Effect of forage to concentrate ratio with sorghum silage as a source of forage on rumen fermentation, N balance, and purine derivative excretion in limit-fed dairy heifers

K. Kljak,* F. Pino,† and A. J. Heinrichs†¹

*Department of Animal Nutrition, Faculty of Agriculture, University of Zagreb, 10000 Croatia

†Department of Animal Science, The Pennsylvania State University, University Park 16802

ABSTRACT

Sorghum silage has been shown to be a good alternative to corn silage for dairy cows; however, studies regarding heifers are insufficiently explored. Therefore, the objective of this study was to evaluate effects of changing forage to concentrate ratio (FOR:CON) in diets based on sorghum silage on N digestibility, rumen fermentation, N balance, C excretion, and microbial N yield in limit-fed dairy heifers. A split-plot 4 × 4 Latin square design with 19-d periods (15 d of adaptation and 4 d of sampling) was conducted with 8 rumen cannulated dairy heifers (age 13.7 ± 0.6 mo and weight 364.8 ± 17.6 kg). Heifers were fed sorghum silage-based diets with 4 FOR:CON (85:15, 75:25, 65:35, and 55:45) balanced for similar metabolizable energy intake per unit of body weight and crude protein concentration. Diets were fed to allow 900 to 1,000 g/d body weight gain and were fed once daily. Total collection of feces and urine was completed on d 15 to 19 to determine N, C, urea N, allantoin, uric acid, and creatinine excretion. Rumen contents were sampled on d 19 at 0, 1.5, 3, 4.5, 6, 9, 13, 17, 21, and 23 h after feeding to measure pH, volatile fatty acid (VFA), ammonia-N, and free AA concentrations. The pH decreased linearly while ammonia-N and free AA levels increased linearly with decreasing FOR:CON of diets. Although mean total VFA did not differ among treatment diets, molar proportions of VFA did. Acetate proportion decreased while propionate and butyrate increased with decreasing FOR:CON. Intake of N and urea N excretion decreased with decreasing forage proportion in diets while total N excretion, apparent N digestibility, and N retention were not different. Intake of C and excretion in feces (g/d) decreased linearly with decreasing FOR:CON in diets. Creatinine, allantoin, and uric acid excretion were not affected by FOR:CON; however, microbial N yield tended to in-

crease linearly with greater concentrate in diets. Heifers limit fed diets based on sorghum silage demonstrated the effect of available ammonia-N and readily fermentable carbohydrates with subsequent effects on nutrient utilization when different FOR:CON were applied. Based on the presented results, FOR:CON 65:35 had the most suitable balance of available ammonia-N and readily fermentable carbohydrates for the most optimal results.

Key words: dairy heifer, sorghum silage, forage to concentrate ratio, limit-fed, protein

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] and corn (*Zea mays* L.) are among the top producing crops in the world, with corn being more prevalent in dairy rations. Corn silage is a key component of ruminant diets because of its high yield and energy content. Compared with cows fed conventional sorghum silage, cows fed with corn silage have greater DMI and greater milk production (Aydin et al., 1999). However, the decline in water resources and extreme weather conditions such as drought or high summer temperatures present considerable risk for corn silage production. When irrigation is not an option, producers are forced to grow crops that are more water efficient than corn. Sorghum is one such crop because of its greater ability to extract water from deeper soil layers compared with corn (Farré and Faci, 2006).

Extensive breeding efforts have been made in recent years to improve sorghum silage quality, including reducing lignification, which leads to greater fiber digestibility. Furthermore, Oliver et al. (2004) showed that the brown midrib trait in sorghum improved fiber digestibility to a level close to that of corn, and cows fed brown midrib sorghum forage hybrids had milk production similar to cows fed with corn silage.

With the development of new hybrids, sorghum silage is becoming increasingly applicable in dairy nutrition, and its utilization has been extensively studied in dairy

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¹Corresponding author: ajh@psu.edu

cows (Grant et al., 1995; Aydin et al., 1999; Oliver et al., 2004; Miron et al., 2007). However, studies that report utilization of sorghum silage in heifer diets are not available. Heifers are typically fed a high-forage, low-energy ration to meet their energy and nutrient requirements.

