

Fermentation of eastern gamagrass (*Tripsacum dactyloides* [L.] L.) by mixed cultures of ruminal microorganisms with or without supplemental corn¹

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ABSTRACT: Five dual-flow fermentors (700 mL) were used to determine the effects of eastern gamagrass (*Tripsacum dactyloides* [L.] L.) diets on microbial metabolism by mixed rumen cultures. Fermentors were incubated with filtered ruminal contents and allowed to adapt for 4 d to diets followed by 3 d of sample collection. Five dietary treatments were tested: 1) gamagrass hay (GH) + no corn (GHNC), 2) gamma grass silage (GS) + no corn (GSNC), 3) GS + low corn (GSLC), 4) GS + medium corn (GSMC); and 5) GS + high corn (GSHC). The experiment was conducted as a randomized complete block design with five treatments and three replications. Total VFA concentrations were not affected by diets. Corn addition linearly decreased ($P < 0.001$) molar proportion of acetate. In contrast, molar proportion of propionate was reduced in GSLC (cubic effect, $P < 0.001$) but remained similar across other diets. Corn supplementation linearly increased molar proportion of butyrate ($P < 0.001$). The acetate + butyrate-to-propionate ratio was highest in cultures offered

GSLC (cubic effect, $P < 0.001$) but similar across other diets. Feeding GSNC resulted in a higher ruminal pH compared with GHNC ($P < 0.03$). Increasing the level of corn supplementation in GS linearly decreased culture pH ($P < 0.001$). All diets resulted in similar methane production, with the exception of GSMC, which lowered methane output (quadratic effect, $P < 0.004$). Total substrate fermented to VFA and gas tended to be greater with GHNC than with GSNC ($P < 0.06$) and linearly increased with the addition of corn ($P < 0.004$). Neutral detergent fiber digestibility was similar between GH and GS and was not affected by supplemental corn. Microbial N flow increased in cultures offered GSHC (quadratic effect, $P < 0.02$). Corn supplementation at the medium and high level linearly decreased C_{18:0} ($P < 0.02$) and increased *trans*-C_{18:1} ($P < 0.004$). Including corn at the high level with GS did not have a detrimental effect on fermentation in dual-flow fermentors.

Key Words: Corn Supplementation, Gamagrass, Microbial Nitrogen

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J. Anim. Sci. 2004. 82:170–178

Introduction

Ensiling results in changes in the chemical composition of forage that typically lower microbial protein synthesis when the silage is fed to animals (Siddons et al., 1985). This is due, in part, to the lack of additional fermentable energy for microbial use. The main factors affecting microbial assimilation of degraded N, when a silage-based diet is fed, are the large amount of nonprotein N in silage, dietary source of carbohydrate, and source and level of dietary N (Dewhurst et al., 2000).

Silages typically contain large amounts of soluble protein but mainly structural carbohydrates that ferment slowly. This mismatch between the rate at which energy and N are supplied to the microbes increases the loss of N in the rumen. Supplementing silage-based diets with moderate levels of starch often increases microbial protein synthesis. However, starch can have negative effects through decreased ruminal pH and fiber degradation, increased energy spilling reaction, and a higher requirement for preformed AA (Russell and Wallace, 1997). Consequently, synchronizing energy and N release has not always benefited the ruminal environment, which supports the argument that perhaps a continuous supply of energy is more important (Maeng et al., 1997).

Eastern gamagrass (*Tripsacum dactyloides* [L.] L.), among several perennial grass forages in the Southeast region of the United States, has been shown to have a high nutritive value when compared with several traditional perennial legumes (Coblentz et al., 1998). The

¹Financial support from North Carolina Dairy Foundation is greatly appreciated. The authors express their thanks to S. McLeod for her excellent assistance in laboratory analysis.

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Received January 7, 2003.

Accepted September 22, 2003

NDF concentration in eastern gamagrass is high and is extensively degraded in the rumen. Also, between 51 and 64% of N in eastern gamagrass is associated with the neutral detergent insoluble fraction (Coblentz et al., 1998). Providing additional energy to microbes fermenting gamagrass might improve the microbial capture of gamagrass N. Our objective was to determine the effects of eastern gamagrass offered as hay or silage without or with supplemental corn on fermentation by mixed cultures of ruminal microorganisms.

