Effect of forage level and replacing canola meal with dry distillers grains with solubles in precision-fed heifer diets: Digestibility and rumen fermentation

F. X. Suarez-Mena,* G. J. Lascano,† D. E. Rico,‡ and A. J. Heinrichs*¹

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

†Department of Animal and Veterinary Sciences, Clemson University, Clemson, SC 29634

‡Department of Animal Science, Université Laval, Quebec, QC, G1V 0A6, Canada

ABSTRACT

Objectives of this study were to determine the effects of feeding differing forage-to-concentrate ratios (F:C) and inclusion rates of corn dry distillers grain with solubles (DDGS) on digestion and rumen fermentation in precision-fed dairy heifer rations. A split-plot design with F:C as whole plot and DDGS inclusion level as sub-plot was administered in a 4-period (19 d) 4 × 4 Latin square. Eight rumen-cannulated Holstein heifers (12.5 ± 0.5 mo of age and 344 ± 15 kg of body weight) housed in individual stalls were allocated to 2 F:C [50:50, low forage, or 75:25 high forage; dry matter (DM) basis] and to a sequence of DDGS inclusion (0, 7, 14, and 21%; DM basis). Forage was a mix of 50% corn silage and 50% grass hay (DM basis). Diets were fed to allow for 800 g/d of body weight gain and fed 1×/d. Rumen contents were sampled at −2, 0, 2, 4, 6, 8, 10, 12, and 20 h after feeding for rumen fermentation measures. Low-forage rations had greater DM and organic matter apparent digestibility. We detected a quadratic effect for DM, organic matter, acid detergent fiber, and neutral detergent fiber apparent digestibility, with the 14% DDGS inclusion level having the highest values. Nitrogen retention decreased with increasing levels of DDGS. Molar proportions of acetate tended to be greater for HF and decreased as DDGS increased; propionate increased as DDGS increased, resulting in the opposite effect on acetate to propionate ratio. Rumen protozoa count decreased as DDGS increased. Moderate levels (14% of DM) of DDGS appear to enhance nutrient utilization and fermentation in precision-fed dairy heifers fed different F:C diets.

Key words: precision feeding, canola meal, dried distillers grains with solubles, heifer diet

INTRODUCTION

Improvements in dairy heifer growth and nutrition programs have resulted in an increase in efficiency through various strategies. One of the strategies to reduce feed cost and increase efficiency is to limit DMI (McLeod and Baldwin, 2000). Controlling DMI increases nutrient efficiency mainly because metabolic nutrient costs of digestion are lower when nutrient amounts are precisely supplied in heifer diets (Zanton and Heinrichs, 2007; Lascano and Heinrichs, 2011). Precision-feeding programs provide the heifer with the amount of nutrients necessary to reach the targeted ADG. Following these results, enhancement in nutrient utilization through manipulation of other nutrient fractions such as fiber, nonstructural carbohydrates, and protein have yielded results that contribute to precision-feeding programs in dairy heifers (Zanton and Heinrichs, 2008; Lascano and Heinrichs, 2011; Lascano et al., 2012). To further increase efficiency and reduce feed costs, a viable option is the inclusion of alternative ingredients and by-products such as canola meal and dry distillers grains (DDGS) to the aforementioned programs.

Using canola meal and DDGS as a protein supplement to replace soybean meal has yielded similar lactation performance in dairy cows (Christen et al., 2010). Both of these ingredients have been minimally investigated under precision-feeding scenarios for dairy heifers. High concentrations of DDGS have been successfully incorporated for growing feedlot cattle rations (Klopfenstein et al., 2008). Although research with feedlot animals is valuable, the objectives are very different compared with those for replacement dairy heifers. Dairy heifer growth needs to be controlled (NRC, 2001; Zanton and Heinrichs, 2009b), whereas feedlot programs target maximum growth in minimum time. Maximal DMI, ADG, and final BW in feedlot finishing diets have been reported with 15% DM inclusion of DDGS (Depenbusch et al., 2009a). However, similar DDGS inclusion levels in research from the same group did not improve ADF or feed efficiency (Depenbusch et

Received March 26, 2015.

Accepted June 27, 2015.

¹Corresponding author: ajh@psu.edu

al., 2008, 2009b) and reduced DM apparent digestibility (Depenbusch et al., 2009b). The conflicting findings could be related to differences in diet composition and the interaction between DDGS and other ingredients in the ration. Moreover, other researchers have observed that ADG and feed efficiency improve at inclusion levels of 50 and 40%, respectively (Firkins et al., 1985; Ham et al., 1994); this increased efficiency was attributed to a reduction in subacute ruminal acidosis because of the lower starch content compared with corn-based diets (Firkins et al., 1985). Additional concerns with feeding high levels of DDGS are the high sulfur concentration and high levels of fat. High levels of sulfur in DDGS come from the sulfur-containing compounds used to control pH and clean equipment in the ethanol plants and can approach the maximum recommended level of sulfur intake (Klopfenstein et al., 2008; Schingoethe et al., 2009). The high fat content in some DDGS may restrict its inclusion level because of the reduction in DMI and ruminal fermentation (NRC, 2001).

These results suggest that the optimum level of DDGS to maximize nutrient utilization depends on diet composition. To our knowledge, the optimum inclusion level at which DDGS replace canola meal in dairy replacement heifers under precision-feeding management has not been explored. Therefore, the hypothesis of this experiment was that DDGS would improve nutrient utilization and rumen fermentation depending on the level of inclusion in high- and low-forage diets precision fed to dairy heifers.

MATERIALS AND METHODS

Animals, Treatments, and Experimental Design

All procedures involving the use of animals were approved by the Pennsylvania State University Institutional Animal Care and Use Committee. Eight Holstein heifers were surgically prepared with a rumen cannula (7.62 cm i.d.; Bar Diamond, Parma, ID) under local anesthesia, 2 mo before the beginning of the experiment and later refitted with larger cannulas (10.16 cm i.d.; Bar Diamond). Heifers (12.5 ± 0.5 mo of age and 344 ± 15 kg of BW at the beginning of the experiment) were randomly assigned to a split-plot 4×4 Latin square experimental design. Whole plot was forage-to-concentrate ratio (**F:C**)—either 50:50 (low forage, **LF**) or 75:25 (high forage, **HF**) on a DM basis—and subplot was level of inclusion of DDGS: 0, 7, 14, or 21% on a DM basis; these levels have been shown to increased nutrient utilization in growing and lactating beef and dairy animals (Depenbusch et al., 2009a; Schingoethe et al., 2009). The DDGS and canola meal levels were chosen to provide the N level (1.67 g of N/kg of BW^{0.75}) that

maximizes N utilization in precision-fed dairy heifers (Zanton and Heinrichs, 2009a); DDGS replaced canola meal at an almost 1:1 ratio. Forage was a mix of 50% corn silage and 50% grass hay on a DM basis. Experimental periods were 19 d long, with 14 d for adaptation and 5 d for sampling. Heifers were housed in individual tiestalls (117×302 cm) with rubber mat flooring in a mechanically ventilated barn with continuous access to fresh water. During nonsampling days, heifers were let out to an outdoor exercise lot for 3 to 4 h/d before feeding, and BW was recorded on their way in and out of the exercise lot. Rations were balanced to provide equal amounts of nutrients and to allow for 800 g of ADG. The amount of feed offered was adjusted weekly based on BW, except just before and during sampling. Single batches of grain ingredients were stored to last for the length of the experiment. Grain ingredients and a mineral-vitamin premix were mixed for each treatment at the beginning of each experimental period, in a drum mixer (Calan Super Data Ranger, American Calan, Northwood, NH); forages were mixed daily using the same equipment. The forage mix, grain mix, and NPN source of each ration were hand mixed (to ensure mixing and quantities were delivered precisely) and delivered once daily at 1200 h.