Traditional ad libitum high-forage, low-energy diets of growing heifers can be replaced with limit feeding when controlled intake for targeted ADG is achieved. Controlled DMI, with the purpose of targeting a set rate of ADG, increases nutrient efficiency (Lascano and Heinrichs, 2011) and reduces manure production (Hoffman et al., 2007; Moody et al., 2007). Additionally, limit feeding enables introduction of high-concentrate diets without negative effects on heifer growth and first-lactation milk production in comparison with high-forage diets (Hoffman et al., 2007; Zanton and Heinrichs, 2007). Traditional corn silage as a source of forage could be replaced with sorghum silage, which has already been shown to be a good alternative to corn silage without detrimental effects on steer performance or ruminal fermentation (Abdelhadi and Santini, 2006). In addition, the often-reported lower DM digestibility of sorghum compared with corn silage might be beneficial in reducing readily available carbohydrates in high-concentrate diets. The extent that sorghum silage could be used in limit feeding dairy heifers is insufficiently explored. Therefore, the objective of this research was to evaluate forage to concentrate ratio (**FOR:CON**) in diets with sorghum silage as the sole forage source in terms of N digestibility, rumen fermentation, N balance, C excretion, and microbial N yield in limit-fed dairy heifers. The hypothesis was that a decreased FOR:CON when sorghum silage is the sole forage source will decrease ruminal pH and N excretion compared with an increased FOR:CON in diets for dairy heifers.

MATERIALS AND METHODS

Sorghum Silage

Brown midrib-6 brachytic dwarf sorghum (AF7102, Altaseed, Amarillo, TX) was grown in fall of 2015 at the Pennsylvania State University. This variety can typically be harvested between 85 and 92 d of growth with high yield and forage quality because brachytic dwarf genes provide strong stalks with good standability. Sorghum was harvested at middough stage with a field chopper (Forage Harvester 8600, John Deere, Moline, IL) with knives adjusted to a 2-cm theoretical length of cut after 2-d wilting because of the high moisture content. Table 1 shows the chemical composition of experimental sorghum silage.

Animals and Feeding

All procedures involving the use of animals were approved by the Pennsylvania State University Institutional Animal Care and Use Committee (IACUC no. 46266). Eight Holstein heifers were surgically prepared with a rumen cannula (10-cm i.d.; Kehl, São Carlos, SP, Brazil) under local anesthesia before the beginning of the experiment. Heifers were allowed a 40-d recovery and pretrial adaptation period for facilities and diets before the start of the experiment. During all periods, heifers were housed in individual tie-stalls with rubber mattress bedding in a mechanically ventilated barn. Heifers had free access to water at all times and were released for exercise 3 to 4 h/d in a paved pen on non-sampling days.

Heifers (13.7 ± 0.6 mo of age and 364.8 ± 17.6 kg of BW at the beginning of experiment) were randomly assigned to a split-plot 4×4 Latin square experimental design. Treatments (Table 2) were 4 TMR containing (DM basis) 85, 75, 65, or 55% sorghum silage as the sole forage plus 15, 25, 35, or 45% of a concentrate mix based on corn grain, canola meal, and slow-release urea (Optigen; Alltech Inc., Nicholasville, KY). Each experimental period was 19 d in length and consisted of 15 d of adaptation and 4 d of sampling. Heifers were weighed on 2 consecutive days at 7-d intervals (d 1, 2, 8, and 9 or d 2, 3, 9, and 10 depending on period), and BW was determined by the average of 2 measurements taken at 0800 and 1130 h on the same day. The amount of TMR offered during the experiment was adjusted on a weekly basis, based on BW, to allow an ADG of 900 to 1,000 g/d. Treatment ration refusals were never present in this system of feeding. Eating patterns were checked at least every 30 min during the day and 2 h during the night; when feeder boxes were empty, time elapsed from feeding time (i.e., eating time) was recorded.

Rations were fed once daily at noon, and the predicted DMI was calculated based on energy intake; diets were formulated to provide 0.22 Mcal of ME/kg of BW^{0.75}

Table 1. Chemical composition (% of DM unless otherwise noted) of experimental sorghum silage

Item	Value
DM, %	26.30
CP	8.06
Soluble CP, % of CP	35.85
RDP, % of CP	67.70
NDF	62.95
ADF	38.10
Starch	1.08
Sugar	5.00
Crude fat	2.99

Table 2. Ingredient and chemical composition (% of DM unless otherwise noted) of treatment rations differing in forage to concentrate ratio (FOR:CON) with sorghum silage as the sole source of forage

Item	FOR:CON				SEM
	85:15	75:25	65:35	55:45	
Ingredient					
Sorghum silage	85.0	75.0	65.0	55.0	
Ground corn	9.4	18.3	27.2	36.1	
Canola meal	2.0	3.2	4.3	5.4	
Optigen ¹	1.9	1.8	1.8	1.8	
Mineral mix ²	1.7	1.7	1.7	1.7	
Chemical composition					
DM, %	31.14	33.95	37.13	41.68	0.37
CP	13.41	13.24	13.26	13.36	0.07
Soluble CP, % of CP	56.41	53.51	52.59	51.40	0.34
RDP, % of CP	63.35	59.84	56.42	53.00	0.45
NDF	55.75	51.15	46.52	41.88	0.37
ADF	33.29	30.03	26.77	23.50	0.15
Starch	8.25	15.12	21.91	28.70	0.03
Sugar	4.56	4.34	4.11	3.88	0.01
Crude fat	3.16	2.30	3.43	3.57	0.01
ME, ³ Mcal/kg	2.39	2.46	2.53	2.60	0.01
ME, ⁴ Mcal/kg of BW ^{0.75}	0.20	0.20	0.20	0.20	

¹Slow-release urea (Alltech, Nicholasville, KY) contains 94.8% DM and 269.9% CP.

