Wheat is more potent than corn or barley for dietary mitigation of enteric methane emissions from dairy cows

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ABSTRACT

Wheat is the most common concentrate fed to dairy cows in Australia, but few studies have examined the effects of wheat feeding on enteric methane emissions, and no studies have compared the relative potencies of wheat, corn, and barley for their effects on enteric methane production. In this 35-d experiment, 32 Holstein dairy cows were offered 1 of 4 diets: a corn diet (CRN) of 10.0 kg of dry matter (DM)/d of single-rolled corn grain, 1.8 kg of DM/d of canola meal, 0.2 kg of DM/d of minerals, and 11.0 kg of DM/d of chopped alfalfa hay; a wheat diet (WHT) similar to the CRN diet but with the corn replaced by single-rolled wheat; a barley diet (SRB) similar to the CRN diet but with the corn replaced by single-rolled barley; and a barley diet (DRB) similar to the CRN diet but with the corn replaced by double-rolled barley. Individual cow feed intakes, milk yields, and milk compositions were measured daily but reported for the last 5 d of the experiment. During the last 5 d of the experiment, individual cow methane emissions were measured using the SF₆ tracer technique for all cows, and ruminal fluid pH was continuously measured by intraruminal sensors for 3 cows in each treatment group. The average DM intake of cows offered the CRN, WHT, SRB, and DRB diets was 22.2, 21.1, 22.6, and 22.6 kg/d. The mean energy-corrected milk of cows fed the WHT diet was less than that of cows fed the other diets. This occurred because the milk fat percentage of cows fed the WHT diet was significantly less than that of cows fed the other diets. The mean methane emissions and methane yields of cows fed the WHT diet were also significantly less than those of cows fed the other diets. Indeed, the CRN, SRB, and DRB diets were associated with 49, 73, and 78% greater methane emissions, respectively, compared with

the emissions from the WHT diet. Methane yield was found to be most strongly related to the minimum daily ruminal fluid pH. This study showed that although the inclusion of wheat in the diet of dairy cows could be an effective strategy for substantially reducing their methane emissions, it also reduced their milk fat percentage and production of milk fat and energy-corrected milk.

Key words: ruminal fluid, pH, cattle, methane, starch

INTRODUCTION

Methane is a potent greenhouse gas, and there is ongoing interest in reducing methane emissions from dairy cows (Moate et al., 2011; O'Mara, 2011). It is well known that feeding ruminants diets containing a high concentration of starch can improve production and reduce their enteric methane emissions, but most published studies have used diets fortified with either corn grain or barley grain (Grainger and Beauchemin, 2011; Sauvant et al., 2011). Many studies have used concentrate mixtures containing corn grain, barley grain, wheat grain, soybean meal, sugar beet pulp, citrus pulp, maize gluten, sunflower meal, calcium soaps of fatty acids, and other ingredients, and the concentrates may have been pelletized or included in a TMR (Kebreab et al., 2003). Wheat is a cereal grain commonly included in the diet of dairy cows, but few studies have examined the effects of wheat feeding on enteric methane emissions from dairy cows (Moate et al., 2016). Substituting wheat into the diet of lactating dairy cows in place of forage reduced methane emissions and methane yield (g/kg of DM) with no negative effect on milk volume, although high levels (i.e., >40% of DMI) of wheat feeding may depress milk fat concentration (Williams et al., 2013; Moate et al., 2014a).

Corn, barley, and wheat generally contain high proportions of starch, and in sacco studies indicate that different methane yields from different grain diets are related to the different rates of degradation of either DM or starch in each grain (Herrera-Saldana et al., 1990). Few in sacco studies have compared the DM

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and starch degradation characteristics of corn, barley, and wheat, but the general consensus is that the DM and starch in wheat is more quickly degraded than the DM and starch in barley (Herrera-Saldana et al., 1990; McDonnell et al., 2017), whereas the DM and starch in barley is more quickly degraded than the DM and starch in corn (Granzin, 2004; Greenwood et al., 2014).

