. The BMRSS (Black Hawk 12 Organic, Blue River Hybrids, Ames, IA) and teff grass were planted on May 28, 2015. The teff grass was planted into a pasture that was BMRSS the prior year and the BMRSS was planted into a pasture that was teff grass the prior year. The fertility input was from manure from cattle rotationally grazing the pastures. The CSP samples were harvested every other day in June by randomly tossing a 0.23 m^2 square into each paddock before grazing and hand clipping to 5 cm above the ground. Three replicates per pasture were collected prior to grazing. Because alfalfa was not grazed, a large quantity of third cutting early-bud alfalfa was harvested at one time using hand clippers at random locations in the field by cutting the forage to 5 cm above the ground. The BMRSS and teff grass samples were harvested on July 14, 2015, before grazing by cutting sample to 5 cm above the ground. Teff was not well established in the pastures at the WCROC, so in order to collect adequate DM for the study, additional teff samples were collected from a University of Minnesota research plot in St. Paul, MN. The teff harvested from the two locations was composited fresh. Samples were dried in an oven at 60 °C for 48 h and ground (2-mm screen; Wiley mill, Thompson Scientific, Philadelphia, PA). Dried, ground forage samples were mixed thoroughly in their respective treatment and pelleted with a CL-5 California Pellet Mill (California Pellet Mill Co., Crawfordsville, IN) to a final dimension of 6 mm diameter × 12 mm long. Pelleting facilitated use of an automated feed delivery system to the fermenters. Pelleted diets were placed in shallow trays and allowed to air dry for 96 h before storing in plastic containers. Ground forage samples were analyzed with near infrared spectroscopy (NIR), and minerals were analyzed using wet chemistry (Rock River Laboratory, Inc., Watertown, WI). Samples were analyzed by AOAC (2005) for CP (method 954.01) and ether extract (method 920.39). The Ca, P, Mg, and K were analyzed using wet chemistry methods (Schalla et al., 2012). Chemical compositions of the four forage treatment diets are in Table 1.

Continuous Culture Operation

An 8-unit, dual-flow, continuous culture fermenter system with a modified pH control and measuring system were used in two consecutive 10-d experimental periods, similar to that described by Hannah et al. (1986) and fermenter operation was similar to Ruiz-Moreno et al. (2015), Carpenter, et al. (2017), and Fessenden et al. (2017). Treatments were randomly assigned and duplicated within experimental period to create a randomized complete block design with four observations per treatment. Fermenter volumes ranged from 1,055 mL to 1,103 mL. Ten liters of ruminal fluid and 1.5 kg of ruminal digesta were collected approximately 4 h after the morning feeding from one ruminally

Table 1. Chemical composition (% DM) of four forage diets (alfalfa, CSP grasses and legumes, BMRSS, and teff grass) used in continuous culture fermentation

Chemical composition, % of DM*	Forage treatment			
	Alfalfa	CSP	BMRSS	Teff
OM	88.1	90.5	89.3	85.5
CP	25.1	18.0	16.9	17.0
NDF	29.8	50.1	50.0	51.2
ADF	22.0	27.2	25.7	26.3
Ether extract	1.61	2.54	2.09	2.94
Lignin	4.5	4.7	5.1	4.2
Ash	11.9	9.5	12.4	14.5
Ca	1.69	0.63	0.71	0.41
Mg	0.51	0.19	0.37	0.30
P	0.38	0.28	0.31	0.42
K	3.84	2.72	3.54	5.78
TTNDFD†	43.9	50.2	56.6	62.4

*Chemical composition results from NIR from Rock River Labs, Watertown, WI analyses; CP, NDF, ADF, and Ash represent results from Stern lab, St. Paul, MN.

†Total tract NDF digestibility from NIR.

[a,b]Means within a row with different superscripts are different at $P < 0.05$.

cannulated lactating cow. At the beginning of each experimental period, ruminal fluid was collected with a pump (Welch model B2585–50; Welch, Niles, IL) with a hose and a 1-mm stainless-steel filter was used. Digesta was collected from the venral, central, and dorsal areas of the rumen. Ruminal fluid was collected into a preheated sealed thermos and transported to the laboratory. Within 20 min of digesta and fluid collection, a 500 mL of liquid and solid rumen sample were mixed using a PT10/3S homogenizer (Kinematica GmbH, Bohemia, NY), squeezed through two layers of cheesecloth, and fermenters were inoculated with 1 liter of ruminal fluid.