Samples and Analyses

The grain mix for each diet was sampled at the beginning and end of each experimental period and composited by diet at the end of the experiment. The forage mix was sampled daily during collection days and composited by period for analyses. Feces and urine were completely collected from d 15 immediately after feeding to d 19 immediately before feeding for 4 d of total collection. Urine was collected using a noninvasive urinary device (Lascano et al., 2010) connected to a container with distilled water and 12 N HCl to maintain pH below 3 and minimize ammonia (NH_3) volatilization. The distilled water was added to avoid the formation of precipitates. Feces were collected hourly from vinyl-covered boards and stored in airtight containers. Every 24 h, urine and feces were weighed, mixed, and sampled. Urine samples were immediately frozen at -20°C . Feces were stored at 4°C until the last day of that collection period, and then composited by period proportionally to daily output; one sub-sample was dried and a fresh sub-sample was frozen at -20°C .

Forage mix, grain mix, and feces were dried in a forced-air oven at 65°C for 48 h and ground through a 1-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA). After drying and grinding, forage was composited by period; composited forage mixes, grain mixes, and feces were analyzed for DM, ash, and CP

(AOAC, 1990), and NDF and ADF (Van Soest et al., 1991). Total FA concentration and FA profile of feed samples were determined by the method of Sukhija and Palmquist (1988) with slight modifications as described by Rico et al. (2014). Briefly, following direct methylation and hexane extraction, methyl esters were quantified using a gas chromatograph equipped with a flame-ionization detector (GC-FID, Agilent 6890A; Agilent Technologies Inc., Santa Clara, CA) and a capillary column (SP-2560; 100 m $\times$ 0.25 mm i.d. with 0.2- μ m film thickness; Supelco Inc., Bellefonte, PA). Frozen fecal samples were thawed and analyzed for CP according to the procedure detailed above. Urine samples were analyzed for N (AOAC, 1990) and purine derivatives (Chen and Gomes, 1992). Feeds and feces were gelatinized with sodium hydroxide for starch concentration analysis by the enzymatic method of Karkalas (1985). Degradable protein was analyzed as described by Krishnamoorthy et al. (1983).

Rumen contents were collected from dorsal, ventral, cranial, caudal, and medial areas of the rumen at -2, -1, 0, 1, 2, 4, 6, 8, 10, 12, 16, 20, and 24 h relative to feeding on d 17. Rumen contents were mixed thoroughly, sampled, and strained through 2 layers of cheesecloth; fluid was immediately analyzed for pH using a hand-held pH meter (HI 98121, Hanna Instruments, Woonsocket, RI). For rumen NH_3N and VFA analyses, 15 mL of filtered rumen fluid was placed into bottles containing 3 mL of 35% metaphosphoric acid and 3 mL of 0.6% 2-ethyl butyric acid (internal standard) and frozen at -20°C until analyzed. After thawing, rumen fluid was centrifuged 3 times at $4,000 \times g$ for 30 min at 4°C to obtain a clear supernatant, which was analyzed for NH_3 according to Chaney and Marbach (1962) and VFA molecular concentration by GC (Yang and Varga, 1989). For estimation of rumen protozoa numbers, 5 mL of rumen fluid was combined with 5 mL of methylgreen-formalin-saline solution (Ogimoto and Imai, 1981) and stored in darkness at 4°C . Counting was done using a Fuchs Rosenthal counting chamber (#3720, Hausser Scientific, Horsham, PA) as in Hristov et al. (1999).

Statistical Analysis

All statistical analysis were conducted in SAS (version 9.2, SAS Institute Inc., Cary, NC) using the MIXED procedure. Data were analyzed as a split-plot, Latin square design with fixed effects of period, forage, DDGS level, and forage $\times$ DDGS level interaction, and a random effect of heifer (forage). Because of unequally spaced rumen sampling, VFA and NH_3 concentrations were determined by calculating the area under the response curve according to the trapezoidal rule (Shipley

and Clark, 1972). Linear and quadratic contrasts of DDGS level were tested using the CONTRAST statement. The sequences of forage and DDGS level were balanced for carryover with respect to previous forage and DDGS level such that all treatments followed every other treatment once; therefore, a fixed effect of previous treatment was included in the analysis. Fixed effect of time and its interaction with other fixed effects were included in the model when multiple observations occurred in a day. Variance homogeneity was evaluated for the main effects of forage and DDGS level and time relative to feeding when repeated measures were analyzed; evidence of significant heterogeneity was determined using the Levene test for equality of variance. Least squares means are presented in tables and evidence for statistical significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

Dietary Nutrient Composition and Intake

Nutrient composition of diets is presented in Table 1. Rations were formulated and fed with the objective of providing similar amounts of energy and N. Dietary NDF and ADF were higher for HF, as was their intake due to the higher level of forage in these rations. The FA concentration in the diet increased with the addition of DDGS (3.4 to 5% of DM) and, consequently, so did ME concentration; therefore, diet DMI decreased as more DDGS was added. Noteworthy, of all dietary FA, DDGS inclusion increased C18:2n-6 in particular (from approximately 37 to 45% of dietary FA) and decreased the concentration of C18:1n-9, a change expected to have negative effects on the growth of several species of ruminal bacteria (Maia et al., 2007).

Nutrient intake is presented in Table 2. Daily DMI was not different for F:C when analyzed as kilograms per day, but when analyzed as grams per kilogram of $\text{BW}^{0.75}$, DMI was higher for HF. Higher DMI for HF were also reported in experiments that controlled nutrient intake at different F:C (Hoffman et al., 2007; Lascano and Heinrichs, 2009; Lascano et al., 2009; Zanton and Heinrichs, 2009a), because the lower nutrient density in HF rations is compensated for by increasing DMI (Hoffman et al., 2007). Intakes of ME and N did not differ by F:C when analyzed as grams per day or megacalories per day, but both were greater for LF when analyzed as intake per kilogram of $\text{BW}^{0.75}$; although differences were marginal. Diets approached the N level recommended by Zanton and Heinrichs (2009a) for optimum N utilization in precision-fed dairy heifer rations (1.67 g/kg of $\text{BW}^{0.75}$).

Table 1. Ingredient and chemical composition (% of DM unless otherwise noted) of low- (LF) or high-forage (HF) dairy heifer diets containing 0, 7, 14, or 21% dry distillers grains with solubles (DDGS)