²Mineral mix (US Feeds, Eldora, IA) contains 94.28% DM, 11.65% CP, 1.7% soluble CP, 5.46% RUP, 8.29% ADF, 19.2% NDF, 5.5% fat, 12.4% Ca, 0.36% P, 2.63% Mg, 0.44% K, 0.39% S, 1,628.87 mg/kg of Mn, 542.71 mg/kg of Cu, 1,639 mg/kg of Zn, 232.94 mg/kg of Fe, 9.90 mg/kg of Se, 9.2 mg/kg of Co, 22.2 mg/kg of I, 70.748 UI/g of vitamin A, 17.637 UI/g of vitamin D, and 1.230 UI/g of vitamin E.

³Estimated as ME = TDN $\times$ 0.04409 $\times$ 0.82.

⁴ME expressed as Mcal/kg of metabolic BW.

and 13.5% CP. Single batches of grain ingredients were stored to last for the length of the experiment. The grain mix was prepared for each treatment at the beginning of each experimental period in a drum mixer (Calan Super Data Ranger, American Calan, Northwood, NH). Using the same equipment, diets were mixed using the grain mix and sorghum silage for each treatment for 3 to 4 d in advance. All diets were kept in closed plastic bags to avoid oxygenation and stored in a cooler room (4°C) until feeding. Dry matter of sorghum silage was measured on mixing day in a microwave as described by Pino and Heinrichs (2014).

Sample Collection and Analysis

Feedstuffs were collected before every period, and TMR was collected during mixing day just prior sampling period, dried in a forced-air oven at 55°C for 48 h, and ground through a 1-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA) for further analysis. Feces and urine were collected in total from d 15 immediately after feeding to d 19 immediately before feeding (4 d total collection). Urine was collected using a noninvasive device (Lascano et al., 2010) in an 18.9-L bucket with 1 L of 10% HCl solution to maintain pH below 3 and minimize NH₃ volatilization. Urine pH was checked several times during each 24-h period, and 10% HCl

solution was added (volume was recorded) if needed. Once daily total urine was weighed, recorded, and subsampled. At the end of each period, urine samples were composited, and a 100-mL subsample was frozen at -20°C until analysis. Feces were collected hourly from waterproof boards and stored in airtight containers. After feeding, daily feces were mixed, and a subsample was saved at 4°C to be composited at the end of each period. The subsample was dried in a forced-air oven at 55°C for 72 h and ground through a 1-mm screen (Wiley mill, Arthur H. Thomas) for further analysis.

Composited and dried feeds and fecal samples were analyzed by Cumberland Valley Analytical Services, Inc. (Hagerstown, MD) to determine DM, CP, soluble protein, and crude fat (AOAC, 1990). Concentrations of NDF and ADF (Van Soest et al., 1991) were analyzed using an Ankom 200 fiber analyzer (Ankom Technology Corp., Fairport, NY). Analysis of NDF included use of heat-stable α -amylase (Ankom Technology Corp.) and sodium sulfite; NDF and ADF values include residual ash. Starch was determined by the method of Hall (2009) using Hazyme enzyme (Centerchem, Norwalk, CT). Rumen degradable protein was calculated based on individual ingredient values. Metabolizable energy intake was estimated for each heifer within each period using the observed OM intake $\times$ 4.409 $\times$ 0.82 (NRC, 2001).

Dried feed and fecal samples and freeze-dried subsamples of thawed urine were analyzed for C and N using Elemental Analyzer (Costech ECS 4010 C/N/S, Costech Analytical Technologies Inc., Valencia, CA). Subsamples of thawed urine were diluted with distilled water to determine allantoin (Chen and Gomes, 1992), uric acid (cat. no. 1045-225, Stanbio Laboratory, Boerne, TX), and creatinine (cat. no. 0430-120, Stanbio Laboratory) contents. Urinary purine derivative (PD; allantoin and uric acid) excretion was used to estimate duodenal microbial N yield (Chen and Gomes, 1992).