As far as we can ascertain, no studies have directly compared the relative potencies of wheat, corn, and barley for their effects on enteric methane production in dairy cows. However, Beauchemin and McGinn (2005) fed finishing beef cattle diets containing 81.4% of either dry-rolled corn or steam-rolled barley and reported different methane yields of 9.2 and 13.1 g of CH₄/kg of DMI for the respective diets. Thus, the type of grain fed has the potential to substantially influence methane emissions. Based solely on assumed differences in the relative rates of starch degradability, one might expect that the barley diet would have been associated with a lower methane yield than the corn diet. However, in the experiment of Beauchemin and McGinn (2005), the barley had a higher NDF concentration than the corn, and the ruminal fluid pH was greater in the barley-fed animals.

The mechanism whereby the DM and starch degradability rates influence methane yields is not well understood but is possibly related to differences in specific features of the daily pH pattern in ruminal fluid, which results when cows are fed cereal grains that differ in their rate of ruminal starch degradability. Low pH has been reported to reduce methanogenesis *in vitro* (Van Kessel and Russell, 1996; Russell, 1998); this effect has also been observed *in vivo* where the methane emission from beef heifers was related to their mean ruminal pH (Doreau et al., 2011; Hünerberg et al., 2015). In contrast, Moate et al. (2012) found methane reductions were reflected in greater duration of pH below 6 and greater area below pH 6 but not in mean ruminal pH.

In addition, increasing the degree of processing (i.e., smaller particle sizes) of cereal grains can increase the rate of ruminal degradation of the grain and decrease ruminal pH (Herrera-Saldana et al., 1990; Yang et al., 2001). Thus, it is possible that differences in degree of processing between the grain types rather than differences between the grains *per se* might have been responsible for differences in methane emissions in some studies that have compared different types of grains for their effects on methane emissions.

The objective of this experiment was to determine the effect of feeding wheat, corn, and barley on methane emissions from lactating dairy cows and to determine whether any observed effect is related to degree of processing of barley, grain type, or ruminal fluid pH

characteristics. We hypothesized (1) that the type of grain would have no effect on milk production or ECM yield; (2) that the type of grain would affect methane emissions, yield, and intensity; (3) that the degree of processing (dry rolling) of barley grain would affect the methane yield; and (4) that methane yield across grain types would be negatively related to their relative rates of degradation of DM and starch and would correlate with specific features of the daily pattern in ruminal fluid pH.

MATERIALS AND METHODS

Cows, Diets, and Management

Thirty-two lactating multiparous Holstein-Friesian cows (including 12 rumen-cannulated cows) producing 32.3 ± 4.38 kg of milk/d (mean $\pm$ SD) with a BW of 537 ± 70.9 kg and that were 71 ± 8.7 DIM were allocated to 4 groups balanced for mean milk yield, BW, age, and DIM according to the method of Baird (1994). Each group of 8 cows (including 3 rumen-cannulated cows) was then randomly allocated to 1 of 4 dietary treatments in which cows were scheduled to individually receive the following: (1) a corn diet (**CRN**) of 10.0 kg of DM/d of single-rolled corn, 1.8 kg of DM/d of canola meal, 0.2 kg of DM/d of minerals, and 11.0 kg of DM/d of chopped alfalfa hay; (2) a wheat diet (**WHT**) similar to the CRN diet but with the corn replaced by single-rolled wheat; (3) a barley diet (**SRB**) similar to the CRN diet but with the corn replaced by single-rolled barley; or (4) a double-rolled barley diet (**DRB**) similar to the CRN diet but with the corn replaced by double-rolled barley. The corn, wheat, and single-rolled barley had been passed once through a roller mill, but the double-rolled barley had been passed twice through the same roller mill to further reduce particle size. The amount of DM offered to each cow in all treatments was the same.

All cows were offered a common diet of 6 kg of DM/d of crushed wheat and 16 kg of DM/d of chopped alfalfa hay during the covariate period (d 1–7), then gradually transitioned to their experimental diets from d 8 to 14. From d 15 to 35, each cow was offered her allocated experimental diet. Days 15 to 28 served as an adaptation period to allow the rumen to adapt to the experimental diets, whereas d 29 to 35 were used as the experiment measurement period. Cows were cared for according to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NHMRC, 2013). Animal use was approved by the Animal Ethics Committee of the Department of Economic Development Jobs Transport and Resources–Victoria.

