Effects of feeding alfalfa hay on chewing, rumen pH, and milk fat concentration of dairy cows fed wheat dried distillers grains with solubles as a partial substitute for barley silage

S. Z. Zhang, G. B. Penner,¹ M. Abdelqader, and M. Oba²

Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, T6G 2P5, Canada

ABSTRACT

The objective of this study was to determine the effects of feeding alfalfa hay on chewing activity, rumen fermentation, and milk fat concentration of dairy cows fed wheat-based dried distillers grains with solubles (DDGS) as a partial replacement of barley silage. Thirty lactating Holstein cows (220 ± 51 DIM), 6 of which were ruminally cannulated, were used in a 3×3 Latin square design with 21-d periods. Cows were fed a control diet [CON; 50% barley silage and 50% concentrate mix on a dry matter (DM) basis], a diet in which barley silage was replaced with DDGS at 20% of dietary DM (DG), or a diet in which barley silage was replaced with DDGS and alfalfa hay at 20 and 10% of dietary DM, respectively (DG+AH). All diets contained approximately 20% crude protein. Compared with the CON diet, cows fed DG and DG+AH diets respectively had greater DM intake (20.1 vs. 23.1 and 22.7 kg/d); yields of milk (24.5 vs. 27.3 and 28.1 kg/d), milk protein (0.88 vs. 0.99 and 1.01 kg/d), and milk lactose (1.11 vs. 1.24 and 1.29 kg/d); and body weight gain (0.25 vs. 1.17 and 1.23 kg/d). However, compared with cows fed the CON diet, cows fed the DG and DG+AH diets respectively had lower chewing time (38.3 vs. 30.7 and 31.5 min/kg of DM intake), mean rumen pH (6.11 vs. 5.88 and 5.84), and minimum rumen pH (5.28 vs. 5.09 and 5.07) and a greater duration that rumen pH was below 5.8 (7.3 vs. 11.2 and 12.0 h/d). However, these response variables did not differ between cows fed the DG and DG+AH diets. Milk fat concentration differed among the 3 diets (3.92, 3.60, and 3.38% for CON, DG, and DG+AH, respectively), but milk fat yield was not affected by treatment. These results indicate that partially replacing barley silage with DDGS can improve productivity of lactating dairy cows but may decrease chewing time, rumen pH, and

milk fat concentration, and that dietary inclusion of alfalfa hay may not alleviate such responses.

Key words: dried distillers grains with solubles, alfalfa hay, barley silage, milk fat

INTRODUCTION

In western Canada, wheat is the main feedstock used for ethanol production and wheat-based dried distillers grains with solubles (DDGS) is a commonly available byproduct feedstuff for animals. Wheat DDGS is high in NDF concentration, ranging from 25.9% (Dong et al., 1987) to 54.1% (McKinnon and Walker, 2008) with an average of 37.4% (Zhang, 2010). In addition to the high NDF concentration, the NDF from DDGS is highly digestible in the rumen (Nuez Ortín and Yu, 2009). Therefore, wheat DDGS can be considered a nonforage fiber source (NFFS) and used as a partial replacement of forage in diets for lactating dairy cows. However, the physical effectiveness of DDGS at stimulating chewing is lower than that of forages (Clark and Armentano, 1993). Penner et al. (2009) observed that dairy cows decreased chewing time and milk fat concentration when barley silage was partly replaced with corn or wheat wet distillers grains.

When dairy cows are fed high NFFS diets, dietary inclusion of alfalfa hay in place of silage often increases chewing time (Allen and Grant, 2000), milk fat concentration (Smith et al., 1993), and milk yield (Mullins et al., 2009). In our companion study (Zhang et al., 2010), cows fed a high NFFS diet in which barley silage was replaced by DDGS at 20% of dietary DM maintained rumen pH and milk fat concentration, and the experimental diets contained alfalfa hay. As such, we hypothesized that feeding alfalfa hay would prevent the reduction in milk fat concentration when cows are fed a high DDGS diet.

The objective of this study was to determine the effects of feeding alfalfa hay on chewing time, rumen fermentation, and milk fat concentration when DDGS was fed as a partial replacement of barley silage in diets for lactating dairy cows.

Received December 17, 2009.

Accepted March 22, 2010.

¹Current address: Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, SK, S7N 5A8, Canada.

²Corresponding author: moba@ualberta.ca

Table 1. Chemical composition and particle size distribution of barley silage, wheat-based dried distillers grains with solubles (DDGS), and alfalfa hay

Item	Barley silage (n = 3)		DDGS (n = 1)		Alfalfa hay (n = 3)	
	Mean	SD	Mean	SD	Mean	SD
Composition (% DM)						
DM, as fed	38.2	2.2	94.9	— ¹	87.3	2.1
OM	89.1	0.3	93.9	—	89.5	2.0
CP	14.4	0.2	45.9	—	20.3	0.8
NDF	54.3	1.6	30.1	—	47.5	2.4
Starch	8.8	0.2	2.7	—	2.1	0.1
Ether extract	3.0	0.1	3.8	—	2.1	0.1
NFC	17.2	1.6	14.2	—	19.9	0.8
Particle size distribution, as fed (%)						
>19 mm	6.1	0.9	0	—	47.2	2.7
19–8 mm	73.8	0.2	0	—	20.1	4.4
<8 mm	20.2	0.7	100	—	32.7	3.9
PEF ²	0.80	0.01	0	—	0.67	0.04

¹Not applicable.²PEF = physical effectiveness factor, determined as the proportion of particles retained on 19- and 8-mm sieves (Lammers et al., 1996).

MATERIALS AND METHODS

Animals, Diets, and Experimental Design

The current study was conducted at the Dairy Research and Technology Center of University of Alberta (Edmonton, AB, Canada). All procedures were pre-approved by the Faculty Animal Policy and Welfare Committee at the University of Alberta and conducted according to the guidelines of the Canadian Council of Animal Care (Ottawa, Ontario, Canada).