Materials and Methods

Apparatus, Diets, and Experimental Design

An *in vitro* study was designed to monitor the effects on ruminal fermentation when eastern gamagrass was fed as hay (**GH**) or silage (**GS**) without or with supplemental corn. Ruminal inoculum was obtained from a nonlactating, ruminally cannulated Holstein cow fed alfalfa (*Medicago sativa* L.) hay. The surgery protocol and animal handling procedures were approved by the North Carolina State University Institution of Animal Care and Use Committee (Approval No. 02-045). Whole ruminal contents (approximately 3.5 L) were obtained 2 h postprandially and squeezed through a double-layered cheesecloth. The strained ruminal fluid (700 mL per fermentor) was used to inoculate five fermentors (Teather and Sauer, 1988). Anaerobic conditions in the culture vessels were maintained by infusion of CO₂ gas at a rate of 20 mL/min. A circulating water bath was used to maintain the temperature of the fermentors at 39°C. Continuous stirring of fermentor contents was achieved with the aid of a central paddle set at a speed of 10 rpm for the duration of the experiment. The liquid dilution rate of the cultures was maintained at 6.3 %/h by regulating the addition of artificial saliva prepared as described by Slyter et al. (1966). The solids dilution rate was 4.6 %/h, which produced a mean solids retention time of 22 h. Alfalfa hay pellets (14 g DM basis) were added to the fermentors twice daily for 2 d during the adaptation period followed by 2 d of gradual transition to the gamagrass treatment diets. On d 5, 100% of the experimental diets were added to the fermentors for 3 d. Between 13.6 and 14.5 g of the experimental diets (DM basis) were placed in each fermentor daily in two equal portions at 0800 and 1700.

A second year stand of "Iuka" eastern gamagrass was used for this experiment. The forage was harvested in the late vegetative stage using a conventional mower-conditioner. The forage for silage was immediately chopped (6 to 12.5 cm in length), blown into a wagon, and bagged for ensiling. The DM of the forage at time of ensiling was 32.7%. The forage preserved as hay was tedded twice daily and baled 3 d later at 87.3% DM using a conventional baler. Before the start of the experiment, the hay was cut into lengths ranging between 7 to 13 cm by passing it through a hydraulic bale processor (Van Dale 5600, J. Starr Industries, Fort Atkinson,

WI), which avoids excessive leaf loss that may occur during grinding. Five dietary treatments were compared in a randomized complete block design with three blocks as repeated runs. Treatments consisted of: 1) GH + no corn (**GHNC**), 2) GS + no corn (**GSNC**), 3) GS + low corn (**GSLC**), 4) GS + medium corn (**GSMC**), and GS + high corn (**GSHC**). Feed samples of gamagrass hay were ground through a Wiley mill (2-mm screen; Thomas Scientific, Philadelphia, PA). Gamagrass silage, which had been stored frozen (-20°C), was thawed and then processed in a food chopper (approximate length = 1 cm; model FC 19, G. S. Blakeslee & Co., Cicero, IL). All diets contained a protein supplement composed of soybean meal (48% CP) and a vitamin and mineral premix. Dietary CP was formulated to be similar across all diets based on CP values from preliminary samples of gamagrass hay and silage. Diets were prepared by weighing and blending each ingredient manually.

Sample Collection and Analytical Procedures

Feed samples were analyzed for DM, ash, and Kjeldahl N according to the methods of the AOAC (1999). Concentrations of NDF and ADF were determined using an Ankom 200 fiber extractor (Ankom Technologies, Fairport, NY) according to the method of Van Soest et al. (1991). Feed lipid was extracted as outlined by Feller et al. (1995). The nonprotein N fraction in the feed was determined using a trichloroacetic acid precipitation (Licitra et al., 1996). Five milliliters of thoroughly mixed culture contents was collected 2 h after feeding twice daily and centrifuged, and the supernatant was analyzed for VFA by GLC (model CP-3380; Varian, Walnut Creek, CA) and for NH₃-N using a colorimetric assay (Beecher and Whitten, 1970). Ten microliters of headspace gas samples from the fermentor was drawn into a gas tight syringe (Hamilton Co., Reno, NV) 2 h after feeding and analyzed for methane using GLC. Daily methane output (mmol/d) was calculated as reported earlier (Jenkins et al., 2002) using the following equation: Methane concentration in fermentor headspace (mmol/mL) × CO₂ gas flow through the fermentor headspace (20 mL/min) × 60 min × 24 h. The pH of the ruminal cultures was recorded when samples were taken for CH₄ analysis.

Separate 5-mL samples of the mixed culture contents were taken on the last day of each period at 4 h after the morning feeding and frozen (-70°C) for long-chain fatty acid analysis. The frozen samples were thawed, methylated (Kramer et al., 1997), and then analyzed for long-chain fatty acid by GLC.

Throughout the experiment, effluent containers were emptied daily. On the last day of each period, effluent containers were drained and 50 mL of the particulate matter was obtained and frozen for subsequent analysis. Frozen samples were thawed, centrifuged at 1,300 × g for 10 min, and analyzed for DM and NDF according to procedures outlined above for feed samples. On the

last day of each period, 50 mL of mixed culture contents was placed in conical tubes and frozen (-20°C). After thawing, the samples were mixed with 25 mL of 0.9% (wt/vol) NaCl and 1.5 mL of 30% (vol/vol) formaldehyde and divided into two aliquots. Both aliquots were independently used to isolate microbial cells by differential centrifugation. They were first centrifuged at $1,074 \times g$ for 5 min to precipitate particles and then at $47,800 \times g$ for 20 min to sediment the bacteria. The resulting microbial pellet was dried for 4 h at 50°C and analyzed for microbial N concentrations.

