Precision-feeding dairy heifers a high rumen-undegradable protein diet with different proportions of dietary fiber and forage-to-concentrate ratios¹

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ABSTRACT: The addition of dietary fiber can alter nutrient and N utilization in precision-fed dairy heifers and may further benefit from higher inclusion levels of RUP. The objective of this experiment was to determine the effects of feeding a high-RUP diet when dietary fiber content was manipulated within differing forage-to-concentrate ratios (F:C) on nutrient utilization of precision-fed dairy heifers. Six rumen-cannulated Holstein heifers (555.4 ± 31.4 kg BW; 17.4 ± 0.1 mo) were randomly assigned to 2 levels of forage, high forage (HF; 60% forage) or low forage (LF; 45% forage), and to a fiber proportion sequence (low fiber: 100% oat hay and silage [OA], 0% wheat straw [WS]; medium fiber: 83.4% OA, 16.6% WS; and high fiber: 66.7% OA, 33.3% WS) administered according to a split-plot 3×3 Latin square design (21-d periods). Similar levels of N intake (1.70 g N/kg BW^{0.75}) and RUP (55% of CP) were provided. Data were analyzed as a split-plot, 3×3 Latin square design using a mixed model with fixed effects of period and treatment. A repeated measures model was used with data that had multiple measurements over time. No differences were observed for DM, OM, NDF, or ADF

apparent digestibility coefficients (dC) between HF- and LF-fed heifers. Heifers receiving LF diets had greater starch dC compared to HF heifers. Increasing the fiber level through WS addition resulted in a linear reduction of OM dC. There was a linear interaction for DM dC with a concurrent linear interaction in NDF dC. Nitrogen intake, dC, and retention did not differ; however, urine and total N excretion increased linearly with added fiber. Predicted microbial CP flow (MP_f) linearly decreased with WS inclusion mainly in LF heifers, as indicated by a significant interaction between F:C and WS. Rumen pH linearly increased with WS addition, although no F:C effect was detected. Ruminal ammonia concentration had an opposite linear effect with respect to MP_f as WS increased. Diets with the higher proportion of fiber benefited the most from a high RUP supply, complementing the substantial reduction in predicted MP_f caused by the incremental dietary fiber concentration. These results suggest that RUP supplementation is a practical method for reestablishing optimal ruminal N balance in the event of increased dietary fiber through forage inclusion in precision-fed dairy heifer diets.

Key words: dietary fiber, heifer, N metabolism, rumen-undegradable protein

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doi:10.2527/jas2017.1974

INTRODUCTION

¹This work was funded in part by the California State University Agricultural Research Initiative. The authors acknowledge support from Katie Olagaray for sampling and data collection and members of the Clemson University and California Polytechnic State University Ruminant Nutrition Team for their contributions to this work.

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Received July 31, 2017.

Accepted October 17, 2017.

Feed costs represent the largest expense of heifer rearing, so it becomes imperative that nutrient efficiency be enhanced in order to optimize production and economic stability (Heinrichs et al., 2013). Typical heifer diets consist of a high proportion of low-quality forage, which can result in suboptimal nutrient efficiency, particularly with protein. Additionally, dietary forage-to-concentrate (F:C) variations have the potential to alter fractional digesta retention time and can result in differential nutrient utilization (Colucci

et al., 1990). Feeding excess protein to dairy heifers is a costly practice, whereas aiming for a lower, yet adequate, CP intake could result in improved nutrient utilization and management (Hoffman et al., 2001). The potential of the rumen to modify protein as a means for improving the efficiency of N utilization for growth and productivity of dairy heifers has received little attention (Ding et al., 2015). Although microbial protein provides the ruminant with some metabolizable AA, additional sources of RUP are needed (Merchen and Titgemeyer, 1992; NRC, 2001). Recently, Lascano et al. (2016) concluded that additional dietary fiber reduced microbial protein flow and nutrient and N utilization under high proportions of RDP (~65%) with diets having 45% or 90% forage concentration, suggesting a higher RUP inclusion is warranted. There is currently limited research on how differing levels of fiber within a high- or low-concentrate diet with high RUP would affect nutrient utilization, rumen fermentation, and N efficiency in ruminants. Therefore, the objective of this experiment is to determine if altering the F:C and increasing the fiber content in diets high in RUP would improve N utilization when precision fed to dairy heifers. We hypothesize that manipulating fiber levels within F:C will increase RUP utilization.

MATERIALS AND METHODS

Animals and Experimental Design

All procedures involving animals were approved by the California Polytechnic State University Institutional Animal Care and Use Committee (protocol ID: 1208). Six rumen-cannulated Holstein heifers aged 17.4 ± 0.1 mo at the start of the study and weighing 555.4 ± 31.4 kg BW were randomly assigned to 2 levels of forage, low forage (LF; 45% forage) or high forage (HF; 60%), and to a forage combination sequence to modify dietary fiber content (low fiber treatment [LFi]: 100% oat hay and silage [OA], 0% wheat straw [WS]; medium fiber treatment [MFi]: 83.4% OA, 16.6% WS; and high fiber treatment [HFi]: 66.6% OA, 33.3% WS) within F:C. Heifers were surgically fitted with a rumen cannula (10.16 cm i.d.; Bar Diamond, Parma, ID) under local anesthesia 5 mo before the initiation of sample collection. Heifers were assigned to the treatments in a split-plot 3×3 Latin square design with 21-d periods. The whole-plot factor was the diet F:C, whereas the subplot factors consisted of the differing fiber proportions within the forage fraction of the diet. Specifically, heifers were offered a basal HF or LF diet containing a LFi, MFi, or HFi forage combination. Dietary ingredients and nutritional composition are presented in Table 1. Similar N intake and RDP levels were provided to supply 1.70 g N/kg BW^{0.75}, which has been observed

