This is a final project report submitted to The Ceres Trust.

Project Title:

Dual flow continuous culture fermentation of organic BMR sorghum-sudangrass and teff grass to determine digestibility of forages in an organic dairy grazing system

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Ceres Trust Funding Awarded: $9,904

Project Period: 2015-2016

Project Summary

Organic dairy cattle must have at least 120 days on pasture and at least 30% of their dry matter intake must come from pasture forage. Typically, perennial cool season grasses are used in pastures in the upper Midwest, but these grasses experience a decreased period of growth in summer. Warm season grasses have been suggested as a solution to pasture productivity during the decreased growth rate of cool season grasses. In this study the warm season grasses BMR sorghum sudangrass and teff grass were compared to a cool season grass pasture mixture and alfalfa in a dual-flow continuous culture rumen fermentation system to determine forage quality.

Problem addressed

Pasture management is a very important component of organic dairy farming. Warm season grasses may provide a solution to the summer slump that occurs with cool season grasses in organic dairy pastures. This project will evaluate effects of organic pasture forage digestibility of warm season grasses (BMR sorghum-sudangrass and teff grass), as well as cool season perennial ryegrass and alfalfa that are used in pasture systems in the Midwest. Results will help organic farmers that graze dairy cows to make better pasture management decisions for their cattle.

Results were disseminated to stakeholders through extension publications, research center field days, and through online educational materials.

Project Objective

Our objective is to evaluate the effects of organic pasture forage digestibility of warm season grasses (BMR sorghum-sudangrass and teff grass) compared to cool season pasture species (perennial ryegrass and alfalfa) that are used in pasture systems in the Midwest.

Methodology

Four experimental treatments were randomly allocated to an 8-unit, dual flow continuous culture rumen fermentation system designed to simulate rumen fermentation and outflow to the small intestine. Four forage dietary treatments were compared during two experimental periods. The organic forage study was conducted at the West Central Research and Outreach Center (Morris, MN) from May to October 2016. Perennial pastures were established in 2012 and grazing began in 2013. BMR sorghum sudangrass (Blackhawk variety, Blue River Hybrids, Ames, IA) and teff grass were planted on May 28, 2016. Perennial pasture samples were harvested every other day in June by randomly tossing a $0.76m^2$ square into each paddock before grazing and hand clipping to 4 cm above the ground. Alfalfa was harvested using hand clippers at random locations in the field. BMR sorghum sudangrass and teff grass samples were harvested before grazing by cutting sample to 4 cm above the ground. Samples were dried in an oven at 60 degrees C for 48 hours and ground (2-mm screen; Wiley mill, Thompson Scientific, Philadelphia, PA). Dried, ground forage sample was mixed thoroughly and pelleted in a pellet mill. Chemical compositions of the four treatment diets can be found in Table 1. BMR sorghum-sudangrass and teff grass were chosen as the warm season grasses to study because these grasses are beginning to be used by organic farmers incorporating warm season grasses into their grazing systems.

Continuous culture operation

An 8-unit, dual flow continuous culture rumen fermenter system, similar to that described by Hannah et al., (1986) was used in the present experiment. Fermenter volumes ranged from 1,055mL to 1,103 ml. Fermenter operation was similar to Binversie, et al (2016). Ten liters of ruminal fluid and three handfuls of ruminal digesta were collected approximately four hours after the morning feeding from one ruminal cannulated lactating Swedish Red/Montbeliarde cow consuming a high forage TMR twice daily, (70% forage, 30% concentrate). Liquid samples were collected from the rumen by hand using a cup. To maintain sample temperature, liquid was transported from the barn to the lab in an insulated thermos container. Within 20 minutes of collection, liquid and whole rumen samples were mixed using a homogenizer, squeezed through 2 layers of cheesecloth, and fermenters were inoculated with about 1 L of rumen fluid. Solid mean retention time, solid dilution rate, and liquid dilution rate were 24 h, 4%/h (Bargo, 2003;), and 10%/h by regulation of the buffer input and filtrate removal.

