Nutrient Utilization of Differing Forage-to-Concentrate Ratios by Growing Holstein Heifers¹

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ABSTRACT

Traditionally, high-forage, low-concentrate diets fed *ad libitum* have been the primary system of feeding dairy heifers. However, high-concentrate diets can be fed at restricted intakes to reach desired rates of gain and increase nutrient efficiency. A total mixed ration containing high corn silage (CS; HCS: 77% CS, 23% concentrate) or low CS (LCS: 67% concentrate, 33% CS) was fed at restricted intakes in 2 trials to evaluate nutrient utilization by growing heifers. In the first trial, 4 ruminally cannulated heifers (298 ± 16 kg of body weight) were fed to study differences in rumen pH, volatile fatty acid and ammonia concentrations, and mass of rumen contents. In situ determinations were made on the total mixed ration and CS. Low CS rations were digested more rapidly in situ when compared with HCS (4.5 vs. $2.3 \pm 0.3\%/h$), and no differences were observed in CS digestibility when incubated in the rumen of heifers fed either ration. Mean rumen pH tended to be lower for LCS than for HCS (5.9 vs. 6.2 ± 0.1). Individual and total rumen volatile fatty acid concentrations and rumen ammonia concentration were not different between treatments. Total mass of rumen contents was lower for LCS. In the second trial, four 6-mo-old heifers (172 ± 14 kg of body weight) and four 12-mo-old heifers (337 ± 10 kg of body weight) were used. Digestibility of dry matter was greater for the LCS than the HCS diet in both age groups (76.3 vs. 71.1% for 12-mo-old heifers; 71.4 vs. 68.9% for 6-mo-old heifers). Apparent digestibility of N was not different between treatments; however, retained N was higher for the LCS diets for both age groups. Fecal output was significantly reduced in the LCS diets for both age groups. Feeding low-forage, high-concentrate diets to growing dairy heifers at restricted intakes, although more highly digestible, re-

sulted in few significant differences in rumen fermentation patterns and lower fecal output.

Key words: dairy heifer, nitrogen digestibility, rumen fermentation, rumen pH

INTRODUCTION

Efficient utilization of feeds by dairy heifers can help to minimize rearing costs. Typically, heifers are fed high-forage diets, which are often inefficiently digested because of high levels of poorly digestible fiber. Concentrates and more highly digestible forages, such as corn silage (CS), can be substituted for lower digestibility forages in heifer rations to improve overall DM digestibility (DMD) of the diet.

In experiments studying high-concentrate diets fed to growing ruminants, heifers consuming high-concentrate diets produced less heat energy, retained more tissue energy, and consumed and digested less DM, N, and energy than heifers fed a forage diet (Reynolds et al., 1991). In addition, concentrate-fed heifers excreted less fecal DM, N, energy, and urinary N than those fed high-forage diets (Reynolds et al., 1991).

Nitrogen utilization is a key component of nutrient metabolism and is an important aspect in reducing nutrient outflow into the environment (Bach et al., 2005). Recent studies have shown that when dairy heifers were limit-fed high-forage (Hoffman et al., 2007) or high-concentrate (Zanton and Heinrichs, 2007) diets, feed efficiency was improved. Reviews of heifers fed traditional high-forage diets showed that heifers produced 3.26 ± 1.0 kg/d per 100 kg of BW just in feces (Wilkerson et al., 1997). Nennich et al. (2005) estimated the total manure produced by heifers to be 5.6 kg/d per 100 kg of BW. Improved digestibility has resulted in a reduction in the total amount of manure production by limit-fed animals on high-forage diets (Hoffman et al., 2007). Feeding restricted intakes of more digestible combinations of forages and concentrates may be beneficial by reducing total output of feces, which would likely reduce costs associated with manure removal and bedding. The objective of this research was to study nutrient utilization and rumen fermentation of CS-based high- and low-concentrate

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Table 1. Ingredient composition, forage-to-concentrate ratio, and particle size of high corn silage (HCS) or low corn silage (LCS) rations fed to growing Holstein heifers in 2 experiments