Thirty multiparous lactating Holstein cows (220 ± 51 DIM; 632 ± 65 kg of BW) were used in this study. Of the 30 cows, 6 were previously fitted with ruminal cannulas. Cows were assigned to 1 of 3 dietary treatments in a 3×3 Latin square design balanced for carryover effects. Each period consisted of an 18-d diet adaptation period and a 3-d data and sample collection period. Data from 1 cannulated cow were removed from the study because she dried off during the second period. One intact cow was removed during the third period for the same reason, but the data collected from this cow during the first and second periods were included for statistical analyses. Each period consisted of an 18-d diet adaptation period and a 3-d data collection period. The treatments were control (**CON**: 50% barley silage and 50% concentrate mix on a DM basis; Tables 1 and 2); a diet in which barley silage was replaced by DDGS at 20% of dietary DM (**DG**); and a diet in which barley silage was replaced by DDGS and alfalfa hay at 20 and 10% of dietary DM, respectively (**DG+AH**). Water was added to the DG and DG+AH diets and mixed evenly to make DM concentration similar across treatments. Diets were formulated according to NRC (2001)

to meet or exceed the nutritional requirements for a 650-kg cow producing 36 kg of milk/d with 3.5% milk fat and 3.2% milk protein. All diets were formulated to contain similar CP concentrations using variable amounts of beet pulp, corn gluten meal, and urea.

Cows were housed individually in tie stalls with sawdust bedding and allowed to exercise for 2 h daily throughout the experiment except for weekends and during sample collection periods. Cows were fed experimental diets as a TMR once daily at 0730 h and had free access to fresh water. Feed was offered at 105 to 110% of actual feed intake on the previous day. Samples of TMR, feed ingredients, and feed refusals were collected daily during sample collection periods and composited by period for TMR and feed ingredients and

Table 2. Ingredients of the experimental diets¹

Component (% of DM)	CON	DG	DG+AH
Barley silage	49.5	30.2	19.8
Alfalfa hay	—	—	10.2
DDGS	—	20.7	20.4
Concentrate mix ²	36.7	36.8	36.7
Beet pulp	4.6	12.1	12.9
Corn gluten meal	8.2	—	—
Urea	1.0	0.2	—

¹CON: control; DG: 20% wheat-based dried distillers grains with solubles (DDGS) replacing barley silage; DG+AH: 20% DDGS + 10% alfalfa hay replacing barley silage.

²Contained 45.9% rolled corn, 45.9% rolled barley, 2.2% limestone, 0.8% dicalcium phosphate, 0.6% magnesium oxide, 1.6% NaCl, and 3.0% premix (contained 0.10% Ca, 0.60% P, 11.50% Na, 0.30% Mg, 10 mg/kg of F, 80 mg/kg of I, 5,000 mg/kg of Zn, 31,000 mg/kg of Mn, 1,170 mg/kg of Cu, 6.2 mg/kg of Co, 1,265 kIU/kg of vitamin A, 142 kIU/kg of vitamin D, and 3,800 IU/kg of vitamin E).

Table 3. Nutrient composition and particle size distribution of the experimental diets¹

Item	CON (n = 3)		DG (n = 3)		DG+AH (n = 3)	
	Mean	SD	Mean	SD	Mean	SD
Composition (% DM)						
DM, as fed	49.3	1.0	49.1	1.4	48.0	1.1
OM	89.9	0.9	91.8	0.9	90.6	0.9
CP	20.3	0.8	20.8	0.5	20.3	0.6
NDF	37.0	0.4	35.6	0.9	34.9	1.1
Forage NDF	26.3	0.3	16.1	0.5	15.2	0.4
Starch	27.4	0.3	25.2	1.4	24.1	1.4
Ether extract	2.6	0.3	2.5	0.3	2.4	0.4
NFC	30.0	1.4	33.0	2.1	33.0	0.3
Particle size distribution, as fed (%)						
>19 mm	3.0	0.6	1.8	0.8	6.0	1.0
19–8 mm	36.5	2.0	22.3	2.5	16.7	1.1
<8 mm	60.5	2.4	75.8	6.0	77.3	5.1
PEF ²	0.39	0.03	0.24	0.02	0.23	0.02

¹CON: control; DG: 20% wheat-based dried distillers grains with solubles (DDGS) replacing barley silage; DG+AH: 20% DDGS + 10% alfalfa hay replacing barley silage.

²PEF = physical effectiveness factor, determined as the proportion of particles retained on 19- and 8-mm sieves (Lammers et al., 1996).

by period and cow for refusals. The DM concentration of barley silage and alfalfa hay was determined twice weekly and dietary formulation was adjusted if necessary. The dietary DM concentration was similar among treatments as water was added to the DG and DG+AH diets (Table 3). Dietary forage NDF concentration (DM basis) was 26.3, 16.1, and 15.2% for the CON, DG, and DG+AH diets, respectively.

Cows were milked twice daily at 0400 and 1600 h. Milk was sampled from both a.m. and p.m. milkings on d 19, 20, and 21 of each period. Cows were weighed after the morning milking on 2 consecutive days immediately before the start of experiment and on the last 2 d of each period. Body condition score was determined by 2 experienced individuals separately at the beginning of the experiment and at the end of each period using a 5-point scale (1 = thin to 5 = fat; Wildman et al., 1982) and was then averaged.

Chewing Activity and Sorting Behavior

Chewing activities were monitored for 24 h on d 20 of each period. Eating and ruminating activities were recorded every 5 min and each activity was assumed to continue for the entire 5-min interval between observations. Total chewing time was calculated as the sum of eating time and ruminating time. Sorting index was calculated as the ratio of actual intake to expected intake for particles retained on each sieve of Penn State Particle Separator (Leonardi and Armentano, 2003). A sorting index of 100, greater than 100, and less than 100 indicate no sorting, selective consumption, and selective refusals, respectively.