to maximize N utilization in dairy heifers (Zanton and Heinrichs, 2009). Targeted RUP levels (~50%) were obtained by modifying the amounts of blood meal, soybean meal, heat-treated soybean meal, and urea, maintaining the same concentrate proportion in the diets (45% and 60% for LF and HF, respectively; Table 1). Diets were offered as total mixed rations (TMR) in a precision-feeding scenario by estimating daily intakes to provide equal amounts of ME (0.20 ± 0.05 Mcal/kg BW^{0.75}; Lascano and Heinrichs, 2011) and were adjusted weekly to reach the targeted 800 g/d of ADG. Adaptation to treatment rations (within each F:C treatment) were made over the first 15 d of each period, and sample collection began on d 16. Heifer BW measurements were taken daily 2 h before feeding, and weighted averages were then used to determine the amount of feed offered for the following 7-d interval. Rations were prepared daily at 1200 h prior to feeding at 1400 h. Heifers were housed in individual stalls (120 × 325 cm) in a naturally ventilated tie-stall barn with rubber mattress bedding and were allowed access to an exercise lot for 2 h before the 1400 h feeding on nonsampling days. Water was available at all times.

Fecal, Urine, and Feed Sample Collection and Analysis

Feces and urine were collected from d 16 to 20 (4 d of total collection). Urine was collected via a modified noninvasive urine device (Lascano et al., 2010), weighed, and subsampled daily before feeding. A 250-mL subsample was frozen at -20°C for later analyses. Urine was acidified to pH < 2 by the addition of 12 N HCl to minimize $\text{NH}_3\text{-N}$ volatilization (Zanton and Heinrichs, 2009). Feces were collected hourly and stored in airtight containers. Every 24 h, total collections of feces were mixed, weighed, recorded, and subsampled. Feedstuffs, TMR, feces (dry basis), and urine and fecal aliquots (wet basis) were composited by period. Feces, TMR, and diet ingredients were dried in a 55°C forced-air oven for 4 d, ground through a 1-mm screen using a Wiley Mill (Arthur H. Thomas, Philadelphia, PA), and analyzed for DM, OM, ash, N (AOAC, 2000), ADF, ADL, and NDF (Van Soest et al., 1991) using an ANKOM²⁰⁰ Fiber Analyzer (ANKOM Technology Corporation, Fairport, NY) with heat-resistant α -amylase and sodium sulfite utilized in the NDF procedure. Starch was analyzed on reground samples (<0.5-mm screen) using a modified procedure reported by Zanton and Heinrichs (2009); modifications included the use of amyloglucosidase and analysis of released glucose monomers validated with procedure 1075 (Stanbio Laboratory Inc., Boerne, TX). Ruminally degradable protein was determined before the start of the experiment at Cumberland Valley Analytical Services Inc. (Wanesboro, PA) by an in vitro incubation with *Streptomyces griseus* (Krishnamoorthy

Table 1. Ingredient and chemical composition of low (LF) and high (HF) forage diets containing 3 levels of fiber through the incorporation of fibrous feedstuffs (0% [LFi], 17% [MFi], or 33% [HFi] in the 45% or 60% forage fraction of the diet)

Item	LF			HF		
	LFi	MFi	HFi	LFi	MFi	HFi
Ingredients						
Oat hay ¹	30	15	0	30	20	10
Oat silage ²	15	22.5	30	30	30	30
Wheat straw ³	0	7.5	15	0	10	20
Cracked corn	43.04	42.44	41.84	31.97	29.20	29.02
Heat-treated soybean meal	0.99	2.49	3.99	0.30	0.35	0.00
Soybean meal	3.70	3.05	2.40	0.30	3.53	2.90
Urea	0.20	0.20	0.20	0.35	0.00	0.00
Blood meal	3.57	3.32	3.07	4.16	4.00	5.16
Molasses	1.00	1.00	1.00	1.00	1.00	1.00
Sodium bicarbonate	0.35	0.35	0.35	0.00	0.00	0.00
Mineral mix ⁴	2.15	2.15	2.15	1.92	1.92	1.92
Chemical composition⁵						
DM, %	54.62	56.44	53.52	52.77	51.43	53.14
CP, %	13.85	14.24	14.64	13.38	13.49	14.01
Soluble, % CP	24.60	25.99	27.30	32.76	26.66	24.67
RDP, % CP	52.57	52.71	52.84	54.79	53.38	50.16
RUP, % CP	47.43	47.29	47.16	45.21	46.62	49.84
Starch	38.24	37.26	36.28	31.20	29.18	28.94
NFC, ⁶ %	42.69	39.58	36.46	36.84	33.43	30.34
NDF, %	35.25	37.62	39.98	41.63	43.95	46.35
Hemicellulose, %	16.78	17.28	17.76	17.49	17.9	18.53
ADF, %	18.47	20.34	22.22	24.14	26.05	27.82
Cellulose, %	10.01	11.22	13.47	13.97	14.93	15.15
ADL, %	6.05	6.30	6.37	6.51	6.89	6.96
Ether extract, %	2.79	2.73	2.67	2.52	2.43	2.42
Ash, %	5.61	6.01	6.41	6.46	6.89	7.14
ME, ⁷ Mcal/kg	3.21	3.25	3.18	3.12	3.17	3.15
Ca, % DM	0.4	0.41	0.42	0.35	0.36	0.35
P, % DM	0.28	0.29	0.30	0.29	0.30	0.30
Na, % DM	0.45	0.42	0.4	0.46	0.41	0.37
K, % DM	1.04	1.12	1.19	1.19	1.27	1.27
Mg, % DM	0.25	0.28	0.27	0.26	0.24	0.25
S, %	0.18	0.19	0.20	0.19	0.20	0.19

¹Oat hay contained 56.1% NDF, 34.2% ADF, and 5.6% CP on a DM basis.