Item	HCS	LCS
Ingredient, % of DM		
Corn silage ¹	76.92	32.98
Ground corn	0.00	27.98
Soybean hulls	7.21	25.00
Sodium bicarbonate	0.67	0.67
Canola meal	5.67	9.62
Expeller soybean meal	7.12	0.00
High mineral mix ²	2.40	0.00
Low mineral mix ³	0.00	3.75
Forage-to-concentrate ratio	77:23	33:67
Particle size, % as fed		
19.0 mm	0.57	0.28
8.0 mm	27.50	9.70
1.18 mm	58.47	65.09
Pan	13.47	24.93

¹Contained 34.5% DM, 40.63% NDF, 24.03% ADF, and 7.27% CP on a DM basis.

²Contained 7.4% vitamin E, 2.5% vitamins A, D, and E, 28.6% distillers corn with solubles, 13.9% plain salt, 34.8% limestone, 6.0% Mg oxide, 5.5% trace mineral premix, and 1.5% Se premix, on a DM basis.

³Contained 7.8% vitamin E, 2.6% vitamins A, D, and E, 28.6% distillers corn with solubles, 14.6% plain salt, 36.5% limestone, 2.6% Mg oxide, 5.7% trace mineral premix, and 1.6% Se premix, on a DM basis.

diets for growing heifers when fed at restricted intakes.

MATERIALS AND METHODS

Two experiments were conducted with Holstein heifers cared for following standard procedures according to The Pennsylvania State University Institutional Animal Care and Use Committee. The TMR contained finely chopped CS as the sole forage source and were supplemented with heat-treated expeller soybean meal, soybean hulls, canola meal, ground corn, and 1 of 2 mineral mixes (Table 1). Animals were fed at 0800 and 2000 h. The high-CS ration (**HCS**) provided a forage-to-concentrate ratio of 77:23, and the low-CS ration (**LCS**) provided a forage-to-concentrate ratio of 33:67. Feed and TMR samples were collected daily and composited every 15 d for analysis. Samples were dried in a forced-air oven at 55°C and analyzed for DM, ash (AOAC, 1990), soluble protein (Krishnamoorthy et al., 1983), ADF, and NDF (Van Soest et al., 1991). Analysis of NDF included heat-stable α -amylase and sodium sulfite (Van Soest et al., 1991). A Leco FP-528 Nitrogen Combustion Analyzer (Leco, St. Joseph, MI) was used to determine CP (AOAC, 1990). Particle size of CS and TMR was determined once per week by using a Penn State Particle Separator (Kononoff et al., 2003).

Heifers were weighed weekly at 2 consecutive feedings, 2 h prior to feeding at 0600 and 1800 h, except

the week immediately prior to sampling. Diets were formulated to provide 2.47 and 2.46 Mcal of ME/kg of DM, 13.41 and 13.64% CP, and were offered at 80.5 and 76.4 g of DM/kg of BW^{0.75} for HCS and LCS, respectively. Heifers were individually housed in tie stalls in an environmentally controlled barn with rubber mats and no supplemental bedding, and lighting was automatically controlled to allow 13.5 h of light/d. Heifers had free-choice access to water and were released 1 h postfeeding for approximately 1 h/d on a paved exercise lot, except on sampling days.