Rumen pH and Rumen Fermentation

Rumen pH was measured every 30 s for 72 h using the LRC rumen pH data logger system (Dascor, Escondido, CA) as described in detail by Penner et al. (2006). Rumen fluid was collected every 9 h over a 72-h period to account for diurnal variation (i.e., 0900 and 1800 h on d 19; 0300, 1200, and 2100 h on d 20; and 0600, 1500, and 2400 h on d 21). For each rumen fluid sampling, rumen digesta was collected from cranial, ventral, and caudal sacs, strained through a perforated material, and placed on ice immediately after collection. The filtrate was centrifuged at $3,000 \times g$ at 4°C for 20 min. Samples were composited for 1 sample per cow for each period and stored at –20°C until analysis.

Sample Analyses

Particle size distribution of feed ingredients and feed refusals was analyzed using Penn State Particle Separator (Lammers et al., 1996). The dietary particle size distribution was calculated from particle size distribution of individual feed ingredients and their dietary allocation. Physically effective factor was defined as the proportion of particles retained on 19- and 8-mm sieves (Lammers et al., 1996) according to Yang and Beauchemin (2006).

The composited samples of TMR, feed ingredients, and feed refusals were dried at 55°C for 48 h in a forced air oven (V-31 STD, Style II, Despatch Industries Inc., Nashua, Mississauga, Ontario, Canada) to determine DM concentration. Dried samples were then ground through a 1-mm screen using a Wiley mill (Thomas-

Wiley, Philadelphia, PA). The samples were analyzed for concentrations of analytical DM (AOAC, 2002; method 930.15), OM (AOAC, 2002; method 942.05), NDF (Van Soest et al., 1991; method A), and starch (Silveira et al., 2007). Concentration of CP was determined using a Leco FP-2000 N Analyzer (Leco Instruments Inc., St. Joseph, MI), and ether extract concentration was determined using a Goldfish extraction apparatus with petroleum ether (Labconco, Kansas City, MO).

Rumen fluid samples were thawed and centrifuged at 4°C at $26,000 \times g$ for 15 min. The supernatant was analyzed for VFA concentration by gas chromatography as described by Khorasani et al. (1996) and for ammonia N concentration using a spectrophotometer (UV/VIS Spectrophotometer, V-530, Jasco Corporation, Easton, MD) as described by Fawcett and Scott (1960). Milk samples were analyzed for milk fat, CP, lactose, and MUN by infrared spectroscopy (AOAC, 2002; method 972.16; MilkoScan 605, Foss North America, Brampton, Ontario, Canada) at the Alberta Central Milk Testing Laboratory (Edmonton, AB, Canada). Milk samples were composited for 1 sample per cow per period based on milk fat yield from each milking and stored at -20°C until fatty acid analysis. Lipids were extracted from the milk samples by the procedure described by Folch et al. (1957). Fatty acids were derivatized using methanolic base (Supelco, Bellefonte, PA) and quantified using a gas chromatography (Varian 3400, Varian Chromatography Systems, Walnut Creek, CA) with a flame ionization detector. Separation of the fatty acid methyl esters was performed using an SP-2560 fused silica capillary column (100 m $\times$ 0.25 mm internal diameter with 0.25 μm film thickness; Supelco). Helium was used as the carrier gas with a head pressure of 241 kPa. The initial column temperature was set at 45°C and held for 4 min, and then increased to 175°C at the rate of $13^{\circ}\text{C}/\text{min}$ and held for 27 min. It was finally increased to 215°C

at the rate of $4^{\circ}\text{C}/\text{min}$ and held for 45 min. The initial injector temperature was set at 50°C and held at 0.2 min, and then increased to 230°C at the rate of $150^{\circ}\text{C}/\text{min}$ and held for 84.2 min. The detector temperature was set at 230°C . Peak integration was performed using the Galaxie Chromatography Data System (Varian Chromatography Systems, Walnut Creek, CA). Individual fatty acids were identified with the fatty acid methyl ester standard number 463 (Nu Chek Prep, Elysian, MN). Conjugated linoleic acid isomers were identified using standards from Matreya (Matreya Inc., Pleasant Gap, PA). Concentration of each fatty acid was reported as g/100 g of total fatty acids.

Statistical Analysis

All data were analyzed using PROC MIXED of SAS (version 9.1; SAS Institute Inc., Cary, NC) according to the following model:

$$Y_{ijkl} = \mu + P_i + T_j + S_k + C(S)_{l(k)} + e_{ijkl},$$

where Y_{ijkl} is the dependent variable, μ is overall mean, P_i is fixed effect of period, T_j is fixed effect of treatment, S_k is fixed effect of square, $C(S)_{l(k)}$ is random effect of cow nested in square, and e_{ijkl} is residual. If overall treatment effect was significant ($P < 0.05$), treatment means were separated by Bonferroni t -test. Treatment effects were declared significant at $P \leq 0.05$ and tendency was declared at $0.05 < P \leq 0.10$.

RESULTS

Feed Intake and Chewing Activity

Dry matter intake was higher ($P < 0.01$) for cows fed the DG and DG+AH diets than for those fed the CON

Table 4. Effects of partially replacing barley silage with wheat-based dried distillers grains with solubles (DDGS) or DDGS plus alfalfa hay in diets for lactating dairy cows on DMI and chewing activity

Item	Diet ¹			SEM	P-value
	CON (n = 29)	DG (n = 29)	DG+AH (n = 28)		
DMI (kg/d)	20.1 ^b	23.1 ^a	22.7 ^a	0.3	<0.01
Time (min/d)					
Eating	280 ^a	234 ^b	234 ^b	9	<0.01
Ruminating	482	468	474	12.1	0.37
Total chewing ²	762 ^a	702 ^b	708 ^b	14.4	<0.01
Time (min/kg of DMI)					
Eating	14.1 ^a	10.2 ^b	10.4 ^b	0.4	<0.01
Ruminating	24.3 ^a	20.5 ^b	21.1 ^b	0.6	<0.01
Total chewing	38.3 ^a	30.7 ^b	31.5 ^b	0.8	<0.01

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

¹CON: control; DG: 20% DDGS replacing barley silage; DG+AH: 20% DDGS + 10% alfalfa hay replacing barley silage.

