Effects of corn-based diet starch content and neutral detergent fiber source on lactation performance, digestibility, and bacterial protein flow in dairy cows

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ABSTRACT

An experiment was conducted to evaluate the effects of corn-based dietary starch content and source of neutral detergent fiber (NDF) on lactation performance, nutrient digestion, bacterial protein flow, and ruminal parameters in lactating dairy cows. Eight ruminally cannulated multiparous Holstein cows averaging 193 ± 11 d in milk were randomly assigned to treatments in a replicated 4×4 Latin square design with 21-d periods. Treatment diets were high corn grain (HCG; 38% corn silage, 19% dry ground corn, and 4% soy hulls), high soy hulls (HSH; 38% corn silage, 11% dry ground corn, and 13% soy hulls), high corn silage (HCS; 50% corn silage, 6% dry ground corn, and 4% soy hulls), and low corn silage (LCS; 29% corn silage, 15% corn, and 19% soy hulls). The HCG, HSH, HCS, and LCS diets contained 29, 23, 24, and 22% starch; 27, 32, 30, and 32% total NDF; and 21, 21, 25, and 17% forage NDF (dry matter basis), respectively. Mean dry matter intake and milk yield were unaffected by treatment. Cows fed LCS had reduced milk fat content compared with HSH and HCS. The concentration of milk urea nitrogen was greater for cows fed HCS compared with the other treatments. Total-tract digestion of NDF was reduced for cows fed the HCG diet. Total-tract starch digestion was increased for cows fed the HSH and HCS compared with HCG and LCS diets. Bacterial protein flow was unaffected by treatment. Ruminal ammonia concentration was reduced in cows fed the HCG and LCS diets compared with the HCS diet. Ruminal propionate increased and the acetate:propionate ratio decreased in cows fed the LCS diet compared with the HCS diet. Ruminal pH was greater for cows fed the HCS diet compared with cows fed the LCS diet. Diet digestibility and performance of mid- to late-lactation cows fed reduced-starch diets by partially replacing corn grain with soy hulls or corn silage was similar to or improved compared with cows fed a normal-starch diet.

Key words: lactation, neutral detergent fiber, reduced-starch diet

INTRODUCTION

The increasing cost of corn grain has led to renewed interest in feeding reduced-starch diets to lactating dairy cows. Reduced-starch diets are typically formulated by partially replacing corn grain with nonforage fiber sources (NFFS) such as citrus pulp, corn gluten feed, soy hulls, or wheat middlings; or with forages such as barley silage, corn silage, hay or haycrop silages. Several studies have investigated the effect of replacing high-moisture corn or dry ground shelled corn with NFFS (Ranathunga et al., 2010; Ferraretto et al., 2012; Akins et al., 2014) or forages (Oba and Allen, 2003a; Dann et al., 2008; Agle et al., 2010) on animal performance. The NDF from NFFS is more digestible than that from forages (Ipharraguerre et al., 2002; Bradford and Mullins, 2012). However, NDF from forage provides more physically effective NDF because of larger particle size and lower particle density, potentially leading to increased rumination activity, improved rumen function, and increased milk fat (Mertens, 1997), but may also reduce DMI due to rumen fill (Allen, 2000). Direct comparisons between the 2 approaches for reducing dietary starch content are limited. Weiss (2012) replaced 25% (DM basis) of concentrates with corn milling product or 25% and 40% of a combination of forage and concentrate with corn milling product. Replacement of concentrate with the corn milling product resulted in an increase in DMI (22.8 to 24.1 kg/d), a tendency for a decrease in milk yield (42.0 to 40.3 kg/d), but an increase in milk fat yield (1.18 to 1.40 kg/d). Replacement of forage and concentrate with the corn milling product resulted in a linear increase in DMI (22.8 to 24.4 and 25.1 kg/d) and a quadratic response in milk fat yield (1.18 to 1.24 and 1.17 kg/d), whereas milk yield was unaffected. Further comparisons of the partial replacement of corn grain or forage with alternative NFFS are needed to identify optimal ration formulation strategies when reduced-starch diets are fed.

Poor weather conditions, such as drought or excessive rainfall, or alfalfa winterkill can result in inadequate

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forage inventories for dairy producers. Depleted forage inventory may require feeding lower amounts of forage and offsetting the forage in diets with purchased feeds. An associated increase in the cost of corn grain and the inability to readily purchase forages may result in the need to partly replace forage and corn grain with NFFS. However, comparisons of partially replacing forage and corn grain with NFFS for lactating dairy cows are limited (Boddugari et al., 2001; Mullins et al., 2010; Sullivan et al., 2012).

Experimental objectives were to compare the effects of partly replacing dietary starch with different sources of NDF on lactation performance, nutrient digestibility, bacterial protein flow, and ruminal parameters. Our hypothesis was that the partial replacement of corn grain with NFFS or corn silage and partial replacement of corn grain and silage with NFFS would maintain milk yield and improve milk fat yield in mid- to late-lactation cows.

MATERIALS AND METHODS

Animal Management and Experimental Design

All experimental protocols were approved by the Animal Care and Use Committee of the College of

Agriculture and Life Sciences at the University of Wisconsin-Madison (protocol no: A01471-0-04-11). Eight multiparous Holstein cows averaging 651 ± 45 kg of BW and 193 ± 11 DIM at trial initiation, fitted with rumen cannulas (Bar Diamond, Parma, ID) measuring 10.2 cm in diameter were randomly assigned to a replicated 4×4 Latin square design. Experimental periods lasted 21 d, with 15 d for adaptation and 6 d for sample collection. Experimental diets included a normal-starch diet containing dry ground shelled corn (**HCG**) and reduced-starch diets formulated by partially replacing dry ground shelled corn with soy hulls (**HSH**) or corn silage (**HCS**) or by partially replacing corn silage and corn grain with soy hulls (**LCS**). Ingredient compositions of the experimental diets are in Table 1. Trace minerals and vitamins were supplemented to meet or exceed NRC (2001) guidelines.