²Oat silage contained 60.1% NDF, 39.9% ADF, 10.7% CP, and 22.1% starch on a DM basis.

³Wheat straw contained 81.5% NDF, 52.5% ADF, and 4.4% CP on a DM basis.

⁴Mineral mix contained 7.8% vitamin E, 2.6% vitamin ADE, 28.6% distillers corn with soluble vitamin D, 14.3% plain salt, 36.5% limestone, 2.6% magnesium oxide, 5.7% trace mineral premix, and 1.6% selenium premix on a DM basis.

⁵*n* = 4 composite samples representing 24 samples per treatment taken daily throughout the collection periods of the experiment. All ingredients and nutrients are expressed on a DM basis unless otherwise specified.

⁶Calculated from ingredients as $NFC = 100 - (NDF + CP + \text{ether extract} + \text{ash})$.

⁷ME = $TDN \times 0.04409 \times 0.82$.

et al., 1983). Urine subsamples and fecal aliquots were thawed and analyzed for N using the Kjeldahl method (AOAC, 2000). The thawed urine samples were diluted (1:10) with distilled water and analyzed for uric acid (catalog number 1045-225, Stanbio Laboratory) and allantoin (Chen and Gomes, 1992). Urinary purine derivative (PD; allantoin and uric acid) excretion was used to estimate duodenal microbial N flow (Chen and Gomes,

1992). Metabolizable energy intake was calculated for each heifer within each period using observed digested OM intake $\times 4.409 \times 0.82$ (NRC, 2001).

Ruminal Sampling

Rumen fluid was sampled from 5 locations in the rumen (dorsal, ventral, anterior, caudal, and central) on

d 20 to 21 at -2, 0, 2, 4, 6, 8, 12, 16, 20, and 24 h after the 1400 h feeding. Rumen fluid was strained through 2 layers of cheesecloth, pH was recorded using a hand-held pH meter (model HI98121, Hanna Instruments Italia Sri, Italy), and 5 mL of strained fluid were placed in 2 15-mL tubes containing 1 mL 2-ethyl butyric and 1 mL metaphosphoric acid and stored at -20°C until VFA and rumen ammonia determination as described by Moody et al., (2007). Additionally, an aliquot of 1 mL was mixed with 4 mL of methyl green formalin-saline solution (35% formaldehyde solution, 100 mL; distilled water, 900 mL; methyl green, 0.6 g; sodium chloride, 8.0 g). Mixed samples were stored at 4°C in a dark room until counted for ruminal ciliate protozoa using a Fuchs-Rosenthal counting chamber (Hausser Scientific, Horsham, PA) as described by Ogimoto and Imai (1981). Protozoa genera distribution was determined from the total number of cells of each genus counted in 100 fields using the Sedgewick-Rafter chamber (Hausser Scientific; Dehority, 1993). At the end of each 21-d period rumen contents were evacuated 4 h after the 1400 h feeding, which was expected to represent the times rumen contents reach maximum fill. Mass and volume of total contents were recorded.

Statistical Analysis

All dependent variables were analyzed as a split-plot, Latin square design with fixed effects of period, F:C, and fiber proportion and their interaction with heifer within F:C included as a random effect. Statistical analyses were conducted using SAS (SAS Inst. Inc., Cary, NC) using the mixed procedure for the following model:

$$Y_{ijklm} = \mu + F_i + S_j + P_k + FS_{ij} + H_{m(i)} + e_{ijklm},$$

where Y_{ijklm} = the dependent variable, μ = the overall mean, F_i = the fixed effect of F:C, S_j = the fixed effect of fiber proportion, P_k = the fixed effect of period, $H_{m(i)}$ = the random effect of heifer within F:C, FS_{ij} = the interaction between F:C and fiber proportion, and e_{ijklm} = the residual error. The sequences of fiber proportions were balanced for carryover effects with respect to previous treatment such that all treatments followed every other treatment once; therefore, fixed effect of previous treatment also was included in the statistical analysis. The F:C effect was assessed with denominator degrees of freedom and the error term as associated with the whole-plot error of heifer within F:C, and the effects of fiber proportion and the interaction were evaluated against the pooled residual error. For observations where multiple measures occurred in a period, the fixed effect of time and its interaction with other fixed effects were included in the model. Repeated measurements (Littell et al., 1998), including

simple, autoregressive 1, and compound symmetry covariance structures, were utilized in the analysis depending on low values received for goodness of fit measures (Akaike's information criterion and Schwartz's Bayesian criterion). Orthogonal polynomials were utilized to determine the linear and quadratic effects of fiber proportion (0%, 16.6%, and 33.3% WS addition) and their interaction with F:C. Normality of residuals was evaluated using the Shapiro-Wilk test for normality. Least squares means are presented in tables, and evidence for statistical significance was declared at $P \leq 0.05$, whereas trends for main effects are discussed at $0.10 \geq P \geq 0.05$ and trends for interactions are discussed at $0.15 \geq P \geq 0.10$.

