Effects of timing of corn silage supplementation on digestion, fermentation pattern, and nutrient flow during continuous culture fermentation of a short and intensive orchardgrass meal

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ABSTRACT

Using a dual-flow continuous culture fermenter system, this study evaluated the effect of timing of corn silage supplementation on ruminal digestion and nutrient flows following a short and intensive orchardgrass herbage meal. Treatments included 28 g dry matter (DM) of corn silage added either 9 h (9BH; 0700 h) or 1 h (1BH; 1500 h) before adding 42 g DM orchardgrass herbage or no corn silage (control; 70 g DM herbage). Herbage was fed as follows: 66% of the total herbage meal at 1600 h, 22% at 1720 h, and the remaining 12% at 1840 h. Effluent was analyzed for organic matter (OM), crude protein (CP), and neutral detergent fiber (NDF). Purine concentrations in effluent and bacterial isolates were used to estimate the partition of effluent N flow into bacterial and nonbacterial fractions, and to calculate true OM digestibility. Fermenters were sampled for pH, volatile fatty acids (VFA), and $\text{NH}_3\text{-N}$ at 0730, 1100, 1530, 1600, 1720, 1840, and 2000 h on d 10. Data were analyzed as a 3×4 Latin square experimental design. True digestibilities for OM (average of 78.5%) and CP (average of 84.6%), and apparent NDF digestibility (average of 82.7%) were not affected by treatment. Mean ruminal pH was lower for 9BH than for 1BH, averaging 5.6 and 6.5, respectively. Molar proportions of acetate were not affected by treatment. Propionate concentration was greater for 9BH than for 1BH, averaging 20.5 and 18.1 mM, respectively. Diurnal patterns of pH, $\text{NH}_3\text{-N}$, and acetate:propionate ratio were affected by treatment: 9BH had the lowest values for all measurements as the day progressed. The $\text{NH}_3\text{-N}$ concentration and effluent $\text{NH}_3\text{-N}$ flow were higher for 1BH (11.4 mg/100 mL and 0.26 g/d, respectively) than for 9BH (8.8 mg/100 mL and 0.20 g/d, respectively). Effluent $\text{NH}_3\text{-N}$ flow (as a % of total N flow) was the lowest for 9BH. Bacterial efficiency was not affected by

treatments, with a mean of 10.5 g of N/kg of OM truly digested. Under the same resource allocation (pasture plus supplement), a simple change in timing of corn silage feeding (9 rather than 1 h before an orchardgrass herbage meal) may alter ruminal fermentation pattern. These changes could increase the glucogenic nutrient supply and improve N utilization by reducing ammonia N losses.

Key words: corn silage, supplementation, herbage digestion, nutrient supply

INTRODUCTION

Pastoral grazing enables cost-effective milk production in temperate climates (Holmes et al., 2002; Bargo et al., 2003a). However, in pastoral systems farmers are faced with the challenge of continuous growth of herbage while maintaining its quality, minimizing feed costs, and providing concentrates or conserved forages when herbage quantity or quality is decreased due to seasonal changes. Nitrogen fertilizer, as urea, is widely and successfully used to stimulate herbage growth; however, excessive urea application has led to N leaching and contamination of ground water, as well as losses of N_2O (Tamminga, 1996; de Klein and Ledgard, 2001). Strategic grazing management that reduces the time at pasture (4–8 h/d) has been proposed to reduce pugging and treading damage in wet conditions to improve pasture production and utilization (Blackwell, 1993; Gregorini et al., 2008; Kennedy et al., 2009). Limited access to pasture forces cattle to consume most of the daily herbage DMI in less than 4 h (Chilibroste et al., 2007; Gregorini et al., 2008, 2009). This type of management has other benefits, including less urinary N deposition, which can reduce nitrates leaching to ground water (McLeod et al., 2009). However, acute restriction of time available to graze has reduced ruminal OM digestion in beef heifers (Gregorini et al., 2008) and lowered herbage DMI and milk production in dairy cows (Mattiauda et al., 2003; McLeod et al., 2009; Pérez-Ramírez et al., 2009) so that supplementation is required to maintain nutrient intake and productivity.