Cows were housed on bedded mattresses in a tiestall barn in the Dairy Cattle Center at the University of Wisconsin-Madison. Cows were milked twice daily in a parlor at 0700 and 1900 h, with yields recorded at each milking for each cow. Milk samples were collected from each cow for 4 consecutive milkings on d 16 and 17 and analyzed for fat, true protein, lactose, and MUN concentrations using infrared analysis (method 972.16; AOAC International, 2012; AgSource Milk Analysis Labora-

Table 1. Dietary ingredients and nutrient composition¹

Item	HCG	HSH	HCS	LCS
Ingredient, % of DM				
Corn silage	38.1	38.1	50.4	29.3
Alfalfa silage	18.0	18.0	18.1	17.9
Concentrate mix, % of DM				
Ground dry corn	19.1	11.0	5.8	14.6
Soy hull pellets	4.0	12.6	4.0	18.9
Soybean meal, 48%	9.3	9.1	9.7	9.0
Soybean meal, expeller ²	9.4	9.2	9.9	8.1
Sodium bicarbonate	0.54	0.54	0.55	0.54
Calcium carbonate	0.45	0.45	0.46	0.45
Monocalcium phosphate	0.18	0.18	0.18	0.23
Magnesium oxide	0.18	0.18	0.18	0.18
Trace mineral salt ³	0.45	0.45	0.46	0.45
Vitamin premix ⁴	0.27	0.27	0.27	0.27
Nutrient				
DM, % as fed	57.2	57.8	51.3	61.7
CP, % of DM	18.5	18.7	18.4	18.9
NDF, % of DM	26.9	31.6	29.9	32.1
Forage NDF, % of DM	20.5	20.5	24.9	17.2
Ether extract, % of DM	3.6	3.6	3.6	3.7
Ash, % of DM	7.2	7.4	7.8	7.3
NFC, ⁵ % of DM	44.2	39.1	40.6	38.4
Starch, % of DM	28.5	22.7	23.8	22.0
Starch:forage NDF ratio	1.39	1.11	0.96	1.28

¹HCG = high corn grain (normal-starch diet); HSH = high soy hulls; HCS = high corn silage; LCS = low corn silage (reduced-starch diet).

²Exceller meal (Quality Roasting Inc., Valders, WI).

³Contained 88% NaCl; 0.002% Co; 0.2% Cu; 0.012% I; 0.18% Fe; 0.8% Mn; 0.006% Se; 1.4% Zn.

⁴Vitamin A 3,300 IU/kg; vitamin D 1,100,000 IU/kg; vitamin E 11,000 IU/kg.

⁵Calculated as $100 - (\text{CP \%} + \text{NDF \%} + \text{ether extract \%} + \text{ash \%})$.

tory, Menomonie, WI) using a Foss Milko-Scan FT6000 (Foss Electric, Hillerød, Denmark). Fat-corrected milk and ECM were calculated from average milk yield during the treatment period and average milk fat and protein content according to NRC (2001). Cows were fed a TMR individually twice daily at 1000 and 1900 h for a target of 5% refusals with the amounts fed and refused recorded daily. The corn silage, alfalfa silage, and concentrate mix DM content was determined weekly with the as-fed proportions adjusted as necessary to maintain the desired DM proportions of the ingredients in the TMR.

Sampling and Laboratory Analysis

Subsamples of TMR and refusals for each cow were collected on d 19 to 21 of each period. The TMR, refusals, and diet ingredients sampled weekly were dried at 60°C for 48 h in a forced-air oven to determine DM content, and ground to pass a 1-mm screen using a Wiley mill (model #4; Thomas Scientific, Swedesboro, NJ). Composites of diet ingredients, TMR for each experimental diet, and refusals for each cow were made by period for analyses at a commercial feed testing laboratory (Dairyland Laboratories Inc., Arcadia, WI). Analyses included DM, OM (method 942.05; AOAC International, 2012), CP (method 990.03; AOAC International, 2012), ether extract (method 2003.05; AOAC International, 2012), NDF using α -amylase and sodium sulfite (Van Soest et al., 1991), and starch (Bach Knudsen, 1997; YSI Biochemistry Analyzer; YSI Inc., Yellow Springs, OH). Composites of alfalfa silage, corn silage, and soy hulls obtained before blending of experimental concentrate mixes were analyzed for *in vitro* NDF digestibility after 30-h incubations (Holden, 1999). Prolamin protein was determined on dry ground corn obtained before blending of experimental concentrate mixes (Larson and Hoffman, 2008). Composites of corn silage and dry ground corn were analyzed for *in vitro* starch digestibility after 7-h incubations (Richards et al., 1995). Particle size of the dry ground corn samples were determined by dry sieving using a Tyler Ro-Tap Shaker (model RX-29, Tyler, Mentor, OH) containing sieves with 4,760-, 2,380-, 1,190-, 595-, 297-, 149-, and 63- μ m apertures plus a bottom pan, with mean particle size calculated using a log normal distribution (Baker and Herrman, 2002).

Digesta flow from the rumen was quantified using the omasal sampling technique (Huhtanen et al., 1997; Ahvenjärvi et al., 2000). The omasal digesta markers included indigestible NDF (**iNDF**; Huhtanen et al., 1994) for the large particle phase (**LP**), YbCl₃ (Siddons et al., 1985) for the small particle phase (**SP**), and Cr-EDTA (Udén et al., 1980) for the fluid phase

(**FP**). The external bacterial marker ¹⁵N was used to measure bacterial N flows from the rumen. Before each infusion, a sample of ruminal digesta was collected from each cow and frozen at -20°C, lyophilized, and ground to pass a 1-mm screen (Udy mill; Udy Corp., Fort Collins, CO) to determine the natural abundance of ¹⁵N. A marker solution containing 3.0 g/L of YbCl₃, 6.0 g/L of Cr-EDTA, and 3.75 g/L of ¹⁵NH₄SO₄ with 10 atom percent excess ¹⁵N (Sigma-Aldrich, St. Louis, MO) dissolved in solution was continuously infused at a constant rate of 2.3 mL/min for 138 h from d 15 to 21 using 2 syringe pumps (model 33; Harvard Apparatus Inc., Holliston, MA).