²The sum of eating time and ruminating time.

diet, with no difference between the DG and DG+AH diets (Table 4). Eating time (min/d) and total chewing time were shorter ($P < 0.01$) for cows fed the DG and DG+AH diets than for those fed the CON diet, but ruminating time was not affected by treatment (Table 4). Eating, ruminating, and total chewing time per unit of DMI were consistently decreased ($P < 0.01$) by feeding the DG or DG+AH diets compared with the CON diet.

Rumen Fermentation and Sorting Behavior

Daily mean rumen pH was lower ($P < 0.01$) for cows fed the DG and DG+AH diets than for those fed the CON diet, but the daily maximum pH was not different across the treatments (Table 5; Figure 1). The durations that rumen pH was below 5.8 and 5.5 were longer ($P < 0.01$) for cows fed the DG and DG+AH diets than for those fed the CON diet. The area that rumen pH was below pH 5.8 ($P < 0.01$) was also higher for cows fed the DG and DG+AH diets compared with those fed the CON diet. The molar proportions of acetate and isobutyrate were lower ($P < 0.01$) and the molar proportion of propionate was higher ($P < 0.01$) for cows fed the DG and DG+AH diets compared with those fed the CON diet. The ratio of acetate to propionate was

higher ($P < 0.01$) for cows fed the CON diet than for those fed the DG and DG+AH diets. The concentration of rumen $\text{NH}_3\text{-N}$ tended to be lower ($P = 0.10$) for cows fed the DG and DG+AH diets than for those fed the CON diet.

Cows fed the CON diet sorted against long particles ($P < 0.05$; Table 6), but cows fed the DG diet did not sort, and those fed the DG+AH diet sorted for long particles ($P < 0.05$).

Performance and Fatty Acids Profile

Milk yield was greater ($P < 0.01$) for cows fed the DG and DG+AH diets compared with those fed the CON diet (Table 7). Milk fat concentration was lower ($P < 0.01$) for cows fed the DG and DG+AH diets compared with those fed the CON diet, but milk fat yield was not affected by treatment. Milk fat concentration was lower ($P < 0.01$) for cows fed the DG+AH diet compared with those fed the DG diet. The yields of milk protein and lactose were greater ($P < 0.01$) for cows fed the DG and DG+AH diets compared with those fed the CON diet, whereas there were no differences in the concentrations of milk protein and lactose compared with those fed the CON diet. The concentration of MUN was lower ($P < 0.01$) for cows fed the DG and DG+AH treatments

Table 5. Effects of partially replacing barley silage with wheat-based dried distillers grains with solubles (DDGS) or DDGS plus alfalfa hay in diets for lactating dairy cows on rumen fermentation of ruminally cannulated cows

Item	Diet ¹			SEM	P-value
	CON (n = 5)	DG (n = 5)	DG+AH (n = 5)		
Rumen pH					
Minimum	5.28	5.09	5.07	0.05	0.06
Mean	6.11 ^a	5.88 ^b	5.84 ^b	0.05	<0.01
Maximum	6.87	6.87	6.77	0.04	0.12
Duration (h/d)					
pH <5.8	7.3 ^b	11.2 ^a	12.0 ^a	0.9	0.01
pH <5.5	3.6 ^b	6.9 ^a	7.4 ^a	0.6	<0.01
pH <5.2	1.2	2.8	2.5	0.5	0.13
Area (pH × h/d)					
pH <5.8	2.4 ^b	4.6 ^a	4.7 ^a	0.4	<0.01
pH <5.5	0.8	1.8	1.8	0.3	0.06
pH <5.2	0.1	0.4	0.3	0.1	0.25
Total VFA (mM)	114.5	123.3	132.5	5.0	0.11
VFA molar proportions (mol/100 mol)					
Acetate	62.1 ^a	59.2 ^b	58.0 ^b	0.8	<0.01
Propionate	20.9 ^b	24.1 ^{ab}	27.3 ^a	1.1	0.02
Isobutyrate	0.9 ^a	0.7 ^b	0.5 ^b	0.04	<0.01
Butyrate	11.8	12.5	10.6	0.8	0.35
Isovalerate	1.6 ^a	1.0 ^{ab}	0.7 ^b	0.2	0.01
Acetate:propionate	3.0 ^a	2.5 ^b	2.2 ^b	0.1	<0.01
Rumen $\text{NH}_3\text{-N}$ (mg/dL)	22.8	15.4	11.6	3.0	0.10

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

¹CON: control; DG: 20% DDGS replacing barley silage; DG+AH: 20% DDGS + 10% alfalfa hay replacing barley silage.

Figure 1. The diurnal fluctuations in rumen pH for cows fed different diets: control (CON), 20% wheat-based dried distillers grains with solubles (DDGS) replacing barley silage (DG), and 20% DDGS + 10% alfalfa hay replacing barley silage (DG+AH).

than for those fed the CON treatment and for those fed the DG+AH treatment compared with those fed the DG treatment.