Fermenters were maintained at a constant temperature of 39 °C and were constantly purged with N_2 gas at a rate of 40 mL/min to maintain anaerobiosis. Amount of diet (as-fed) was adjusted on days 0, 4, and 7 for DM content to attain a feeding rate of 60 g of diet DM/fermenter daily (de Veth and Kolver, 2001). Fermenters were fed throughout the day with the automated feeding system, and the pelleted forage diet was slowly fed into the fermenter over eight equally spaced, 90-min periods. An automated feeding device (Hannah et al., 1986) controlled by a timer (DT 17, Intermatic, Spring Grove, IL) was used to regulate feeding duration and schedule. Each 90-min feeding period was followed by 90 min of rest. Artificial saliva was prepared according to Weller and Pilgrim (1974) and infused continuously into fermenters and contained 0.4 g/L of urea to simulate N recycling. The pH was maintained within a range between 5.0 to 6.7 with automated influx of 3*M* HCl and 5*M* NaOH as needed and recorded using Daqboard and Dasylab software (Daisy Lab, National Instrument Services, Austin, TX). Optimal fiber digestion occurs when pH is greater, typically above 6.0 (Bach et al., 1999). Culture pH was recorded every 5 min. Solids dilution rate were adjusted daily to 4% per hour, similar to Bargo et al. (2003), by regulating artificial saliva input. Liquid dilution rate was 10% per hour, similar to Cerrato-Sánchez et al. (2007). Dilution rates were attained by regulation of artificial saliva input and filtrate removal. Anaerobic conditions were maintained with constant infusion of N_2 at a rate of 20 mL/min using a digital flow meter (Aalborg GFM 17, Orangeburgh, NY).

Sample Collection and Analysis

Fermenters were operated for two consecutive 10-d periods consisting of a 7-d diet-adaptation period followed by a 3-d sample collection period. Fermenter pH was recorded automatically every 5 min, and mean, minimum, and maximum pH were analyzed for the 3-d sampling time by fermenter for each period.

On days 8 to 10 of each experimental period, a water bath maintained the temperature of the effluent containers at 2 °C to prevent further microbial and enzymatic activity. Solids and liquid effluent samples were collected on days 8, 9, and 10 and homogenized (PT10/3S homogenizer, Kinematica GmbH) for 2 min. A subsample of 500 mL of effluent was taken each day, and the three sample days were combined within fermenter. This sample was kept frozen at −20 °C until analysis for total N, NH_3-N, and VFA. A 500 mL of the combined solids and liquid effluent sample per fermenter representing 3 d of collection in each period was lyophilized and used for analysis of DM, OM, NDF, ADF, ash, and purines. On the final day of sampling at the end of each period, fermenter contents were squeezed through two layers of cheesecloth, and the liquid was centrifuged at 1,000 × *g* for 10 min at 4 °C to remove feed particles. The supernatant was then centrifuged at 20,000 × *g* for 20 min at 4 °C to isolate the microbial pellet. The microbial pellet was suspended in distilled water, frozen at −20 °C and then lyophilized prior to analysis of DM, ash, total N, and purines.

Pelleted forage samples (dietary forage treatments), effluent, and microbial pellets were