Using the omasal sampling technique, 200-mL spot samples were collected from the omasal canal 4 times daily at 2-h intervals on d 18 to 21 to cover a 24-h feeding cycle. Sampling was at 0600, 0800, 1000, and 1200 h on d 18; 1400, 1600, 1800, and 2000 h on d 19; and 2200, 0000, 0200, and 0400 h on d 20 to 21. Omasal samples were pooled by period for each cow.

The 2.4-L pooled omasal composites were thawed at room temperature and separated into 3 digesta phases as described by Reynal and Broderick (2005). Samples were squeezed through 1 layer of cheesecloth and particles retained on the cheesecloth were defined as LP. The filtrate was centrifuged at 1,000 $\times$ g (5°C, 5 min) and the supernatant poured off. The supernatant was defined as the FP and the pellet was defined as the SP. The separated phases were then frozen, lyophilized at a commercial laboratory (Anteco Pharma, Lodi, WI), and ground to pass a 1-mm screen (Wiley mill). Concentrations of Cr (method 2006.03; AOAC International, 2012) and Yb (method 985.01; AOAC International, 2012) were determined by inductively coupled plasma optical emission spectrometry at a commercial laboratory (Analabs, Fulton, IL) using a Varian Vista-MPX spectrophotometer (Agilent Technologies Inc., Santa Clara, CA). Indigestible NDF was determined in LP, SP, TMR, and refusals (but not FP; Ahvenjärvi et al., 2000) according to Krizsan and Huhtanen (2013) with modifications. One-gram samples were weighed into F57 fiber bags (Ankom Technology, Macedon, NY) in quadruplicate. Samples were incubated *in situ* for 288 h in 2 ruminally cannulated Holstein multiparous lactating dairy cows fed a TMR containing (DM basis) alfalfa silage (44.5%), corn silage (26.8%), alfalfa hay (10.7%), wheat straw (6.5%), and concentrate mixture (11.5%). After removal from the rumen, bags were washed in a washing machine for 5 min, dried at 50°C for 48 h, and analyzed for NDF as described above. Concentrations of Cr, Yb, and iNDF were used to reconstitute the omasal true digesta flowing out of the rumen based on the triple-marker approach of France and Siddons (1986). Concentrations of Cr, Yb, and iNDF were greater than

those of the other markers in FP, SP, and LP, respectively, allowing for application of the triple-marker approach. Total-tract nutrient digestibilities were determined using iNDF as an internal marker. Six total fecal grab samples were collected from each cow per period on d 18 to 21 during omasal sampling every 4 h to cover a 24-h feeding cycle. Total-tract nutrient digestibilities were calculated from iNDF and nutrient concentration in the refusals-adjusted diet and feces. Ruminal and total-tract digestibility of potentially digestible NDF (**pdNDF**) was calculated as $\text{NDF (\% of DM)} - \text{iNDF (\% of DM)}$ in omasal digesta or feces, respectively.

Ruminal fluid was collected for ruminal pH, VFA concentration, and $\text{NH}_3\text{-N}$ concentration immediately after omasal sample collections. Ruminal fluid was collected from the posterior and anterior ventral rumen. Ruminal pH was determined immediately using a Cardy Twin pH meter (model #B-213, Spectrum Technologies Inc., Plainfield, IL). Duplicate 1-mL aliquots of ruminal fluid were added to microcentrifuge tubes containing 0.02 mL of 50% H_2SO_4 acid for later VFA analysis. Ruminal VFA concentrations were measured by GLC (Supelco, 1998). Concentrations of individual VFA were measured on a Clarus 500 gas chromatograph (PerkinElmer, Norwalk, CT) using a 4% Carbowax 20 M on 80/120 mesh Carbowax-B-DA, 1.8 mm $\times$ 2 mm column (Supelco Inc., Bellefonte, PA) with an oven temperature of 160°C and an N flow rate of 24 mL/min. Separate duplicate 1-mL aliquots of ruminal fluid samples were added to microcentrifuge tubes containing 0.02 mL of 50% TCA solution for later analysis of $\text{NH}_3\text{-N}$ according to Bal et al. (2000).

During omasal spot sampling, an additional 500 mL was collected per day (125 mL per spot sampling) for isolation of fluid- (**FAB**) and particle-associated (**PAB**) bacteria. At the end of each sampling day, the 500-mL omasal composites were squeezed through 1 layer of cheesecloth. Solids retained on the cheesecloth were rinsed with 400 mL of 0.85% (wt/vol) NaCl solution and squeezed again. Filtrates were placed in a refrigerator (5°C) until processed for isolation of FAB. The solids retained on the cheesecloth were placed in a 1-L bottle containing 350 mL of cold (5°C) solution containing 0.85% (wt/vol) NaCl and 0.1% (vol/vol) Tween-80. Bottle contents were mixed thoroughly and placed in a refrigerator (5°C). Filtrates for FAB isolation were centrifuged ($1,000 \times g$, 5°C, 5 min). Pellets were added to the bottle contents containing PAB, and supernatants were decanted and recentrifuged ($11,330 \times g$, 5°C, 30 min). These supernatants were discarded and the pellets were resuspended in 100 mL of McDougall's buffer and recentrifuged ($11,330 \times g$, 5°C, 30 min). Supernatants were then discarded and the bottles containing FAB were stored at -20°C until lyophilized.

Contents of the PAB bottles were blended for 20 s in a one-speed Waring blender (Waring Products Division, New Hartford, CT), transferred back to the bottles and stored at 5°C overnight. Contents of PAB bottles were then squeezed through 2 layers of cheesecloth and the filtrates were centrifuged ($1,000 \times g$, 5°C, 5 min). The pellets were discarded and the supernatant was recentrifuged ($11,330 \times g$, 5°C, 30 min). Supernatants and final PAB pellets were processed in the same way as FAB.