Feed, Milk, and BW

Diets were offered to cows in 2 equal portions each day, one from 0700 to 1100 h and the other from 1600 to 2000 h. Cows were fed in individual stalls in a well-ventilated animal house (Williams et al., 2011) and were offered their concentrate and minerals first, then alfalfa once they had finished their concentrate. Quantities of grain offered to individual cows were weighed and then mixed by hand before being offered. Refusals of concentrate were collected and weighed, with proportions of each concentrate component being assumed to be the same as that offered on a wet basis. Concentrate components offered were representatively subsampled each Monday of the experiment. Alfalfa was representatively subsampled each day. The DM concentration was determined by drying feed subsamples in a forced draft oven at 105°C for 24 h. Subsamples of each feed were kept frozen at -18°C, separately bulked over the duration of the experiment, and then subsequently freeze-dried and ground to pass through a 0.5-mm screen. Feeds were analyzed for CP, soluble protein, ADF, NDF, lignin, NFC, starch, ash, and crude fat (ether extract) according to the procedures of Dairy One (2015).

Particle size determinations were made on the grains and the canola meal. The grains and canola meal were first dried at 60°C for 24 h and then 100 g of each feed was placed in the top sieve of a 6-sieve set (2.0, 1.0, 0.5, 0.25, 0.15, and 0.053 mm) with a pan at the bottom. The sieving procedure involved 30 horizontal 17-cm manual movements; the sieve apparatus then turned a quarter turn and this process was repeated for a total of 120 movements. Rotating the apparatus prevented particle agglomeration. Each sample was analyzed in duplicate. Amounts of sample on each sieve were expressed as a percentage of sample size. It was assumed that samples had a logarithmic-normal distribution with a geometric mean and geometric standard deviation, estimated according to the procedures of ASAE (2001).

When not in the animal house, cows were either at the dairy parlor being milked (0630–0700 h and 1500–1530 h) or held in an open-air loafing pad with no access to additional feed. At all times, water was available to cows ad libitum.

Milk yield was measured for each cow at each milking using a milk metering system (MM25; DeLaval International, Tumba, Sweden). Milk samples were collected from individual cows during morning and afternoon milkings on d 5 to 7 (covariate period) and d 29 to 35 (measurement period). Fat, protein, and lactose in these milk samples were measured using a near-infrared milk analyzer (model 2000; Bentley Instruments, Chaska, MN). Somatic cells were counted by a cell counter

(Bentley SC500; Bentley Instruments). The ECM, standardized to 4.0% fat and 3.3% protein, was calculated using the equation of Tyrrell and Reid (1965):

$$\text{ECM (kg/cow per day)} = \frac{\text{milk yield (kg)} \times (376 \times \text{fat \%} + 209 \times \text{protein \%} + 948)}{3,138}$$

Milk fatty acids were determined by first extracting milk fat from fresh samples using a method based on the Röse-Gottlieb gravimetric method (International Dairy Federation, 1987). Samples of extracted milk fat were stored at -18°C until analyzed for fatty acid composition. Concentrations of FAME in milk fat were determined by GC following methylation with sodium methoxide (Slover and Lanza, 1979). Analyses were conducted using a gas chromatograph with auto-sampler and flame ionization detector (Agilent 6890; Agilent Technologies, Santa Clara, CA) and with an RTX-2330 column (105 m × 0.25 mm i.d., 0.2-μm film thickness), using helium as the carrier gas at a rate of 1.4 mL/min. Peak identification was by retention time comparisons with fatty acid standards (189-19, 37 FAME 4:0-24:0; Sigma Chemical Corp., St. Louis, MO) included every 15 samples. Cows were weighed daily over the last 14 d of the experiment to enable estimation of the average daily BW change for each cow.