Concentrations of short- and medium-chain fatty acids ($C < 16$) were not affected by treatment, but the concentration of long-chain fatty acids ($C > 16$) was increased ($P < 0.01$) by feeding the DG and DG+AH diets. The concentration of C16 was lower ($P < 0.01$) for cows fed the DG and DG+AH diets than for those fed the CON diet. The concentration of *trans*-10 C18:1 in milk fat was higher ($P = 0.03$) when cows were fed the DG+AH diet compared with when cows were fed the CON diet, but was not affected by feeding the DG diet (Table 8). Cows fed the DG+AH diet tended to have greater ($P = 0.06$) concentrations of unsaturated fatty acids in milk than those fed the CON diet, but there was no difference in the concentrations of unsaturated fatty acids between cows fed the DG and CON diets. The concentration of *trans*-10,*cis*-12 conjugated linoleic acid (CLA) in milk was not different among treatments.

DISCUSSION

Effects of DDGS as a Partial Replacement of Barley Silage

Nonforage fiber sources are high in NDF content but have a low physical effectiveness at stimulating chewing activity because of their small particle size (Clark and Armentano, 1993). Many studies have been conducted to investigate effects of feeding NFFS as a partial forage replacement for lactating dairy cows. Partial replacement of forage with NFFS often reduces milk fat concentration (Weidner and Grant, 1994; Boddugari et al., 2001) by decreasing chewing time and rumen pH (Boddugari et al., 2001; Harvatine et al., 2002). Penner et al. (2009) reported that chewing time and milk fat concentration were decreased by feeding wet distillers grains as a partial replacement of barley silage.

In our study, particles retained on the 19- and 8-mm sieves were reduced for diets containing DDGS in place of barley silage, and DG and DG+AH treatments decreased chewing time, rumen pH, and milk

Table 6. Effects of partially replacing barley silage with wheat-based dried distillers grains with solubles (DDGS) or DDGS plus alfalfa hay in diets for lactating dairy cows on sorting behavior

Sorting index ¹	Diet ²			SEM	P-value
	CON (n = 29)	DG (n = 29)	DG+AH (n = 28)		
>19.0 mm	90.9 ^c	100.5 ^b	104.9 ^a	1.3	<0.01
19.0 to 8.0 mm	96.6 ^b	98.5 ^a	96.0 ^b	0.5	<0.01
<8.0 mm	102.6 ^a	100.5 ^b	100.5 ^b	0.2	<0.01

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

¹A sorting index above 100 indicates sorting for particles, and a sorting index below 100 indicates sorting against particles (Leonardi and Armentano, 2003).

²CON: control; DG: 20% DDGS replacing barley silage; DG+AH: 20% DDGS + 10% alfalfa hay replacing barley silage.

Table 7. Effects of partially replacing barley silage with wheat-based dried distillers grains with solubles (DDGS) or DDGS plus alfalfa hay in diets for lactating dairy cows on milk yield and milk composition, change of BW and BCS

Item	Diet ¹			SEM	P-value
	CON (n = 29)	DG (n = 29)	DG+AH (n = 28)		
Yield (kg/d)					
Milk	24.5 ^b	27.3 ^a	28.1 ^a	1.1	<0.01
Fat	0.95	0.96	0.92	0.04	0.53
CP	0.88 ^b	0.99 ^a	1.01 ^a	0.03	<0.01
Lactose	1.11 ^b	1.24 ^a	1.29 ^a	0.05	<0.01
Milk composition					
Fat (%)	3.92 ^a	3.60 ^b	3.38 ^c	0.12	<0.01
CP (%)	3.66	3.67	3.64	0.05	0.76
Lactose (%)	4.52	4.52	4.55	0.03	0.55
MUN (mg/dL)	21.3 ^a	16.0 ^b	13.9 ^c	0.52	<0.01
BW change (kg/d)	0.25 ^b	1.17 ^a	1.23 ^a	0.11	<0.01
BCS change (per 21 d)	0.06 ^b	0.23 ^a	0.11 ^a	0.04	0.02

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

¹CON: control; DG: 20% DDGS replacing barley silage; DG+AH: 20% DDGS + 10% alfalfa hay replacing barley silage.

fat concentration. Because particle size of diets affects chewing activity (Mertens, 1997), decreased chewing time for cows fed DDGS diets can be attributed to the smaller particle size of DDGS relative to barley silage. Decreased chewing activity may have resulted in less secretion of saliva (Bowman et al., 2003) and low rumen pH for cows fed the DG diet. In addition, greater DMI for cows fed the DG diet, despite reduced eating time, is expected to increase fermentation acid production in the rumen, decreasing the rumen pH further. Duration of rumen pH below 5.8 was 3.9 h longer for cows fed the DG diet than for those fed the CON diet. Milk fat yield was not affected by treatment, but milk fat concentration was decreased by feeding the DG diet compared with the CON diet. Although concentrations of short- and medium-chain fatty acids (C <16) were not affected by treatment, concentration of C16, which partially comes from the de novo synthesis (Grummer, 1991), was decreased by feeding the DG diet. Low concentrations of C16 may be caused by the inhibition of de novo fatty acid synthesis or result from the increased concentrations of long-chain fatty acids in milk. However, in the present study, the concentration of *trans*-10,*cis*-12 CLA in milk fat, an inhibitor for de novo synthesis of fatty acids in the mammary gland (Peterson et al., 2003; Lock et al., 2007), was not affected by feeding the DDGS diet compared with the CON diet.

Effects of Inclusion Alfalfa Hay in the DG Diet

Dietary inclusion of alfalfa hay in place of silage often increased chewing time (Allen and Grant, 2000) and

milk fat concentration (Smith et al., 1993) when cows were fed high NFFS diets. In our companion study (Zhang et al., 2010), feeding DDGS in place of barley silage at 20% of dietary DM did not affect rumen pH or milk fat concentration, but the experimental diets contained alfalfa hay. Therefore, we hypothesized that dietary inclusion of alfalfa hay would alleviate the reductions in chewing time, rumen pH, and milk fat concentration that are often observed when cows were fed high NFFS diets (Smith et al., 1993; Allen and Grant, 2000). However, in the present study, addition of alfalfa hay in place of barley silage did not increase chewing time and rumen pH. The milk fat concentration was even reduced by feeding the DG+AH diet compared with the DG diet.