Item	LF				HF			
	0	7	14	21	0	7	14	21
Ingredient								
Grass hay	25.00	25.00	25.00	25.00	37.50	37.50	37.50	37.50
Corn silage	25.00	25.00	25.00	25.00	37.50	37.50	37.50	37.50
DDGS ¹	0.00	7.00	14.00	21.00	0.00	7.00	14.00	21.00
Ground corn	18.55	17.91	17.24	16.58	2.55	1.81	1.05	0.29
Soy hulls	8.00	8.00	8.00	8.00	—	—	—	—
Canola meal	20.45	13.84	7.26	0.67	19.45	12.95	6.48	—
Mineral-vitamin mix ²	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Optigen ³	—	0.25	0.50	0.75	—	0.24	0.48	0.72
Chemical composition								
DM	69.38	69.28	68.93	68.48	59.33	59.11	58.49	57.76
CP	15.31	14.59	15.02	15.12	13.96	13.90	14.15	14.19
RDP, % of CP	43.59	43.78	45.15	45.32	54.47	55.28	52.63	54.77
Soluble P, ⁴ % of CP	23.46	25.50	23.53	21.27	31.64	28.70	26.53	24.99
Soluble P, ⁵ % of CP	23.46	28.57	29.49	30.15	31.64	31.77	32.56	34.02
ADF	26.17	26.82	26.02	25.23	29.33	28.88	28.93	28.23
NDF	39.94	41.81	42.12	41.69	46.27	46.47	47.17	47.60
Starch	23.12	21.45	21.86	22.53	18.66	17.89	17.79	17.44
Total FA ⁶	3.35	3.74	4.32	4.99	3.15	3.53	4.01	4.67
Ash	6.07	6.09	6.10	5.78	6.48	6.63	6.37	5.98
Ca	0.82	0.87	0.82	0.80	0.80	0.83	0.74	0.72
P	0.22	0.21	0.18	0.15	0.22	0.21	0.19	0.17
Mg	0.39	0.37	0.36	0.29	0.33	0.33	0.32	0.31
K	0.88	0.92	0.92	0.90	1.01	1.00	1.01	1.01
Na	0.22	0.29	0.26	0.26	0.23	0.28	0.28	0.25
S	0.14	0.14	0.14	0.13	0.13	0.13	0.14	0.13
Mn, mg/kg	75.10	87.62	78.01	73.10	88.99	91.65	85.28	75.25
TDN ⁷	70.84	70.30	71.15	72.59	67.36	67.73	68.49	69.54
NFC ⁸	37.38	34.73	35.03	35.63	31.41	30.85	30.93	30.94
ME, ⁹ Mcal/kg	2.56	2.54	2.57	2.62	2.44	2.45	2.48	2.51
FA profile, % of total FA								
C14:0	0.32	0.30	0.29	0.27	0.40	0.38	0.37	0.37
C16:0	16.07	16.42	16.71	16.94	16.77	17.07	17.26	17.35
C16:1n-7	0.51	0.39	0.30	0.23	0.46	0.36	0.30	0.26
C18:0	2.12	2.07	2.05	2.00	2.00	1.98	1.96	1.96
C18:1n-9	29.42	25.78	23.28	21.50	24.97	22.14	20.19	19.11
C18:2n-6	37.06	41.86	44.80	46.99	38.25	41.62	44.00	45.16
C18:3n-3	7.39	7.42	7.29	7.19	10.04	10.05	9.97	9.92
Unidentified	7.11	5.76	5.28	4.88	7.12	6.40	5.95	5.88

¹Contained 88.1% DM, 31.4% NDF, 12.9% ADF, 29.1% CP, and 11.8% ether extract on DM basis, and soluble protein 16% of CP.

²Contained 3.9% vitamin E, 0.59% vitamin ADE, 48.5% distillers corn with soluble vitamin D, 10.0% plain salt, 29.4% limestone, 4.6% magnesium oxide, 1.3% trace mineral premix, and 1.6% selenium premix.

³Optigen is an NPN source (256% CP, DM basis) from Alltech Inc. (Lexington, KY).

⁴Optigen soluble protein assumed to be 25% of CP.

⁵Optigen soluble protein assumed to be 95% of CP.

⁶Does not include Optigen FA contribution to total FA.

⁷Calculated.

⁸Calculated from ingredients as $NFC = 100 - (NDF + CP + \text{ether extract} + \text{ash})$.

⁹Estimated: $ME = TDN \times 0.04409 \times 0.82$.

We detected linear reductions of DM, ADF, NDF, ash, and TDN intake as DDGS were incorporated in the rations. This adjustment was necessary to maintain isocaloric diets and reflected the higher estimated ME as DDGS replaced canola meal in the diets. However, when ME intake was calculated with the actual digestibility of diets, its intake decreased as more DDGS were added to the rations; although differences were minor, this reflects the depression in OM digestibility

as DDGS were included at higher rates in the diets. Similarly, N intake slightly decreased as DDGS concentration increased.

Digestibility

Apparent digestibility (**AD**) of nutrients is presented in Table 3. The AD of DM and OM was greater for LF rations. Similar observations have been reported

Table 2. Nutrient intake of low- (L) or high-forage (H) dairy heifer diets containing 0, 7, 14, or 21% dry distillers grains with solubles (DDGS)

Item	F:C ¹	DDGS, % in diet					Contrast, <i>P</i> -value				
		0	7	14	21	SE	F:C	DDGS		Interaction	
								Linear	Quadratic	Linear	Quadratic
Intake, kg/d											
As fed	L	9.37	9.16	9.02	8.91	0.20	<0.01	<0.01	0.13	0.05	0.64
	H	11.33	10.94	10.81	10.60						
DM	L	6.50	6.35	6.21	6.10	0.13	0.52	<0.01	0.45	0.03	0.87
	H	6.72	6.47	6.32	6.12						
ADF	L	1.70	1.70	1.62	1.54	0.04	<0.01	<0.01	0.02	0.01	0.02
	H	1.97	1.87	1.83	1.73						
NDF	L	2.60	2.65	2.62	2.54	0.06	<0.01	<0.01	0.06	0.00	0.00
	H	3.11	3.01	2.98	2.91						
Ash	L	0.39	0.39	0.38	0.35	0.01	0.03	<0.01	<0.01	<0.01	0.16
	H	0.44	0.43	0.40	0.37						
TDN ²	L	4.60	4.46	4.42	4.43	0.09	0.42	<0.01	0.02	0.11	0.38
	H	4.53	4.38	4.33	4.26						
Water, voluntary	L	31.29	24.89	30.49	24.26	4.80	0.57	0.64	0.96	0.39	0.98
	H	24.00	24.47	24.65	25.50						
Water, total	L	34.16	27.71	33.29	27.06	4.78	0.80	0.62	0.94	0.39	0.98
	H	28.61	28.94	29.14	29.98						
ME, ³ Mcal/d	L	16.48	15.93	15.68	14.70	0.39	0.29	<0.01	0.10	0.39	0.89
	H	15.74	15.34	15.04	14.27						
DM, g/kg of BW ^{0.75}	L	12.50	12.28	11.95	11.76	0.04	<0.01	<0.01	0.70	0.03	0.39
	H	12.75	12.47	12.15	11.81						
ME, ³ g/kg of BW ^{0.75}	L	0.20	0.19	0.19	0.18	<0.01	0.01	<0.01	0.02	0.12	0.86
	H	0.19	0.18	0.18	0.17						
FA intake, g/d											
Total	L	198.53	231.32	258.89	299.47	5.15	0.17	<0.01	<0.01	0.36	0.18
	H	189.79	213.18	252.04	290.60						
C14:0	L	0.63	0.70	0.75	0.82	0.02	<0.01	<0.01	<0.01	<0.01	<0.01
	H	0.75	0.82	0.94	1.07						
C16:0	L	31.91	37.98	43.25	50.73	0.87	0.72	<0.01	<0.01	0.60	0.30
	H	31.83	36.39	43.49	50.41						
C16:1n-7	L	1.01	0.91	0.78	0.69	0.02	0.03	<0.01	<0.01	<0.01	<0.01
	H	0.88	0.77	0.75	0.74						
C18:0	L	4.22	4.80	5.31	6.00	0.10	0.03	<0.01	<0.01	0.02	0.05
	H	3.79	4.22	4.95	5.71						
C18:1n-9	L	58.41	59.64	60.27	64.38	1.14	<0.01	<0.01	<0.01	<0.01	0.10
	H	47.39	47.20	50.90	55.55						
C18:2n-6	L	73.59	96.82	115.98	140.73	2.29	0.10	<0.01	0.05	<0.01	0.32
	H	72.59	88.72	110.89	131.24						
C18:3n-3	L	14.68	17.16	18.88	21.53	0.44	<0.01	<0.01	<0.01	<0.01	0.01
	H	19.05	21.42	25.13	28.82						

¹Forage- to-concentrate ratio.²Analyzed by Cumberland Valley Analytical Services Laboratory (Maugansville, MD).³Estimated: ME = TDN × 0.04409 × 0.82.

for limit-fed dairy (Hill et al., 2007; Zanton and Heinrichs, 2009a; Lascano and Heinrichs, 2011) and beef heifers (Reynolds et al., 1991). Greater digestibility of LF rations can be attributed to an increased retention time in the rumen of rations with lower F:C (Leaver et al., 1969; Colucci et al., 1990) and to the greater digestibility of the ingredients in these rations (NRC, 2001). Starch AD did not differ with F:C or DDGS level. The AD of ADF and NDF was not different for F:C, which conflicts with other studies where AD was greater for lower F:C. Zanton and Heinrichs (2009a) observed greater NDF but similar ADF digestibility for lower F:C and concluded that hemicellulose was better

digested in rations with lower F:C. Lascano and Heinrichs (2011) observed similar hemicellulose digestibility but greater cellulose digestibility at lower F:C; NDF and ADF digestibility decreased for higher F:C. Forage sources were different between the 2 studies, which may account for differences in fiber fraction AD. In the present study, F:C difference between treatments was 25 percentage units, less than in Lascano and Heinrichs (2011) and Zanton and Heinrichs (2009a), where the F:C difference was 60 and 50 percentage units, respectively. Narrower treatment differences in the present study may explain the lack of statistical differences in fiber digestibility.