Rumen fluid samples were taken on d 19 from 5 locations in the rumen (dorsal, ventral, anterior, caudal, and central) at 0, 1.5, 3, 4.5, 6, 9, 13, 17, 21, and 23 h relative to the feeding time. Rumen fluid was strained through a 0.28-mm fiberglass mesh screen (New York Wire, Mt. Wolf, PA), and pH was recorded (pH meter, model M90, Corning Inc., Corning, NY). For VFA analysis, 5 mL of filtered rumen fluid was pipetted in tubes containing 1 mL of 0.6% 2-ethylbutyric and 1 mL of 25% metaphosphoric acid (Yang and Varga, 1989). For ammonia-N and free AA analysis, the same volume of rumen samples was pipetted in tubes containing one drop of concentrated HCl (Chaney and Marbach, 1962). For all rumen analyses, tubes were frozen at -20°C until analyzed. After thawing, rumen fluid was centrifuged at $28,000 \times g$ for 20 min at 4°C to obtain clear supernatant and analyzed by GC (Agilent 7890A, Santa Clara, CA) for determination of VFA concentration using a column (2 m long $\times$ 2 mm i.d.) packed with Carbowax 20 M on 80/100 Carbowax BPA (Sulpeco Inc., Bellefonte, PA; Yang and Varga, 1989). Working conditions were back inlet temperature of 220°C , front detector temperature of 225°C , and oven temperature of 125°C , and N_2 was used as the carrier with an overall total flow rate of 36 mL/min. Thawed samples for ammonia-N and free AA were centrifuged at $4,000 \times g$ for 20 min at 4°C and directly used for determination of ammonia-N concentration (Chaney and Marbach, 1962). An aliquot of prepared supernatant was incubated on ice for 30 min with 3-trichloroacetic acid (5% content in final solution) to precipitate any soluble proteins that could interfere with free AA determination. The mixtures were centrifuged at $28,000 \times g$ for 20 min at 4°C to obtain clear supernatant and analyzed by the method described by Snell and Snell (1954) to determine free AA concentration; results were corrected for ammonia-N concentration.

Statistical Analysis

All statistical analyses were conducted in SAS (version 9.3, SAS Institute Inc., Cary, NC) using the MIXED procedure. Dependent variables were analyzed

as a split-plot 4×4 Latin square design with fixed effects of period and FOR:CON and a random heifer effect. Because of unequally spaced rumen sampling, pH, VFA, ammonia-N, and free AA were analyzed as repeated measures with SP(POW) as the covariance structure for time intervals. Residual variances were assumed to be normally distributed, and all data are presented as least squares means. The least squares means and standard errors were determined using the LSMEANS statement, and differences of least squares means were determined using the PDIF statement. The P -values for the linear, quadratic, and cubic effects and their interactions were analyzed using orthogonal polynomial contrasts. Cubic effects were not significant and thus are not shown in the tables. When needed, analysis of slopes for rumen data was performed using orthogonal contrasts using GLM procedure. Differences were declared significant at $P \leq 0.05$ and tendencies at $P \leq 0.10$ for main effects.

RESULTS AND DISCUSSION

Treatments (Table 2) were balanced for CP and ME (on the basis of $\text{BW}^{0.75}$). As a direct result of differing FOR:CON, diets differed mainly in fiber and starch accompanied with increased ME content with increasing concentrate proportion. However, the opposite trend was found for soluble CP and RDP; their content in CP decreased with increasing concentrate proportion in the diets. Dry matter intake increased linearly with increasing FOR:CON in diets to keep the same amount of ME per kilogram of $\text{BW}^{0.75}$ ($P < 0.01$; average $\pm$ SD): 7.29 ± 0.09 kg for FOR:CON 85:15; 7.12 ± 0.40 kg for FOR:CON 75:25; 6.93 ± 0.33 kg for FOR:CON 65:35; and 6.73 ± 0.53 kg for FOR:CON 55:45.

Average BW of heifers over the study was 390.8, 389.8, 386.6, and 385.4 ± 6 kg, and hence DMI % BW was 1.87, 1.83, 1.79, and 1.74 for FOR:CON 85:15, 75:25, 65:35, and 55:45, respectively. Heifers were fed once daily, and their eating patterns varied. In general, heifers consumed rations with greater concentrate proportion more rapidly. As a result, eating time increased with increasing forage proportion in diet ($P < 0.01$; average $\pm$ SD): 17.16 ± 5.29 h for FOR:CON 85:15; 12.86 ± 6.03 h for FOR:CON 75:25; 6.00 ± 2.62 h for FOR:CON 65:35; and 3.31 ± 0.96 h for FOR:CON 55:45. These eating patterns likely influenced rumen fermentation characteristics.