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Whole-plant corn (*Zea mays* L.) silage is often used as a supplement in pastoral systems because it provides energy with low inputs of N, diluting the excessive N often supplied by herbage. Few studies have focused on the importance of the timing of corn silage or concentrate supplementation. For example, Adams (1985) showed evidence of greater ADG in beef cattle supplemented in late morning when cattle normally do not graze. Mitani et al. (2005) reported greater milk N output by dairy cows when corn silage was fed immediately before rather than after a grazing period. In addition, Gekara et al. (2005) found that DMI, grazing time, and pasture utilization were greater when lactating beef cows were supplemented in the morning (0700 h) instead of the evening (1800 h). From these studies emerges the concept that timing of corn silage supplementation may alter herbage digestion and ruminal metabolism, changing the nutrient supply to the animal. These studies reported direct effects on DMI, ADG, and milk production, but there is little information concerning the ruminal effects of timing of supplementation.

Continuous culture fermentation systems have been successfully used for rapid evaluations of ruminal responses to hypothetical feeding scenarios (Kolver and de Veth, 2002; Bargo et al., 2003b; Wales et al., 2004). The objective of this study was to use a dual-flow continuous culture fermenter system to measure the effect of timing of corn silage supplementation, either 9 or 1 h before a short and intensive herbage meal, on rumen microbial fermentation and nutrient flows.

MATERIALS AND METHODS

Site, Experimental Design, Treatments, and Diets

The study was conducted at the USDA-ARS Pasture Systems and Watershed Management Research Unit (University Park, PA). Three treatments were randomly applied to a 4-unit dual-flow continuous culture fermenter system using a replicated Latin square design with 2 squares and 3 periods. The first square was complete and used 3 fermenters, whereas the second square was incomplete, using only the fourth fermenter. For each period, there was 1 fermenter per treatment, and the fourth fermenter was used to duplicate one of the treatments in each run (period). Each period consisted of a 7-d diet adaptation period followed by a 3-d sampling period. Treatments included 28 g DM of corn silage fed 9 (9BH) or 1 h (1BH) before 42 g DM of orchardgrass (*Dactylis glomerata* L.) herbage or no corn silage (control; CON, 70 g DM of herbage). Herbage was fed to simulate the ingestion rate dynamics during a grazing bout (Gregorini et al., 2008); 66% (27.7 g) of the total herbage meal was fed at 1600 h, 22% (9.4 g)

at 1720 h, and the remaining 12% (4.9 g) at 1840 h. Corn silage was fed in 2 equal portions 30 min apart. Herbage was harvested from orchardgrass pastures at the Russell Larson Agricultural Research Center (Rock Springs, PA; 40°48' N, 77°52' W; 330 m above sea level). All the orchardgrass herbage for the experiment was harvested at one time during the afternoon (1500 h), frozen at −20°C, freeze-dried (Ultra 35 Super ES, Virtis, Gardiner, NY), and then ground through a 2-mm mesh screen (Wiley mill, Thompson Scientific, Philadelphia, PA). Corn silage was obtained from The Pennsylvania State University Dairy Research Center (University Park, PA), immediately frozen at −20°C, freeze-dried, and ground through a 2-mm mesh screen as above. Samples of herbage and corn silage were chemically analyzed for NDF and ADF (Ankom A200 Filter Bag Technique, Ankom Technology, Macedon, NY), CP (method 990.03; AOAC, 2000), total digestible nutrients (Weiss et al., 1992), and NSC (starch; YSI 2700 Select Biochemistry Analyzer, YSI, Yellow Springs, OH; ethanol-soluble carbohydrates; YSI; Hall et al., 1999).