RESULTS AND DISCUSSION

Nutrient Intakes

Dietary ingredients and chemical composition are presented in Table 1. Diets were formulated to differ in dietary fiber, and when WS replaced OA, NDF concentration increased linearly in the diet. The addition of WS and OA to LF and HF resulted in 6 different proportions of NDF (Table 1). Heifers were fed diets that provided 18.4 and 17.45 ± 1.34 Mcal ME/d for LF and HF, respectively. Dry matter intake did not differ in heifers fed LF or HF diets (Table 2), but DMI tended ($P = 0.07$) to linearly increase with the addition of fiber to the diet. This was a result of the proportionally higher planned dietary fiber increase (lower energy concentration) to maintain isocaloric diets. Per design, mean heifer BW in the treatment groups did not differ between F:C or with increasing fiber proportions throughout the experiment. Starch intake tended to be greater for LF-fed heifers compared to HF-fed heifers ($P = 0.08$) and decreased linearly as fiber increased in the diets. Intake of NDF ($P = 0.06$) and ADF ($P = 0.04$) tended to be or was higher for the HF group and linearly increased from LFi to HFi ($P < 0.01$). On the basis of diet cellulose concentration and intake, this fraction was the most affected by increasing WS in the 2 F:C treatments (Tables 1 and 2). This observation is consistent with results reported by Ding et al. (2015), where higher levels of orchard grass hay and WS resulted in greater intakes of ADF. As planned, similar caloric intakes (ME intake: 0.20 ± 0.12 Mcal/kg of BW^{0.75}) and targeted ADG levels (817 ± 34.1 g/d) were achieved in all treatments throughout the experiment. This outcome reflects that the intention to maintain isocaloric diets and growth rates of a precision feeding system was achieved.

Apparent Digestibility and N Dynamics

Apparent total tract nutrient digestibility coefficients (dC) and N dynamics are presented in Table 3.

Table 2. Feed intake of dairy heifers fed differing forage-to-concentrate ratios as low (LF) or high (HF) forage diets containing 3 levels of fiber through the incorporation of fibrous feedstuffs (0% [LFi], 17% [MFi], or 33% [HFi] in the 45% or 60% forage fraction of the diet)

						Contrasts, <i>P</i> -value ¹				
Item	Forage	Fiber			SE	Forage	Fiber		Interaction	
		LFi	MFi	HFi			L	Q	L	Q
Initial BW, kg	LF	554	556	556	31.38	0.99	0.47	0.61	0.92	0.58
	HF	554	556	556						
Intake, % BW	LF	1.69	1.64	1.72	0.12	0.26	0.08	0.58	0.92	0.45
	HF	1.7	1.71	1.78						
Intake, kg/d										
As fed	LF	13.26	14.00	16.07	0.64	0.07	<0.01	0.03	0.40	0.70
	HF	16.40	16.14	16.91						
DM	LF	9.09	9.36	9.58	0.44	0.66	0.07	0.50	0.81	0.42
	HF	9.52	9.44	9.91						
OM	LF	7.71	8.09	8.09	0.4	0.82	0.2	0.82	0.84	0.50
	HF	8.02	7.98	8.26						
Ash	LF	0.47	0.49	0.55	0.04	0.12	0.01	0.02	0.76	0.97
	HF	0.55	0.57	0.65						
Starch	LF	3.34	2.92	3.09	0.15	0.08	0.04	0.92	0.18	0.49
	HF	2.66	2.58	2.66						
NDF	LF	2.96	2.92	3.39	0.18	0.06	<0.01	0.03	0.60	0.23
	HF	3.56	3.64	3.94						
ADF	LF	1.52	1.61	1.85	0.11	0.04	<0.01	0.59	0.56	0.15
	HF	1.93	2.10	2.20						
ADL	LF	0.61	0.59	0.55	0.05	0.27	0.94	0.85	0.34	0.22
	HF	0.62	0.65	0.69						
Hemicellulose	LF	1.44	1.31	1.55	0.09	0.16	0.06	0.10	0.95	0.79
	HF	1.64	1.53	1.74						
Cellulose	LF	0.91	1.05	1.29	0.08	0.03	<0.01	0.83	0.30	0.13
	HF	1.33	1.43	1.48						
ME, ² Mcal/d	LF	19.59	17.69	18.02	1.34	0.61	0.74	0.14	0.29	0.61
	HF	17.06	17.08	18.24						

¹L = linear; Q = quadratic.²Calculated as digestible OM $\times$ 0.82 $\times$ 4.409. Intakes of ME were calculated to allow 800 g/d ADG and 0.20 $\pm$ 0.12 Mcal/kg BW^{0.75}.

Dry matter, OM, NDF, ADF, cellulose, and hemicellulose dC did not differ between LF and HF diets. Frequently, when modifying F:C in diets precision fed to dairy heifers, significant increases in dC are observed (Moody et al., 2007; Zanton and Heinrichs, 2009). In the present experiment, forage represented 45% and 60% of the diets, respectively, which is a smaller difference in F:C than what has been reported in the literature (25% vs. 75%, 80% vs. 20%, and 90% vs. 45%; Zanton and Heinrichs, 2009; Lascano and Heinrichs 2011; Lascano et al., 2016). Apparent DM and ADF dC resembled results of Zanton and Heinrichs (2009) when heifers were fed diets composed of a combination of WS and corn silage with N intake similar to the present study (1.70 g N/kg BW^{0.75}) but were higher with a higher level of RDP (~62%). These dC were also similar to those in a study assessing the effects of RDP (62% to 81% RDP) on diets containing 85% forage, with no F:C manipulation, in which lower-quality for-

ages and higher NDF proportions were fed (Ding, et al., 2015). It is possible that these dC (DM, OM, NDF, ADF, cellulose, and hemicellulose) in precision-fed dairy heifers are most responsive when the separation in F:C is more than 40% between diets. However, highly digestible carbohydrates do not seem to require such extreme changes in F:C to be affected, as reflected by starch dC being greater for the LF diets. The increase in starch dC is likely due to increased retention time, and therefore a higher digestibility rate, rather than F:C modification which can be attributed to its higher digestibility rate, along with increased ($P < 0.01$) retention time (Colucci et al., 1990; Reynolds et al., 1991).