In experiment 1, 4 Holstein heifers were obtained that had been fitted with a rumen cannula (10.16 cm i.d.; Bar Diamond, Parma, ID) under local anesthesia for a previous experiment. Heifers (298 ± 16 kg of BW and 376 ± 3.5 d of age) were randomly assigned to 1 of 2 treatments in a 2-period crossover design. Each experimental period was 21 d in length. Rumen contents were sampled on d 19 at 0, 1, 2, 3, 4, 5, 6, 9, 12, and 24 h following the 0800-h feeding. Rumen fluid was strained through 4 layers of cheesecloth, pH was recorded (pH meter, model M90, Corning Inc., Corning, NY), and 15 mL of fluid was placed into bottles containing 3 mL of 25% metaphosphoric acid and 3 mL of 0.6% 2-ethyl butyric acid (internal standard). Samples were stored at -20°C until VFA and NH₃ analyses were conducted. Later, samples were centrifuged 3 times at $4,000 \times g$ for 30 min at 4°C to obtain a clear supernatant. The supernatant was analyzed for rumen NH₃ according to Chaney and Marbach (1962) and for molecular concentration of VFA by gas chromatography (Yang and Varga, 1989). Whole-rumen evacuation was completed 6 h postfeeding on d 21 to determine the mass of rumen contents. In situ digestibility was determined on d 15 to 21 for CS, HCS, and LCS in heifers fed both HCS and LCS, using CS in situ digestibility as a standard in all heifers. Dried samples were initially ground through a 2-mm screen with a Wiley mill (Arthur H. Thomas, Philadelphia, PA), and 10×20 cm in situ bags with a pore size of 50 ± 15 μ m were filled with approximately 5 g of feed and attached to cords with weights and snaps, to be affixed to the outer rims of the cannulas. Bags were placed in 39°C distilled water for 15 min prior to insertion into the rumen, incubated in heifers for 72, 48, 36, 24, 16, 12, 8, 4, 2, and 1 h and simultaneously removed 4 h postfeeding. Bags were separated, rinsed manually, and put through a 2-min cold-water rinse cycle of a washing machine 3 times, then rolled and dried in a 55°C forced-air oven for 4 d before being weighed to determine DMD (AOAC, 1990). In situ data were fit to the model of Orskov and McDonald (1979) for each feed (CS or diet) within heifer within period curve, with coefficients estimated in the NLIN proce-

dures of SAS (SAS Institute, 2006), using the Marquardt compromise as the iterative method. Least squares means, standard errors, and statistical tests were then calculated by using the statistical procedures described below, with the coefficients derived from the NLIN procedure serving as the observed values.

In experiment 2, two groups of heifers approximately 6 mo (172 ± 14 kg of BW; 171 ± 5 d) and 12 mo of age (337 ± 10 kg of BW; 368 ± 10 d) were randomly assigned to either the HCS or LCS and were allowed to adapt to a tie-stall facility for 2 wk prior to initiation of the trial. Heifers were fed in a split-plot design, with age of heifers as the whole plot and treatment administered in a 2-period Balaam's design (Balaam, 1968) as the subplot. Two 21-d periods were used, with 17 d of adaptation and 4 d of total fecal and urine collection. Urine was collected via indwelling catheters (no. 14 French balloons, Rusch Inc., Duluth, GA) inserted prior to feeding on d 17 and removed on d 21. Urine samples were collected into a carboy, and 12 N HCl was added to maintain pH below 3.0. Every 24 h, feces and urine were weighed, recorded, and subsampled. Urine was subsampled and frozen at -20°C for later analysis. Fecal subsamples were dried in a 55°C forced-air oven for 4 d, then ground through a 1-mm screen (Wiley mill, Arthur H. Thomas). All samples were analyzed for DM (AOAC 1990) and CP (AOAC, 1990), and fecal samples were analyzed for NDF (Ankom²⁰⁰ Fiber Analyzer, Ankom Technology Corporation, Fairport, NY) by using heat-treated α -amylase and sodium sulfite.

The 2 trials were analyzed and are reported separately. All statistical analyses were conducted in SAS (SAS Institute, 2006) with the MIXED procedure. Period was a fixed design effect, and fixed treatment effects included diet in experiment 1, as well as age category and the diet by age interaction in experiment 2. Heifer was included as a random effect in experiment 1, and heifer within age was included as a random effect in experiment 2. Because of unequally spaced rumen sampling, mean pH, VFA, and ammonia-N concentrations were determined by calculating the area under the response curve according to the trapezoidal rule. All denominator degrees of freedom for *F*-tests were calculated according to Kenward and Roger (1997). Residual variances were assumed to be normally distributed, and all data are presented as least squares means. A treatment effect was declared significant when $P < 0.05$, and trends were reported at $P < 0.10$.