Table 3. Apparent nutrient digestibility of low- (L) or high-forage (H) dairy heifer diets containing 0, 7, 14, or 21% dry distillers grains with solubles (DDGS)

Digestibility, %							Contrast, <i>P</i> -value							
							DDGS, % in diet							
							F:C ¹					DDGS		Interaction
0	7	14	21	SE	F:C	Linear		Quadratic	Linear	Quadratic				
DM	L	66.92	67.16	68.16	64.74	0.96	0.02	0.13	0.01	0.32	0.31			
	H	62.96	63.61	63.61	62.57									
OM	L	70.17	69.41	69.86	66.68	0.78	<0.01	0.01	0.02	0.03	0.85			
	H	64.81	65.62	65.76	64.50									
Starch	L	98.58	98.55	98.58	98.42	0.24	0.37	0.27	0.93	0.62	0.74			
	H	98.98	98.61	98.82	98.52									
NDF	L	48.34	52.98	54.62	47.81	1.63	0.32	0.57	<0.01	0.59	0.04			
	H	47.34	49.39	50.71	48.22									
ADF	L	41.20	48.39	50.84	43.86	2.16	0.15	0.06	<0.01	0.76	0.02			
	H	39.90	42.05	43.52	41.97									

¹Forage-to-concentrate ratio.

We detected a quadratic AD response to increasing levels of DDGS for DM, OM, ADF, and NDF, with 14% DDGS having the greatest AD in both F:C treatments. Depenbusch et al. (2009a) fed increasing levels of DDGS (0, 15, 30, 45, 60, and 75%, DM basis) in the diets to beef heifers and observed a quadratic effect for DMI, ADG, and final BW, maximized at 15% DDGS. The quadratic effect in the present study and in Depenbusch et al. (2009a) could be in response to FA intake increasing with DDGS. When fat levels in the diet are low, rumen fermentation is not affected because rumen microbes are able to saturate FA; at higher levels, this capacity can be exceeded and FA can accumulate in the rumen and interfere with fermentation (NRC, 2001). When high levels of fat interfere with rumen fermentation, digestibility of nonlipid energy sources is reduced (Jenkins, 1993). Wu et al. (1991) observed a quadratic response in FA AD as fat was increased from 0 to 6% DM in mid-lactation dairy cow diets. This phenomenon may partly explain the quadratic effects on DM and OM AD, increasing along with the addition of DDGS up to 14% inclusion but decreasing at 21% DDGS, likely because of negative effects of unsaturated lipids on microbial growth (Jenkins, 1993; Maia et al., 2007).

In the present study, a quadratic interaction was observed for ADF and NDF AD; the effects of DDGS on AD were greater at lower F:C. Also, OM AD was lower at higher F:C but this difference diminished as the DDGS level linearly increased. These interactions could be because the adverse effects of fat are greater in LF rations (Grant and Weidner, 1992). Higher digestibility at lower levels of DDGS (before FA concentration affects fermentation) could be due to the high NDF digestibility of distillers grains. Fiber digestibility of distillers grains is high because of their low lignin content (NRC, 2001).

Nitrogen Dynamics

Nitrogen intake, AD, and dynamics are presented in Table 4. Daily intakes of N did not differ by F:C when analyzed as grams per day or megacalories per day but was greater for LF when analyzed as intake per kilogram of BW^{0.75}. Diets approached the N level recommended by Zanton and Heinrichs (2009a) for optimum N utilization in precision-fed dairy heifer rations (1.67 g/kg of BW^{0.75}). Nitrogen AD was not different for F:C, similar to previous studies (Moody et al., 2007; Lascano et al., 2009; Lascano and Heinrichs, 2011). In contrast, Hill et al. (2007) and Zanton and Heinrichs (2009a) observed higher N digestibility at lower F:C. Zanton and Heinrichs (2009a) attributed the improved N digestibility of LF diets to greater levels of N excreted in the feces of HF, which agrees with Hill et al. (2007). In the studies where nitrogen AD was similar, N excreted in feces and urine was also similar (Moody et al., 2007; Lascano et al., 2009; Lascano and Heinrichs, 2011); N retention was greater only in the study of Moody et al. (2007) because of greater N intake. In the present study, urine N excretion tended ($P = 0.08$) to be higher (6 g/d) for LF, which corresponded to a numerically higher N intake ($P = 0.13$; 7 g/d) for LF; therefore, retained N was similar. Conflicting results in nitrogen AD could be due to N intake level; Zanton and Heinrichs (2009a) observed a tendency ($P = 0.10$) for N intake to interact with F:C in N digestibility coefficients.

Level of DDGS did not affect nitrogen AD, but N retention (% of intake and % digested) decreased as DDGS level increased in the diets, even when N excreted in feces linearly decreased as DDGS levels increased. Reduction in N retention was mainly attributed to the linear increase in and higher rate of N urine excretion. As mentioned earlier, this is likely a result of the NPN

Table 4. Nitrogen intake, apparent digestibility, and dynamics of dairy heifers fed low- (L) or high-forage (H) diets containing 0, 7, 14, or 21% dry distillers grains with solubles (DDGS)

Item							Contrast, <i>P</i> -value							
							DDGS, % in diet				DDGS		Interaction	
							F:C ¹	0	7	14	21	SE	F:C	Linear
Intake g/d	L	159.18	148.23	149.32	147.57	2.98	0.13	<0.01	<0.01	0.90	0.02			
	H	150.21	143.84	143.16	138.94									
g/kg of BW ^{0.75}	L	1.91	1.79	1.79	1.78	0.01	<0.01	<0.01	<0.01	0.05	<0.01			
	H	1.78	1.73	1.72	1.67									
Digestibility, %	L	64.66	63.26	67.47	64.46	1.27	0.22	0.13	0.91	0.50	0.32			
	H	63.19	62.21	64.42	65.47									
Fecal N, g/d	L	56.25	54.49	48.66	52.55	2.12	0.60	<0.01	0.57	0.51	0.20			
	H	55.38	54.51	50.96	47.90									
Urinary N, g/d	L	61.10	63.67	64.11	67.28	3.08	0.08	0.02	0.46	0.79	0.56			
	H	54.80	57.09	55.82	63.07									
Retained N g/d	L	41.83	30.07	36.56	27.74	4.36	0.98	0.01	0.81	0.86	0.70			
	H	40.03	32.24	36.38	27.97									
% of intake	L	26.23	20.35	24.41	18.88	2.81	0.68	0.03	0.89	0.94	0.81			
	H	26.67	22.60	25.43	20.10									
% of digested	L	40.08	31.50	36.18	29.25	3.91	0.54	<0.01	0.92	0.87	0.64			
	H	42.05	35.70	39.39	30.49									

¹Forage-to-concentrate ratio.

added to rations along with DDGS and a lack of synchrony of N and energy available for microorganisms in the rumen. It appears that substantial proportions of the NPN were rapidly hydrolyzed to NH₃N in the rumen, where it can be absorbed, metabolized to urea in the liver, and lost in the urine (Bach et al., 2005). The fact that AD of DM and NDF were depressed as the inclusion rate of DDGS increased, together with the linear increase in unsaturated FA and its toxicity to microbial populations, could explain the effects on N retention. It remains unclear whether these observations are caused by changes in rumen microbial populations, dietary NPN, or by lack of synchrony in carbohydrate and N availability. In spite of the decrease in N retention as DDGS increased, retention at 14% DDGS may be enough to support growth. At this level, N retention was similar to that observed by Zanton and Heinrichs (2009a), at similar N intake and in heifers with similar age and BW.