Dry matter and OM dC showed a linear interaction ($P = 0.05$) from LFi to HFi diets. There was a linear reduction in DM and OM dC following a similar pattern for the LF-fed heifers (73.2%, 71.9%, and 62.6% for DM dC in LFi, MFi, and HFi, respectively), whereas there was no effect in the HF group. Increasing the amount

Table 3. Nutrient digestibility parameters of dairy heifers fed differing forage-to-concentrate ratios as low (LF) or high (HF) forage diets containing 3 levels of fiber through the incorporation of fibrous feedstuffs (0% [LFi], 17% [MFi], or 33% [HFi] in the 45% or 60% forage fraction of the diet)

Item	Forage					Contrasts, <i>P</i> -value ¹				
		Fiber			SE	Forage	Fiber		Interaction	
		LFi	HFi	HFi			L	Q	L	Q
Digestibility, %										
DM	LF	73.2	71.9	62.6	2.94	0.94	0.06	0.12	0.05	0.05
	HF	67.3	69.3	70.3						
OM	LF	75.1	73.1	65.1	2.98	0.94	0.07	0.14	0.04	0.05
	HF	68.8	72.4	71.1						
NDF	LF	59.4	57.0	51.9	4.32	0.68	0.23	0.17	0.10	0.15
	HF	56.7	55.1	59.5						
ADF	LF	47.3	52.7	49.3	7.02	0.86	0.58	0.33	0.94	0.69
	HF	43.0	53.7	49.3						
Cellulose	LF	79.1	78.8	60.6	7.44	0.63	0.48	0.61	0.16	0.17
	HF	67.8	76.8	86.9						
Hemicellulose	LF	74.8	63.3	58.7	4.31	0.83	0.06	0.33	0.05	0.17
	HF	71.5	64.3	65.0						
Starch	LF	93.0	93.7	94.0	1.28	<0.01	0.26	0.71	0.37	0.71
	HF	87.7	86.3	89.0						
Ash	LF	52.3	51.7	48.9	4.01	0.94	0.68	0.69	0.30	0.37
	HF	47.4	49.1	55.6						
N										
Digestibility, %	LF	61.1	66.3	59.1	4.20	0.76	0.58	0.59	0.31	0.95
	HF	60.9	61.4	64.4						
Intake, g/kg BW ^{0.75}	LF	1.6	1.6	1.7	0.11	0.83	0.14	0.88	0.71	0.74
	HF	1.7	1.5	1.7						
Intake, g/d	LF	189.7	186.9	202.6	9.74	0.77	0.11	0.20	0.97	0.73
	HF	183.6	184.5	199.8						
Fecal N, g/d	LF	67.9	70.4	77.0	5.43	0.42	0.83	0.71	0.22	0.26
	HF	79.3	78.6	73.8						
Urine N, g/d	LF	49.4	53.3	54.9	4.19	0.39	0.03	0.63	0.05	0.40
	HF	47.5	57.1	66.0						
Total excreted N, g/d	LF	117.9	123.1	131.7	13.63	0.85	0.01	0.64	0.95	0.61
	HF	125.4	135.6	139.5						
Retained N, g/d	LF	72.4	63.1	70.7	10.93	0.32	0.70	0.65	0.67	0.28
	HF	58.3	48.7	59.9						
Retained N, % Intake	LF	36.7	33.7	35.0	4.49	0.28	0.93	0.63	0.68	0.27
	HF	25.7	32.3	30.3						

¹L = linear; Q = quadratic.

of WS increased NDF content, possibly resulting in the observed response for OM digestibility (Firkins et al., 2001). The greater reduction in DM and OM dC in LF diets differs slightly from that reported in previous experiments (Lascano and Heinrichs, 2011; Lascano et al., 2016), where fiber was sequentially increased by adding corn stover or WS, respectively, resulting in a linear reduction in both LF and HF diets. Replacing OA with WS in the diet within the same F:C resulted in similar hemicellulose concentrations in the diets (~17%; Table 1), but ADF content was increased and reduced OM and DM dC to a greater extent in the LF heifers because of an intrinsic lower dC and higher intake of cellulose. There was a linear interaction for hemicellulose dC consistent

with the OM dC pattern previously discussed. This interaction may be due to a greater sensitivity of hemicellulose to higher passage rates as more dietary fiber is introduced under precision-feeding scenarios with reduced intakes in dairy heifers (Lascano et al., 2016).

Intake of N was similar among treatments (1.70 ± 0.11 g/kg BW^{0.75}; Table 3). Nitrogen intake and apparent dC did not differ with F:C or fiber increase in the diet, in agreement with Lascano et al. (2016), where RDP was higher (~65% CP) and yielded higher dC, although differences among treatments were not observed. Fecal and urine N did not differ between F:C, but the addition of WS resulted in a linear increase in urine N and total N excreted that was more pronounced in the HF groups

Table 4. Excretion parameters of dairy heifers fed differing forage-to-concentrate ratios as low (LF) or high (HF) forage diets containing 3 levels of fiber through the incorporation of fibrous feedstuffs (0% [LFi], 17% [MFi], or 33% [HFi] in the 45% or 60% forage fraction of the diet)

						Contrasts, <i>P</i> -value ¹				
Item, kg/d	Forage	Fiber			SE	Forage	Fiber		Interaction	
		LFi	MFi	HFi			L	Q	L	Q
Wet feces	LF	11.21	10.89	15.91	1.67	0.20	<0.01	0.91	0.93	0.10
	HF	14.77	10.43	15.24						
Dry feces	LF	2.10	2.11	3.04	0.33	0.59	<0.01	0.64	0.87	0.11
	HF	2.81	2.03	3.05						
Fecal water ²	LF	9.10	8.81	12.85	1.38	0.70	<0.01	0.97	0.91	0.12
	HF	11.97	8.40	12.19						
Urine	LF	13.31	14.15	13.35	3.17	0.81	0.15	0.89	0.17	0.11
	HF	10.50	12.09	14.12						
Total water excreted	LF	22.37	22.65	26.23	3.65	0.93	<0.01	0.06	0.04	0.73
	HF	22.34	20.26	30.08						