Calculations and Statistical Analysis

Nonfibrous carbohydrates were calculated as $100 - (\text{ash} + \text{CP} + \text{fat} + \text{NDF})$. The total substrate degraded was partitioned into the amount fermented to VFA, gas ($\text{CH}_4 + \text{CO}_2$), and microbial biomass. Both the direct (from the fermentation of glucose) and the indirect (release of CO_2 as VFA enter a bicarbonate-based buffer medium) CO_2 production amounts were estimated (Beuvinck and Spoelstra, 1992). The substrate fermented to VFA, CH_4 , and CO_2 was calculated based on the moles of individual VFA produced, daily methane output, and direct and indirect CO_2 released from fermentation and buffer addition, respectively (Wolin, 1960; Van Soest, 1994; Blümmel et al., 1997). The moles of ATP produced were estimated by assigning 2, 3, and 3 mol of ATP per mole of acetic, propionic, and butyric acids, respectively, and 1 mol of ATP per mole of methane (Groot et al., 1998). Energy and dilution rate are among the several factors that can influence the efficiency of microbial growth (Hespell and Bryant, 1979). With mixed cultures of ruminal microorganisms, the effect of varying energy concentration had little effect on the efficiency of microbial protein synthesis (Isaacson et al., 1975). Microbial synthesis ranged between 10 and 13.6 g of cells/mol of ATP, across several different concentrations of energy, with an average of 11.7 g of cells/mol of ATP (Isaacson et al., 1975). At dilution rates similar to those used in this study, microbial efficiency averaged 11.6 g of cells/mol of ATP (Hespell and Bryant, 1979). In the present study, the yield of microbial biomass per mole of ATP was set at 11.7 mg/mmol ATP, and, because 80% of the bacterial components are synthesized from glucose (Groot et al., 1998), microbial biomass (g of DM/d) was calculated as follows: $[(0.8 \text{ YATP (2 acetate, mmoL/d)} + (3 \text{ propionate, mmoL/d)} + (3 \text{ butyrate, mmoL/d)} + (\text{CH}_4, \text{ mmoL/d})]/1,000$. Assigning a fixed value for ATP production can be misleading because the exact amount of ATP produced, particularly in continuous cultures, is not known and can vary. Assumed ATP values, however, provide a basis for the discussion of variables like the microbial N that were measured directly. In addition to estimating the amount of substrate converted to microbial biomass using stoichiometric equations outlined above, microbial N flow (g of DM/d) was calculated directly from the total microbial DM percentage obtained from the

microbial pellets isolated from culture contents and the mean flow rate of fermentor contents using the following equation:

$$\text{Microbial N flow, g/d} = (\text{microbial DM flow, g/d}) \times (\text{microbial N \%}/100)$$

The efficiency of microbial synthesis was calculated as follows:

$$\text{Microbial efficiency, g N/kg of apparent OM fermented} = [(\text{Microbial N flow, g/d}) \div (\text{OM fermented, g/d})] \times 1,000.$$

Both the microbial N flow (g/d) that was estimated directly and the conversion of substrate to microbial biomass (g of DM/d) that was estimated using stoichiometric equations are reported.

Data for GHNC and GSNC were analyzed separately as repeated measures according to a randomized complete block design using the PROC MIXED procedures of PC SAS (SAS Inst., Inc., Cary, NC). The model included the effect of treatment and the random effect of run. A separate analysis for increasing levels of corn supplementation was conducted using the same design as described above. Linear, quadratic, and cubic contrasts were calculated for treatments to determine the ruminal fermentation response to increasing levels of corn.

Results

A protein supplement was included to formulate diets (Table 1) with similar CP concentration. The GHNC and GSNC treatments, however, tended to have higher CP concentration compared to GS with additional corn. This occurred because the CP value for GH and GS in the final diets was higher than the value obtained from samples taken earlier (13.7 vs. 11.0%). Concentrations of nonprotein N, NDF, and ADF decreased with increasing levels of corn. Corn addition increased the fat concentration of the diet as well as calculated nonfibrous carbohydrate concentration. Linolenic acid made up almost 50% of total FA in GHNC and GSNC diets. Both $\text{C}_{18:0}$ and *trans*- $\text{C}_{18:1}$ FA were present at less than 3% of the total FA. Including corn in GS diets decreased $\text{C}_{18:3}$ and increased $\text{C}_{18:2}$ FA concentrations.