Fermenters were maintained at a constant temperature of 39^{o} C and were constantly purged with N_2 gas at a rate of 40 ml/min to maintain anaerobic conditions. Total DM intake for fermenters was maintained at 60g DM/fermenter daily (de Veth and Kolver, 2001) and was fed to the fermenters in eight equal proportions throughout the day with a motorized auger which slowly pushed feed into the fermenter over eight equally spaced, 1.5 hour periods per day. PH was maintained in a range of 5.6-6.7 with automatic influx of HCl and NaOH as needed, and recorded using Daqboard and Dasylab software.

Sample collection and analysis

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

Effluent was collected for the first 7 days in 4-L containers and weighed at 1500 h and discarded. On days 8 to 10, a water bath maintained the temperature of the effluent containers at 2^{o} C to prevent further microbial fermentation. Solid and liquid effluent samples were collected on d 8 to 10 and homogenized (PT10/3S homogenizer, Kinematica GmbH, Bohemia, NY) for 2 min, a subsample of 500 mL was taken each day, and the three sample days were composited. This sample was kept frozen at -20^{o} C until analysis for total N, Ammonia-N, and VFA. A subsample, approximately 500 mL of the composited 1500 mL effluent from each fermenter was lyophilized and used for analysis of DM, OM, NDF, ADF, ash and purines. On the final day of sampling, the fermenter contents were squeezed through cheesecloth, the liquid was centrifuged at 1,000 x g for 10 minutes to remove feed particles and then centrifuged at 20,000 x g for 20 minutes to isolate the microbial pellet. The microbial pellet was suspended in distilled water, frozen at -20^{o} C and then lyophilized prior to analysis of DM, Ash, total N, and purines.

Pelleted forage samples (dietary treatments), effluent, and microbial pellets were analyzed for DM by drying at 105^{o}C for 24 h. Ash was determined by the weight difference after 24 h combustion

at ^{550oC} (AOAC, 1984; method 967.04). The total N content of the diets, effluent, and bacteria and ammonia N of diets and effluent were determined by steam distillation using a 2300 Kjeltec Analyser Unit (Foss Tecator AB, Hogonas, Sweden). Sequential fiber analyses (Ankom) were used to determine NDF and ADF concentrations of the diet and effluents. Purine concentrations were determined by the method of Zinn and Owens (1986). Purine contents of the effluent and bacteria were used to calculate nitrogen metabolism and microbial efficiency.

Statistical analysis

Data were analyzed using the MIXED procedure in SAS (SAS Inst. Inc., Cary NC, 2013). Forages were analyzed as a fixed effect and period was a random effect. Contrasts of the warm season grasses compared to the cool season perennial pastures (BMRSS, Teff vs Cool), and warm season grasses compared to alfalfa and cool season perennial pasture (AC vs BT) were conducted. All treatment results were reported with least squares means, with significance declared at $P<0.05$, and a trend was declared at $P<0.10$. PH was analyzed for mean, maximum, and minimum pH for the three-day sampling period. PH was also analyzed using the MIXED procedure in SAS for amount of time each fermenter spent below pH 5.8 and above pH 6.4 (de Veth and Kolver, 2001).

Results

The chemical composition of the forage treatments is shown in Table 1. Ground forage samples were analyzed with NIR and minerals were analyzed with wet chemistry for the dietary composition (Rock River Labs, Watertown, WI.) The warm season grasses (BMRSS and teff grass) have lower CP than alfalfa and cool season perennial pasture diets. Dietary protein levels can affect ruminal fermentation patterns and digestibility and create confounding results (Bach, 1999), which is why many in vitro studies feed isonitrogenous diets when investigating different treatments. However, as our goal of this study was to investigate the differences in ruminal fermentation between these different grasses, 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, 1999). This difference in N content of the diets was accounted for by expressing results as a percentage of total N intake (Bach, 1999).