Nutrient Excretion

Excretion parameters are presented in Table 5. Wet, dry, and water fecal outputs were higher for HF. This is comparable to previous reports where precision-fed heifers were offered diets with different F:C (Hill et al., 2007; Moody et al., 2007; Zanton and Heinrichs, 2009a; Lascano and Heinrichs, 2011). The increase of wet fecal output is proportional to the increase in F:C and consistent with the decrease in AD of DM and OM (Lascano and Heinrichs, 2011). In addition, as-

fed intake and DMI (as g/kg of BW^{0.75}) were higher for HF, which may have contributed to greater fecal output. Higher fecal water excretion of HF could be in response to greater NDF intake and lower AD of these rations; Zanton and Heinrichs (2009a) determined that fecal water output was highly correlated with fecal NDF excretion. Urine output was statistically similar ($P = 0.23$) for HF and LF despite large numerical differences; LF output was 76% greater than HF output. The lack of difference in urine output is congruent with the observations of Moody et al. (2007), Zanton and Heinrichs (2009a), and Lascano et al. (2009); however, numerical increases for LF were observed: 2 kg/d in 12-mo-old heifers by Moody et al. (2007) and 3.4 kg/d by Zanton and Heinrichs (2009a). Other experiments reported significantly greater urine output at lower F:C (Hill et al., 2007; Lascano and Heinrichs, 2011). Greater urine excretion should be related to higher voluntary water intake because LF rations have a greater DM percentage. Lascano and Heinrichs (2011) reported a 5.9 L/d numerical increase in voluntary water intake for LF, similar to the numerically ($P = 0.57$) greater 3 kg/d observed in the present study. Manure output was similar for HF and LF, which is comparable to the report by Lascano and Heinrichs (2011), who determined that this was due to the inverse relationship between fecal water and urine output. Hill et al. (2007) observed greater manure excretion for LF, whereas Lascano et al. (2009) observed greater manure excretion for HF rations. Differences among the later trials are likely because urine output for LF in Hill et al. (2007) was 3

Table 5. Excretion parameters of dairy heifers fed low- (L) or high-forage (H) diets containing 0, 7, 14, or 21% dry distillers grains with solubles (DDGS)

							Contrast, <i>P</i> -value					
		DDGS, % in diet					DDGS				Interaction	
Item	F:C ¹	0	7	14	21	SE	F:C	Linear	Quadratic	Linear	Quadratic	
Wet feces, kg/d	L	11.66	11.07	11.01	11.82	0.29	<0.01	0.02	0.01	<0.01	0.30	
	H	15.03	14.49	13.67	13.76							
Dry feces, kg/d	L	2.15	2.09	1.98	2.16	0.06	<0.01	0.02	0.01	0.06	0.42	
	H	2.49	2.36	2.30	2.29							
Fecal water, kg/d	L	9.51	8.98	9.03	9.66	0.27	<0.01	0.02	0.02	<0.01	0.30	
	H	12.54	12.14	11.37	11.46							
Urine, kg/d	L	15.44	10.04	13.35	9.58	3.73	0.23	0.51	0.97	0.38	0.72	
	H	5.97	7.82	6.59	7.10							
Manure, kg/d	L	27.1	21.11	24.36	21.4	3.74	0.57	0.37	0.78	0.54	0.65	
	H	21.01	22.31	20.26	20.85							

¹Forage-to-concentrate ratio.

times that of HF, whereas urine output was similar in Lascano et al. (2009).

Wet, dry, and water fecal outputs responded quadratically to DDGS level, with lower excretions at the 14% level. This was in response to AD of DM, OM, and fiber and was an inverse relationship, with higher AD at 14% DDGS. Leupp et al. (2009) observed that fecal output decreased linearly as DDGS level increased, and they attributed it to a similar trend in OM intake. Linear interactions for wet, dry, and water fecal outputs, where differences due to DDGS level were greater in HF rations, could be due to the higher fiber content of these diets. Urine and manure output were not affected by F:C.

Rumen Fermentation and Microbial Population

Rumen fermentation parameters are presented in Table 6 and estimated bacterial CP flow and protozoa population are given in Table 7. Rumen pH was similar for F:C and DDGS and was reported in a companion study (Suarez-Mena et al., 2013). Rumen NH₃N concentration tended ($P = 0.07$) to be higher for HF, likely because of lower microbial activity and growth. Estimated bacterial CP flow was numerically greater for LF (339 vs. 256 g/d; $P = 0.39$). Ammonia is used by bacteria for protein synthesis (NRC, 2001), and a decrease in their population would reduce NH₃N uptake and increase its concentration in the rumen. When protein degradation exceeds microbial capacity to assimilate amino acids and NH₃, ammonia accumulates in the rumen (NRC, 2001). The assimilation of N by bacteria depends on the supply of fermentable carbohydrates (Nocek and Russell, 1988; Bach et al., 2005), and the synchrony at which N and carbohydrates become available determines microbial growth (Bach et al., 2005). In the present study, HF diets had more RDP and

soluble CP and less NFC than LF diets (Table 1); thus, synchrony of nutrient availability of HF may have negatively affected microbial synthesis. The differences in VFA molar proportions in the present study, although marginal, suggest a shift in bacterial species population. The greater proportion of acetate for HF is consistent with previous reports in limit-fed heifers (Lascano and Heinrichs, 2009) and steers (Sudweeks, 1977) and corresponds to a greater population of cellulolytic bacteria. Acetate results from the fermentation of structural carbohydrates by cellulolytic bacteria, whereas propionate results from the fermentation of NFC by amylolytic bacteria (Enjalbert et al., 1999). The decrease in acetate proportion in LF diets is compensated for by an increase in propionate when the total concentration of VFA remain similar; so the acetate-to-propionate ratio (A:P) decreases at lower F:C (Lascano and Heinrichs, 2009). In the present study, propionate proportion and A:P were similar for F:C, but the increase in acetate proportion in HF was compensated for by a decrease in the isoacids. Higher isoacids in LF suggest a greater population of amylolytic bacteria. Isoacids are a product of branched-chain AA deamination (Lascano and Heinrichs, 2009). Protein deamination in the rumen and the consequent release of isoacids decreases when RDP availability is low (Mansfield et al., 1994; Yang et al., 2004).