Methane Emissions

The modified SF₆ tracer technique as fully described by Deighton et al. (2014) was used to estimate methane emissions from individual cows. In this experiment, the permeation tubes were filled with about 2.8 g of 99.999% pure SF₆ (Advanced Specialty Gases, Reno, NV). The release rate of SF₆ from permeation tubes was determined by incubating the permeation tubes in an oven set at 39.0°C and weighing the permeation tubes twice per week for 4 wk. The SF₆ release rate from permeation tubes was 4.1 ± 0.31 mg/d (average ± SD) and ranged from 3.8 to 4.8 mg/d. Cows were dosed by mouth with SF₆ permeation tubes on d 24. Each permeation tube was encapsulated in a clear gelatin capsule (size no. 10, Torpac, Fairfield, NJ) and then administered using a balling gun. From d 30 to 35, evacuated canisters of 800-mL capacity were used to continuously sample eructated gases. These evacuated canisters were attached to a saddle mounted on the back of each cow (Deighton et al., 2014). For breath samples, one end of a flexible sampling tube incorporating a flow restrictor was attached to the evacuated canister and the other end was fixed to a sampling point on a leather muzzle attached to the cow's head using

a leather halter (Deighton et al., 2014). This arrangement resulted in a loss of just 3% of gas samples during the course of the experiment. The appropriate rate of flow of gases into canisters was achieved by means of orifice plates (Lenox Laser, Glen Arm, MD; Deighton et al., 2014). An initial sampling rate of approximately 0.2 mL/min was used to continuously sample eructated gases. Samples of background gases were collected from the right flank of each individual cow into evacuated 800-mL canisters. Canisters were exchanged once per day before the morning feeding at approximately 0700 h for a total of 5 d of collection. Analysis of collected gas samples was carried out the day following collection using GC (Williams et al., 2011). Column temperature was 65°C, and the run duration was 4.2 min. A piston extractor (NIWA, Wellington, New Zealand) was used to extract a sample from the canister and then inject it into the GC. With the GC inlet valve closed, the front and back chambers of the piston were evacuated to approximately 98 kPa vacuum. The canister was then connected to the piston and the sample was allowed to fill the front chamber of the piston. Compressed air (~10 kPa) was then admitted to the back chamber of the piston to pressurize the sample in the front chamber. The GC cycle was then initiated, and sample flowed into the GC. Once the GC valve closed the sample remaining in the front chamber of the piston was returned to the canister, which was then disconnected, and the process was repeated with the next canister. Methane emissions for each day were calculated using Equation 2 of Williams et al. (2011):

$$R_{\text{CH}_4} = R_{\text{SF}_6} \frac{[\text{CH}_4]_{\text{M}} - [\text{CH}_4]_{\text{BG}}}{[\text{SF}_6]_{\text{M}} - [\text{SF}_6]_{\text{BG}}} \times \frac{\text{MW}_{\text{CH}_4}}{\text{MW}_{\text{SF}_6}} \times 1,000.$$

In this equation, R_{CH_4} is the calculated emission rate of enteric methane (g/animal per day); R_{SF_6} is the measured release rate of SF_6 from the permeation tube (mg/d); $[\text{CH}_4]_{\text{M}}$ and $[\text{SF}_6]_{\text{M}}$ are the measured concentrations of methane and SF_6 , respectively, in the breath sample; $[\text{CH}_4]_{\text{BG}}$ and $[\text{SF}_6]_{\text{BG}}$ are the concentrations of methane and SF_6 , respectively, measured in the background sample; MW_{CH_4} is the molecular mass of methane (16); and MW_{SF_6} is the molecular mass of SF_6 (146). For convenience, the concentrations of methane are generally expressed in parts per million (ppm) and the concentrations of SF_6 in parts per trillion (ppt). The factor of 1,000 in the equation is a unit converter taking into account the disparate units for $[\text{CH}_4]$ in parts per million, $[\text{SF}_6]$ in parts per trillion, and R_{SF_6} in milligrams per day so that R_{CH_4} will have the desired units of grams per day. In this experiment, the average $\pm$ standard deviation of $[\text{CH}_4]_{\text{BG}}$ and $[\text{SF}_6]_{\text{BG}}$ was 10.3

± 2.68 ppm and 16.4 ± 2.83 ppt, respectively, whereas for $[\text{CH}_4]_{\text{M}}$ and $[\text{SF}_6]_{\text{M}}$ the values were 192.4 ± 61.8 ppm and 196.5 ± 34.5 ppt, respectively. In this experiment, the SF_6 technique was used on both rumen-fistulated and nonfistulated cows because we found in yet-unpublished studies that rumen fistulation does not influence the accuracy of the SF_6 technique when cannulas are fitted to minimize leakage.