Our findings indicate that physical effectiveness of high NFFS diets may not be increased by dietary inclusion of alfalfa hay. However, Allen and Grant (2000) reported increased chewing time by replacing alfalfa silage with alfalfa hay at 19% of dietary DM for cows fed high wet corn gluten feed diet. In the present study, the dietary inclusion of alfalfa hay was 10% and might not be high enough to stimulate chewing activity. The lack of stimulatory effect of alfalfa hay on chewing time may be also attributed to similar dietary physically effective factor between the DG and DG+AH diets. In our study, although the proportion of diet particles retained on the 19-mm sieve of the Penn State Particle Separator was greater for the DG+AH diet compared with the DG diet (6.0 ± 0.96 vs. $1.8 \pm 0.75\%$), these long particles may have little effect on chewing time compared with those retained on the 8-mm sieve. This observation is in agreement with Allen (1997); forage

Table 8. Effects of partially replacing barley silage with wheat-based dried distillers grains with solubles (DDGS) or DDGS plus alfalfa hay in diets for lactating dairy cows on milk fatty acids profile of ruminally cannulated cows

Item (g/100 g of total fatty acids)	Diet ¹			SEM	P-value
	CON (n = 5)	DG (n = 5)	DG+AH (n = 5)		
C4:0	1.39	0.94	1.25	0.22	0.40
C5:0	0.32	0.31	0.30	0.02	0.64
C6:0	1.85	1.79	1.53	0.15	0.35
C7:0	0.05	0.07	0.05	0.01	0.33
C8:0	1.12	1.17	1.04	0.15	0.82
C9:0	0.08	0.07	0.14	0.02	0.05
C10:0	3.60	3.44	3.26	0.21	0.57
C11:0	0.39	0.39	0.37	0.02	0.63
C12:0	4.27	4.14	3.82	0.20	0.33
C14:0	12.6	12.3	12.0	0.30	0.10
C14:1	1.51	1.33	1.55	0.14	0.47
C15:0	1.55	1.65	1.76	0.11	0.37
C16:0	31.9 ^a	29.0 ^b	28.5 ^b	0.4	<0.01
C16:1 <i>trans</i>	0.43	0.49	0.54	0.03	0.05
C16:1 <i>cis</i>	2.38	1.68	2.08	0.47	0.42
C18:0	7.30	8.49	6.96	0.50	0.11
<i>trans</i> -6 or <i>trans</i> -8 C18:1	0.18	0.28	0.32	0.08	0.55
<i>trans</i> -9 C18:1	0.20	0.32	0.31	0.06	0.36
<i>trans</i> -10 C18:1	0.36 ^b	0.60 ^{ab}	1.14 ^a	0.15	0.03
<i>trans</i> -11 C18:1	1.14	1.52	2.65	0.46	0.13
<i>cis</i> -12 C18:1	0.27 ^b	0.43 ^{ab}	0.56 ^a	0.04	<0.01
<i>cis</i> -9 C18:1	18.6	19.3	18.5	0.40	0.38
<i>cis</i> -7 C18:1	0.51	0.58	0.78	0.08	0.11
C19:0	0.18	0.23	0.26	0.02	0.17
<i>trans</i> -9, <i>trans</i> -12 C18:2	0.15	0.14	0.13	0.02	0.68
<i>cis</i> -9, <i>cis</i> -12 C18:2	3.00 ^b	4.03 ^a	4.36 ^a	0.21	<0.01
<i>cis</i> -9, <i>trans</i> -11 C18:2	0.56	0.62	0.94	0.13	0.13
<i>trans</i> -10, <i>cis</i> -12 C18:2	0.03	0.03	0.04	0.01	0.11
C18:3 n-3	0.28 ^b	0.34 ^b	0.41 ^a	0.02	<0.01
C18:3 n-6	0.05	0.06	0.15	0.06	0.46
C20:0	0.16 ^a	0.15 ^{ab}	0.12 ^b	0.01	0.03
C20:1 n-12	0.18	0.16	0.15	0.01	0.04
C20:1 n-15	0.05 ^b	0.07 ^{ab}	0.09 ^a	0.01	0.02
C20:3 n-6	0.13	0.15	0.17	0.01	0.07
C22:0	0.06 ^a	0.05 ^b	0.04 ^b	0.01	<0.01
C20:4	0.17	0.16	0.15	0.01	0.34
Short- and medium-chain fatty acids (C < 16)	30.2	28.9	28.3	0.6	0.18
C16 ²	35.2 ^a	31.8 ^b	31.6 ^b	0.4	<0.01
Long-chain fatty acid (C > 16)	34.6 ^b	39.2 ^a	40.1 ^a	0.6	<0.01
SFA ³	68.3	65.6	62.6	1.3	0.06
UFA ⁴	31.7	34.4	37.4	1.3	0.06

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

¹CON: control; DG: 20% DDGS replacing barley silage; DG+AH: 20% DDGS + 10% alfalfa hay replacing barley silage.

²C16: the sum of C16:0, C16:1 *trans*, and C16:1 *cis*.

³SFA: saturated fatty acids.

⁴UFA: unsaturated fatty acids.

particle size affects total chewing time to a lesser extent if a mean sieve aperture size exceeds 3 mm.