Samples of FAB, PAB, FP, and SP + LP were prepared for total NAN and ^{15}N analyses according to Brito et al. (2006). Approximately 3 mg of bacteria or 6 mg of LP + SP, FP, and background ruminal contents were weighed into tin capsules (Elemental Microanalysis Ltd., Okehampton, UK) followed by addition of 50 μL of 72 mM K_2CO_3 . Capsules were placed in 96-well microtiter plates and dried at 55°C overnight to volatilize ammonia. Samples were analyzed for total N and ^{15}N using a Costech 4010 elemental analyzer (Costech Analytical Technologies Inc., Valencia, CA) interfaced to a Thermo-Finnigan Delta-Plus Advantage isotope ratio mass spectrometer (Thermo-Electron GmbH, Bremen, Germany). Calculations of FAB NAN, PAB NAN, total bacterial NAN, and RUP flows at the omasum were made according to Brito et al. (2006). The mean ($\pm$ SD) background ^{15}N was 0.36717 ± 0.00014 atom %.

Statistical Analysis

Data were analyzed using the mixed procedure of SAS (2004; SAS Institute Inc., Cary, NC) for a replicated 4×4 Latin square design according to the following model:

$$Y_{ijkl} = \mu + S_i + P_j + C_{k(i)} + T_l + ST_{il} + E_{ijkl},$$

where Y_{ijkl} was the dependent variable, μ was the overall mean, S_i was the fixed effect of square i , P_j was the fixed effect of period j , $C_{k(i)}$ was the random effect of cow k (within square i), T_l was the fixed effect of treatment l , ST_{il} was the interaction between square i and treatment l , and E_{ijkl} was the residual error. Ruminal data were analyzed with time as repeated measures using the first-order autoregressive covariance structure, which provided the best fit according to Sawa's Bayesian information criterion. Degrees of freedom were calculated using the Kenward-Roger option. Means were determined using the least squares means statement, and treatment means were compared using the Bonferroni t -test option after a significant overall treatment F -test. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Nutrient composition of the diets and ingredients are in Tables 1 and 2, respectively. Partially replacing dry ground corn grain with soy hulls or corn silage resulted in a reduction of dietary starch from 28.5% (DM basis) for the HCG diet to 22.7, 23.8, and 22.0% starch for the HSH, HCS, and LCS diets, respectively. Dietary CP levels were greater than 18.4% for all treatments due to the high CP content in the alfalfa silage (Table 2). Forage NDF content of the HCG and HSH diets was 20.5%. Forage NDF content for the HCS diet increased to 24.9%, but was reduced to 17.2% on the LCS diet. Mean particle size of the corn grain was (mean $\pm$ SD) 828 ± 31 μ m and prolamin content of the corn grain was 32.0 g/100 g of CP (data not provided in Table 2).

Treatment effects for DMI, milk yield, and milk composition are in Table 3. Dry matter intake was unaffected by treatment (mean = 24.9 kg/d). Dry matter intake was also unaffected when soy hulls replaced 10 to 40% of corn grain in a Latin square design (Ipharraguerre et al., 2002). In continuous lactation trials lasting 12 wk, however, results are variable, with reports of similar (Ferraretto et al., 2012; Akins et al. 2014) or greater (Gencoglu et al., 2010) intake when soy hulls partially replaced corn grain, but decreased intake when dried distillers grains and soy hulls replaced corn grain (Ranathunga et al., 2010). The partial replacement of dry ground corn with corn silage resulted in similar (Dann et al., 2008; Agle et al., 2010) or decreased DMI (Oba and Allen, 2000a, 2003a; Weiss et al., 2011). The NDF content of the diets ranged from 26.9 to 32.1%, and these concentrations may have been less than required to elicit a negative response on voluntary DMI due to ruminal distension (Allen, 2000). Furthermore, the HSH and LCS diets contained greater concentrations of NDF from NFFS that have a faster passage rate out of the rumen compared with NDF from forage (Firkins,

1997), further limiting the negative effects of ruminal distension on voluntary DMI.

Actual milk yield, FCM, and ECM were unaffected by treatment and averaged 36.0, 37.3 and 37.0 kg/d, respectively. Akins et al. (2014) found that cows fed a normal-starch diet containing 26.9% starch (% of DM) produced more milk compared with a reduced-starch diet containing 20.4% starch (% of DM). However, several studies have reported that milk yield is unaffected by dietary starch content when corn grain is replaced with soy hulls (Gencoglu et al., 2010; Ranathunga et al., 2010; Ferraretto et al., 2012). Dann et al. (2008) reported that partially replacing corn grain with corn silage resulted in similar milk yield, whereas several studies have reported decreased milk yield (Oba and Allen, 2003a; Agle et al., 2010; Weiss et al., 2011). Across all dietary treatments, the similar DMI and concentration of total VFA (Table 7) suggest that nutrient supply to support milk synthesis was similar. Furthermore, although a greater amount of starch (kg/d) was digested by cows fed the HCG diet, a greater amount of NDF (kg/d) was digested by cows fed the HSH, HCS, and LCS diets, resulting in greater total-tract DM digestion and nutrient supply for milk production.

Milk fat content was increased on the HSH and HCS diets compared with LCS (Table 3; $P = 0.02$), whereas milk fat yield was unaffected by dietary treatment. Ferraretto et al. (2012) reported a tendency for increased milk fat content for cows fed a reduced-starch diet. Milk fat content was unaffected in several other studies (Ipharraguerre et al., 2002; Gencoglu et al., 2010; Ranathunga et al., 2010; Akins et al., 2014). Milk fat content was similar (Oba and Allen, 2003a; Dann et al., 2008; Weiss et al., 2011) or increased (Oba and Allen, 2000a; Agle et al., 2010) when dry ground corn was partially replaced by corn silage. In the present study, forage NDF content of the LCS diet was 17.2% (DM basis), and physically effective NDF may have been

Table 2. Ingredient nutrient composition (% of DM unless otherwise noted)¹

Nutrient	Corn silage	Alfalfa silage	HCG mix	HSH mix	HCS mix	LCS mix	Ground corn	Soy hull pellets
DM, % as fed	41.4	33.9	85.5	85.2	86.0	85.3	91.6	90.9
CP	6.9	23.5	26.4	26.9	34.0	24.1	9.0	15.0
NDF	36.2	37.0	14.7	25.3	15.9	28.2	7.1	57.6
Ether extract	3.3	3.5	3.9	3.8	4.0	3.7	3.5	2.8
Ash	4.1	10.5	8.5	9.1	12.2	8.0	1.3	5.0
Starch	38.2	—	31.7	18.5	14.3	20.5	71.2	—
NFC	49.9	26.6	46.4	34.9	33.9	36.0	79.6	20.4
IVNDFD, ² % of NDF	54.3	49.3	—	—	—	—	—	76.0
IVSD, ³ % of starch	68.4	—	—	—	—	—	47.6	—

¹HCG = high corn grain (normal-starch diet); HSH = high soy hulls; HCS = high corn silage; LCS = low corn silage (reduced-starch diet).