Ruminal Fermentation

Ruminal fluid samples (~400 mL) were collected from each cow by mouth after the morning feeding period (1100–1145 h) on d 35 using a sampling tube (with a 2-mm screen) and a vacuum pump (Moate et al., 2014b). The pH of the ruminal fluid was immediately measured using a pH meter (FG2; Mettler-Toledo, Schwerzenbach, Switzerland). A 0.5-mL representative subsample of ruminal fluid was transferred to a 12-mL plastic vial and then diluted with 4.5 mL of a solution containing 4% formalin, 0.9% saline, and 0.4% methylene blue. These samples were stored at 4°C until they were counted for ciliate protozoa in a counting chamber (Mod-Fuchs-Rosenthal; Hawksley and Sons Ltd., Lancing, UK) using a microscope (Leitz Laborlux S; Leica Microsystems Pty Ltd., North Ryde, NSW, Australia). The protozoa were categorized into 3 main categories because there was not a great diversity of different types of protozoa. A 4-mL subsample of ruminal fluid was diluted with 1 mL of 25% orthophosphoric acid and then frozen for subsequent analysis of VFA according to the method of Packer et al. (2011). The concentrations of D- and L-lactate in ruminal fluid were measured according to the method of Gawehn (1988). Ammonia concentrations in ruminal fluid were measured by the following spectrophotometric method (QuikChem method 12-107-06-2-A; Lachat Instruments, Loveland, CO). A 0.5-mL representative subsample of ruminal fluid was transferred to a 12-mL plastic vial and then diluted with 5.0 mL of an aqueous solution containing 1% formic acid and 1% orthophosphoric acid. This subsample was then stored at -18°C until analysis. These samples were thawed and centrifuged at $1,500 \times g$ for 10 min, and 1.0 mL of supernatant was diluted with 9.0 mL of 0.1 M HCl. These samples were then analyzed and quantified by comparison with standard ammonia-N solutions in the range 0.20, 0.50, 2.0, 4.0, 8.0, and 20.0 mg/L at 660 nm using a series 8000 Flow Injection Analyzer (Lachat Instruments).

The pH of ruminal fluid in cannulated cows (3 cows/diet) was continuously monitored by intraruminal boli (KB5; Kahne Ltd., Auckland, New Zealand) from d 27 to 35. In the week before insertion, a pH calibration was undertaken according to the manufacturer's

Table 1. Composition of main dietary ingredients

Item	Single-rolled corn	Single-rolled wheat	Single-rolled barley	Double-rolled barley	Canola meal	Alfalfa hay
Composition (g/kg of DM unless noted)						
CP	103	145	100	104	387	178
Soluble protein (% CP)	25.5	33.0	38.5	38.5	24.0	38.6
ADF	23	32	66	68	209	369
NDF	65	87	173	169	309	460
Lignin	9	14	15	15	72	93
NFC	779	725	673	672	127	244
Starch	691	603	532	545	5	15
Ash	15	21	25	24	65	90
Crude fat	37.5	21.8	29.0	31.5	113	27.6
Gross energy (MJ/kg of DM)	18.35	18.12	18.22	18.27	19.44	18.21
Grain particle size (% of DM retained on sieves)						
Coarse (2 mm)	62.2	71.0	86.5	57.4	5.3	—
Medium (1 mm)	27.3	21.5	12.3	34.4	30.9	—
Fine (<1 mm)	10.5	7.5	1.2	8.1	63.8	—
Fatty acid (% of total fatty acids)						
C14:0	0.82	2.32	0.34	0.37	0.97	2.74
C16:0	13.3	17.9	21.4	22.0	5.5	28.11
C16:1	0.14	0.12	0.13	0.12	0.52	0.20
C18:0	1.90	0	0	0	1.95	0.00
C18:1 <i>cis</i> -9	22.5	14.9	13.0	12.2	56.9	2.98
C18:1 <i>cis</i> -11	0.84	0.89	0.74	0.68	5.88	0.42
C18:2n-6	58.2	58.5	57.3	58.3	18.7	18.8
C18:3	1.36	3.86	5.00	4.33	7.39	31.47
Other	0.85	1.44	2.16	2.06	2.19	15.31

instructions. Validation of pH was done in the week after removal, and a linear interpolation was used to correct for any drift in individual boli. All boli were fitted with a 750-g weight so that they resided at the bottom of the rumen.