Continuous Culture Operation

This experiment used a 4-unit dual-flow continuous culture system similar to that described by Hoover et al. (1989) with the following modifications: pH was not controlled, neither pasture nor corn silage was pelleted, and urea was added to the mineral buffer solution (Weller and Pilgrim, 1974) at a rate of 0.4 g/L. Fermenter volumes were 1,140 mL, fed 70 g of DM/d, and operation was similar to Soder et al. (2007). Fermenters were fed as described above. The rumen fluid donor, a lactating Holstein dairy cow, was cared for according to the guidelines stipulated by The Pennsylvania State University Animal Care and Use Committee (IACUC #14829), and fed a TMR ad libitum (40% concentrate, 60% forage). Ruminal fluid was siphoned by using a hand pump from the mid rumen and held anaerobically at 39°C until the time of fermenter inoculation. Within 15 min after collection, each fermenter was inoculated with 1,000 mL of rumen fluid sieved through 2 layers of cheesecloth. In addition, approximately 25 g of whole ruminal digesta from the central and dorsal regions of the rumen was added to each fermenter. Solid and liquid dilution rates were adjusted to approximate 5 and 8%/h, respectively. Values for solids and liquid dilution rates were chosen based on studies conducted with cattle managed similarly to the treatments of the present experiment (Gregorini et al., 2008). The solids and liquid dilution rates were regulated by adjusting the buffer input rate and the liquid removal rate (filter rate). These rates were calculated using the volume of

the fermenter vessels and the desired solid retention time and liquid dilution rate. Fermenters were constantly purged with N₂ gas to maintain positive gas flow to preserve anaerobiosis. Temperature was maintained at 39°C.

Sample Collection and Analyses

Collection of effluent samples for VFA, NH₃-N, pH, and bacterial harvest was similar to the methods of Soder et al. (2007). On d 7 to 10, 4-L plastic effluent jugs containing 20 mL of 50% sulfuric acid and submerged approximately one-third of the depth in a water bath at 4°C were used to collect the effluent. The solid and liquid effluent weights were recorded daily at 1100 h. On d 8 to 10 of each period, the liquid and solid effluents were composited for each fermenter (approximately 2,000 mL per fermenter per period) and homogenized using a 3-L Waring Blender (Waring, New Hartford, CT). Then, a 600-mL subsample was collected and stored at 4°C for further DM determination by centrifuging the subsample of effluent at 15,000 × *g* for 45 min and oven-drying it for 24 h at 102°C (Hoover et al., 1989). An additional 50-mL effluent sample was squeezed through 8 layers of cheesecloth, and a 15-mL aliquot of fluid was preserved with 3 mL of 25% metaphosphoric acid and 3 mL of 0.6% 2-ethylbutyric acid (internal standard), swirled, and then frozen at −20°C for later NH₃-N (Bargo et al., 2003b) and VFA analyses (Yang and Varga, 1989). The remaining effluent was freeze-dried and ground through a 1-mm screen sieve (USA Standard Testing Sieve, VWR, West Chester, PA) for OM (methods 930.15 and 942.05; AOAC, 2000), CP (micro-Kjeldahl digestion; method 976.06; AOAC, 2000; using 75-mL calibrated tubes with CuSO₄/K₂SO₄ catalyst), and NDF (Van Soest et al., 1991) analyses. The methods of Van Soest et al. (1991) for NDF analysis were used with amylase and sodium sulfite (inclusive of ash) using the Ankom A200 Filter Bag Technique (Ankom Technology).