analyzed for DM by drying at 105 °C for 24 h. Ash was determined by the weight difference after 24-h combustion at 550 °C (AOAC, 1984; method 967.04). Total N content of diets, effluent, and bacteria and NH_3-N of diets and effluent were determined using the Kjeldahl method (AOAC, 1984; method 984.13). Ammonia-N concentration in the fermenters was determined on the supernatant of a centrifuged (5,000 × *g* for 15 min at 4 °C) subsample of liquid effluent by steam distillation (Bremner and Keeney, 1965) with magnesium oxide using a 2,300 Kjeltec Analyzer Unit (Foss Tecator AB, Höganäs, Sweden). Sequential fiber analyses (Van Soest, 2015) were used to determine NDF and ADF concentrations of the diet and effluents using an ANKOM A200 fiber analyzer with F57 fiber bags (ANKOM Corp, Fairport, NY) and lignin content of the diet was measured gravimetrically after hydrolysis of acid detergent residue using 12 *M* H_2SO_4 (Van Soest, 2015). Purine concentrations were determined by the method of Zinn and Owens (1986). Purine concentrations of the effluent and bacteria were used to calculate N metabolism and efficiency of microbial protein synthesis. Effluent samples were prepared for VFA analysis using the procedure for rumen fluid preparation described by Erwin et al. (1961). Ruminal fluid effluent was centrifuged to remove heavy feed particles and clarified, then 2.0 mL was mixed with a 25% meta-phosphoric acid solution (0.5 mL) and centrifuged at 10,000 *g* for 15 min until supernatant was clear. The supernatant was stored at −20 °C until analyzed. Effluent VFA concentration was performed via gas chromatography (Agilent 7890B GC-FID with a G4567A Autosampler). The Agilent DB-FFAP column was 30 m length, 0.25 mm diameter, and had a film thickness of 0.15 µm. Chromatographic conditions were 1 µL injection with an inlet temperature of 240 °C, helium as carrier gas at 1 mL/min constant flow, initial oven temperature of 60 °C with a 2-min hold time, ramp at 20 °C/min to 220 °C and a 1-min hold time at 220 °C. The flame ionization detector was set at 250 °C. Standard solutions with known concentrations of VFA were analyzed to calibrate chromatograph.

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS software 9.4 (SAS Inst. Inc., 2016). Data from fermenters were analyzed as a randomized complete block (period) design. Period served as block with all treatments equally represented within block. Forages were analyzed as a fixed effect and period (block) was a random effect (Ruiz-Moreno et al., 2015; Binversie et al., 2016; Carpenter et al., 2017). All treatment results were reported with least squares means, with significance declared at $P < 0.05$. The pH was analyzed for mean, maximum, and minimum pH for the 3-d sampling period as repeated measures with an autoregressive of order 1 structure of covariance on the basis of the minimum values of Akaike's information criterion. All treatment results were reported with least squares means, with significance declared at $P < 0.05$ and trends declared at $P < 0.10$. Differences among treatments were tested using LSMEANS with the Tukey adjustment for multiple means comparison. Results of the current study are reported as least squared means from four observations per forage treatment.

RESULTS AND DISCUSSION

The chemical composition of the forage treatments is provided in Table 1. Forage analyses by NIR were performed by Rock River Labs Inc., while CP, NDF, and ADF were analyzed using the same procedures used to analyze fermenter effluent. Dietary protein levels may affect ruminal fermentation patterns and digestibility and create confounding results (Bach et al., 1999), which is why many in-vitro studies feed isonitrogenous diets when investigating alternative treatments. However, the current study investigated the differences in ruminal fermentation between CSP and warm-season annual grasses, and it was important to keep the treatments at their original protein levels, with the understanding that this may ultimately affect fermentation, and may be of interest to reflect a grazing situation (Bach et al., 1999). This difference in N content of the diets was accounted for by expressing results as a percentage of total N intake (Bach et al., 1999).

Digestibility

Apparent and true (corrected for contribution of bacterial flow) digestibility and pH flow for forage treatments are provided in Table 2. The CSP, BMRSS, and teff grass had lesser ($P < 0.05$) apparent DM and OM digestibility than alfalfa. The NDF digestibility for CSP and teff was lesser ($P < 0.05$) than alfalfa, and BMRSS was similar

Table 2. Nutrient digestibility and pH of four forage diets (alfalfa, CSP grasses and legumes, BMRSS, and teff grass) during continuous culture fermentation

	Forage				
Item	Alfalfa	CSP	BMRSS	Teff	SEM
Apparent digestibility					
	----------%----------				
DM	69.4[a]	47.1[b]	52.6[b]	49.8[b]	5.2
OM	54.1[a]	32.5[b]	38.1[b]	29.4[b]	4.7
NDF	75.5[a]	52.6[b]	65.9[ab]	56.6[b]	5.3
ADF	75.5[a]	55.4[b]	67.5[ab]	59.4[ab]	5.3
True digestibility*					
DM	85.8[a]	64.0[b]	66.2[b]	65.9[b]	5.7
OM	69.2[a]	47.0[b]	50.4[b]	44.1[b]	4.1
pH					
Mean	6.38	6.18	6.19	6.22	0.10
Minimum	5.88	5.14	5.41	5.92	0.31
Maximum	7.26	6.76	7.23	7.01	0.25

*Corrected for contribution of bacterial flow.

[a,b]Means within a row with different superscripts are different at $P < 0.05$.