Although rumen pH was similar between the DG and DG+AH treatments, milk fat concentration was lower for cows fed the DG+AH diet. Because there was no difference in milk fat yield, the treatment effect can be partly attributed to the dilution of milk fat by numerically higher milk yield for cows fed the DG+AH diet. In milk fat, there was an increase in the concen-

tration of *trans*-10 C18:1 for cows fed the DG+AH diet compared with those fed the CON diet. *Trans*-10 C18:1 was previously considered to inhibit de novo fatty acid synthesis in the mammary gland and depress milk fat concentration (Griinari et al., 1998). However, a recent study (Lock et al., 2007) demonstrated that *trans*-10 C18:1 has no effect on milk fat synthesis. But, the greater concentration of *trans*-10 C18:1 in milk fat may indicate greater accumulation of *trans*-10 C18:1

in the rumen, suggesting that the biohydrogenation pathway was altered for cows fed the DG+AH diet. However, the concentration of *trans*-10,*cis*-12 CLA in milk fat was not affected by treatment. Therefore, it is not certain whether de novo fatty acid synthesis in the mammary gland was inhibited by feeding the diets containing DDGS.

In a previous study (Weidner and Grant, 1994), cows fed a diet containing soy hulls and alfalfa hay decreased milk fat concentration compared with those fed a diet containing soy hulls without alfalfa hay. Milk fat concentration often decreases for cows fed diets containing long-chopped forages, which was attributed to sorting against long particles (Kononoff and Heinrichs, 2003; Onetti et al., 2004). The greater DM concentration of DDGS relative to barley silage diets would have caused sorting against long particles; however, in the current study, we added water to the DG and DG+AH diets, which likely allowed the fine particles to stick to larger particles (Miller-Cushon and DeVries, 2009) and thus decreased sorting for fine particles. Leonardi et al. (2005) reported that the extent of sorting against long particles was reduced when the DM concentration of a hay-based diet was decreased from 80.8 to 64.4% by addition of water. In contrast, sorting against long particles was not reduced as DM concentration of a silage-based diet decreased from 57.6 to 47.9% by addition of water (Miller-Cushon and DeVries, 2009). These results imply that the addition of water likely prevents sorting if an initial diet is dry and easily sorted. In the present study, DM concentration of the DG+AH diet was decreased from 70 to 48% by addition of water, and cows fed the DG+AH diet did not sort against long particles, and sorting behavior does not explain lower milk fat concentration for the DG+AH treatment compared with the DG treatment. It is also noteworthy that cows fed the DG+AH diet actually sorted for long particles. This might be explained as an effort to alleviate low rumen pH. This is in agreement with the report that cows sorted for long feed particles as an attempt to meet physically effective fiber requirement when cows experience low rumen pH (Keunen et al., 2002; Beauchemin and Yang, 2005; DeVries et al., 2008).

CONCLUSIONS

Partial replacement of barley silage with wheat DDGS may increase DMI and milk and milk protein yields of lactating dairy cows, but may decrease chewing time, rumen pH, and milk fat concentration. Compared with the DG diet, cows fed the DG+AH diet decreased milk fat concentration but did not affect other response variables measured in this study. Dietary inclusion of alfalfa hay may not alleviate the reductions in chewing

time, rumen pH, and milk fat concentration that occur when DDGS partially replaces forage in diets for lactating dairy cows.

ACKNOWLEDGMENTS

The authors of this study gratefully acknowledge A. Reiz and H. Gerardo Cuervo (University of Alberta, Edmonton, AB, Canada) for technical assistance, and the staff of the Dairy Research and Technology Center (University of Alberta, Edmonton, AB, Canada) for general animal care. Financial support from the Alberta Livestock and Meat Agency, Alberta Milk (both in Edmonton, AB, Canada), and the Agricultural Bioproducts Innovation Program (Agriculture and Agri-Food Canada, Ottawa, ON, Canada) is gratefully acknowledged.

REFERENCES

- Allen, D. M., and R. J. Grant. 2000. Interactions between forage and wet corn gluten feed as sources of fiber in diets for lactating dairy cows. *J. Dairy Sci.* 83:322–331.
- Allen, M. S. 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. *J. Dairy Sci.* 80:1447–1462.
- AOAC. 2002. Official Methods of Analysis. 17th ed. Association of Official Analytical Chemists, Gaithersburg, MD.
- Beauchemin, K. A., and W. Z. Yang. 2005. Effects of physically effective fiber on intake, chewing activity, and ruminal acidosis for dairy cows fed diets based on corn silage. *J. Dairy Sci.* 88:2117–2129.
- Boddugari, K., R. J. Grant, R. Stock, and M. Lewis. 2001. Maximal replacement of forage and concentrate with a new wet corn milling product of lactating dairy cows. *J. Dairy Sci.* 84:873–884.
- Bowman, G. R., K. A. Beauchemin, and J. A. Shelford. 2003. Fibrolytic enzymes and parity effects on feeding behavior, salivation, and ruminal pH of lactating dairy cows. *J. Dairy Sci.* 86:565–575.
- Clark, P. W., and L. E. Armentano. 1993. Effectiveness of neutral detergent fiber in whole cottonseed and dried distillers grains compared with alfalfa haylage. *J. Dairy Sci.* 76:2644–2650.
- DeVries, T. J., F. Dohme, and K. A. Beauchemin. 2008. Repeated ruminal acidosis challenges in lactating dairy cows at high and low risk for developing acidosis: Feed sorting. *J. Dairy Sci.* 91:3958–3967.
- Dong, F. M., B. A. Rasco, and S. S. Gazzaz. 1987. A protein quality assessment of wheat and corn distillers dried grains with solubles. *Cereal Chem.* 64:327–332.
- Fawcett, J. K., and J. E. Scott. 1960. A rapid and precise method for the determination of urea. *J. Clin. Pathol.* 13:156–159.
- Folch, J., M. Lees, and G. H. S. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226:477–480.
- Griinari, J. M., D. A. Dwyer, M. A. McGuire, D. E. Bauman, D. L. Palmquist, and K. V. V. Nurmela. 1998. *Trans*-octadecenoic acids and milk fat depression in lactating dairy cows. *J. Dairy Sci.* 81:1251–1261.
- Grummer, R. R. 1991. Effect of feed on the composition of milk fat. *J. Dairy Sci.* 74:3244–3257.
- Harvatine, D. I., J. E. Winkler, M. Devant-Guille, J. L. Firkins, N. R. St-Pierre, B. S. Oldick, and M. L. Eastridge. 2002. Whole linted cottonseed as a forage substitute: Fiber effectiveness and digestion kinetics. *J. Dairy Sci.* 85:1988–1999.
- Keunen, J. E., J. C. Plaizier, L. Kyriazakis, T. F. Duffield, T. M. Widowski, M. I. Lindinger, and B. W. McBride. 2002. Effects of