On the last day of each sampling period, pH was recorded at 0730, 1100, 1530, 1600, 1720, 1840, and 2000 h directly from the fermenters (model 360, Beckman Instruments, Fullerton, CA). At the same times, 15-mL aliquots were collected by pipette (Pipetman model P-10ML, Rainin Instruments, Woburn, MA), passed through 8 layers of cheesecloth, and preserved with 3 mL each of 0.6% 2-ethylbutyric acid (internal standard) and 25% m-phosphoric acid (preservative) for later VFA and NH₃-N analyses. Both solid- and liquid-attached microbes were harvested (Griswold et al., 1996; de Veth and Kolver, 2001) by mixing fermenter contents in a blender and straining through 53-μm Nitex cloth (Wildco, Buffalo, NY). Strained contents

were centrifuged at 1,000 × *g* for 10 min to remove feed particles. Microbes were isolated by centrifuging the supernatant at 20,000 × *g* for 30 min (Beckman J2-21, Beckman Instruments, Palo Alto, CA) twice, first using saline and then using 50% methanol (Griswold et al., 1996), and prepared for analysis by freeze-drying. Purine concentrations (Zinn and Owens, 1986, as modified by Makkar and Becker, 1999) in effluent and bacterial isolates were used to partition effluent N flow into bacterial and nonbacterial fractions and to calculate true OM digestibility and flows (Stern and Hoover, 1990).

Statistical Analyses

True OM and CP digestibilities, NDF and ADF digestibilities, and nutrient flows were analyzed using a mixed model. The model included the fixed effects of treatment and period and the random effect of fermenter. Least squares means were compared by least square minimum difference, when protected by a significant ($P < 0.10$) treatment effect. The diurnal pattern of rumen microbial fermentation was analyzed in a repeated measures analysis using cubic smoothing spline models within the linear mixed model framework as described in Verbyla et al. (1999). These models were fitted using REML. For all variables except acetate:propionate ratio, there was a significant ($P < 0.05$) interaction of treatment with time of sampling; then the times were analyzed separately to further investigate the treatment effects. The data at each time point were analyzed using linear models. A value of $P < 0.05$ was considered significant. GenStat 11.1 was used for all statistical analysis (Payne et al., 2008).

RESULTS

Diet Composition and Nutrient Digestibility

Dietary inputs and chemical composition of orchardgrass herbage and corn silage and the total diet are presented in Table 1. True OM and CP digestibilities were not affected by treatment (Table 2). Apparent NDF digestibility was similar for 1BH and 9BH, but tended to be higher ($P < 0.10$) for CON (84.9%) than for 9BH (mean 80.5%).

Mean Fermenter pH and VFA

Mean ruminal pH was highest for CON and lowest for 9BH ($P < 0.05$; Table 2). Total VFA concentration was lower ($P < 0.05$) for CON than for supplemented treatments, and there was no difference between 1BH and 9BH. Acetate, valerate, isobutyrate, and isovalerate concentrations did not differ between treatments.

Table 1. Chemical composition (% of DM) of ingredients and total diet (corn silage plus herbage) composition supplied to the continuous culture fermenters

Parameter	Corn silage	Herbage	Corn silage plus herbage
OM	96.1	93.2	94.4
CP	9.8	23.5	17.7
ADF	24.2	32.5	29.2
NDF	43.6	55.5	50.3
Lignin	3.9	4.5	4.3
NSC	43.8	17.1	27.3
Lipids	2.8	3.9	3.4

Molar proportions of propionate were highest ($P < 0.05$) for 9BH, whereas butyrate concentrations tended to be the highest ($P < 0.10$) for 1BH.

Diurnal Patterns of Ruminal pH, Ammonia, and VFA

Ruminal pH was lowest ($P < 0.05$) for 9BH at the 1100 and 1600 h sampling times (Figure 1a). The CON diet had the highest ($P < 0.05$) pH at the 1600, 1720, and 1840 h sampling times. For both 1BH and CON, pH decreased when either corn silage or orchardgrass herbage was fed. Despite similar circadian patterns, there were distinct differences ($P < 0.05$) in $\text{NH}_3\text{-N}$ between the 3 treatments, with lower concentrations for 9BH than for 1BH for most of the 24-h period (Figure 1b).