($P > 0.10$) to the other forages. The CSP was lesser ($P < 0.05$) for ADF digestibility than alfalfa but was similar ($P > 0.10$) to BMRSS and teff. True DM and OM digestibilities were lesser ($P < 0.05$) in grasses compared with alfalfa. Digestibilities among BMRSS and teff grass were similar ($P > 0.10$). A study by de Veth and Kolver (2001) found a range of apparent DM digestibility (44.7% to 56.4%) and OM digestibility (48.1% to 58.7%) from ryegrass pastures, which was similar to results from the current study for DM and OM digestibility. A study by Soder et al. (2013b) found much greater apparent digestibility of DM, OM, and NDF for 100% orchardgrass in a dual-flow continuous culture fermenter system, which could have been due to a greater CP level or the greater level of daily diet, which was greater than the daily diet level of 60 g DM/d in the current study. The current study has only 20% orchardgrass in the diet, and differences between Soder et al. (2013b) and the current study may be due to the composition of orchardgrass in the diet. A study evaluating alfalfa in continuous culture found lesser apparent NDF and ADF digestibility than results from the current study for alfalfa (Ribeiro et al., 2005). Recently, Dillard et al. (2017) studied mixes of cool-season (orchardgrass) and warm-season grasses (sorghum × sudangrass and Japanese millet) and found similar DM digestibilities, greater OM digestibilities, and great NDF and ADF digestibility compared to the current study. Overall, findings of apparent digestibility are consistent with previous research comparing alfalfa to grass in vivo, in which alfalfa disappeared more quickly from the rumen than the perennial ryegrass because of a faster rate of digestion and faster particle size reduction of alfalfa (Waghorn et al., 1989). Results from Ribeiro et al. (2005) were different than results from the current study because although there was greater nutrient digestibility in alfalfa, bacterial OM flow was similar across all treatments (Table 4).

Volatile Fatty Acids

Least square means of VFA for the forage treatments are in Table 3. Total VFA amount (mM) were similar ($P > 0.10$) among the forage diets. The amount of total VFA for CSP was similar to amount of total VFA found in a previous study for pasture intake in continuous culture (Bargo et al., 2003) but greater than total VFA with a ryegrass only diet in continuous culture fed at 60 g DM/d (de Veth and Kolver, 2001). Total VFA for the current study for the grass species was much greater than reported by Dillard et al. (2017) for mixes of cool- and warm-season grass species. Quite possibly, differences between studies were observed, because the current study utilized warm-season grasses as the only forage source, and Dillard et al. (2017) utilized alternative combinations of cool-season and warm-season grasses.

There were some differences in individual VFA concentrations and in molar proportions of VFA. These changes may indicate differences in a shift of the rumen microbial population when alfalfa and alfalfa hay are studied (Ribeiro et al., 2005). Similar results of individual VFA production for CSP were found for a smooth bromegrass and orchardgrass pasture diets in continuous culture (Bargo et al., 2003). Molar proportions of acetate, propionate, and butyrate were similar to previous results of a CSP grass and legume diets in continuous culture (Bach et al., 1999).

Bargo et al. (2003) showed an inverse correlation between pH patterns and VFA production from smooth bromegrass and orchardgrass pasture diets. Forage treatments were similar ($P > 0.10$) in mean pH among the forage treatments in this study and differences in individual VFA do not match the pattern of pH. Quite possibly, this insignificant differences reported in this study may be due to the fact that pH was controlled for in the study. However, another study found no large differences of individual proportions of VFA with change in pH (de Veth and Kolver, 2001). The molar proportion of

Table 3. VFA concentration of 4 forage diets (alfalfa, CSP grasses and legumes, BMRSS, and teff grass) in continuous culture fermentation

		Forage			
VFA	Alfalfa	CSP	BMRSS	Teff	SEM
Total VFA, mM	78.6	77.6	75.6	82.3	13.1
Individual VFA, mol/100 mol					
Acetate	72.1[a]	71.2[ab]	67.7[b]	75.0[a]	1.3
Propionate	16.5[b]	20.0[a]	18.4[ab]	17.1[b]	0.54
Butyrate	7.9[b]	7.2[b]	10.1[a]	6.7[b]	0.58
Isobutyrate	0.69[a]	0.27[b]	0.25[b]	0.17[b]	0.03
Isovalerate	0.82[ab]	0.30[ab]	1.2[a]	0.09[b]	0.31
Valerate	1.7[a]	0.90[b]	2.1[a]	0.83[b]	0.17
Caproate	0.29[a]	0.14[b]	0.32[a]	0.10[b]	0.03
A:P ratio	4.4[a]	3.6[b]	3.7[b]	4.4[a]	0.18

[a,b]Means within a row with different superscripts are different at $P < 0.05$.