²IVNDFD = ruminal in vitro NDF digestibility after a 30-h incubation.

³IVSD = ruminal in vitro starch digestibility after a 7-h incubation.

Table 3. Effects of dietary starch content and NDF source on lactation performance¹

Item	HCG	HSH	HCS	LCS	SEM	<i>P</i> -value
DMI, kg/d	25.5	23.9	24.3	25.7	1.27	0.43
Milk, kg/d	36.4	35.8	35.3	36.4	2.88	0.92
Milk/DMI, kg/kg	1.42	1.50	1.42	1.41	0.07	0.27
FCM, ² kg/d	37.7	37.5	37.6	36.5	2.36	0.91
FCM/DMI, kg/kg	1.47 ^{ab}	1.58 ^a	1.52 ^{ab}	1.41 ^b	0.05	0.02
ECM, kg/d	37.5	37.1	36.9	36.5	2.28	0.96
ECM/DMI, kg/kg	1.46 ^{ab}	1.56 ^a	1.49 ^{ab}	1.42 ^b	0.04	0.04
Fat, %	3.74 ^{ab}	3.85 ^a	4.00 ^a	3.54 ^b	0.31	0.02
Fat, kg/d	1.35	1.35	1.37	1.27	0.08	0.60
Protein, %	3.31	3.26	3.26	3.33	0.12	0.28
Protein, kg/d	1.19	1.16	1.11	1.19	0.07	0.59
Lactose, %	4.82	4.75	4.76	4.85	0.08	0.27
Lactose, kg/d	1.76	1.70	1.69	1.77	0.14	0.79
MUN, mg/dL	16.0 ^b	16.4 ^b	19.8 ^a	15.8 ^b	0.79	0.001
BW, kg	663.4	662.3	679.8	673.3	10.8	0.31
BW change, kg/d	0.14	0.18	0.51	1.01	0.38	0.37
BCS	2.95	2.94	2.91	2.98	0.11	0.22

^{a,b}Least squares means within rows with different superscripts differ ($P < 0.05$).

¹HCG = high corn grain (normal-starch diet); HSH = high soy hulls; HCS = high corn silage; LCS = low corn silage (reduced-starch diet).

²4.0% FCM yield = $(0.4 \times \text{kg of milk}) + (15.0 \times \text{kg of fat})$; ECM yield = $(12.86 \times \text{kg of fat}) + (7.04 \times \text{kg of protein}) + (0.325 \times \text{kg of milk})$.

limiting even though NDF content was 32.1% (Table 1). Low physically effective NDF can lead to reduced rumination, decreased saliva flow, reduced buffering capacity, lower ruminal pH, and decreased milk fat yields (Mertens, 1997). Milk protein content and yield were similar across dietary treatments. Milk protein content decreased (Akins et al., 2014), tended to decrease (Gencoglu et al., 2010), or was unaffected (Ipharraguerre et al., 2002; Ferraretto et al., 2012) when corn grain was partially replaced by soy hulls. Milk protein content was similar (Dann et al., 2008; Agle et al., 2010; Weiss et al., 2011), decreased (Oba and Allen, 2003a), or tended to decrease (Oba and Allen, 2000a) when corn grain was partially replaced by corn silage.

The increase in milk fat content and numerically lower DMI on the HSH diet resulted in improved FCM feed conversion (kg of FCM/kg of DMI; Table 3; $P = 0.02$) for the HSH diet compared with the LCS diet. Replacing corn grain with soy hulls typically results in numerically lower feed conversion (Gencoglu et al., 2010; Ferraretto et al., 2012; Akins et al., 2014). Sullivan et al. (2012) partially replaced dry corn grain, corn silage, and alfalfa hay with wet corn gluten feed and found that ECM feed conversion was unaffected by diet.

Treatment effects for digestion of DM, OM, and starch are in Table 4. Total-tract digestion of DM (% of intake) was increased for cows fed the HSH and HCS diets compared with the HCG diet ($P = 0.01$). Similar results were observed by others (Gencoglu et al., 2010; Ferraretto et al., 2012) when replacing corn grain with soy hulls and are thought to be related to negative

associative effects of starch on ruminal fermentation (Firkins, 1997). However, pH was similar between the HCG and HSH diets in the current trial. Total-tract digestion of DM was similar (Oba and Allen, 2000b) or decreased (Oba and Allen, 2003b) when corn grain was partially replaced by corn silage.