Total VFA concentrations averaged 46 mM and were not different between GHNC and GSNC (Table 2). Supplemental corn linearly increased ($P < 0.03$) total VFA concentrations. Concentrations of acetate, propionate, and butyrate were similar between GHNC and GSNC ($P > 0.10$). There was a linear increase in ruminal propionate ($P < 0.002$) and butyrate ($P < 0.001$) with increasing levels of corn.

Molar percentages of acetate were higher with GHNC ($P < 0.001$) compared with GSNC (Table 2). Corn addition linearly decreased ($P < 0.001$) molar percentage of acetate. Feeding gamagrass as hay or

Table 1. Ingredient and chemical composition of diets

Item	Diet ^a				
	GHNC	GSNC	GSLC	GSMC	GSHC
Ingredient	% of DM				
Gama grass hay	78.0	—	—	—	—
Gama grass silage	—	81.5	70.6	56.7	29.7
Ground corn	—	—	12.6	27.8	54.6
Protein supplement ^b	22.0	18.5	16.8	15.5	15.7
Composition	DM				
DM, %	89.1	38.9	40.6	44.3	58.3
CP, %	19.5	18.4	16.9	16.4	15.7
Nonprotein N, % of total N	28.2	34.3	31.5	31.6	28.7
ADF, %	28.1	30.6	26.9	21.4	12.2
NDF, %	60.2	61.9	55.2	45.7	30.2
Fat, %	1.9	1.8	2.1	2.5	3.3
Ash, %	10.1	11.9	10.9	9.0	7.6
NFC, % ^c	8.3	6.0	14.9	26.4	43.2
Fatty acid profile	g/100 g of total fatty acid				
C _{16:0}	24.6	22.9	17.4	13.9	10.8
C _{18:0}	2.6	2.9	1.9	2.7	2.3
<i>trans</i> -C _{18:1}	2.7	2.9	0.8	1.1	1.2
<i>cis</i> -C _{18:1}	—	0.8	13.8	15.0	19.1
C _{18:2}	17.2	18.6	24.5	34.7	46.3
C _{18:3}	47.8	46.4	36.5	27.2	15.7
C _{20:0}	1.1	0.9	1.1	0.8	0.7

^aGHNC = gamagrass hay + no corn; GSNC = gamagrass silage (GS) + no corn; GSLC = GS + low level of corn; GSMC = GS + medium level of corn; GSHC = GS + high level of corn.

^bProtein supplement included (DM basis) 78.6% soybean meal (48% CP), 8.5% defluorinated rock phosphate, 2.5% calcitic limestone, 2.7% salt, 1.6% magnesium oxide, 5.4% sodium bicarbonate, 0.6% vitamin and trace mineral premix (McNess 1401), which contained 21.5% Ca, 5.5% S, 3.9% Zn, 3.9% Mn, 1.2% Cu, 1.0% Fe, 0.07% I, 0.06% Co, 0.03% Se, 1200 IU of vitamin A/g, 300 IU of vitamin D₃/g, and 4 IU of vitamin E/g.

^cNFC (nonfibrous carbohydrate) = 100 - (CP + NDF + fat + ash).

Table 2. Concentration and molar ratio of VFA in continuous cultures receiving gamagrass hay (GH) or silage (GS) with or without corn^a

Item	GHNC	GSNC	SE	<i>P</i> <	GS with or without corn ^b				SE	Probability of greater <i>F</i>		
					GSNC	GSLC	GSMC	GSHC		Contrasts		
										Linear	Quadratic	Cubic
Total, mM	47.0	44.9	2.07	0.48	44.9	49.0	49.1	52.4	2.1	0.03	0.66	0.47
Individual, mM												
Acetate	30.0	27.7	1.4	0.21	27.7	30.0	28.9	30.3	1.3	0.25	0.72	0.28
Propionate	8.73	8.21	0.48	0.30	8.21	8.33	9.23	9.77	0.36	0.002	0.84	0.41
Butyrate	5.38	5.91	0.23	0.13	5.91	7.46	7.72	9.05	0.48	0.001	0.43	0.29
Valerate	0.98	0.97	0.06	0.83	0.97	0.97	1.02	0.96	0.05	0.91	0.38	0.60
Isobutyrate	0.50	0.53	0.04	0.55	0.53	0.56	0.52	0.50	0.03	0.30	0.68	0.50
Isovalerate	1.38	1.68	0.12	0.09	1.68	1.67	1.76	1.88	0.16	0.29	0.85	0.84
Individual, mol/100 mol												
Acetate (A)	63.9	61.5	0.4	0.001	61.5	61.3	58.8	57.9	0.7	0.001	0.52	0.13
Propionate (P)	18.6	18.3	0.4	0.51	18.3	17.0	18.8	18.7	0.4	0.05	0.58	0.001
Butyrate (B)	11.5	13.1	0.3	0.001	13.1	15.2	15.7	17.1	0.6	0.001	0.17	0.21
Valerate	2.09	2.16	0.08	0.39	2.16	1.99	2.07	1.82	0.07	0.001	0.57	0.03
Isobutyrate	1.04	1.17	0.08	0.18	1.17	1.14	1.06	0.94	0.05	0.002	0.85	0.69
Isovalerate	2.91	3.73	0.22	0.003	3.73	3.38	3.54	3.54	0.27	0.79	0.52	0.43
A:P	3.45	3.37	0.07	0.28	3.37	3.61	3.13	3.11	0.08	0.01	0.74	0.001
(A + B):P	4.08	4.09	0.09	0.91	4.09	4.51	3.97	4.03	0.11	0.06	0.45	0.001

^aEach value is the mean of three runs.