RESULTS AND DISCUSSION

Treatment rations with CS as the sole forage are described in Table 1. The particle size of the CS was

much smaller than industry normal (Heinrichs et al., 1999) and was considered to be a challenge for the concept of high-concentrate, limit-fed diets. Particle size determination showed that LCS had a much smaller particle size than HCS ($P < 0.01$), which was expected, given the intentional disparity in forage-to-concentrate ratio. As planned, both diets would be considered short in particle size compared with more conventional dairy heifer diets. Nutrient analysis of rations is shown in Table 2. Dry matter, CP, N, and ME intakes are shown in Table 3 for heifers during experiments 1 and 2. Normally, low-forage, high-concentrate diets differ drastically in composition from traditional high-forage diets, especially in NDF and NFC. In these studies, the LCS and HCS diets did not show statistical differences because of the use of a high-quality CS as well as replacement of the CS in the LCS diets with a combination of corn, a high-NFC concentrate, and soybean hulls, a high-NDF by-product. In addition, the particle size distribution was extremely low as compared with normal CS values (Heinrichs et al., 1999).

In experiment 1, there was a trend for mean rumen pH to be lower in heifers fed LCS ($P < 0.08$), and for pH to remain below 6 for a longer duration ($P < 0.06$; Table 4). Maximum rumen pH was lower ($P < 0.04$) in heifers consuming LCS, but minimum pH was not affected by diet ($P > 0.24$). The variation in pH during the day (Figure 1) was similar between treatments and can be explained by the rapid consumption of a restricted amount of readily fermentable feedstuffs for both diets.

Mean concentrations of total VFA, individual VFA, and ammonia were not influenced by dietary treatment (Table 4). Figure 2 presents the variation in rumen ammonia after the morning feeding, showing the peak rumen ammonia concentration 1 to 2 h after feeding for both diets. Satter and Slyter (1974) noted that 2 mg/dL of ammonia-N is near the limiting concentration needed to provide for rumen microbial growth in lactating cattle and that 5 mg/dL provided a margin of safety. Both diets maintained rumen ammonia production of <5 mg/dL for extended periods during the day, which may be due to the lower than intended dietary CP concentrations and differences in N intakes observed between diets. Both rumen ammonia and total VFA concentrations were quite low for some parts of the 12-h cycle, but because heifers were fed twice per day, these periods of lesser rumen activity were broken up by periods of increased rumen VFA and NH_3 concentrations.

The wet and dry masses of rumen contents are described in Table 4. Heifers fed LCS had a reduced mass of rumen contents compared with HCS heifers ($P <$

Table 2. Nutrient composition of high corn silage (HCS) or low corn silage (LCS) rations fed to growing Holstein heifers during experiments 1 and 2 (DM basis)

Nutrient ¹	Experiment 1			Experiment 2		
	HCS	LCS	SE	HCS	LCS	SE
DM, %	40.15	58.57	—	40.15	58.57	—
CP, %	10.18	12.31	0.05	10.48	12.22	0.32
Soluble CP, % of CP	40.51	24.99	1.62	38.13	26.60	4.44
ADF, %	24.50	24.75	1.88	24.85	25.04	1.14
NDF, %	41.60	38.31	2.65	41.94	38.31	2.41
Starch, %	28.43	29.70	3.68	28.13	30.70	2.79
NFC, %	40.79	40.80	3.01	39.96	41.19	2.14
TDN, ² %	68.30	68.03	1.22	67.94	67.74	1.18
ME, ³ Mcal/kg of DM	2.47	2.46	0.04	2.46	2.45	0.04
Ca, %	0.50	0.58	0.03	0.53	0.52	0.03
P, %	0.29	0.33	0.02	0.29	0.31	0.01
Mg, %	0.27	0.28	0.01	0.26	0.27	0.01
K, %	1.04	1.20	0.06	1.05	1.18	0.03

¹n = 2 composite samples, representing 42 samples/treatment taken daily throughout the trial.²Total digestible nutrients (calculated from ingredients).³Estimated: ME = TDN × 0.04409 × 0.82.