As expected, rumen pH decreased linearly with increasing concentrate in diets because of the increased amount of rapidly fermentable starch ($P < 0.01$; Table 3). Detected values were comparable to previous studies in which limit feeding of dairy heifers was evaluated with various FOR:CON and various forage sources

(Gabler and Heinrichs, 2003; Moody et al., 2007; Zanton and Heinrichs, 2008). In the study by Gabler and Heinrichs (2003), forage source in all diets was a mixture of corn silage and grass hay (proportions in diets were 34.4 and 25.6%, respectively). Moody et al. (2007) evaluated nutrient utilization of high and low corn silage rations (FOR:CON were 77:23 and 33:67, respectively), and Zanton and Heinrichs (2008) investigated nutritional efficiency of different DMI in high forage ration (34.93% of grass silage, 22.06% of grass hay, and 23.70% of corn silage in rations DM). Additionally, studies by Gabler and Heinrichs (2003) and Moody et al. (2007) reported similar minimum pH values; in this study minimum pH values were affected by diets in the same manner as the mean value ($P < 0.01$). However, contrary to our results with sorghum silage, Lascano and Heinrichs (2009) reported that FOR:CON with corn silage as the sole source of forage did not affect ruminal pH of precision-fed dairy heifers (18 mo of age and 449 kg BW), and the minimum pH values were less than ours. In the present study, heifers fed all diets except FOR:CON 85:15 achieved a minimum pH value 6 h after feeding, although for diets with FOR:CON 75:35 and 65:35, the 6-h values were similar to the ones achieved 4.5 h after feeding ($P > 0.10$). Heifers fed a diet with FOR:CON 85:15 had similar minimum pH values at 4.5, 6, and 9 h after feeding ($P > 0.10$). Variation in rumen pH during the day (Figure 1) reflected variations in amounts of rapidly fermentable starch in diets, but the rumen pH of heifers fed diets with FOR:CON 85:15 and 75:25 was not lower than pH 6 at any time point.

Mean concentrations of total VFA were not influenced by dietary treatment (Table 3). Obtained values were slightly lower compared with corn silage, but Las-

cano and Heinrichs (2009) did not observe an effect of FOR:CON on total VFA either. Although total VFA in the present study was not affected, molar proportions of major VFA (acetate, propionate, and butyrate) differed among dietary treatments. Acetate decreased linearly ($P < 0.01$) and propionate and butyrate increased linearly ($P < 0.01$) as FOR:CON decreased in diets, reflecting changes in the substrate available for rumen fermentation and fiber digestion depression. The increase in the concentrate proportion potentiates propionate and butyrate formation by fermentation of NSC, and as a result, the acetate to propionate ratio decreased linearly ($P < 0.01$) with decreased FOR:CON. However, this pattern was not observed for heifers fed with FOR:CON 55:45; propionate molar proportion decreased and the obtained value was similar to the 75:25 diet ($P > 0.10$). These results imply that the rapidly consumed diet by heifers fed with FOR:CON 55:45 caused a lower pH (4.5) at 8 h after feeding that could affect processes in the rumen. Decreased production of all acids was therefore expected, but it was not detected for butyrate. Similar data were shown by Murphy et al. (2000), who reported increased butyrate production in dairy cows fed diets with decreased FOR:CON with either hay or silage as a forage source regardless of pH depression. Compared with other heifer studies with various forage sources and FOR:CON (Gabler and Heinrichs, 2003; Moody et al., 2007; Lascano and Heinrichs, 2009), the present study with sorghum silage as a forage source yielded greater rumen acetate molar proportion regardless of the FOR:CON. Molar proportions of valerate, isovalerate, and isobutyrate were not affected by changing FOR:CON in dietary treatments.

Ruminal ammonia-N concentrations differed among dietary treatments ($P < 0.01$; Table 3), with a linear in-

Table 3. Rumen fermentation and free amino acids of dairy heifers fed differing forage to concentrate ratio (FOR:CON) with sorghum silage as the sole source of forage

Item	FOR:CON				SEM	<i>P</i> -value ¹	
	85:15	75:25	65:35	55:45		L	Q
pH							
Mean	6.78	6.69	6.61	6.57	0.04	<0.01	0.44
Minimum	6.49	6.06	5.94	5.71	0.06	<0.01	0.14
Ammonia N, mM	6.96	7.75	8.15	8.51	0.39	<0.01	0.57
Free amino acids, mM	1.23	1.49	2.06	2.31	0.10	<0.01	0.98
Total VFA, mM	110.6	108.0	107.8	105.5	2.42	0.03	0.92
VFA, molar proportions							
Acetate	68.99	68.23	67.38	66.82	0.32	<0.01	0.62
Propionate	16.30	16.76	17.24	16.85	0.19	<0.01	0.01
Butyrate	8.93	9.16	9.61	10.64	0.16	<0.01	0.04
Valerate	2.34	2.48	2.36	2.30	0.09	0.31	0.08
Isobutyrate	1.66	1.77	1.74	1.72	0.08	0.30	0.41
Isovalerate	1.78	1.62	1.65	1.63	0.07	0.15	0.13
Acetate:propionate	4.31	4.15	3.98	4.04	0.08	<0.01	0.06