Table 1. Chemical composition (% DM) of four forage diets (Alfalfa, Cool season mixed perennial pasture, BMR sorghum sudangrass, and teff grass) used in continuous culture fermentation.				
	Dietary Treatment			
Chemical Composition	Alfalfa	Cool	BMRSS	Teff
OM	88.1	89.1	89.3	87.8
CP	20.7	22.96	18.51	17.54
NDF	33.2	50.0	51.9	52.1
ADF	33.1	32.2	33.3	34.0
Milk 2006 Milk/ton	3280	2653	2578	2555
Ether extract	1.61	2.50	2.13	2.18
Ash	11.9	10.9	10.7	12.2
NFC	33.7	17.3	19.6	18.7
Ca	1.69	0.67	0.60	0.56
Mg	0.51	0.23	0.33	0.30
P	0.38	0.33	0.34	0.40
K	3.84	3.10	3.25	3.71
TTNDFD	43.9	54.6	54.2	55.6

Digestion and microbial flow

The results in Table 2 show apparent and true digestibility and pH with the different forage diets. Cool perennial grasses and teff grass had lower apparent OM, NDF, and ADF digestibility than alfalfa, while BMRSS was intermediate. This is 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 factors like a faster rate of digestion and faster particle size reduction (Waghorn, 1989). The cool season perennial pasture and both warm season grasses were significantly lower than alfalfa for apparent DM digestibility, and true digestibility for DM and OM. Ribeiro found that higher nutrient digestibility resulted in higher bacterial OM flow (Ribeiro, 2005). This was different than results from this study because as there were higher nutrient digestibilities in alfalfa, bacterial OM flow was similar across all treatments. (Interpretation) There were no significant effects observed in the contrasts of either warm grasses versus cool season perennials, or warm grasses versus cool season grass and alfalfa. So, the warm season grasses are not significantly different than cool season grasses and legumes for apparent or true digestibility.

Table 2. Nutrient digestibility and pH of four forage diets (Alfalfa, Cool season mixed perennial pasture, BMR sorghum sudangrass, and teff grass) during continuous culture fermentation

	Diet					Contrast (*P*-value)	
Item	Alfalfa	Cool	BMRSS	Teff	SEM	BMRSS Teff vs Cool	AC vs BT
Apparent digestibility							
DM, %	69.4[a]	47.1[b]	52.6[b]	49.8[b]	5.2	0.53	0.20
OM, %	31.1[a]	9.8[b]	16.4[ab]	11.9 [b]	4.7	0.47	0.21
NDF, %	75.5[a]	52.6[b]	65.9[ab]	56.6[b]	5.3	0.21	0.61
ADF, %	75.5[a]	55.4[b]	67.5[ab]	59.4[ab]	5.3	0.24	0.72
True digestibility							
DM, %	85.8[a]	64.0[b]	66.2[b]	65.9[b]	5.7	0.74	0.09
OM, %	53.3[a]	29.3[b]	33.1[b]	30.2[b]	4.8	0.70	0.07
pH							
Mean	6.38	6.18	6.19	6.22	0.10	0.8395	0.4612
Minimum	5.8825	5.1375	5.41	5.9225	0.31	.1931	0.6257
Maximum	7.2575	6.7625	7.2325	7.005	0.25	0.2721	0.6739

Table 3 shows nitrogen metabolism of fermenters fed the different forages. Nitrogen intake was highest in fermenters fed alfalfa, next highest in fermenters fed cool season mixed pastures, and lowest for fermenters fed either of the warm season grasses. Warm season grasses do have lower crude protein levels than cool season pastures, and it is well documented that alfalfa has high protein. Both contrasts were significant ($P<0.001$) for amount of N intake per day. Past results show that N-intake can affect rates of microbial growth and ultimately digestibility in in vitro systems like the continuous culture system and in cows, so diets are traditionally formulated to be isonitrogenous when determining effects of treatments. However, for this study we were interested in seeing the effect of the forage on fermentation. Farmers grazing these warm season grasses of interest as complementary grasses to cool season grasses in a grazing system would most likely not be supplementing protein to be making their cow's grazing diets isonitrogenous, so for this study we chose to look at the effects of the grasses with their natural nitrogen/protein levels. Crude protein degradation was similar for all dietary treatments in the fermenters.