Calculations

Estimates of ME intake (**MEI**) were made using appropriate dietary and cow data from Tables 1 and 2 and inputting these data into CPM-Dairy software (Boston et al., 2000). Predictions of daily methane emissions were made based on the estimates of MEI using Equation 1d from Ellis et al. (2007) and assuming an energy density of 55.5 MJ/kg of methane:

$$\text{CH}_4 \text{ (MJ/d)} = 4.08 (\pm 1.32) + 0.0678 (\pm 0.00905) \times \text{MEI (MJ/d)}.$$

The mean melting points (**MMP**) of milk fat were estimated by the method described by Jensen and Patton (2000) using melting points of individual fatty acids as reported by Weast (1975). The pH bolus data for each cow were logged over 5 d. These data were first averaged for 15-min intervals, and then the data for the corresponding intervals over the 5 d were averaged for each cow to produce a 24-h diurnal pattern of ruminal fluid pH for each cow. These data were then summarized

in several ways: average duration below pH 6, average area below pH 6, average time to nadir after a.m. feeding and p.m. feeding, average nadir pH after a.m. feeding and p.m. feeding, mean pH, average minimum pH, average maximum pH, and average pH range. In addition, a graph of the 24-h diurnal pattern of ruminal fluid pH for each dietary treatment was produced by averaging the data from the relevant individual cows.

Statistical Analyses

Milk production data (milk yields; fat, protein, and lactose concentrations and yields; ECM) and DMI during the covariate period, and separately in the final week of the experimental period and for the methane-related data from the 5-d methane measurement period, were expressed as daily averages for each animal before data analysis. The resulting averages for the treatment period were analyzed by analysis of covariance with diet as treatment structure, animal as blocking structure, and the corresponding covariate averages as covariate. Data on milk fatty acids, ruminal fluid data on pH, ammonia, VFA, lactate, and protozoa, and methane data were analyzed by ANOVA using the same treatment and blocking structures but without covariate. Means presented in Tables 2 through 5 are the least squares means from the model. The standard errors of the differences of the means are presented in each table in the

Results section. Statistical significance for difference in means was declared when $P < 0.05$.

The individual-cow summarized pH parameters (bolus data; 3 cows/dietary treatment) were linearly regressed against methane yield. All statistical analyses were performed using Genstat 18 software (VSN International Ltd., Hemel Hempstead, UK).

RESULTS

The chemical compositions of the feeds and the percentages of grain particles of different sizes are shown in Table 1. The particle sizes (geometric mean $\pm$ geometric SD) were 0.9 ± 1.50 , 1.1 ± 1.21 , 1.3 ± 1.05 , 1.0 ± 1.36 , and 0.4 ± 1.75 mm for corn, wheat, single-rolled barley, double-rolled barley, and canola meal, respectively. Total feed DMI, milk fat concentration, ECM, and milk fat yield were less ($P \leq 0.01$) for cows fed the WHT diet than for cows fed the other diets (Table 2). No treatment differences in average daily BW change were measured over the last 14 d of the experiment. For 17 of the 37 individual fatty acids or groups of fatty acids examined, the percentages of individual fatty ac-

ids or groups of fatty acids in the milk fat of cows fed the WHT diet were different ($P < 0.05$) than those in the milk fat of cows fed the other diets (Table 3). In particular, the milk fat from cows fed the WHT diet had a lower mean concentration of C18:0 and higher mean concentrations of CLA *trans*-10,*cis*-12 and of total PUFA than the milk fat from cows fed the other diets. The milk fat from cows fed the CRN diet had a lower ($P = 0.005$) estimated MMP than that from cows fed the barley-based diets.

The mean methane emission (expressed as g/d) and methane yields (expressed as g/kg of DMI or as a percentage of gross energy intake) of cows fed the WHT diet were less ($P < 0.001$) than those of cows fed the other diets (Table 4). Similarly, the mean methane intensity (g/kg of ECM) of cows fed the WHT diet was less ($P < 0.001$) than that of cows fed the other diets.