Rumen NH₃N concentration had a linear tendency ($P = 0.08$) to increase as DDGS levels increased; in spite of the marginal but statistically significant linear N intake (g/kg BW^{0.75}) decrease with increasing levels of DDGS. This could be a response of dietary NPN supply added along with DDGS levels to balance N intake. The NPN fed was a controlled-release urea product (Optigen; Alltech Inc., Lexington, KY). Urea rapidly hydrolyzes to NH₃N in the rumen resulting in a peak concentration of ruminal NH₃N within 1 h after feeding

Table 6. Rumen fermentation parameters and weight of dairy heifers fed low- (L) or high-forage (H) diets containing 0, 7, 14, or 21% dry distillers grains with solubles (DDGS)

							Contrast, <i>P</i> -value				
DDGS, % in diet											
Item	F:C ¹	0	7	14	21	SE	F:C	DDGS		Interaction	
								Linear	Quadratic	Linear	Quadratic
NH ₃ , mg/dL	L	6.25	5.72	6.54	7.41	0.88	0.07	0.08	0.49	0.79	0.69
	H	6.67	7.97	7.20	8.87						
VFA, mM	L	103.61	101.87	98.29	93.49	4.78	0.80	0.02	0.29	0.40	0.69
	H	101.25	104.77	100.16	97.08						
VFA, molar % of total VFA											
Acetate	L	64.94	64.45	64.02	62.59	0.68	0.07	<0.01	0.14	0.18	0.99
	H	65.94	66.73	65.33	65.17						
Propionate	L	18.36	19.01	19.28	21.15	0.36	0.29	<0.01	0.01	0.02	0.45
	H	19.20	18.37	18.87	20.00						
Butyrate	L	12.38	12.70	12.51	11.80	0.53	0.29	0.96	0.13	0.14	0.86
	H	11.34	11.36	12.47	11.66						
Valerate	L	1.73	1.56	2.09	1.79	0.22	0.10	0.69	0.71	0.26	0.81
	H	1.36	1.30	1.33	1.24						
Isobutyrate	L	1.09	1.08	0.99	1.16	0.05	0.01	0.45	0.29	0.16	0.16
	H	0.97	0.93	0.91	0.86						
Isovalerate	L	1.50	1.20	1.11	1.51	0.12	0.16	0.37	0.08	0.45	0.02
	H	1.19	1.30	1.09	1.07						
Branched-chain	L	4.32	3.84	4.19	4.46	0.26	0.02	0.71	0.35	0.13	0.13
	H	3.52	3.54	3.33	3.16						
Acetate:propionate	L	3.54	3.40	3.33	2.96	0.08	0.12	<0.01	0.01	0.03	0.42
	H	3.44	3.64	3.47	3.27						
Rumen contents ²											
Weight, kg	L	37.36	36.48	37.86	38.78	2.40	0.03	0.70	0.45	0.04	0.66
	H	47.81	47.21	45.69	45.56						
Volume, L	L	42.60	40.21	40.96	42.33	2.75	0.03	0.59	0.66	0.60	0.13
	H	51.75	51.94	52.08	50.19						

¹Forage-to-concentrate ratio.²Determined by whole rumen contents evacuation.

(Taylor-Edwards et al., 2009). Optigen has a vegetable oil coating (Inostroza et al., 2010) that protects it from ruminal degradation and controls N release (Garcia-Gonzales et al., 2007). However, the only time point at

which NH₃N concentration was different for DDGS level in this study was 2 h after feeding (Figure 1). Canola meal, the ingredient that was replaced by DDGS, has more RDP than DDGS (NRC, 2001).

Table 7. Urinary purine derivatives (PD) excretion, estimated bacterial CP flow, and rumen protozoa population of dairy heifers fed low- (L) or high-forage (H) diets containing 0, 7, 14, or 21% dry distillers grains with solubles (DDGS)

							Contrast, <i>P</i> -value				
							DDGS		Interaction		
DDGS, % in diet											
Item	F:C ¹	0	7	14	21	SE	F:C	Linear	Quadratic	Linear	Quadratic
Allantoin, mmol	L	96.54	77.59	88.78	83.76	12.28	0.42	0.89	0.73	0.34	0.36
	H	67.56	80.39	70.93	77.44						
Uric acid, mmol	L	9.96	8.75	8.58	7.92	1.76	0.21	0.65	0.99	0.21	0.72
	H	5.24	6.19	5.96	6.32						
PD, mmol	L	106.50	86.34	97.36	91.68	13.84	0.39	0.85	0.75	0.30	0.38
	H	72.80	86.58	76.89	83.76						
Microbial CP, ² g/d	L	398.18	291.31	349.26	319.13	75.33	0.39	0.87	0.77	0.29	0.38
	H	215.44	291.92	239.71	277.04						
Allantoin, % of PD	L	90.85	90.04	91.22	91.61	0.93	0.09	0.82	0.52	0.30	0.73
	H	92.86	92.89	92.01	92.39						
Protozoa, ³ ×10 ⁴ /mL	L	27.32	28.80	24.29	11.41	4.46	0.68	<0.01	<0.01	0.25	0.96
	H	21.51	25.88	22.84	12.48						

¹Forage-to-concentrate ratio.²Estimated according to the methods and equations of Chen and Gomes (1992).³Actual protozoa counts were log₁₀-transformed for the statistical analysis.

Figure 1. Mean rumen ammonia concentration after feeding in heifers fed rations containing 0, 7, 14, or 21% corn dry distillers grains with solubles (DDGS). $P < 0.05$ for DDGS $\times$ time interaction. Error bars indicate standard error. Color version available online.

It is also possible that NH_3N release was similar across DDGS levels but because ME intake decreased linearly with the addition of DDGS, microbial use of NH_3N was negatively affected and it accumulated in the rumen. The effect of lower ME intake as DDGS increased could have been exacerbated by a greater proportion of energy coming from FA as DDGS increased, providing less carbohydrates for bacteria. In addition, the effect of lipids on bacterial growth is generally negative (Doreau and Ferlay, 1995). In particular, PUFA are known to be toxic to bacteria by negatively affecting the integrity of their membranes and thus the growth of numerous species (Maia et al., 2007, 2010). Total VFA concentration decreased linearly with increasing DDGS levels, which suggests that the bacteria population declined with the addition of DDGS. However, this is not supported by the estimated bacterial CP flow (Table 7), which remained similar for different DDGS levels.

Molar proportions of acetate decreased and propionate increased linearly as DDGS levels increased; consequently, A:P decreased with increasing levels of DDGS. Butyrate, valerate, and isobutyrate were unaffected by DDGS level, whereas isovalerate had a quadratic interaction with forage level ($P = 0.08$). The observations for total VFA; acetate, propionate, and butyrate molar proportions; and A:P are comparable to those reported by Leupp et al. (2009), who fed steers ad libitum diets containing 30:70 F:C and increasing levels of DDGS (0, 15, 30, 45, and 60% of DM). A lower A:P ratio can oc-

cur in response to a reduction in digestibility of nonlipid energy sources at greater fat intakes (Jenkins, 1993), possibly related to lower protozoa population and the reduction on fiber digestion (Demeyer, 1981; Orpin, 1984). Mendoza et al. (1993) reported a decreased A:P ratio in response to defaunation in sheep. Linear interactions of propionate and A:P that were affected to a greater degree in LF could be due to the numerically greater bacterial population in LF and different species in HF compared with LF. These fermentation changes suggest that bacterial species populations changed with levels of DDGS. Previous studies have reported changes in bacterial population in response to DDGS. Callaway et al. (2010) utilized a quantitative molecular method (bacterial tag-encoded FLX amplicon pyrosequencing) and observed that ruminal *Succinivibrio* genera proportions decreased with the addition of DDGS. Fron et al. (1996), utilizing in vitro and in vivo techniques, suggested that distillers by-products increase the relative numbers of lactic acid-utilizing bacteria. Because of the confounding effects of less ME and more FA with increasing levels of DDGS, it remains unclear if one of them, or the added effect of both, or another nutrient coming from DDGS is responsible for the changes in ruminal fermentation.

The rumen protozoa population responded quadratically to DDGS level; the greatest population was observed at the 7% DDGS level. A large reduction in protozoa counts occurred between the 14 and 21% DDGS

levels and was consistent in both F:C treatments, with the latter having around half the protozoa count of the other 3 DDGS levels. The increase of total FA, in particular 18:2n-6, with increasing DDGS proportions likely explains the decline in protozoa population, because PUFA negatively affect rumen protozoa populations, although the effect is greater for 18:3n-3 oils relative to 18:2n-6 rich oils (Jenkins, 1993; Doreau and Ferlay, 1995). Similar to our observations, Ivan et al. (2001) reported a reduction in the concentrations of ciliate protozoa in ruminal fluid of sheep as dietary PUFA, fed as sunflower oil, increased. The large decrease in protozoa numbers from the 14 to 21% DDGS level may be responsible in part for the lower fiber digestibility and N retention at the 21% level. The reduction in protozoa numbers may have reduced fiber breakdown in the rumen, as ruminating time and feces particle size increased with increasing levels of DDGS (Suarez-Mena et al., 2013).