^bGHNC = GH + no corn; GSNC = GS + no corn; GSLC = GS + low level of corn; GSMC = GS + medium level of corn; GSHC = GS + high level of corn.

Table 3. Ruminal pH, concentration, and production of ammonia-N (NH₃-N), and methane (CH₄) output in continuous cultures receiving gamagrass hay (GH) or silage (GS) with or without corn^a

Item	GHNC	GSNC	SE	P <	GS with or without corn ^b					Probability of greater P		
					GSNC	GSLC	GSMC	GSHC	SE	Contrasts		
										Linear	Quadratic	Cubic
Culture pH	6.10	6.27	0.10	0.03	6.27	6.05	6.00	5.91	0.07	0.001	0.006	0.13
NH ₃ -N, mg/100 mL	29.2	28.1	1.5	0.54	28.1	29.4	27.0	25.8	1.7	0.08	0.62	0.28
NH ₃ -N, g/d ^c	0.31	0.29	0.02	0.50	0.29	0.31	0.28	0.27	0.02	0.08	0.61	0.27
CH ₄ , mmol/d	21.0	19.0	1.3	0.39	19.6	19.2	16.2	20.6	0.9	0.56	0.004	0.08

^aEach value is the mean of three runs.

^bGHNC = GH + no corn; GSNC = GS + no corn; GSLC = GS + low level of corn; GSMC = GS + medium level of corn; GSHC = GS + high level of corn.

^cNH₃-N, g/d = [NH₃-N concentration, mg/100 mL × fermentor volume (700 mL) × 1.5 (turnover rate of fermentor)]/1,000.

silage did not affect the molar percentage of propionate. Feeding GSLC lowered molar proportions of ruminal propionate compared with GSNC, GSMC, and GSHC (cubic effect, $P < 0.001$). The percentage of butyrate was higher in GSNC compared with GHNC ($P < 0.001$). Corn supplementation linearly increased molar percentage of butyrate ($P < 0.001$) and decreased molar percentage of valerate ($P < 0.001$). Ruminal isovalerate increased ($P < 0.003$) with GSNC compared with GHNC. The addition of corn had no effect ($P > 0.10$) on the percentage of ruminal isovalerate but linearly decreased ($P < 0.002$) the concentration of isobutyrate. The acetate-to-propionate (A:P) ratio was similar between GHNC and GSNC. The A:P ratio was highest with GSLC compared with GSNC, GSMC, and GSHC (cubic effect, $P < 0.001$). The lipogenic-to-gluconic VFA ratio ([acetate + butyrate]:propionate) was also similar among GSNC, GSMC, and GSHC but increased (cubic effect, $P < 0.001$) with GSLC.

Feeding GSNC resulted in a higher ruminal pH ($P < 0.03$) compared with GHNC (Table 3). Increasing the level of corn in GS linearly decreased culture pH ($P < 0.001$). Concentrations and flow of NH₃-N did not differ between GHNC and GSNC ($P > 0.10$) and linearly decreased ($P < 0.08$) with corn supplementation. Methane output did not differ between GHNC and GSNC. Including corn resulted in a quadratic effect ($P < 0.004$) on methane production, with GSMC being lowest compared to GSNC, GSLC, and GSHC.

Feeding GHNC or GSNC did not affect the amount of substrate that was converted to VFA, gas (CH₄ + CO₂), or microbial biomass (Table 4). Corn inclusion linearly increased the amount of substrate fermented to VFA ($P < 0.02$), microbial biomass ($P < 0.04$), and to gas ($P < 0.07$). When expressed as a percentage of DM fed, total substrate fermented was greater ($P < 0.06$) with GHNC compared with GSNC (Table 4) and increased ($P < 0.004$) linearly with the addition of corn. Neutral detergent fiber digestibility was similar between GHNC and GSNC and was not affected ($P > 0.10$) by the addition of corn. Feeding gamagrass as hay or silage did not affect ($P > 0.10$) the daily flow of microbial N. There was a quadratic effect on microbial

N flow ($P < 0.02$) and efficiency of microbial protein synthesis ($P < 0.006$) with corn inclusion.