The patterns of total VFA concentrations, acetate:propionate ratio, and individual concentrations of acetate and propionate are shown in Figure 1 c, d, e, and f, respectively. Both supplemented treatments differed ($P < 0.05$) from CON in propionate concentra-

tions (Figure 1f); 9BH showed the highest values for all the measurement times except for 0730 h, when it did not differ from CON. The propionate concentrations for the CON and 1BH treatments differed only at the first 3 sampling times (Figure 1f). The acetate:propionate ratio was lower ($P < 0.05$) for 9BH than for 1BH (Figure 1d). The 9BH treatment had the greatest ($P < 0.05$) butyrate concentration from 1100 to 1530 h, but treatments caused little difference in butyrate concentrations during the herbage meal (data not shown).

Nitrogen Metabolism

Results from N metabolism are presented in Table 3. The $\text{NH}_3\text{-N}$ concentration and the effluent flow of $\text{NH}_3\text{-N}$ from the fermenters were lowest for 9BH, intermediate for 1BH, and highest for CON ($P < 0.05$). Total N flows were similar for 9BH and 1BH, but the distribution of $\text{NH}_3\text{-N}$ and non- $\text{NH}_3\text{-N}$ in effluents differed ($P < 0.05$) between treatments. Bacterial N and efficiency of bacterial protein synthesis per kilogram of OM truly digested were not affected by treatment.

DISCUSSION

OM and NDF Digestibility

The true OM digestibility in the present study was slightly lower than other comparable continuous culture fermentation studies in which orchardgrass was the main component of the diet. For example, Bargo et al. (2003b) reported true OM digestibilities values around 83%; however, the level of NDF of their orchardgrass

Table 2. Nutrient digestibility and ruminal fermentation of orchardgrass herbage only (CON) and orchardgrass herbage plus corn silage fed 1 (1BH) or 9 (9BH) h before a single herbage meal, during continuous culture fermentation

Parameter	Treatment			SEM	P-value
	CON	9BH	1BH		
Nutrient ruminal digestibility					
True OM digestibility, %	78.2	77.7	79.6	1.56	0.71
Apparent NDF digestibility, %	84.9 ^x	80.5 ^y	82.8 ^{xy}	0.08	0.08
True CP digestibility, %	85.3	83.1	86.1	2.41	0.72
Ruminal pH	6.6 ^a	5.6 ^c	6.5 ^b	0.02	<0.01
VFA, mM					
Total	88.0 ^b	92.6 ^a	93.3 ^a	1.74	0.09
Acetate	45.1	44.8	44.9	2.94	0.99
Propionate	18.7 ^b	20.4 ^a	18.2 ^b	0.34	0.01
Butyrate	17.2 ^y	18.1 ^y	21.7 ^x	1.32	0.06
Valerate	5.65	8.07	7.39	1.724	0.42
Isobutyrate	0.62	0.49	0.44	0.06	0.14
Isovalerate	0.59	0.51	0.69	2.71	0.83
Acetate:propionate	2.39 ^a	2.02 ^b	2.45 ^a	0.134	0.03

^{a-c}Means with different superscripts within rows are different ($P < 0.05$).

^{x,y}Means with different superscripts within rows are different ($P < 0.10$).

Table 3. Nitrogen metabolism of orchardgrass herbage only (CON) and orchardgrass herbage plus corn silage fed either 1 (1BH) or 9 (9BH) h before a single herbage meal, during continuous culture fermentation