Table 3. Nitrogen metabolism of four forage diets (Alfalfa, Cool season mixed perennial pasture, BMR sorghum sudangrass, and teff grass) in continuous culture fermentation							
	Diet					Contrast (*P*-value)	
Item	Alfalfa	Cool	BMRSS	Teff	SEM	BMRSS Teff vs Cool	AC vs BT
N intake g/d	3.09[a]	2.31[b]	2.18[c]	2.20[c]	0.01	<0.0001	<0.0001
NH_3-N mg/dl	22.5[a]	7.5[b]	7.4[b]	8.9[b]	0.75	0.47	<0.0001
CP degradation, %	79.8	77.2	69.1	65.9	4.6	0.12	0.04
N flows g/d							
Total N	1.99[a]	1.50[b]	1.51[b]	1.70[ab]	0.11	0.43	0.18
NH_3-N	0.52[a]	0.17[b]	0.17[b]	0.20[b]	0.02	0.65	<0.0001
NAN	1.46	1.33	1.34	1.50	0.12	0.52	0.84
Bacterial N	0.84	0.81	0.67	0.75	0.14	0.57	0.42
Dietary N	0.62	0.53	0.67	0.75	0.12	0.23	0.27
N flows, % of total N flow							
NH_3-N	26.5[a]	11.6[b]	11.4[b]	11.8[b]	1.8	1.0	0.001
NAN	73.5[a]	88.4[b]	88.6[b]	88.2[b]	1.8	1.0	0.001
Bacterial N	41.3	54.1	44.4	43.8	6.1	0.2097	0.5662
Dietary N	32.1	34.3	44.2	44.4	6.0	0.2017	0.0924
Efficiency of microbial protein synthesis							
g N/kg DM truly digested	15.0	19.9	16.0	18.7	2.7	0.446	0.9563
g N/kg OM truly digested	40.2	69.6	49.5	67.6	11.7	0.4536	0.7607

Cool season grass and BMRSS had similar total N flow g/d, while alfalfa had the highest total N flow and teff was intermediate in total N flow. Ammonia-N was significantly higher in alfalfa than for the cool and warm season grasses. There was no difference in nitrogen flows for non-ammonia N, bacterial N, or dietary N. As a percent of total N flows, alfalfa was significantly higher for ammonia nitrogen and non-ammonia nitrogen than the grasses, with no change in bacterial nitrogen or dietary nitrogen flows as a percent of total nitrogen. Even though alfalfa had higher ammonia nitrogen concentrations, there were no differences in the efficiency of microbial protein synthesis between any of the treatments, on either a DM basis or OM basis. This is interesting, but shows that although there was significantly lower Ammonia-N in all the grass treatments than alfalfa, there were still adequate amounts of Ammonia-N for microbial protein synthesis. The minimum amount of Ammonia-N required for microbial protein synthesis has been 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.

There was no significant difference between mean, minimum, or maximum pH between any dietary treatments. A continuous culture rumen fermenter study researching effects of pH levels

determined that the optimal pH for high quality pasture forage is 6.35 (de Veth et al., 2001), which is very close to the mean pH for our treatments, 6.38, 6.18, 6.19, and 6.22 for alfalfa, cool season pasture, BMRSS, and teff grass respectively. Some studies indicate that decreased fermentation may take place beginning at a pH of lower than 6.25 (Sauvant, 1999), but a large decrease in digestibility may not occur until below a ruminal pH of 5.8 in a high quality pasture diet (Bargo et al., 2001; de Veth et al., 2001). We therefore also analyzed the amount of time spent below 5.8 as well as the amount of time spent above 6.4 for this study. Time spent below pH 5.8 ranged from 1 to 5 minutes/day. Sauvant 1999 found that it may be possible to experience a transitory reduction of pH below 6.0 for 4 hours without affecting microbial fermentation. We analyzed the pH of lower than 5.8 because this was a high quality pasture forage from spring pastures (de Veth, 2001) but did not see the amount of time in this lower range anywhere near four hours. Time spent above pH of 6.4 was analyzed, assuming the optimal pH level for these diets would be 6.35. Treatments averaged 4, 6, 7, and 12 hours per day above pH 6.4 for Cool, BMRSS, Teff, and alfalfa, respectively.