¹L = linear, Q = quadratic.²Weight lost on drying at 55°C.

detected by the significant linear interaction ($P = 0.05$). Nitrogen retention was not affected by F:C or fiber proportions; however, a numerical increase was observed from LFi to HFi in the HF group. These retention coefficients align with the results reported by Lascano et al. (2016), but in the present experiment (RUP ~ 50% CP), as dietary fiber was increased, no reduction in N retention was detected. Additionally, Lascano et al. (2016) observed a pronounced linear decrease in N retention with a high RDP diet. Thus, it seems that additional RUP was able to maintain N retention even when NDF levels increased in these diets.

Nutrient Excretion

Excretion parameters are presented in Table 4. Excretion parameters did not differ between LF and HF diets. This result differs from previous experiments where dairy heifers were precision fed and had increased fecal excretion as dietary forage proportions increased (Zanton and Heinrichs, 2009). All excretion parameters, except urine, increased linearly with WS inclusion, which is consistent with the linear reduction in DM dC for LF diets. No effect on urine output is counter to what was reported in a similar experiment with high RDP (Lascano et al., 2016) in which urine decreased linearly (from LFi to HFi) and water output shifted toward fecal output in a fashion similar to that reported by Lascano and Heinrichs (2011). Other experiments have attributed water excretion to minerals such as P and K, but in the present experiment concentrations of these minerals were similar among treatments (Table 1). These results suggest the possibility that when F:C differences between treatments are not as extreme (i.e., 20 vs. 80 F:C), NDF increases wa-

ter excretion in feces while the effect of shifting water from urine to feces is not as pronounced.

Microbial Protein

Estimated microbial N flow (MP_f) to the duodenum is presented in Table 5. There was a trend detected for total PD ($P = 0.13$), and allantoin ($P < 0.01$) and uric acid ($P < 0.01$) were significantly affected by F:C, which resulted in higher MP_f ($P = 0.05$) for the LF group. Protein and carbohydrate interactions, specifically amount and source, can alter this flow (Clark et al., 1992). Similar results were reported by Kljak et al. (2017) when dairy heifers were limit fed different F:C ranging from 45:55 to 90:10. These results confirm the observation that up to 60% concentrate incorporation in the diet enhances MP_f because of the higher energy availability contained in readily fermentable carbohydrates (Pina et al., 2009; Lascano et al., 2016).

Microbial CP flow linearly decreased as fiber increased in the diets, which is similar to the observations made by Valadares et al. (1999) when dairy cows were fed diets containing 20%, 35%, 50%, or 65% concentrate. Pina et al. (2009) also reported a numerical reduction in MP_f as NDF in diets (35% vs. 45% DM) fed to Nellore heifers increased using a higher F:C and constant levels of RUP (~44% RUP; similar to the present experiment). This effect was more pronounced in the LF group, as indicated by a linear interaction ($P < 0.01$), whereas in the HF group the reduction was significant only when comparing LFi to MFi or HFi. Another experiment reported that MP_f was increased as dietary fiber was incorporated into LF diets (Lascano and Heinrichs, 2011). In this experiment, the LF diet contained only 20% forage, and it was concluded that additional fiber included in these F:C

Table 5. Daily urinary excretion of purine derivatives (PD) and estimated microbial CP flow of dairy heifers fed differing forage-to-concentrate ratios as low (LF) or high (HF) forage diets containing 3 levels of fiber through the incorporation of fibrous feedstuffs (0% [LFI], 17% [MFI], or 33% [HFI] in the 45% or 60% forage fraction of the diet)

						Contrasts, <i>P</i> -value ¹				
Item	Forage	Fiber			SEM	Forage	Fiber		Interaction	
		LFi	MFi	HFi			L	Q	L	Q
Allantoin, mmol	LF	65.7	92.8	85.5	14.14	0.10	0.35	0.07	0.15	0.91
	HF	85.8	91.5	122.1						
Uric acid, mmol	LF	10.5	11.6	11.5	0.76	<0.01	<0.01	<0.01	<0.01	0.04
	HF	6.5	7.7	13.0						
Total PD, mmol	LF	76.2	104.4	97.0	13.91	0.13	0.25	0.05	0.11	0.98
	HF	92.3	99.1	135.1						
Allantoin, % PD	LF	90.8	87.8	86.8	0.35	<0.01	0.18	0.15	0.10	0.02
	HF	88.8	92.9	93.2						
Microbial CP, ² g/d	LF	685.7	350.6	195.1	13.80	0.05	<0.01	0.16	<0.01	0.17
	HF	435.9	353.8	344.3						

¹L = linear; Q = quadratic.

²Estimated according to the methods and equations of Chen and Gomes (1992).

improves N utilization and retention. However, Lascano et al. (2016) also reported a linear reduction in MP_f as NDF proportion increased, which was accompanied by a linear increase in N excreted in urine with a numerical reduction in N retention in treatments with the same F:C as the LF group in this experiment (45:55). Under controlled intake conditions, increases in fluid and solid passage rates (**kp**) have been reported to be greater with higher F:C and higher dietary fiber concentrations (Colucci et al. 1990; Lascano et al., 2016). Thus, LF diets could have increased the RDP fraction available because of a higher ruminal retention time, but with incremental fiber, not enough fermentable carbohydrates were available for microbial protein synthesis. However, this was not the case in the HF group, resulting in similar MP_f even when there was an increased total N excretion as fiber went from LFI to HFI. It is noteworthy that in the present experiment the linear reduction observed in MP_f did not result in reduced N retention, suggesting that the inclusion of higher RUP is complementary to higher levels of NDF in both F:C treatments reported for this study.