- a subacute ruminal acidosis model on the diet selection of dairy cows. *J. Dairy Sci.* 85:3304–3313.
- Khorasani, G. R., E. K. Okine, and J. J. Kennelly. 1996. Forage source alters nutrient supply to the intestine without influencing milk yield. *J. Dairy Sci.* 79:862–872.
- Kononoff, P. J., and A. J. Heinrichs. 2003. The effect of reducing alfalfa haylage particle size on cows in early lactation. *J. Dairy Sci.* 86:1445–1457.
- Lammers, B. P., D. R. Buckmaster, and A. J. Heinrichs. 1996. A simple method for the analysis of particle sizes of forage and total mixed rations. *J. Dairy Sci.* 79:922–928.
- Leonardi, C., and L. E. Armentano. 2003. Effect of quantity, quality, and length of alfalfa hay on selective consumption by dairy cows. *J. Dairy Sci.* 86:557–564.
- Leonardi, C., F. Giannico, and L. E. Armentano. 2005. Effect of water addition on selective consumption (sorting) of dry diets by dairy cattle. *J. Dairy Sci.* 88:1043–1049.
- Lock, A. L., C. Tyburczy, D. A. Dwyer, K. J. Harvatine, F. Destailats, Z. Mouloungui, L. Candy, and D. E. Bauman. 2007. *Trans*-10 octadecenoic acid does not reduce milk fat synthesis in dairy cows. *J. Nutr.* 137:71–76.
- McKinnon, J. J., and A. M. Walker. 2008. Comparison of wheat-based dried distillers' grain with solubles to barley as an energy source for backgrounding cattle. *Can. J. Anim. Sci.* 88:721–724.
- Mertens, D. R. 1997. Creating a system for meeting the fiber requirements of dairy cows. *J. Dairy Sci.* 80:1463–1481.
- Miller-Cushon, E. K., and T. J. DeVries. 2009. Effect of dietary dry matter concentration on the sorting behavior of lactating dairy cows fed a total mixed ration. *J. Dairy Sci.* 92:3292–3298.
- Mullins, C. R., K. N. Grigsby, and B. J. Bradford. 2009. Effects of alfalfa hay inclusion rate on productivity of lactating dairy cattle fed wet corn gluten feed-based diets. *J. Dairy Sci.* 92:3510–3516.
- NRC. 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. Natl. Acad. Press, Washington, DC.
- Nuez Ortín, W. G., and P. Yu. 2009. Nutrient variation and availability of wheat DDGS, corn DDGS and blend DDGS from bioethanol plants. *J. Sci. Food Agric.* 89:1754–1761.
- Onetti, S. G., S. M. Reynal, and R. R. Grummer. 2004. Effect of alfalfa forage preservation method and particle length on performance of dairy cows fed corn silage-based diets and tallow. *J. Dairy Sci.* 87:652–664.
- Penner, G. B., K. A. Beauchemin, and T. Mutsvangwa. 2006. An evaluation of the accuracy and precision of a stand-alone submersible continuous ruminal pH measurement system. *J. Dairy Sci.* 89:2132–2140.
- Penner, G. B., P. Yu, and D. A. Christensen. 2009. Effects of replacing forage or concentrates with wet or dry distillers' grains on the productivity and chewing activity of dairy cattle. *Anim. Feed Sci. Technol.* 153:1–10.
- Peterson, D. G., E. A. Matitashvili, and D. E. Bauman. 2003. Diet-induced milk fat depression in dairy cows results in increased *trans*-10, *cis*-12 CLA in milk fat and coordinate suppression of mRNA abundance for mammary enzymes involved in milk fat synthesis. *J. Nutr.* 133:3098–3102.
- Silveira, C., M. Oba, K. A. Beauchemin, and J. Helm. 2007. Effect of grains differing in expected ruminal fermentability on productivity of lactating dairy cows. *J. Dairy Sci.* 90:2852–2859.
- Smith, W. A., B. Harris Jr., H. H. Van Horn, and C. J. Wilcox. 1993. Effect of forage type on production of dairy cows supplemented with whole cottonseed, tallow, and yeast. *J. Dairy Sci.* 76:205–215.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharide in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597.
- Weidner, S. J., and R. J. Grant. 1994. Soyhulls as a replacement for forage fiber in diets for lactating dairy cows. *J. Dairy Sci.* 77:513–521.
- Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Boman, H. F. Trout Jr., and T. N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. *J. Dairy Sci.* 65:495–501.
- Yang, W. Z., and K. A. Beauchemin. 2006. Effects of physically effective fiber on chewing activity and ruminal pH of dairy cows fed diets based on barley silage. *J. Dairy Sci.* 89:217–228.
- Zhang, S. Z. 2010. Evaluation of dried distillers grains with solubles as a partial replacement of barley silage or barley grain in diets for lactating dairy cows. MSc Thesis. University of Alberta, Edmonton, Canada.
- Zhang, S. Z., G. B. Penner, W. Z. Yang, and M. Oba. 2010. Effects of partially replacing barley silage or barley grain with dried distillers grains with solubles on rumen fermentation and milk production of lactating dairy cows. *J. Dairy Sci.* 93:3231–3242.