Apparent ruminal digestion of OM tended to be greater on the HCG and LCS diets compared with the HSH and HCS diets ($P = 0.06$); however, total-tract digestion of OM was increased for the HSH and HCS diets compared with the HCG diet ($P = 0.001$). Oba and Allen (2000b) found that replacing corn grain with corn silage resulted in an increase in apparent ruminal digestion of OM, but total-tract digestion of OM was unaffected (Oba and Allen, 2000b). However, dietary starch contents of the high- and reduced-starch diets were 37.6 and 26.8% in Oba and Allen (2000b). The high amount of dietary starch may have resulted in low rumen pH and unfavorable conditions for ruminal NDF digestion. In contrast, Oba and Allen (2003b) reported that ruminal digestion of OM was unaffected, whereas total-tract digestion was decreased when corn grain was replaced with corn silage, where the normal- and reduced-starch diets contained 32.2 and 21.3% starch. Furthermore, total-tract digestibility of DM and OM was unaffected in several studies where corn grain was partially replaced by corn silage in diets containing either 27.8 or 30.4% starch (Dann et al., 2008), 21.3 or 29.6% starch (Agle et al., 2010), or 26.0 or 30.6% starch (Weiss et al., 2011). The relatively small reduction in dietary starch in the studies by Dann et al. (2008) and Weiss et al. (2011) likely explains the similarity in OM

Table 4. Effects of dietary starch content and NDF source on digestion of DM, OM, and starch¹

Item	HCG	HSH	HCS	LCS	SEM	<i>P</i> -value
DM						
Apparent total-tract digestion, kg/d	17.40	17.46	17.83	18.47	0.93	0.64
Apparent total-tract digestion, %	68.2 ^b	72.9 ^a	73.6 ^a	71.8 ^{ab}	1.09	0.01
OM						
Intake, kg/d	23.70	22.14	22.42	23.89	1.17	0.37
Apparent ruminal digestion, kg/d	9.34 ^{ab}	7.55 ^b	7.44 ^b	9.80 ^a	0.61	0.01
Apparent ruminal digestion, %	39.5	33.8	34.2	40.8	2.13	0.06
Flow at omasum, kg/d	14.36	14.59	14.98	14.08	0.86	0.82
Apparent total-tract digestion, kg/d	16.69	16.55	16.93	17.44	0.87	0.74
Apparent total-tract digestion, %	70.5 ^b	74.7 ^a	75.8 ^a	73.2 ^{ab}	0.87	0.001
Starch						
Intake, kg/d	7.26 ^a	5.50 ^b	5.72 ^b	5.91 ^b	0.30	0.001
Apparent ruminal digestion, kg/d	6.23 ^a	4.62 ^b	4.77 ^b	4.88 ^b	0.29	0.001
Apparent ruminal digestion, %	85.8	84.0	81.3	85.1	2.07	0.43
Flow at omasum, kg/d	1.03	0.88	1.14	0.83	0.12	0.28
Apparent total-tract digestion, kg/d	6.95 ^a	5.35 ^b	5.83 ^b	5.47 ^b	0.29	0.001
Apparent total-tract digestion, %	95.7 ^b	97.3 ^a	98.8 ^a	95.8 ^b	0.41	0.001
Fecal starch concentration, %	3.9 ^a	2.3 ^{bc}	1.3 ^c	3.3 ^{ab}	0.31	0.001

^{a-c}Least squares means within rows with different superscripts differ ($P < 0.05$).

¹HCG = high corn grain (normal-starch diet); HSH = high soy hulls; HCS = high corn silage; LCS = low corn silage (reduced-starch diet).

digestibilities between the normal- and reduced-starch diets in those studies. In the study by Agle et al. (2010), the normal-starch diet resulted in lower ruminal pH, decreased total-tract NDF digestibility, and decreased milk fat content, indicating that starch in the normal-starch diet decreased ruminal digestibility of fiber.

Starch intake was greater for cows on the HCG diet compared with the other diets ($P = 0.001$). Apparent ruminal digestion of starch (%) was unaffected by treatment ($P = 0.43$), whereas total-tract digestion of starch was increased for cows fed the HSH and HCS diets compared with the HCG diet ($P = 0.001$). Total-tract digestion is typically increased for cows fed reduced-starch diets when corn grain is partially replaced by NFFS because a greater portion of starch is from more highly digestible sources such as corn silage (Table 2); Gencoglu et al. (2010) reported that total-tract starch digestion increased from 94.9 to 97.2%, and Ferraretto et al. (2012) found that total-tract starch digestion increased from 93.2 to 95.3%. However, Oba and Allen (2003b) reported that total-tract starch digestion was increased from 93.0 to 94.2% for cows fed a normal-starch diet compared with a diet that partially replaced corn grain with corn silage. Similar to the partial replacement of corn grain with NFFS, replacement with corn silage increases the portion of more highly digestible starch in the silage.

Treatment effects for intake and digestion of NDF are in Table 5. Intake of NDF was greater for cows fed the LCS diet compared with the HCG diet ($P = 0.02$) but was not different for the HSH and HCS diets. Apparent ruminal digestion of NDF (% of intake) tended to be increased for cows fed the HSH diet compared

with the HCG diet ($P = 0.07$). Apparent total-tract digestion of NDF (% of intake) was reduced for cows fed the HCG diet ($P = 0.001$). Apparent ruminal digestion of pdNDF was unaffected ($P = 0.35$), whereas total-tract digestion of pdNDF was increased for cows fed the HCS diet and reduced for cows fed the HCG diet ($P = 0.001$). Increased total-tract NDF digestibility by cows fed the HSH and LCS diets compared with HCG may be related to the high fiber digestibility of soy hulls (Table 2). Total-tract NDF digestibility was increased for cows fed reduced- compared with normal-starch diets in Gencoglu et al. (2010) and Ferraretto et al. (2012). Oba and Allen (2000b) and Agle et al. (2010) reported that total-tract digestion was increased for cows fed diets where corn grain was partially replaced with corn silage; however, several studies found that total-tract NDF digestion was unaffected (Oba and Allen, 2003b; Dann et al., 2008; Weiss et al., 2011).