As this is an in vitro system, diets may be affected differently when actually consumed by cows (Mansfield, et al, 1995). Although "fresh pasture grass" was used, it underwent heating in the drying and pelleting processes, which could change 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 the actual pasture forage grasses that cows would be consuming in a grazing system. Fresh forages have higher concentrations of rapidly fermented sugars as well as higher concentrations of more digestible protein, which could have been lost during some of the heating processes of our forages (Van Soest, 1994). This continuous culture system excludes protozoa from the system, which could also affect some results, as this leaves fermentation to be completed only by bacteria. (Hannah, et al, 1986; Mansfield, et al., 1995). In addition, not all grazing cows consume all-grass diets, so there could be more complex interactions if grazing cows would also consume concentrate or are provided some processed forages in a TMR supplement while grazing pasture. Previous studies have shown alterations in bacterial growth rates with different combinations of carbohydrates, which may decrease fiber digestibility (Russel and Baldwin 1978) and improved N utilization when grazing cows were supplemented with TMR (Vibart, 2010). Minimum effective fiber requirements may be different for cows grazing high quality pasture than for cows fed mixed forage and concentrate diets because of interactions that occur in a mixed diet. (De Veth and Kolver, 2001; Kolver, et al., 1998a). There may also be an interaction if cows graze one type of grass, then sequentially another type of grass, and it would be interesting to see what kind of response this interaction may elicit in the rumen. A grazing study with beef cattle found that it was less advantageous to move cattle to warm season grasses because of the lower nutritive value of the grasses, and increased maturity of cool season grasses in the system when doing this (Moore, 2004). It would be interesting to see how this shift would affect ruminal fermentation in cattle as well, and how it would affect dairy cows if pastures were more closely managed for maturity of forage. Unpublished research from a concurrent study shows that cows on BMRSS have significantly lower levels of rumination than cows grazing cool season perennial grasses, so this may affect microbial activity in the rumen when grazing this grass (unpublished Ruh, Production/Rumination/Activity data, et al 2016).

Conclusion

Preliminary results of this research will be presented at:

- MOSES Organic Conference (February, 2017, LaCrosse, WI)
- American Dairy Science Association/American Society of Animal Science meetings (June 2017, Pittsburgh, PA)

In the future, scientific results will be published in the Journal of Dairy Science, and an extension fact-sheet will be published with on the University of Minnesota Dairy Extension website. These results will be a portion of Kathryn Ruh's Master's thesis.

Publications authored by Investigators

Ruh, Kathryn and Brad Heins. 2016. Study looks at impact of warm season annual grasses for grazing organic dairy cows. Organic Broadcaster. May/June 2016. Page 9,16

Kathryn Ruh, Brad Heins, and James Paulson. Forage quality of two different pasture systems incorporating warm and cool season forages for grazing organic dairy cattle. MOSES Organic Conference Research Poster Session February, 2016

Brad Heins and Kathryn Ruh. 2016. Forage Quality of Two Different Pasture Systems Incorporating Warm and Cool Season Forages for Grazing Organic Dairy Cattle. In Proc. Four-State Dairy Nutrition and Management Workshop. Dubuque, IA June 2016 Page 55-59.

Ruh, K., B. Heins, and J. Paulson. 2016. Forage quality of two different pasture systems incorporating warm and cool season forages for grazing organic dairy cattle. Abstract 634 JAM 2016. Salt Lake City Utah.

Ruh, K., B. Heins, and J. Paulson. 2016. Milk production, rumination and body condition score of organic dairy cattle grazing two pasture systems incorporating warm and cool season forages. Abstract 661 JAM 2016. Salt Lake City Utah.