¹L = linear; Q = quadratic.

crease in average daily concentration as the amount of concentrate in the diets was increased. Peak ammonia-N concentrations were 1.5 h after feeding for all dietary treatments (Figure 2), and a distinct difference existed among treatments that could also be a result of the eating pattern. Heifers ate TMR with a greater proportion of concentrate at a faster rate, and hence, a greater amount of ammonia from slow-release urea occurred in a shorter period. After maximum values, all diets had a decrease in ammonia-N concentrations, and the slope was steeper for diets with greater FOR:CON ($P < 0.01$). In addition, 9 and 13 h after feeding, concentrations of ammonia-N for the diet with FOR:CON 55:45 was less than the 1.4 mM ammonia-N concentration recommended by Satter and Slyter (1974) as the minimum necessary to maintain ruminal bacterial growth in adult cows; however, it was similar to that shown by Gabler and Heinrichs (2003). The gradual slope of ammonia-N decrease for heifers fed with FOR:CON 85:15 most likely reflected the slow-eating pattern of this diet. In other treatments with steeper slopes of ammonia-N decrease, sources of ammonia might be exhausted by bacterial activity or excreted as urea (Huntington and Archibeque, 2000).

Free AA concentration increased with decreased FOR:CON in diets (Table 3; $P < 0.01$). These variations follow the increased proportion of canola meal with increasing concentrate in diets, but differences in

eating patterns among treatments could prevail when diurnal fluctuations of free AA concentration (Figure 3) are considered. Maximum values were achieved 1.5 h after feeding for all dietary treatments, reflecting ruminal degradation of CP from canola meal. Peak free AA values followed the same trend as determined for peak ammonia-N; with lower FOR:CON in TMR, heifers ate a greater amount of soluble CP in a shorter period of time. However, this trend did not occur with the diet having FOR:CON 55:45 whose peak free AA values were similar ($P > 0.05$) to values from FOR:CON 65:35 up to 6 h after feeding. This observation might be a result of less protein degradation due to the predominant starch substrate (Cardozo et al., 2002). The subsequent drop in free AA concentration around 4.5 and 6 h after feeding in all diets except FOR:CON 85:15 was accompanied by a depression in pH, which might reduce protein degradation (Bach et al., 2005). While diets with FOR:CON 85:15 and 75:25 had continuous flow 6 h after feeding and later, free AA in diets with FOR:CON 65:35 and 55:45 increased again. This increase started after heifers had already eaten their rations, and therefore, it could be a result of degradation of proteins released after carbohydrate degradation in the fiber and starch matrix (Bach et al., 2005).

Nitrogen balance data are presented in Table 4. Values determined in the present study were similar to results of a study by Lascano and Heinrichs (2011) in

Figure 1. Rumen pH of dairy heifers fed differing forage to concentrate ratio (FOR:CON) with sorghum silage as the sole source of forage. Error bars represent SE. *Statistical difference ($P < 0.05$) among treatment rations at given time point.

Figure 2. Ammonia-N concentration (mM) of dairy heifers fed differing forage to concentrate ratio (FOR:CON) with sorghum silage as the sole source of forage. Error bars represent SE. *Statistical difference ($P < 0.05$) among treatment rations at given time point.

which 4 levels of corn stover replaced corn silage in high- and low-concentrate diets precision fed to dairy heifers of similar age and BW. In the present study,

treatment diets were balanced for CP, but N intake decreased linearly ($P < 0.01$) with decreasing FOR:CON in diets. Although sorghum silage had low N content

Figure 3. Free amino acid concentration (mM) of dairy heifers fed differing forage to concentrate ratio (FOR:CON) with sorghum silage as the sole source of forage. Error bars represent SE. *Statistical difference ($P < 0.05$) among treatment rations at given time point.

(average 1.17% DM), loss of N with decreasing forage proportion was not compensated by N from concentrate, which was also accompanied by a decrease in DMI. However, dietary treatments were similar in N excretion, both in urine and feces, apparent N digestibility, and N retention ($P > 0.10$). Only the amount of apparently digested N expressed as grams per day tended ($P = 0.07$) to decrease linearly with decreasing diet FOR:CON. Similarly, Moody et al. (2007) reported greater N intake in diets with high corn silage rations compared to those with low corn silage rations in heifers regardless to their age and without the effect of diet on N excretion in feces and urine. However, FOR:CON affected N retention, contrary to findings in the present study. Retained N (g/d) for all treatments was greater than the values reported by Hoffman et al. (2001) for heifers fed with similar N intakes.