Item	Treatment			SEM	P-value
	CON	9BH	1BH		
NH ₃ -N, mg/100 mL	18.69 ^a	8.88 ^c	11.37 ^b	0.417	<0.01
Effluent N flows, g/d					
Total N	1.19 ^a	0.91 ^b	0.87 ^b	0.052	0.02
NH ₃ -N	0.42 ^a	0.20 ^c	0.26 ^b	0.009	<0.01
Non-NH ₃ -N	0.78	0.71	0.62	0.041	0.17
Bacterial N	0.60	0.54	0.50	0.031	0.25
Dietary N	0.18	0.17	0.12	0.054	0.75
Effluent N flows, % of total N flow					
NH ₃ -N	36.3 ^a	22.7 ^b	31.39 ^a	1.77	0.02
Non-NH ₃ -N	63.7 ^b	77.3 ^a	68.6 ^b	1.70	0.02
Bacterial N, % of total non-NH ₃ -N flow	78.3	75.6	82.1	6.20	0.83
Dietary N, % of total non-NH ₃ -N flow	21.7	24.4	17.9	6.50	0.83
Bacterial efficiency g of N/kg of OM truly digested	11.6	10.4	9.5	0.56	0.14

^{a-c}Means with different superscripts within rows are different ($P < 0.05$).

was considerably lower (37%) compared with that used in the present study (56%). Kolver et al. (1998) reported values of true OM digestibility averaging 87%. Both Kolver et al. (1998) and Bargo et al. (2003b) supplemented the orchardgrass herbage with a corn-based concentrate without affecting OM digestibility. The lack of a timing of supplementation effect on OM digestibility is consistent with previous results of Valk (1994), who did not find any effect of timing of corn silage feeding on in vivo or in vitro OM digestibility. Valk (1994) fed dairy cows with grass-based diets, allocating the corn silage either after milking times or in the evening (1830 h).

The NDF digestibility reported here is slightly lower than that reported by Kolver et al. (1998) and Bargo et al. (2003b) when supplementing orchardgrass herbage under continuous culture fermentation studies, but consistent with the in vivo results of Valk (1994). The latter study also reported a null effect of timing of corn silage supplementation on NDF digestibility, which supports the present results. The tendency for lower NDF digestibility in 9BH compared with 1BH could be associated with differences in pH (Table 2) and its patterns.

Ruminal Fermentation

A significant effect of the 9BH treatment on both mean and diurnal variations in ruminal pH (Table 2) was consistent with a large body of literature (Bargo et al., 2003a). The diurnal fluctuations in pH relate to the feeding pattern (Figure 1a) of each treatment. Although the postfeeding decline in pH is expected, the pattern of the decrease when corn silage was fed alone (0700 and 1500 for 9BH and 1BH, respectively, Figure 1a) suggests a significant change in fermentation that

might also have affected the ruminal microflora population dynamics (Van Soest and Demeyer 1979; Leedle et al., 1982). Although 9BH affected pH and NH₃-N concentration, overall effects on concentrations of total VFA and OM digestibility were not significant.

The differences in total VFA concentration between CON and supplemented treatments relate mainly to the greater concentration of propionate for 9BH and butyrate for 1BH (Table 2). Despite the lack of difference in total VFA concentration between 9BH and 1BH, the timing of feeding corn silage had a significant effect on the pattern of fermentation. With the same acetate concentration, 9BH had a greater propionate concentration throughout the day, suggesting that 9BH feeding may supply more glucogenic precursors for the animal compared with 1BH or CON treatments. Generally, modifications of the nutrients supplied by the rumen to the host animal are achieved through modifications to dietary inputs that affect the ruminal fermentation patterns (Van Soest, 1982; France and Dijkstra, 2005) and microbial dynamics (Van Soest and Demeyer, 1979; Leedle et al., 1982). The changes observed for 1BH and 9BH in rumen microbial fermentation patterns were achieved without any change in the diet or in the amount of each feed source allocation, but by altering the ruminal metabolism through changes in the timing of feeding. Such a change in pattern of nutrient supply is also supported by the difference in butyrate concentrations (Table 2) and patterns of valerate and isobutyrate concentrations at different times of the day (data not shown).