Treatment effects for mean N intake, N total-tract digestibility, and flow of FAB, PAB, total bacteria, and RUP are in Table 6. Apparent total-tract N digestibility (% of intake) was reduced for cows fed the HCG diet compared with the other 3 diets ($P = 0.01$). Flow of FAB, total bacterial NAN, and RUP (g/d) were similar for all diets ($P > 0.10$). Flow of PAB (g/d) tended to be greater for cows fed the HCS compared with the LCS diet ($P = 0.07$). Flow of total bacterial NAN averaged 559 g/d. According to an equation developed by Broderick et al. (2010), using OM intake as a predictor of microbial N flow, microbial N flow should average 432 g/d across all treatments. The predicted estimate is 29% lower than the observed average (559 g/d). The greater than expected bacterial and NAN flow is a re-

Table 5. Effects of dietary starch content and NDF source on digestion of NDF¹

Item	HCG	HSH	HCS	LCS	SEM	<i>P</i> -value
NDF						
Intake, kg/d	7.05 ^b	7.60 ^{ab}	7.35 ^{ab}	8.35 ^a	0.38	0.02
Apparent ruminal digestion, kg/d	3.16 ^b	4.10 ^a	3.60 ^{ab}	4.30 ^a	0.23	0.01
Apparent ruminal digestion, %	45.0	54.0	50.0	51.5	2.48	0.07
Flow at omasum, kg/d	3.89	3.50	3.76	4.05	0.29	0.41
Apparent total-tract digestion, kg/d	3.44 ^b	4.39 ^a	4.15 ^a	4.62 ^a	0.20	0.001
Apparent total-tract digestion, %	49.0 ^b	57.8 ^a	56.8 ^a	55.6 ^a	1.34	0.001
Potentially digestible NDF ²						
Intake, kg/d	5.31 ^c	6.20 ^{ab}	5.62 ^{bc}	6.92 ^a	0.30	0.001
Apparent ruminal digestion, kg/d	3.16 ^b	4.10 ^a	3.60 ^{ab}	4.30 ^a	0.23	0.01
Apparent ruminal digestion, %	59.8	66.2	65.1	61.9	3.07	0.35
Flow at omasum, kg/d	2.15	2.10	2.02	2.62	0.22	0.14
Apparent total-tract digestion, kg/d	3.44 ^b	4.39 ^a	4.15 ^a	4.62 ^a	0.20	0.001
Apparent total-tract digestion, %	64.9 ^c	70.9 ^{ab}	74.1 ^a	66.9 ^{bc}	1.39	0.001
Indigestible NDF						
Intake, kg/d	1.74 ^a	1.40 ^b	1.73 ^a	1.43 ^b	0.10	0.01
Flow at omasum, kg/d	1.74 ^a	1.40 ^c	1.73 ^{ab}	1.43 ^{bc}	0.10	0.01

^{a-c}Least squares means within rows with different superscripts differ ($P < 0.05$).

¹HCG = high corn grain (normal-starch diet); HSH = high soy hulls; HCS = high corn silage; LCS = low corn silage (reduced-starch diet).

²Potentially digestible NDF = NDF – indigestible NDF.

sult of the overestimated omasal OM flow (Table 4) due to potential issues with marker flow, analytical error, or a combination of both.

Treatment effects for mean ruminal pH, NH₃-N concentration, total VFA concentration, and VFA molar proportions are in Table 7. Ruminal pH was reduced on the LCS diet compared with the HCS diet ($P = 0.01$) and may be due to inadequate dietary physically effective NDF and a corresponding decrease in rumination and buffering capacity (Mertens, 1997). Mullins et al.

(2010) and Sullivan et al. (2012) found that partial replacement of corn grain and forage with wet corn gluten feed decreased ruminal acetate concentration, whereas ruminal pH was decreased and propionate concentration was increased (Mullins et al., 2010). Rumen NH₃-N concentration was increased on the HCS diet compared with the HCG and LCS diets ($P = 0.001$). The increase in NH₃-N concentration agrees with Hristov et al. (2005), who reported that intraruminal starch decreased NH₃-N concentrations to a greater extent than

Table 6. Effects of dietary starch content and NDF source on omasal flow of nitrogen fractions in lactating dairy cows¹

Item	HCG	HSH	HCS	LCS	SEM	<i>P</i> -value
Nitrogen						
Intake, g/d	737	700	698	769	38.4	0.25
Flow of NAN at omasum, g/d	889	962	963	881	60.2	0.50
Apparent total-tract digestion, g/d	477	500	495	558	30.6	0.08
Apparent total-tract digestion, %	64.7 ^b	71.2 ^a	71.2 ^a	72.5 ^a	1.5	0.01
FAB ² NAN flow						
g/d	228	251	240	227	16.2	0.58
% of total bacteria NAN	41.8	42.7	39.9	44.6	1.5	0.10
PAB ³ NAN flow						
g/d	311	336	361	283	21.2	0.07
% of total bacteria NAN	58.2	57.3	60.1	55.4	1.5	0.10
Total bacterial NAN flow						
g/d	539	587	601	510	33.9	0.16
% of NAN	60.9	61.2	63.9	58.0	2.6	0.41
RUP ⁴ flow						
g/d	2144	2295	2265	2209	257.8	0.97
% of NAN	39.1	38.8	36.1	42.0	3.0	0.81
% of DMI	8.3	9.5	9.0	8.6	0.9	0.72

^{a,b}Least squares means within rows with different superscripts differ ($P < 0.05$).

¹HCG = high corn grain (normal-starch diet); HSH = high soy hulls; HCS = high corn silage; LCS = low corn silage (reduced-starch diet).

²FAB = fluid-associated bacteria.

³PAB = particle-associated bacteria.

⁴Ruminally undegraded N $\times$ 6.25.

Figure 1. Treatment effects for molar proportions of ruminal acetate (mol/100 mol) over time. Normal-starch diet: high corn grain = solid black line (■); reduced-starch diets: high soy hulls = dashed black line (■); high corn silage diet = solid gray line (▲); low corn silage diet = dashed gray line (▲). Treatment differences ($P < 0.05$) are represented by (**); trends ($0.05 \leq P < 0.10$) are represented by (*); error bars represent the standard error of the mean. Cows were fed 50% of their daily ration at 1000 and 1900 h.