0.02 and $P < 0.05$ for wet and dry rumen contents, respectively). This result seems logical because of the reduced DMI of heifers fed LCS. In addition, the rate of ration DMD in situ was increased for the LCS treatment ($P < 0.05$). To provide a control, CS was incubated in situ in heifers consuming both diets. The rate of CS digestibility in situ was not influenced by treatment, suggesting that increased LCS in situ digestibility was not due to drastically differing rumen environments.

In experiment 2, the LCS treatment had greater apparent DMD ($P < 0.01$) in both age groups studied (Table 5), although both diets were highly digestible (>68.9%). Apparent NDF digestibility (Table 5) was greater for HCS than LCS ($P < 0.01$) for both age groups. The in situ rate or extent of diet or CS NDF digestion was not different between diets in experiment 1 (data not shown). The significantly reduced total tract NDF digestibility observed in LCS may therefore be due to the faster rate of passage of soy-

hulls compared with forage (Nakamura and Owen, 1989). Nitrogen intake was increased for the LCS diet in both age groups studied ($P < 0.01$). Heifers on both treatments consumed less N than recommended by NRC (2001) requirements. Intake of N was 112.8 g for HCS and 125.9 g for LCS compared with the 130 g recommended by NRC for 12-mo-old heifers. For 6-mo-old heifers, intake was 69.4 g for HCS and 76.0 g for LCS vs. the NRC recommendation of 107 g. Even though N intake was greater for LCS heifers, fecal N excretion was not different between treatments ($P > 0.20$). However, 12-mo-old heifers excreted a greater amount of fecal N ($P < 0.01$) than their younger counterparts. Similarly, there was no diet effect on urine excretion of N for either group of heifers. Rotger et al.

Table 3. Daily intake of DM and N, and estimated ME intake of growing Holstein heifers fed high corn silage (HCS) or low corn silage (LCS) diets

Experiment	HCS	LCS	SE	P-value
Experiment 1				
DMI, kg	6.1	5.8	0.01	<0.01
N, g	105.5	114.8	0.49	<0.01
ME, Mcal/d	15.1	14.2	0.28	<0.01
Experiment 2, 12-mo-old heifers				
DMI, kg	6.5	6.2	0.07	0.13
N, g	112.5	126.1	1.17	0.04
ME, Mcal/d	15.9	15.1	0.08	0.03
Experiment 2, 6-mo-old heifers				
DMI, kg	4.0	3.8	0.04	0.09
N, g	68.9	76.4	1.36	0.08
ME, Mcal/d	9.7	9.2	0.05	0.02

Table 4. Rumen fermentation measurements, mass of rumen contents, and in situ digestibility of high corn silage (HCS) or low corn silage (LCS) diets fed to 12-mo-old Holstein heifers in experiment 1

Item	HCS	LCS	SE	P-value
Mean pH	6.2	5.9	0.1	0.08
Max pH	7.1	6.6	0.1	0.04
Min pH	5.4	5.3	1.5	0.24
Max – min pH	1.7	1.3	0.2	0.19
Time pH <6.0, h	3.8	7.1	1.1	0.06
Rumen NH ₃ -N, mg/dL	4.3	5.9	0.5	0.13
Total VFA, mM	126.4	139.8	5.8	0.25
Acetate, mM	81.0	87.9	3.5	0.23
Propionate, mM	23.0	26.7	1.6	0.12
Butyrate, mM	16.4	19.3	0.8	0.25
Acetate-to-propionate ratio	3.5	3.3	0.1	0.41
Wet rumen mass, kg	38.5	32.2	0.5	0.02
Dry rumen mass, kg	5.0	4.1	0.2	0.05
DM turnover rate, per d	0.82	0.71	0.03	0.10
In situ DM digestion rate, %/h				
TMR	2.3	4.5	0.3	0.05
Corn silage	1.5	1.1	0.2	0.08

Figure 1. Mean rumen pH in 12-mo-old Holstein heifers fed high corn silage (◆) or low corn silage (■) diets in experiment 1. $P > 0.10$ for the treatment $\times$ time interaction; bars on the graph indicate standard errors.