CONCLUSIONS

Replacing canola meal with DDGS in precision-fed dairy replacement diets had profound effects on ruminal fermentation, as evidenced by the increase in ammonia and reduction in acetate-to-propionate ratios. In addition, DDGS affected nutrient digestibility quadratically; this, coupled with the changes in fermentation, N dynamics, and protozoa counts, suggests changes in microbial populations. Results followed the same pattern in both F:C treatments, with minimum interaction effects observed. However, LF diets consistently resulted in higher nutrient utilization. The present results may reflect a combination of effects of changes in dietary ME and increasing PUFA as DDGS increased. We conclude that DDGS can be successfully incorporated in rations for precision-fed dairy heifers up to 14% inclusion before a decrease in apparent digestibility occurs; however, nitrogen retention may be decreased as dietary inclusion of DDGS increases.

ACKNOWLEDGMENTS

The authors thank Maria Long and Yun Ying (Penn State University, University Park) for technical assistance during the experiment and Coleen Jones (Penn State University) for helpful editorial assistance. Likewise, we thank Kevin Harvatine (Penn State University) for kindly providing the gas chromatograph for fatty acid analyses.

REFERENCES

AOAC. 1990. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Arlington, VA.

- Bach, A., S. Calsamiglia, and M. D. Stern. 2005. Nitrogen metabolism in the rumen. *J. Dairy Sci.* 88:E9–E21.
- Callaway, T. R., S. E. Dowd, T. S. Edrington, R. C. Anderson, N. Krueger, N. Bauer, P. J. Kononoff, and D. J. Nisbet. 2010. Evaluation of bacterial diversity in the rumen and feces of cattle fed different levels of dried distillers grains plus solubles using bacterial tag-encoded FLX amplicon pyrosequencing. *J. Anim. Sci.* 88:3977–3983.
- Chaney, A. L., and E. P. Marbach. 1962. Modified reagents for determination of urea and ammonia. *Clin. Chem.* 8:130–132.
- Chen, X. B., and M. J. Gomes. 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives—An overview of the technical details. International Feed Resources Unit, Rowett Research Institute, Occasional Publication, Aberdeen, UK.
- Christen, K. A., D. J. Schingoethe, K. F. Kalscheur, A. R. Hippen, K. K. Karges, and M. L. Gibson. 2010. Response of lactation dairy cows to high protein distillers grains or 3 other protein supplements. *J. Dairy Sci.* 93:2095–2104.
- Colucci, P. E., G. K. MacLeod, W. L. Grovum, I. McMillan, and D. J. Barney. 1990. Digesta kinetics in sheep and cattle fed diets with different forage to concentrate ratios at high and low intakes. *J. Dairy Sci.* 73:2143–2156.
- Demeyer, D. I. 1981. Rumen microbes and digestion of plant cell walls. *Agric. Environ.* 6:295–337.
- Depenbusch, B. E., C. M. Coleman, J. J. Higgins, and J. S. Drouillard. 2009a. Effects of increasing levels of dried corn distiller's grains with solubles on growth performance, carcass characteristics, and meat quality of yearling heifers. *J. Anim. Sci.* 87:2653–2663.
- Depenbusch, B. E., E. R. Loe, M. J. Quinn, M. E. Corrigan, M. L. Gibson, K. K. Karges, and J. S. Drouillard. 2008. Corn distillers grains with solubles derived from a traditional or partial fractionation process: Growth performance and carcass characteristics of finishing feedlot heifers. *J. Anim. Sci.* 86:2338–2343.
- Depenbusch, B. E., E. R. Loe, J. J. Sindt, N. A. Cole, J. J. Higgins, and J. S. Drouillard. 2009b. Optimizing use of distillers grains in finishing diets containing steamflaked corn. *J. Anim. Sci.* 87:2644–2652.
- Doreau, M., and A. Ferlay. 1995. Effect of dietary lipids on nitrogen metabolism in the rumen: A review. *Livest. Prod. Sci.* 43:97–110.
- Enjalbert, F., J. E. Garrett, R. Moncoulon, C. Bayourthe, and P. Chocoteau. 1999. Effects of yeast culture (*Saccharomyces cerevisiae*) on ruminal digestion in non-lactating dairy cows. *Anim. Feed Sci. Technol.* 76:195–206.
- Firkins, J. L., L. L. Berger, and G. C. Fahey Jr.. 1985. Evaluation of wet and dry distillers grains and wet and dry corn gluten feeds for ruminants. *J. Anim. Sci.* 60:847–860.
- Fron, M., H. Madeira, C. Richards, and M. Morrison. 1996. The impact of feeding condensed distillers byproducts on rumen microbiology and metabolism. *Anim. Feed Sci. Technol.* 61:235–245.
- Garcia-Gonzales, R., J. M. Tricárico, G. A. Harrison, M. D. Meyer, K. R. McLeod, D. L. Harmon, and K. A. Dawson. 2007. Optigen is a sustained release source of non-protein nitrogen in the rumen. *J. Dairy Sci.* 90(Suppl.1):98. (Abstr.)
- Grant, R. J., and S. J. Weidner. 1992. Effect of fat from whole soybeans on performance of dairy cows fed rations differing in fiber level and particle size. *J. Dairy Sci.* 75:2742–2751.
- Ham, G. A., R. A. Stock, T. J. Klopfenstein, E. M. Larson, D. H. Shain, and R. P. Huffman. 1994. Wet corn distillers byproducts compared with dried corn distillers grains with solubles as a source of protein and energy for ruminants. *J. Anim. Sci.* 72:3246–3257.
- Hill, S. R., K. F. Knowlton, R. E. James, R. E. Pearson, G. L. Bethard, and K. J. Pence. 2007. Nitrogen and phosphorus retention and excretion in late-gestation dairy heifers. *J. Dairy Sci.* 90:5634–5642.
- Hoffman, P. C., C. R. Simson, and M. Wattiaux. 2007. Limit feeding of gravid Holstein heifers: Effect on growth, manure nutrient excretion, and subsequent early lactation performance. *J. Dairy Sci.* 90:946–954.
- Hristov, A. N., T. A. McAllister, F. H. Van Herk, K. J. Cheng, C. J. Newbold, and P. R. Cheeke. 1999. Effect of *Yucca schidigera* on