NDF. Ruminal concentration of total VFA tended to increase for cows fed the LCS diet ($P = 0.06$). Acetate tended to increase for cows fed the HCS diet ($P = 0.07$), and a diet by time interaction was observed for ruminal acetate, resulting in an increase in ruminal acetate ($P < 0.05$) for cows fed the HCS diet at 0800, 2000, 0000, 0200, and 0400 h (Figure 1) and a decrease in acetate for HCG and LCS at 0800 and 0400 h. Propionate was decreased for cows fed the HCS diet compared with the LCS diet ($P < 0.01$), resulting in an increase in the acetate:propionate ratio for the HCS diet compared with the LCS diet ($P = 0.01$). We detected an increase

in ruminal propionate ($P < 0.05$) for cows fed the LCS diet at 0000 h and reduced ruminal propionate for cows fed the HCS diet at all times except 0600 and 1000 h (Figure 2). These results led to an increase in the acetate:propionate ratio ($P < 0.05$) for cows fed the HCS at all times except for 1000 and 1200 h, and a decreased ratio for cows fed the LCS diet at time 0000 h (Figure 3). The molar proportions of acetate and propionate for cows fed the HCS diet compared with cows fed the HCG diet is in agreement with the results for ruminal starch digestion (kg/d). The lesser amount of starch and greater amount of forage NDF in the

Table 7. Effects of dietary starch content and NDF source on ruminal pH, NH_3 concentration, and VFA concentrations and molar proportions¹

Item	HCG	HSH	HCS	LCS	SEM	P-value	
						Diet	Diet × hour
Mean pH	6.14 ^{ab}	6.10 ^{ab}	6.25 ^a	6.04 ^b	0.07	0.01	0.61
NH_3 , mg/dL	9.6 ^b	10.0 ^{ab}	12.1 ^a	8.0 ^b	1.32	0.001	0.65
Total VFA, mM	105.2	105.0	99.8	112.6	3.69	0.06	0.88
VFA molar proportions, mol/100 mol							
Acetate	61.1	62.2	63.6	61.2	0.92	0.07	0.01
Propionate	24.3 ^{ab}	24.5 ^{ab}	21.8 ^b	25.4 ^a	0.90	0.01	0.001
Isobutyrate	0.39 ^{ab}	0.35 ^{ab}	0.46 ^a	0.34 ^b	0.03	0.02	0.66
Butyrate	11.4	10.3	11.2	10.2	0.45	0.19	0.26
2-Methylbutyrate	0.59 ^{ab}	0.54 ^b	0.70 ^a	0.52 ^b	0.04	0.01	0.77
Isovalerate	0.48 ^{ab}	0.41 ^b	0.54 ^a	0.40 ^b	0.03	0.01	0.68
Valerate	1.76 ^{ab}	1.74 ^{ab}	1.63 ^b	1.95 ^a	0.10	0.03	0.38
Acetate:propionate ratio	2.57 ^{ab}	2.58 ^{ab}	2.94 ^a	2.45 ^b	0.13	0.01	0.01

^{a,b}Least squares means within rows with different superscripts differ ($P < 0.05$).

¹HCG = high corn grain (normal-starch diet); HSH = high soy hulls; HCS = high corn silage; LCS = low corn silage (reduced-starch diet).

Figure 2. Treatment effects for molar proportions of ruminal propionate (mol/100 mol) over time. Normal-starch diet: high corn grain = solid black line (■); reduced-starch diets: high soy hulls = dashed black line (▣); high corn silage diet = solid gray line (▲); low corn silage diet = dashed gray line (▴). Treatment differences ($P < 0.05$) are represented by (**); trends ($0.05 \leq P < 0.10$) are represented by (*); error bars represent the standard error of the mean. Cows were fed 50% of their daily ration at 1000 and 1900 h.

HCS diet likely resulted in greater acetate and lower propionate production in the rumen. Treatment effects were observed for all branched-chain VFA ($P \leq 0.03$). Cows fed the HCS diet had increased concentrations of all branched-chain VFA, and the HSH and LCS diets resulted in lower concentrations of 2-methylbutyrate

and isovalerate, whereas the LCS diet resulted in lower concentrations of isobutyrate and valerate. Rumen pH was increased (Oba and Allen, 2003a; Agle et al., 2010), $\text{NH}_3\text{-N}$ was increased (Agle et al., 2010), and propionate concentration decreased (Oba and Allen, 2000a, 2003a; Agle et al., 2010) when corn grain was replaced

Figure 3. Treatment effects for ruminal acetate:propionate ratio over time. Normal-starch diet: high corn grain = solid black line (■); reduced-starch diets: high soy hulls = dashed black line (▣); high corn silage diet = solid gray line (▲); low corn silage diet = dashed gray line (▴). Treatment differences ($P < 0.05$) are represented by (**); trends ($0.05 \leq P < 0.10$) are represented by (*); error bars represent the standard error of the mean. Cows were fed 50% of their daily ration at 1000 and 1900 h.

by corn silage. Unexpectedly, data in the present study and from Sullivan et al. (2012) and Mullins et al. (2010) suggest that the partial replacement of corn grain and forage with NFFS decreases ruminal pH and ruminal acetate concentration and increases propionate concentration, even though these diets would contain reduced amounts of starch and greater amounts of NDF on a DM basis. It is unknown if these changes in ruminal parameters can negatively affect rumen function and animal performance over time and further research may be warranted.

CONCLUSIONS

Milk yield and DMI were unaffected by treatment. Cows fed LCS had reduced milk fat content compared with HSH and HCS. The concentration of MUN was greater for cow fed HCS compared with the other treatments. Ruminal ammonia concentration was lower for HCG and LCS compared with HCS. Total-tract digestion of NDF was reduced for cows fed the HCG diet. Total-tract starch digestion was increased in cows fed the HSH and HCS compared with HCG and LCS diets. Bacterial protein and NAN flows were similar for all dietary treatments. However, NAN flows were greater than N intake, likely because of overestimated OM flows. Diet digestibility and performance of mid- to late-lactation cows fed reduced-starch diets by partially replacing corn grain with soy hulls or corn silage were similar or improved compared with cows fed a normal-starch diet. Under the conditions of this experiment, the decrease in milk fat content and low ruminal pH due to low forage NDF content for cows fed the LCS diet suggest that partial replacement of corn silage and corn grain with soy hulls is not a viable ration formulation strategy to improve forage inventory on dairy farms. Similar lactational responses for cows fed reduced-starch diets by partly replacing corn grain with soy hulls or corn silage can improve financial returns for dairy farms when the price of corn grain is increased.

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