(2005) found that the rate and extent of N degradation increased from 13 to 41 wk of age, suggesting an increase in proteolytic activity with age. For the current

trial, total N excretion was not altered by treatment, even though N intakes were greater for heifers consuming LCS. Apparent N digestibility was not affected

Figure 2. Mean rumen ammonia variation after feeding in 12-mo-old Holstein heifers fed high corn silage (◆) or low corn silage (■) diets in experiment 1. $P > 0.51$ for the treatment $\times$ time interaction; bars on the graph indicate standard errors.

Table 5. Apparent total tract digestibility, N dynamics, and manure excretion for 12-mo-old and 6-mo-old Holstein heifers fed high corn silage (HCS) and low corn silage (LCS) diets in experiment 2

Digestibility, %	12-mo-old		6-mo-old		SE	P-value		
	HCS	LCS	HCS	LCS		Age	Diet	Age × diet
DM	71.1	76.3	68.9	71.5	1.4	0.07	0.01	0.31
N	58.1	59.9	52.2	55.0	2.6	0.10	0.34	0.84
NDF	67.6	56.4	68.6	57.5	1.1	0.34	<0.01	0.95
N intake, g/d	112.8	125.9	69.4	76.0	4.5	<0.01	0.01	0.25
N excretion in feces, g/d	47.3	49.9	32.9	33.8	1.5	<0.01	0.26	0.58
N excretion in urine, g/d	33.9	32.4	15.7	10.8	3.0	<0.01	0.29	0.58
Total N excretion, g/d	81.0	82.6	48.6	44.5	3.7	<0.01	0.74	0.42
Retained N, g	32.1	43.0	22.3	30.0	2.9	<0.01	0.01	0.58
Retained N, % of N intake	26.8	34.7	30.4	40.6	2.8	0.09	<0.01	0.69
Wet feces, kg/d	12.4	7.8	8.0	5.5	0.3	<0.01	<0.01	<0.01
Dry feces, kg/d	2.0	1.5	1.3	1.1	0.1	<0.01	<0.01	0.03
Urine, kg/d	10.9	12.8	4.9	5.0	2.2	0.06	0.13	0.22
Manure, kg/d	23.0	20.0	12.7	10.6	2.2	0.02	0.03	0.69
Manure, kg/100 kg of BW	6.4	5.6	6.6	5.8	0.7	0.87	0.03	0.91

by diet or by age. Retained N (as a percentage of N intake and grams of N retained) was greater for heifers receiving LCS ($P < 0.01$) than for heifers receiving HCS in both age groups.

Total excretion of manure components is detailed in Table 5. Heifers in both age groups that received LCS produced less wet and dry feces than heifers fed HCS (both $P < 0.01$). The magnitude of this reduction in fecal production is striking. A 6-mo-old heifer receiving the HCS treatment produced approximately the same amount of wet feces as a heifer twice her age and twice her BW receiving the LCS treatment (Table 5). Any reduction in fecal production can help dairy heifers become less environmentally detrimental, simply by reducing the mass of manure produced. Urine production (kg/d) was not different between heifers receiving HCS or LCS.

In summary, feeding low-forage, high-concentrate diets (LCS) at restricted intakes resulted in minimal changes in ruminal fermentation patterns compared with heifers receiving a high-forage diet (HCS). Although the rate of ration DMD in situ was increased for LCS, the rate of CS DMD in situ was not different for heifers consuming LCS and HCS. Apparent DMD was significantly greater for LCS, but both diets were highly digestible. Although N intake was greater for LCS than HCS in heifers at 6 and 12 mo of age, total N excretion was not affected by diet. Total fecal excretion was reduced for both 6- and 12-mo-old heifers consuming LCS. These data reinforce the potential of the non-traditional strategy of feeding restricted amounts of high-concentrate diets to increase nutrient utilization and efficiency and minimize manure wastes, and indicate that further research into the N requirements of heifers prior to breeding is warranted.

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