The fatty acid composition of ruminal cultures that received GHNC was similar to cultures that received GSNC (Table 5). More than 60% of the total FA consisted of C_{18:0} in cultures offered GHNC and GSNC. The concentration of the *trans*-isomer of C_{18:1} averaged less than 5.2% in cultures fed GHNC and GSNC. Adding corn linearly decreased ($P < 0.02$) the C_{18:0} content of the ruminal cultures. A decrease in C_{18:0} was accompanied by a linear increase in the *trans*-C_{18:1} concentration ($P < 0.004$), which made up nearly 20% of the total FA in ruminal cultures receiving GSMC and GSHC. However, concentration of *cis*-C_{18:1} remained unchanged across all treatments. The concentration of linoleic acid was less than 2% of the total fatty acids in cultures receiving GHNC and GSNC. The addition of corn resulted in a linear increase ($P < 0.01$) in the linoleic acid content of ruminal cultures. The percentage of saturated FA was lower ($P < 0.06$) and that of unsaturated FA higher ($P < 0.06$) in GSNC than in GHNC. The addition of corn linearly decreased ($P < 0.005$) saturated FA concentration and linearly increased ($P < 0.005$) unsaturated FA concentration.

Discussion

Lowering the forage-to-concentrate ratio by the addition of corn at the medium and high levels lowered the acetate-to-propionate ratio, which is consistent with previous findings (Siciliano-Jones and Murphy, 1989). The decrease in the A:P ratio was due primarily to a decrease in ruminal acetate because ruminal propionate was not affected. In contrast, the addition of corn at the low level increased the A:P ratio due to a decrease in ruminal propionate, which was unexpected and is difficult to explain. Higher molar percentages of butyrate in cultures receiving GSNC compared to GHNC may be due to a higher concentration of protozoa. Huhtanen (1992) and Jaakkola and Huhtanen (1993) reported that an increase in the number of protozoa is accompanied by an increase in ruminal butyrate. Ushida et al. (1986) also showed that faunated

Table 4. Partitioning of substrate, fermentability, and microbial growth in continuous cultures receiving gamagrass hay (GH) or silage (GS) with or without corn^a

Item	GHNC	GSNC	SE	P <	GS with or without corn ^b				SE	Probability of greater F		
					GSNC	GSLC	GSMC	GSHC		Contrasts		
										Linear	Quadratic	Cubic
DM fed, g/d	13.7	14.5	—		14.5	14.0	13.6	13.6	—			
Substrate used, g of DM/d												
For VFA ^c	3.07	2.93	0.12	0.36	2.93	3.23	3.25	3.51	0.12	0.02	0.57	0.37
For CH ₄ + CO ₂ ^d	2.26	2.22	0.07	0.73	2.22	2.31	2.13	2.51	0.08	0.07	0.13	0.13
For microbial biomass	1.29	1.22	0.03	0.18	1.22	1.32	1.30	1.44	0.04	0.01	0.92	0.24
Substrate fermented, % ^e	48.3	43.9	1.2	0.06	43.9	49.0	49.2	54.8	1.6	0.004	0.73	0.24
NDF digestibility, %	42.9	42.7	1.7	0.94	42.7	42.6	39.9	44.2	2.0	0.48	0.12	0.55
Microbial efficiency ^f	5.5	5.8	0.37	0.35	5.8	4.6	4.6	6.4	0.37	0.78	0.006	0.24
Microbial N flow, g/d	0.28	0.25	0.03	0.17	0.25	0.22	0.24	0.38	0.03	0.04	0.02	0.08

^aEach value is the mean of three runs.

^bGHNC = GH + no corn; GSNC = GS + no corn; GSLC = GS + low level of corn; GSMC = GS + medium level of corn; GSHC = GS + high level of corn.

^c(acetate, mol/d × 60.05) + (propionate, mol/d × 74.08) + (butyrate, mol/d × 88.10).

^dSubstrate used for (CO₂, mol/d × 44) + (CH₄, mol/d × 16) + (2H₂O, mol/d × 36).

^e[(Substrate used for VFA, CO₂ + CH₄ + 2H₂O, and microbial biomass) ÷ (DM fed)] × 100.

^fEfficiency of microbial synthesis expressed as g of N/kg of apparent OM fermented.

sheep had higher molar percentages of butyrate than did defaunated sheep. Jaakkola and Huhtanen (1993) reported a tendency for larger numbers of protozoa in cultures fed grass silage compared with grass hay diets, which may, in part, explain the present increase in butyrate in cultures receiving GSNC.

The decrease in acetate percentages in cultures receiving the medium or high supplemental corn seems to be compensated for by an increase in the percentage of butyrate. This resulted in a similar (acetate + butyrate):propionate ratio when compared to cultures receiving gamagrass hay or silage without supplemental corn. Sutton et al. (1988) concluded that ruminal (ace-

tate + butyrate):propionate ratio is probably more important for milk fat production than the A:P ratio because acetate and butyrate are each independently and positively correlated with milk fat concentration. Propionate, however, is negatively correlated with milk fat concentration. Cultures, in this study, fed gamagrass silage with low level of additional corn had the highest (acetate + butyrate):propionate ratio.