Nitrogen Metabolism

As expected, N intake was significantly reduced by corn silage supplementation, and this accounted for

Figure 1. Diurnal patterns of a) ruminal pH, b) $\text{NH}_3\text{-N}$, c) total VFA, d) acetate:propionate ratio, e) acetate, and f) propionate during continuous culture fermentation of orchardgrass herbage only (CON; —) and orchardgrass herbage plus corn silage fed 1 (1BH; - - -) or 9 (9BH; ·····) h before a single herbage meal.

the decrease in ruminal $\text{NH}_3\text{-N}$ concentration because bacterial N were not affected by treatment (Table 3). The decrease in ruminal $\text{NH}_3\text{-N}$ for the treatments receiving corn silage was sustained throughout the day (Figure 1b). The magnitude of such a decrease was much greater when the corn silage was fed 9 h before the intensive orchardgrass meal (9BH vs. 1BH), which might relate to steadier microbial growth over a 24-h period. A potential steadier microbial growth probably relates to the higher frequency of nutrient supply to the microbial population in 9BH compared with 1BH and CON. Similar effects on ruminal $\text{NH}_3\text{-N}$ concentration were reported by Graf et al. (2005) with grazing dairy cows, where the effect of corn silage feeding on ruminal $\text{NH}_3\text{-N}$ concentration was evident approximately 10 h after the corn silage meal. In contrast, Mitani et al. (2005) did not find any effect of timing of corn silage

supplementation on ruminal $\text{NH}_3\text{-N}$ of grazing dairy cows. In their experiment, Mitani et al. (2005) fed cows corn silage 2 h before or immediately after a 5-h grazing period. Although Mitani et al. (2005) attempted to prepare the rumen microbial population for a single herbage meal, it appears that 2 h was not sufficient time to affect ruminal microbial populations and products of fermentation (i.e., $\text{NH}_3\text{-N}$ concentrations). The work of Mitani et al. (2005) matches the results of $\text{NH}_3\text{-N}$ concentrations obtained with the treatment 1BH in the present study, which evidences the benefit of better timing of corn silage supplementation as in 9BH.

The distribution of $\text{NH}_3\text{-N}$ in the effluent (Table 3) indicates an important effect of corn silage supplementation on ruminal utilization of N by reducing $\text{NH}_3\text{-N}$ concentration (Valk, 1994; Romera et al., 2007). Lower $\text{NH}_3\text{-N}$ losses from the 9BH treatment, compared with

1BH, are consistent with the results mentioned above, indicating that strategic timing of corn silage supplementation (e.g., 9BH) might reduce N losses as $\text{NH}_3\text{-N}$ (e.g., gaseous NH_3 excretions) from cows grazing lush pastures, and to some extent reduce N excretion in urine (Romera et al., 2007; Pinares-Patiño et al., 2009).

Previous studies using continuous culture fermenter systems that replaced herbage with starch (Kolver et al., 1998) or corn-based concentrates (Bach et al., 1999; Bargo et al., 2003b) did not report changes in bacterial N flow (g/d). Although timing of supplementation (9BH vs. 1BH) did not have a significant effect on bacterial efficiency, in the context of the present results the numerical increase (9.5%) for 9BH merits further study, because such a potential difference may enable changes to be made in rumen microbial population dynamics.

CONCLUSIONS

Timing supplementation of corn silage at 9 h rather than 1 h before a short and intensive herbage meal improved N utilization by reducing $\text{NH}_3\text{-N}$ concentration and flow, which was supported by the reduction in ruminal $\text{NH}_3\text{-N}$ concentration by 30%. Moreover, supplementing corn silage 9 h instead of 1 h before a short and intensive herbage meal appears to increase the supply of glucogenic nutrients (propionate) by 13%. Under the same herbage allocation, a simple change in timing of supplementation may improve utilization of nutrients supplied by pasture. In conjunction with grazing management including restricted access to pasture to avoid damage in wet conditions, appropriate timing of supplementation might help to mitigate pollutant losses of N. In vivo studies are required to confirm the benefits of timing of corn silage supplementation on the release of urea N to the environment.

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