A tendency ($P = 0.07$) was detected for the amount of apparently digested N (g/d) to decrease with decreasing FOR:CON in diets that followed a reduction in N intake of treatment rations. However, regardless of the starch, soluble CP, or RDP concentrations in diets, apparent N digestibility (% N intake) was similar among treatments. This observation suggests that in all diets heifers exceeded their N requirements, but also that either N retention was not as efficient considering starch concentrations in diets with greater concentrate proportions or that N utilization was more efficient in diets with greater forage proportions due to postruminal degradation. Lower starch concentrations in diets (i.e., less available fermentable substrate) and greater urea excretion (Table 5) in diets with greater forage

proportion in treatment rations could indicate the latter.

Urea N excretion decreased linearly ($P < 0.05$) with decreasing FOR:CON in diets (i.e., with decreasing N intake; Table 5). When expressed as a percentage of total urine N, urea N excretion tended ($P = 0.09$) to decrease linearly in the same manner as excretion expressed as grams per day. The greatest values of urea excretion (both g/d and % of total urine N) were similar to values reported by Zanton and Heinrichs (2009) for similar N intake per BW^{0.75} of heifers (41.45 to 54.64 g/d and 79.96 to 68.19% of total urine N, respectively) in high- or low-forage diets with a mixture of corn silage and wheat straw as a source of forage. Although our findings in relation to a linear relationship of urea N excretion and N intake were similar to those for younger heifers reported by Gabler and Heinrichs (2003; 146 d of age and 153 kg of initial BW) and heifers of similar age reported by Zanton and Heinrichs (2009; 12.3 mo of age and 362 kg of initial BW), the latter authors found that urine N excretion was not affected by forage amount. Therefore, the urea N excretion in our study was likely primarily determined by N intake, which is analogous to the different FOR:CON of treatment rations.

Urinary excretion of allantoin and uric acid was found to be similar ($P > 0.10$) among treatment rations with constant relative contributions to total PD (Table 5). Urinary excretion of allantoin constituted the majority (around 91% of total PD excreted), which is less than the 97% reported by Gabler and Heinrichs (2003) or the range from 92.1 to 96.42% reported by Lascano and Heinrichs (2011). Although not significant, a lin-

Table 4. Nitrogen balance of dairy heifers fed differing forage to concentrate ratio (FOR:CON) with sorghum silage as the sole source of forage

Item	FOR:CON				SEM	<i>P</i> -value ¹	
	85:15	75:25	65:35	55:45		L	Q
N intake, g/d	155.8	151.2	146.6	143.9	1.72	<0.01	0.55
N excretion, g/d							
Urine	54.97	56.89	52.97	55.02	4.72	0.85	0.98
Feces	61.12	61.70	56.50	57.13	1.99	0.07	0.99
Total	116.1	118.6	109.5	112.1	5.22	0.38	0.99
N excretion, % of total N excretion							
Urine	47.06	47.64	47.45	48.45	2.14	0.67	0.92
Feces	52.94	52.36	52.55	51.55	2.14	0.67	0.92
N excretion, % of N intake							
Urine	35.54	37.74	35.93	38.32	3.16	0.62	0.99
Feces	39.27	40.84	38.45	39.95	1.41	0.95	0.98
Apparent N digested							
g/d	94.70	89.53	90.10	85.73	2.57	0.02	0.86
% of N intake	60.73	59.16	61.55	60.05	1.41	0.95	0.98
N retained							
g/d	39.07	32.64	38.34	31.97	4.91	0.43	0.94
% of N intake	25.35	21.45	26.42	22.56	3.25	0.82	0.99
% of digested N	41.55	35.18	41.87	37.29	5.21	0.80	0.87

¹L = linear; Q = quadratic.

Table 5. Daily urinary excretion of urine, purine derivatives (PD), estimated bacterial CP flow, and creatinine of dairy heifers fed differing forage to concentrate ratio (FOR:CON) with sorghum silage as the sole source of forage

Item	FOR:CON				SEM	P-value ¹	
	85:15	75:25	65:35	55:45		L	Q
Urea N							
g/d	41.10	38.99	35.10	33.14	1.96	<0.01	0.97
% of urine N	76.22	69.50	69.95	62.26	3.81	0.02	0.89
Allantoin, mmol	107.3	114.6	120.4	116.4	4.74	0.12	0.21
Uric acid, mmol	10.43	10.56	10.88	11.55	0.60	0.20	0.66
Total PD, mmol	117.8	125.1	131.3	128.1	4.89	0.09	0.69
Allantoin, % of PD	91.02	91.48	91.77	90.95	0.54	0.97	0.27
Uric acid, % of PD	8.98	8.52	8.23	9.09	0.54	0.97	0.27
Daily purine absorption, ² mmol	97.67	106.7	114.1	110.3	5.79	0.08	0.25
Microbial N yield, g of N/d	71.01	77.60	82.95	80.22	4.21	0.08	0.26
Creatinine							
g/d	10.14	10.79	11.02	10.87	3.38	0.18	0.31
mg/kg of BW	25.13	27.23	27.75	25.29	1.51	0.86	0.09

¹L = linear; Q = quadratic.²Estimated according to the methods and equations of Chen and Gomes (1992).

ear trend in increased PD excretion was observed with decreased FOR:CON. However, an increasing trend for allantoin and total PD excretion halted at the greatest concentrate proportion in diets, and the greatest detected value was excreted in heifers fed with FOR:CON 65:35.