Fermentable carbohydrates, when added to forage diets, lower ruminal pH, thereby reducing cellulolytic activity and limiting fiber digestion. Although, the addition of corn lowered ruminal pH, the decrease was not as great as one would expect with the amount of corn

Table 5. Fatty acid (FA) composition of culture contents in continuous cultures receiving gamagrass hay (GH) or silage (GS) with or without corn^a

Item	GHNC	GSNC	SE	P <	GS with or without corn ^b				SE	Probability of greater F		
					GSNC	GSLC	GSMC	GSHC		Contrasts		
										Linear	Quadratic	Cubic
					g/100 g of total FA							
C _{14:0}	0.37	0.52	0.23	0.70	0.52	0.79	0.71	0.68	0.18	0.49	0.18	0.25
C _{16:0}	23.0	23.7	0.7	0.39	23.7	21.7	22.9	21.0	0.9	0.08	0.99	0.10
C _{18:0}	64.4	61.4	3.2	0.07	61.4	60.4	50.8	49.6	3.2	0.02	0.46	0.28
<i>trans</i> -C _{18:1}	4.47	5.17	0.75	0.32	5.2	9.0	18.0	18.7	2.6	0.004	0.13	0.30
<i>cis</i> -C _{18:1}	4.04	5.47	1.95	0.14	5.47	4.05	3.89	5.46	2.47	0.81	0.15	0.81
C _{18:2}	1.32	0.73	0.58	0.55	0.73	1.57	1.61	3.02	0.44	0.01	0.83	0.39
C _{18:3}	0.32	0.32	0.17	0.97	0.32	0.22	0.46	0.26	0.18	0.97	0.62	0.41
C _{20:0}	1.53	1.49	0.14	0.88	1.49	1.41	1.10	1.08	0.08	0.008	0.20	0.22
C _{22:0}	0.51	0.73	0.19	0.33	0.73	0.78	0.54	0.20	0.14	0.004	0.33	0.39
Saturated (S)	89.9	87.8	3.2	0.06	87.8	85.1	76.1	72.5	3.3	0.005	0.43	0.37
Unsaturated (U)	10.2	12.2	3.2	0.06	12.2	14.9	23.9	27.5	3.3	0.005	0.43	0.37
Monounsaturated	8.5	10.6	2.7	0.19	10.6	13.1	21.8	24.1	2.9	0.006	0.34	0.30
Polyunsaturated	1.64	1.55	0.73	0.92	1.55	1.87	2.07	3.33	0.58	0.03	0.57	0.78

^aEach value is the mean of three runs.

^bGHNC = GH + no corn; GSNC = GS + no corn; GSLC = GS + low level of corn; GSMC = GS + medium level of corn; GSHC = GS + high level of corn.

included at the high level. This may, in part, be due to the buffering effect of protozoa associated with the feeding of gamagrass silage. Structural carbohydrates, in particular the high concentration of NDF in gamagrass silage and its relatively slow rate of degradation, may permit sequestration of protozoa. Protozoa accumulate polysaccharides from engulfed starch and soluble carbohydrates and may decrease the rate of acid production. They also allow a more gradual fermentation, reducing rapid increases in the concentration of lactic acid (Jaakkola and Huhtanen, 1993), and thereby preventing a rapid decline in ruminal pH. The lower methane production in cultures fed gamagrass silage with medium corn was unexpected. However, methane output did not change with increased fermentability by corn supplementation. Neutral detergent fiber digestibility was not affected by the lower ruminal pH that was also reflected in the maintenance of methane production. All of these indicators support the viability of protozoal populations at the high level of corn inclusion.

De Visser et al. (1998b) observed a decrease in both the rate and extent of NDF digestibility in diets that were based primarily on grass silage in the presence of supplemental ruminal degradable starch. In another study, De Visser et al. (1998a) reported a numerical decrease in ruminal pH from 6.2 to 5.9 with increasing flaked corn starch in the diet. The authors could not explain the reduction in the NDF digestibility with the small change in pH. They speculated that there might be competition between cellulolytic and amylolytic bacteria for growth factors including N (A. M. van Vuuren, LeLystad, The Netherlands, personal communication). In this study, NDF digestibility was similar across all diets, and culture pH ranged from 6.3 to 5.9. The higher concentrations of fermentable carbohydrate from corn added to gamagrass silage, even at the high level, in the current study did not reduce the activity of cellulolytic microorganisms. Under normal circumstances, ammonia is the most abundant N compound available for microbial growth, and most cellulolytic organisms have an absolute requirement for ammonia. Polan et al. (1976) reported that ammonia might be limited when rapidly fermentable carbohydrates are fed, thus reducing cellulose digestion (Stern et al., 1978). Since the concentrations of $\text{NH}_3\text{-N}$ and NDF digestibility were not decreased in the cultures offered gamagrass silage supplemented with the high level of corn, ammonia must not have limited the growth of the cellulolytic microorganisms.