Rumen Protozoa

Rumen protozoa number and genera are presented in Table 6. Total protozoa counts were not different between treatments. However, there was a consistent numerical difference throughout the day between LF and HF groups that resulted in a significant time effect ($P < 0.01$; Fig. 1). Protozoa have been shown to increase protein digestion compared with digestion by bacteria alone, particularly if dietary protein is of low degradability (Ushida and Jouany, 1985; Hoover and Stokes, 1991). *Entodinium* spp. populations represented around 95% of the total count in both F:C treatments, which is in agreement with

what has been reported in other experiments (Ushida and Jouany, 1985). *Entodinium* spp. have been reported to be the major genus of rumen ciliates, increasing from 30% to 70% in Holstein heifers as concentrate increases in the diet (Dennis et al., 1983), and were significantly greater in number ($P = 0.01$) in the LF-fed heifers compared to HF-fed heifers. This observation can be explained by the inherent starch granule engulfing action and high amylase activity of entodiniomorphid protozoa when LF diets are fed (Jouany and Ushida, 1999). Peak protozoa concentration of *Entodinium* spp. was observed at 4 h postfeeding for LF and HF treatment groups, which is consistent with the postprandial total protozoa count variation. Protozoa have high deaminase activity, and the contribution of ammonia from protozoa is high (Hino and Russell, 1987), which supports the greater count at 4 h and is consistent with peak NH_3 -N production at 4 h (Fig. 2). *Epidinium* spp. and *Diplodinium* spp. were significantly higher in the LF group, and *Dasytricha* spp. tended ($P = 0.06$) to be higher in the LF group, but dietary fiber did not affect genera counts or relative abundance. These observations suggest that protozoa and genera counts are modulated to a higher degree by F:C and not affected by increasing dietary fiber levels under high-RUP conditions in the rumen.

Rumen Fermentation

Rumen VFA profile, NH_3 -N, and pH are presented in Table 7. There were no differences between F:C or fiber proportions for total ruminal VFA concentration. Acetate, propionate, and butyrate concentrations were similar across treatments. The lack of differences in acetate and propionate are reflected in the acetate-to-propionate ratio, which also did not differ among F:C or increasing fiber proportions. Isovalerate tended to be

Table 6. Protozoal population total counts and genera counts (relative abundance, %) of dairy heifers fed differing forage-to-concentrate ratios as low (LF) or high (HF) forage diets containing 3 levels of fiber through the incorporation of fibrous feedstuffs (0% [LFi], 17% [MFi], or 33% [HFi] in the 45% or 60% forage fraction of the diet)

						Contrasts, <i>P</i> -value ¹				
Protozoa ² , 10 ³ /mL	Forage	Fiber				Forage	Fiber		Interaction	
		LFi	MFi	HFi	SEM		L	Q	L	Q
Total protozoa	LF	199.7	266.3	216.2	35.8	0.45	0.44	0.42	0.55	0.61
	HF	198.6	211.2	204.8						
<i>Entodinium</i> spp.	LF	60.17 (94.4)	62.64 (94.6)	60.52 (94.0)	3.39	0.01	0.24	0.66	0.67	0.31
	HF	51.98 (95.2)	57.68 (94.9)	56.34 (95.4)						
<i>Dasytricha</i> spp.	LF	1.02 (1.6)	1.16 (1.8)	1.21 (1.9)	0.14	0.06	0.98	0.4	0.68	0.43
	HF	0.94 (1.7)	0.98 (1.6)	0.93 (1.6)						
<i>Epidinium</i> spp.	LF	0.36 (0.6)	0.32 (0.5)	0.51 (0.8)	0.11	0.03	0.31	0.71	0.54	0.89
	HF	0.18 (0.3)	0.18 (0.3)	0.23 (0.4)						
<i>Ophryoscolex</i> spp.	LF	0.57 (0.9)	0.53 (0.8)	0.57 (0.9)	0.12	0.44	0.77	0.87	0.88	0.64
	HF	0.47 (0.9)	0.52 (0.9)	0.53 (0.9)						
<i>Diplodinium</i> spp.	LF	1.6 (2.5)	1.55 (2.3)	1.57 (2.4)	0.14	0.01	0.21	0.48	0.16	0.29
	HF	1.01 (1.9)	1.42 (2.3)	1.04 (1.8)						

¹L = linear; Q = quadratic.²Estimated according to the methods of Dehority (1993).**Table 7.** Rumen fermentation of dairy heifers fed differing forage-to-concentrate ratios as low (LF) or high (HF) diets containing 3 levels of fiber through the incorporation of fibrous feedstuffs (0% [LFi], 17% [MFi], or 33% [HFi] in the 45% or 60% forage fraction of the diet)