Table 4. Nitrogen metabolism of four forage diets (alfalfa, CSP grasses and legumes, BMRSS, and teff grass) in continuous culture fermentation

	Forage				
Variable	Alfalfa	CSP	BMRSS	Teff	SEM
N intake, g/d	3.09[a]	2.31[b]	2.18[c]	2.20[c]	0.01
NH3-N, mg/dL	22.5[a]	7.5[b]	7.4[b]	8.9[b]	0.75
CP degradation, %	79.8	77.2	69.1	65.9	4.6
N flows, g/d					
Total N	1.99[a]	1.50[b]	1.51[b]	1.70[ab]	0.11
NH_3-N	0.52[a]	0.17[b]	0.17[b]	0.20[b]	0.02
NAN	1.46	1.33	1.34	1.50	0.12
Bacterial N	0.84	0.81	0.67	0.75	0.14
Dietary N	0.62	0.53	0.67	0.75	0.12
N flows, % of total N flow					
NH3-N	26.5[a]	11.6[b]	11.4[b]	11.8[b]	1.8
NAN	73.5[a]	88.4[b]	88.6[b]	88.2[b]	1.8
Bacterial N	41.3	54.1	44.4	43.8	6.1
Dietary N	32.1	34.3	44.2	44.4	6.0
Efficiency of Microbial protein synthesis					
g N/kg DM truly digested	15.0	19.9	16.0	18.7	2.7
g N/kg OM truly digested	21.3	30.4	24.0	33.3	4.9

[a,b]Means within a row with different superscripts are different at $P < 0.05$.

butyrate was greatest for BMRSS compared to the other forage treatments.

Isobutyrate was greater in alfalfa than the CSP, BMRSS, and teff grass treatments, which is similar to the pattern of true and apparent digestibility of DM and OM of the dietary treatments (Table 2). The CP degradation (Table 4) was similar ($P > 0.10$) among treatments. Molar proportions of acetate, propionate, and butyrate of alfalfa from this study were similar to previously reported values of acetate, propionate, and butyrate for fresh alfalfa in continuous culture (Ribeiro et al., 2005).

There are no previous studies that evaluated warm-season grass as the only forage in continuous culture. One study investigated warm-season and cool-season grasses fed to cannulated steers and reported a significant effect for warm-season vs. cool-season grasses for the molar proportions of propionate, butyrate, and valerate in ruminal fluid (Bohnert et al., 2011), which was in agreement to the current study. Individual concentrations from the current study of acetate and propionate were greater and lesser for butyrate for warm-season grasses (BMRSS) reported by Dillard et al. (2017). Differences observed between studies may be due to warm-season grass being analyzed as the only forage source compared to warm-season grasses composited with orchardgrass in Dillard et al. (2017).

Nitrogen Metabolism

The N metabolism of fermenters fed the various forages is found in Table 4. The N intake was highest for alfalfa, intermediate for CSP, and lowest for warm-season grasses based on the dietary CP of the forage treatments. The N intake can affect rates of microbial growth and ultimately digestibility measured in vitro and in vivo (Bach et al., 1999). CP degradation was similar ($P > 0.05$) among dietary treatments in the fermenters. This observation is slightly lesser than CP digestibility found in a previous study using pasture diets in continuous culture (Soder et al. 2013b).