The level of readily fermentable carbohydrates in the diet for optimal microbial growth has been extensively researched but is difficult to define (Kim et al., 1999). Scollan et al. (1996) reported that the microbial N flow to the duodenum was not affected by various sources of carbohydrates when supplemented at 10% of silage intake to grass silage-based diets fed to growing steers. The efficiency of utilization of energy from fermented OM by ruminal microbes is estimated at 60 to 70% of that from unfermented feeds (ARC, 1984). The reduced

efficiency with the ensiled material is attributed primarily to the lesser fermentable fraction (Charmley, 2001) and greater absorption of VFA across the rumen wall (Thomas and Thomas, 1985). Furthermore, silages typically contain a high proportion of nonprotein N compounds that are more rapidly degraded in the rumen, resulting in less than optimal capture of feed N by microbes. In gamagrass, however, a large proportion of the N is associated with the neutral detergent insoluble fraction (51 to 64% of total N; Coblenz et al., 1998). Restricted fermentation of this fraction during ensiling may result in an unusually low concentration of nonprotein N (48.2%) when compared with other grass silages (60 to 70% of total N; Van Soest, 1994).

Contrary to our expectation, microbial N flow in the present study decreased when corn was included at the low and medium level, compared with GSNC, but increased when included at the high level. Chamberlain et al. (1985) reported an increase in the number of total protozoa with starch supplementation to grass silage diets. Jaakkola and Huhtanen (1993) also showed an increase in protozoal populations with an increase in the level of concentrate in silage-based diets. We did not directly measure protozoal populations in the present study. However, protozoa were visually discernable as large white bands, within the fermentors. The relatively slow rate at which culture contents are stirred (10 to 12 rpm) allows protozoal populations to remain in cultures over extended periods (Teather and Sauer, 1988). Over a period of 1 to 2 d, they tended to localize at either the base or the bottom of the overflow port, areas that seemed to have the slowest turnover rate. If silages do indeed support increased population of protozoa, some of the lack of response to additional corn, in the present study, may be due to the increase in culture fauna. An increase in protozoa can result in an increase in the intraruminal recycling of N, thereby reducing microbial efficiency (Firkins et al., 1992). Consequently, enhancing microbial capture of dietary N by providing additional energy to grass silages may result in an increase in the recycling of ruminal N, thereby limiting the potential impact of increased energy supply in reducing ruminal ammonia-N. With the GSHC diet, however, culture pH may have been low enough to affect protozoal populations and favor the growth of the amylolytic microbes.

Grass is rich in $\text{C}_{18:3}$ FA, and preservation as silage should not decrease its concentration (Doreau and Poncet, 2000). Both gamagrass hay and silage contained a similar concentration of $\text{C}_{18:3}$, suggesting that there was no oxidation damage during the ensiling process. Because corn contains high amounts of polyunsaturated FA, which are precursors for *trans*- $\text{C}_{18:1}$, gamagrass silage with medium or high level of supplemental corn resulted in increased production of *trans*- $\text{C}_{18:1}$ in the culture contents, a consequence of incomplete biohydrogenation (Harfoot and Hazlewood, 1997). An increase in the *trans*- $\text{C}_{18:1}$ concentration of milk fat across a wide range of diets has been correlated with a reduction in

milk fat yield. Recent work has shown that the decrease in milk fat yield is associated with a specific increase in *trans*-10 C_{18:1} rather than an increase in the total *trans*-C_{18:1} concentration of milk fat (Griinari et al., 1998; Piperova et al., 2000). Although we observed an increase in total *trans*-C_{18:1} content we did not measure individual *trans*-isomers. Gamagrass silage-based diets supplemented with corn even at the high level, however, did not seem to decrease the (acetate + butyrate):propionate ratio.

Gamagrass silage supported ruminal fermentation similar to gamagrass hay. Addition of corn to gamagrass silage lowered culture pH but increased the amount of substrate fermented to VFA and gas. Contrary to expectation, the addition of large amounts of corn to gamagrass silage did not have a negative effect on microbial fermentation.

Implications

Feeding eastern gamagrass conserved as hay or silage had similar effects on fermentation by mixed cultures of ruminal microorganisms. The addition of corn to gamagrass silage diets lowered ruminal pH but did not affect fiber digestibility. Including corn at greater than 50% of dietary dry matter increased the daily outflow of microbial nitrogen. The large proportion of the extensively degradable fiber in gamagrass may make it suitable for corn supplementation as an effective strategy to increase the passage of microbial protein and a potential base perennial forage for the dairy enterprise.

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