						Contrasts, <i>P</i> -value ¹				
Item	Forage	Fiber			SEM	Forage	Fiber		Interaction	
		LFi	MFi	HFi			L	Q	L	Q
Total VFA, mM	LF	101.29	109.88	98.31	9.87	0.70	0.80	0.45	0.24	0.73
	HF	95.58	99.47	106.96						
Individual VFA, mol/100 mol										
Acetate	LF	57.48	59.89	58.53	2.12	0.33	0.98	0.21	0.42	0.96
	HF	58.93	60.16	61.44						
Propionate	LF	21.59	20.66	20.41	1.82	0.24	0.89	0.44	0.97	0.95
	HF	22.85	22.01	21.86						
Butyrate	LF	16.14	14.36	15.42	1.65	0.23	0.93	0.22	0.22	0.84
	HF	14.65	13.95	12.35						
Valerate	LF	1.38	1.56	1.64	0.33	0.65	0.36	0.66	0.64	0.33
	HF	1.41	1.20	1.45						
Isobutyrate	LF	1.09	1.27	1.25	0.20	0.17	0.61	0.33	0.51	0.88
	HF	0.92	0.95	1.14						
Isovalerate	LF	1.40	1.51	1.34	0.13	0.08	0.12	0.50	0.99	0.34
	HF	1.36	1.30	1.14						
Isoacids	LF	2.91	1.59	3.21	0.99	0.97	0.27	0.97	0.44	0.51
	HF	2.25	2.56	2.85						
Acetate:propionate	LF	2.75	3.00	3.03	0.24	0.40	0.88	0.11	0.97	0.96
	HF	2.61	2.87	2.90						
NH ₃ -N, mg/dL	LF	7.14	9.92	13.14	0.90	0.16	0.01	0.09	0.02	0.37
	HF	8.20	8.05	9.66						
pH	LF	6.24	6.48	6.58	0.14	0.31	0.04	0.48	0.98	0.38
	HF	6.52	6.58	6.68						

¹L = linear; Q = quadratic.

Figure 1. Postprandial total rumen protozoa counts in dairy heifers fed differing forage-to-concentrate ratios as low (LF) or high (HF) forage diets. Arrow represents feeding time.

higher in LF-fed heifers ($P = 0.08$), but valerate was not different. Valerate production in the rumen is derived from the degradation of AA and carbohydrates, and subsequent decreases in valerate production can also be attributed to increases in the liquid fraction kp accompanied by decreased fermentation time (Eun et al., 2004). Isoacid molar proportions were not different among F:C treatments. The concentration of total VFA of the LF treatments were not different than HF treatments and were lower than that reported by Fuentes et al. (2009) and Lascano et al. (2016). Kljak et al. (2017) reported a linear reduction in total VFA concentration as F:C decreased, and values reported are similar to the ones reported in this experiment with F:C of 45:55 and 60:40. Total rumen VFA concentration influences pH, particularly when F:C is manipulated. The amount of fermentable carbohydrates in LF diets results in greater VFA production coupled with a reduction in pH (Calsamiglia et al., 2008). In the present study, rumen pH did not differ among treatments ($P = 0.31$), consistent with no differences observed in total VFA. These data are in agreement with those of Lascano and Heinrichs (2009), where pH was not different across the F:C treatments when VFA concentration was not altered in heifers precision fed F:C of 80:20, 60:40, and 40:60. This result can be explained through the reduction of the highly fermentable carbohydrate pool available to produce VFA and reduce rumen pH when intake is controlled.

Mean ruminal $\text{NH}_3\text{-N}$ concentration did not differ among F:C ($P = 0.16$). However, there was a linear interaction as fiber went from LFi to HFi in the LF group, with no significant changes in $\text{NH}_3\text{-N}$ concentration in the HF group (Fig. 2). All the HF treatments followed a similar pattern throughout the day regardless of the fiber proportion, whereas as dietary fiber increased in the LF group, $\text{NH}_3\text{-N}$ concentration increased proportionally with NDF. Rumen microbes require N to meet requirements for protein synthesis (Fuentes et al., 2009).

Figure 2. Postprandial rumen $\text{NH}_3\text{-N}$ concentration of dairy heifers fed differing forage-to-concentrate ratios as (A) high (HF) or (B) low (LF) forage diets containing 3 levels of fiber through the incorporation of fibrous feedstuffs (0% [LFi], 17% [MFi], or 33% [HFi] in the 45% or 60% forage fraction of the diet). Arrow represents feeding time.

Additionally, if fermentable carbohydrates are not available for microbes to capture N, an accumulation of $\text{NH}_3\text{-N}$ is observed, which limits microbial growth (Pina et al., 2009). The overall greater $\text{NH}_3\text{-N}$ levels in the LF-fed heifers as NDF concentration increased in the diet through the addition of WS is consistent with the reduction of MP_f discussed above. These results are in agreement with observations made by Lascano et al. (2016) for the LF treatment that resembles this group in the current experiment, with the difference that N retention was incrementally reduced with higher fiber levels and a fixed amount of RDP (~65%). In the current experiment additional RUP (~50%) yielded similar N retentions among different fiber treatments (LFi, MFi, and HFi). Thus, it seems the RDP was not incorporated into microbial CP and increased ruminal $\text{NH}_3\text{-N}$ concentration as lower amounts of fermentable carbohydrates were available as energy sources for microbial growth.

Conclusions

To optimize altered N dynamics in precision-fed dairy heifers consuming fibrous diets, a common suggestion is to offer higher dietary RUP levels. Results from this experiment demonstrate that higher dietary RUP levels, regardless of F:C (60:40 vs 45:55), do not impact apparent total tract digestibility of most nutrients, fermentation parameters, or N dynamics in precision-fed dairy heifers. In addition, diets with higher propor-

tions of fiber (Mfi and Hfi) demonstrated a benefit from the high RUP supply, suggesting a complementation for the substantial reduction of predicted microbial protein flow caused by the incremental fiber dietary concentrations. This effect was primarily observed in the LF diet treatment containing 45% forage and resulted in similar coefficients of N retention across treatments. An increased accumulation of ruminal $\text{NH}_3\text{-N}$ suggests that as more structural carbohydrate sources are incorporated in the diets, additional dietary RUP could complement microbial protein flow. When dairy heifers were fed a HF diet (60% forage), benefits from the higher RUP supply were not detected, although there was an increase in N excretion. Therefore, we can conclude that additional dietary fiber depressed microbial protein flow and N utilization, but RUP supply was instrumental in reestablishing N balance primarily under LF conditions in the present experiment.

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