The NH_3-N was greater ($P < 0.05$) in alfalfa-fed fermenters than for CSP, BMRSS, and teff fermenters. Similar results of NH_3-N were found for cool-season grasses in continuous culture compared to CSP in the current study (Bach et al., 1999). The CSP and BMRSS had similar total N flow, while alfalfa had the greatest ($P < 0.05$) total N flow and teff was intermediate in total N flow. As a percent of total N flows, NH_3-N was greater ($P < 0.05$) in alfalfa than the grasses, reflecting the pattern of total NH_3-N for each treatment. Treatments were similar ($P > 0.10$) for non–NH_3-N, bacterial N, or dietary N flows as a percent of total N. Even though alfalfa had greater NH_3-N concentrations, treatments were similar ($P > 0.10$) in efficiency of microbial protein synthesis among any of the treatments, on either a DM or OM basis. This demonstrates that although there was lesser NH_3-N in all the grass treatments than in alfalfa, there were still adequate amounts of NH_3-N for microbial protein synthesis. The minimum amount of NH_3-N required for microbial protein synthesis

was estimated to be 5 mg/dL for in-vitro systems (Satter and Slyter, 1974), and all treatments in this study were well above that value. Efficiency of microbial protein synthesis on an OM basis in the current study was lesser to previous results for alfalfa (Ribeiro et al., 2005) and pasture in continuous culture (de Veth and Kolver, 2001; Cerrato-Sánchez et al., 2007; Soder et al., 2013b, 2016).

Alfalfa, CSP, BMRSS, and teff grass were similar ($P > 0.10$) for mean, minimum, or maximum pH (Table 2). One continuous culture fermenter study researching effects of pH levels determined that the optimal pH for high-quality pasture forage is 6.35 (de Veth and Kolver, 2001). We analyzed the amount of time spent below 5.8 as well as the amount of time spent above 6.4. Time spent below pH 5.8 ranged from 1 to 5 min/d. Sauvant et al. (1999) found that it may be possible to experience a pH below 6.0 for 4 h without affecting microbial fermentation. pH was analyzed at 5.8 because we expected lesser values from the CSP examined in our study. Our results suggest that fermenters spent almost no time below a pH of 5.8.

Because the study was in-vitro system, diets may be affected differently when actually consumed by cows (Mansfield et al., 1995). Although fresh grass was used, it underwent heating and pressure in the drying and pelleting processes which could alter some components of the grass. These changes should have been uniform across all treatments; however, it is important to note that dietary values of pelleted grasses may be different than actual pasture forage grasses that cows would be consuming in a grazing system. Fresh forages have greater concentrations of rapidly fermented sugars as well as greater concentrations of more digestible protein, which could have been lost during heat processing of our forages (Van Soest, 1994). In addition, most grazing cows do not consume forage-only diets, so there may be more complex interactions if grazing cows were to be supplemented with concentrate or processed forages while grazing pasture. Previous studies have shown alterations in bacterial growth rates with different combinations of carbohydrates, which may decrease fiber digestibility (Russell and Baldwin 1978). Vibart et al. (2010) demonstrated improvement in N utilization when grazing cows are supplemented with a total mixed ration. Minimum effective fiber requirements may be different for cows grazing high-quality cool-season grasses than for cows fed mixed forage and concentrate diets because of interactions that occur in a mixed diet (Kolver and Muller, 1998; De Veth and Kolver, 2001).

It is known that grazing cows will experience changes in their diet throughout the grazing season due to weather, the gradually increasing maturity of cool season grasses, as well as changes in season in temperate regions of the United States. Cows in a complementary grazing system may have additional disruptions to their diet because they often graze one type of grass, then sequentially another type of grass in a different pasture. Results from the current study found several changes in ruminal fermentation among forage treatments of different species. Future research could investigate how this shift in microbial population, possibly every few days or every few weeks, affects a grazing dairy cow. It would be interesting to determine what kind of effect a sequential pasture rotation may have on the rumen microbial population and fermentation. Further research dealing with warm-season grasses in continuous culture systems should include variations of concentrate and total mixed ration supplementation. Research on fermentation characteristics of warm-season grasses should be studied further, because there had not been much previous research conducted.

Dairy farmers that utilize warm-season grasses in pasture rotations for lactating dairy cattle, may benefit from utilizing BMRSS and teff grass in their rotations. The BMRSS and teff grass had similar digestibility when compared to cool-season pasture grasses and legumes. No differences for total VFA were found for CSP compared to warm-season grasses; however, specific individual VFA differed among alfalfa, CSP, and warm-season grasses. Forage quality of the forages utilized in this study may have profound effect on the results, and results may be different depending on weather conditions throughout the year (i.e., drought or heavy rains). Overall, fermentation of warm-season grasses was similar to the CSP. Results of this study indicate that warm-season grasses may be successfully grazed by dairy cows in monoculture and may be included in a complementary grazing system with CSP grasses and legumes, without concerns about negative impact on rumen or animal health.

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