- ruminal fermentation and nutrient digestion in heifers. *J. Anim. Sci.* 77:2554–2563.
- Inostroza, J. F., R. D. Shaver, V. E. Cabrera, and J. M. Tricarico. 2010. Effect of diets containing a controlled-release urea product on milk yield, milk composition, and milk component yields in commercial Wisconsin dairy herds and economic implications. *Prof. Anim. Sci.* 26:175–180.
- Ivan, M., P. S. Mir, K. M. Koenig, L. M. Rode, L. Neill, T. Entz, and Z. Mir. 2001. Effects of dietary sunflower seed oil on rumen protozoa population and tissue concentration of conjugated linoleic acid in sheep. *Small Rumin. Res.* 41:215–227.
- Jenkins, T. C. 1993. Lipid metabolism in the rumen. *J. Dairy Sci.* 76:3851–3863.
- Karkalas, J. 1985. An improved enzymic method for the determination of native and modified starch. *J. Sci. Food Agric.* 36:1019–1027.
- Klopfenstein, T. J., G. E. Erickson, and V. R. Bremer. 2008. Board-invited review: Use of distillers by-products in the beef cattle feeding industry. *J. Anim. Sci.* 86:1223–1231.
- Krishnamoorthy, U., C. J. Sniffen, M. D. Stern, and P. J. Van Soest. 1983. Evaluation of a mathematical model of rumen digestion and an in vivo simulation of rumen proteolysis to estimate rumen undegradable nitrogen content of feedstuffs. *Br. J. Nutr.* 50:555–568.
- Lascano, G. J., and A. J. Heinrichs. 2009. Rumen fermentation pattern of dairy heifers fed restricted amounts of low, medium, and high concentrate diets without and with yeast culture. *Livest. Sci.* 124:48–57.
- Lascano, G. J., and A. J. Heinrichs. 2011. Effects of feeding different levels of dietary fiber through the addition of corn stover on nutrient utilization of dairy heifers precision-fed high and low concentrate diets. *J. Dairy Sci.* 94:3025–3036.
- Lascano, G. J., A. J. Heinrichs, and J. M. Tricarico. 2012. Substitution of starch by soluble fiber and *Saccharomyces cerevisiae* dose response on nutrient digestion and blood metabolites for precision-fed dairy heifers. *J. Dairy Sci.* 95:3298–3309.
- Lascano, G. J., G. I. Zanton, A. J. Heinrichs, and W. P. Weiss. 2010. Technical note: A noninvasive urine collection device for female cattle: Modification of the urine cup collection method. *J. Dairy Sci.* 93:2691–2694.
- Lascano, G. J., G. I. Zanton, F. X. Suarez-Mena, and A. J. Heinrichs. 2009. Effect of limit feeding high- and low-concentrate diets with *Saccharomyces cerevisiae* on digestibility and on dairy heifer growth and first-lactation performance. *J. Dairy Sci.* 92:5100–5110.
- Leaver, J. D., R. C. Campling, and W. Holmes. 1969. The effect of level of feeding on the digestibility of diets for sheep and cattle. *Anim. Prod.* 11:11–18.
- Leupp, J. L., G. P. Lardy, K. K. Karges, M. L. Gibson, and J. S. Caton. 2009. Effects of increasing level of corn distillers dried grains with solubles on intake, digestion, and ruminal fermentation in steers fed seventy percent concentrate diets. *J. Anim. Sci.* 87:2906–2912.
- Maia, M. R., L. C. Chaudhary, C. S. Bestwick, A. J. Richardson, N. McKain, T. R. Larson, I. A. Graham, and R. J. Wallace. 2010. Toxicity of unsaturated fatty acids to the biohydrogenating ruminal bacterium, *Butyrivibrio fibrisolvens*. *BMC Microbiol.* 10:52.
- Maia, M. R., L. C. Chaudhary, L. Figueres, and R. J. Wallace. 2007. Metabolism of polyunsaturated fatty acids and their toxicity to the microflora of the rumen. *Antonie van Leeuwenhoek* 91:303–314.
- Mansfield, H. R., M. D. Stern, and D. E. Otterby. 1994. Effects of beet pulp and animal by-products on milk yield and in vitro fermentation by rumen microorganism. *J. Dairy Sci.* 77:205–216.
- McLeod, K. R., and R. L. Baldwin. 2000. Effects of diet forage:concentrate ratio and metabolizable energy intake on visceral organ growth and in vitro oxidative capacity of gut tissues in sheep. *J. Anim. Sci.* 78:760–770.
- Mendoza, G. D., R. A. Britton, and R. A. Stock. 1993. Influence of ruminal protozoa on site and extent of starch digestion and ruminal fermentation. *J. Anim. Sci.* 71:1572–1578.
- Moody, M. L., G. I. Zanton, J. M. Daubert, and A. J. Heinrichs. 2007. Nutrient utilization of differing forage-to-concentrate ratios by growing Holstein heifers. *J. Dairy Sci.* 90:5580–5586.
- Nocek, J. E., and J. B. Russell. 1988. Protein and energy as an integrated system. Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. *J. Dairy Sci.* 71:2070–2107.
- NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th ed. Natl. Acad. Press, Washington, DC.
- Ogimoto, K., and S. Imai. 1981. Atlas of Rumen Microbiology. Japan Scientific Societies Press, Tokyo, Japan.
- Orpin, C. G. 1984. The role of ciliate protozoa and fungi in the rumen digestion of plant cell walls. *Anim. Feed Sci. Technol.* 10:121–143.
- Reynolds, C. K., H. F. Tyrrell, and P. J. Reynolds. 1991. Effects of diet forage-to-concentrate ratio and intake on energy metabolism in growing beef heifers: Whole body energy and nitrogen balance and visceral heat production. *J. Nutr.* 121:994–1003.
- Rico, D. E., Y. Ying, and K. J. Harvatine. 2014. Effect of a high palmitic acid fat supplement on milk production and apparent total-tract digestibility in high- and low-milk yield dairy cows. *J. Dairy Sci.* 97:3739–3751.
- Schingoethe, D. J., K. F. Kalscheur, A. R. Hippen, and A. D. Garcia. 2009. Invited review: The use of distillers products in dairy cattle diets. *J. Dairy Sci.* 92:5802–5813.
- Shipley, R. A., and R. E. Clark. 1972. Tracer Methods for In Vivo Kinetics. Academic Press, New York, NY.
- Suarez-Mena, F. X., G. J. Lascano, and A. J. Heinrichs. 2013. Chewing activities and particle size of rumen digesta and feces of precision-fed dairy heifers fed different forage levels with increasing levels of distillers grains. *J. Dairy Sci.* 96:5184–5193.
- Sudweeks, E. M. 1977. Chewing time, rumen fermentation and their relationship in steers as affected by diet composition. *J. Anim. Sci.* 44:694–701.
- Sukhija, P. S., and D. L. Palmquist. 1988. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. *J. Agric. Food Chem.* 36:1202–1206.
- Taylor-Edwards, C. C., G. Hibbard, S. E. Kitts, K. R. McLeod, D. E. Axe, E. S. Vanzant, N. B. Kristensen, and D. L. Harmon. 2009. Effects of slow-release urea on ruminal digesta characteristics and growth performance in beef steers. *J. Anim. Sci.* 87:200–208.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597.
- Wu, Z., O. A. Ohajuruka, and D. L. Palmquist. 1991. Ruminal synthesis, biohydrogenation, and digestibility of fatty acids by dairy cows. *J. Dairy Sci.* 74:3025–3034.
- Yang, C.-M. J., and G. A. Varga. 1989. Effect of three concentrate feeding frequencies on rumen protozoa, rumen digesta kinetics, and milk yield in dairy cows. *J. Dairy Sci.* 72:950–957.
- Yang, W. Z., K. A. Beauchemin, D. D. Vedres, G. R. Ghorbani, D. Colombatto, and D. P. Morgavi. 2004. Effects of direct-fed microbial supplementation on ruminal acidosis, digestibility, and bacterial protein synthesis in continuous culture. *Anim. Feed Sci. Technol.* 114:179–193.
- Zanton, G. I., and A. J. Heinrichs. 2007. The effects of controlled feeding of a high-forage or high-concentrate ration on heifer growth and first-lactation milk production. *J. Dairy Sci.* 90:3388–3396.
- Zanton, G. I., and A. J. Heinrichs. 2008. Rumen digestion and nutritional efficiency of dairy heifers limit-fed a high forage ration to four levels of dry matter intake. *J. Dairy Sci.* 91:3579–3588.
- Zanton, G. I., and A. J. Heinrichs. 2009a. Digestion and nitrogen utilization in dairy heifers limit-fed a low or high forage ration at four levels of nitrogen intake. *J. Dairy Sci.* 92:2078–2094.
- Zanton, G. I., and A. J. Heinrichs. 2009b. Review: Limit-feeding with altered forage-to-concentrate levels in dairy heifer diets. *Prof. Anim. Sci.* 25:393–403.