Microbial N predicted to flow to the duodenum was estimated from total urine excretion of PD (Table 5). Microbial N yield followed the same trend as PD excretion; the peak value was determined for heifers fed with FOR:CON 65:35. Rather high variability for both PD excretion and microbial N yield cannot be excluded as the explanation for determined similarity of treatment rations. Our results are similar to Pina et al. (2009) who found that increasing concentrate proportion in diets with corn silage as a forage source increased microbial N yield. However, lower values determined for heifers fed with FOR:CON 55:45, the greatest concentrate proportion among diets, does not correspond with the greatest determined available ammonia-N in the rumen. This observation might be related to the low ammonia-N concentration between 9 and 13 h after feeding determined for FOR:CON 55:45; the concen-

tration was less than the 1.4 mM assumed minimum necessary to maintain ruminal bacterial growth (Figure 2). Because low ammonia-N concentrations were not observed for FOR:CON 65:35 to the same extent, this diet seems to have achieved a more balanced supply of ammonia-N and readily fermentable carbohydrates.

Urinary creatinine excretion was similar among treatment rations whether expressed as total daily excretion or per unit of BW (Table 5). These results suggest muscle mass was constant regardless of the FOR:CON in treatment rations (Costa e Silva et al., 2014; Hobson, 1939). Urinary creatinine values obtained in our study (10.14 to 11.02 g/d or 25.13 to 27.75 g/kg of BW) are slightly greater than the ranges reported by Zanton and Heinrichs (2009; 9.03 to 10.16 g/d or 22.03 to 25.25 g/kg of BW) for heifers of similar BW and age.

Carbon intake and excretion in urine and feces are presented in Table 6. Similar to N intake, C intake decreased with decreasing FOR:CON in diets ($P < 0.05$). Excretion of C in urine was similar ($P > 0.10$), while excretion in feces varied among treatment rations. Carbon excretion in feces (g/d) decreased linearly ($P < 0.05$) with decreasing FOR:CON in diets, following the

Table 6. Carbon intake and excretion of dairy heifers fed differing forage to concentrate ratio (FOR:CON) with sorghum silage as the sole source of forage

Item	FOR:CON				SEM	P-value ¹	
	85:15	75:25	65:35	55:45		L	Q
C intake, g/d	3,244	3,204	3,099	3,003	38.86	<0.01	0.08
C excretion, g/d							
Urine	112.2	112.0	108.8	101.9	6.41	0.22	0.58
Feces	1,249	1,190	1,061	985.2	18.63	<0.01	0.66
Total	1,361	1,302	1,169	1,087	19.51	<0.01	0.56

¹L = linear; Q = quadratic.

same trend as for C intake. Because C was mostly excreted in feces (around 90%), total excretion in manure (g/d) also decreased linearly in the same manner.

CONCLUSIONS

Results from this study showed that decreased FOR:CON in diets with sorghum silage as a sole source of forage had an effect on some of the evaluated parameters in limit-fed dairy heifers. The overall effect was beneficial compared with increasing FOR:CON, though the latter did not have a negative effect. Decreasing FOR:CON in limit-fed dairy heifers decreased ruminal pH value; however, this decrease did not impair ruminal processes. Decreasing FOR:CON increased ammonia-N and free AA concentration in the rumen. Although soluble CP and RDP decreased with decreasing FOR:CON, heifers ate diets with a greater concentrate proportion more rapidly, which resulted in greater available ammonia-N and free AA in the rumen. Total VFA concentration in the rumen was similar among treatments, but VFA molar proportions varied because of the different amount of readily fermentable carbohydrate. Nitrogen intake decreased with decreasing FOR:CON; therefore, the effect of FOR:CON on N balance and microbial N yield could not be separated from the effect of variations in N intake. In spite of decreased N intake with decreasing FOR:CON, N excretion, apparent digestibility, and retention were similar among treatments, and total PD excretion tended to increase linearly. Hence, reduction in N intake did not have a negative effect on microbial N yield. Our results show that sorghum silage could be used as an adequate forage source in dairy heifer limit feeding compared with corn silage or mixture of corn silage and hay as previously studied. Furthermore, our results also show that FOR:CON 65:35 had the most optimal balance of available ammonia-N and readily fermentable carbohydrates.

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