The means for fermentation parameters measured in a sample of ruminal fluid collected postfeeding are shown in Table 5. For many of these parameters, the mean for the cows fed the WHT diet was significantly different from the corresponding means for cows fed the other diets. In particular, the pH in the ruminal fluid of

Table 2. Quantity of feeds eaten and milk yields from cows on each treatment¹

Item	CRN	WHT	SRB	DRB	SED ²	P-value
No. of cows	8	8	8	8	—	—
Feed intake ³ (kg of DM/d)						
Alfalfa hay	10.3 ^{ab}	9.7 ^a	10.8 ^b	10.8 ^b	0.43	0.032
Crushed corn grain	9.9	0	0	0	—	—
Crushed wheat	0	9.5	0	0	—	—
Single-rolled barley	0	0	9.8	0	—	—
Double-rolled barley	0	0	0	9.9	—	—
Canola meal	1.8	1.7	1.8	1.8	—	—
Minerals	0.2	0.2	0.2	0.2	—	—
Total DMI	22.2 ^b	21.1 ^a	22.6 ^b	22.7 ^b	0.44	0.002
Total CP	3.55	3.79	3.60	3.66	—	—
Total NDF	5.97	5.85	7.24	7.23	—	—
Total starch	6.96	5.93	5.43	5.59	—	—
Total fat	0.86	0.67	0.79	0.82	—	—
Gross energy intake (MJ/d)	404	384	412	414	—	—
Grain ⁴ (%)	44.6	45.0	43.5	43.6	1.10	0.371
Milk (kg/d)	32.1	32.3	31.3	30.6	0.80	0.16
ECM (kg/d)	31.2 ^b	27.6 ^a	30.7 ^b	30.7 ^b	1.10	0.010
Milk composition (%)						
Fat	3.82 ^b	2.83 ^a	3.95 ^b	4.14 ^b	0.238	<0.001
Protein	3.17	3.19	3.27	3.16	0.052	0.162
Lactose	5.01	4.96	4.96	4.93	0.039	0.252
Log ₁₀ SCC	1.68	1.23	1.48	1.50	0.277	0.421
Fat (kg/d)	1.23 ^b	0.91 ^a	1.22 ^b	1.26 ^b	0.079	<0.001
Protein (kg/d)	1.01	1.03	1.01	0.96	0.025	0.065
Lactose (kg/d)	1.61	1.61	1.53	1.50	0.048	0.071
BW change (kg/d)	0.89	0.95	1.38	0.92	0.325	0.685

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

¹CRN = single-rolled corn; WHT = single-rolled wheat; SRB = single-rolled barley; DRB = double-rolled barley.

²SED = standard error of the difference.

³Feed intakes measured during the last week of the experiment.

⁴The percentage of corn, barley, or wheat in each diet.

cows fed the WHT diet was lower ($P = 0.013$) than the pH in the ruminal fluid of cows fed the other diets. The ruminal fluid mean concentration of acetic acid (expressed as a molar percentage of total VFA) was lower ($P = 0.004$) for cows fed the WHT diet than for cows fed the other diets, whereas the concentrations of propionic and D- and L-lactic acids were greater ($P < 0.01$) in the ruminal fluid of cows fed the WHT diet compared with the other diets. The counts of entodinia, epidinia, and total protozoa were all substantially reduced ($P < 0.001$) in the ruminal fluid of cows fed the WHT diet compared with the other diets. Graphs of the average

diurnal patterns of ruminal fluid pH (as measured by intraruminal boli) of cows in the 4 dietary treatments were all W shaped, and in the period between 1000 and 2000 h, the ruminal fluid pH in cows fed the WHT diet was approximately 0.3 pH units less than in the other treatments (Figure 1). The relationships between pH variables (mean pH, minimum pH, maximum pH, pH range, duration below pH 6, area below pH 6) and methane yield of individual cows are shown in Figure 2. Among these relationships, the strongest relationship ($R^2 = 0.81$) was between minimum daily pH and methane yield.

Table 3. Means of individual milk fatty acids (g/100 g of total fatty acids unless otherwise stated) and estimated mean melting points (MMP) of the milk fat from cows offered each treatment¹

Item	CRN	WHT	SRB	DRB	SED ²	P-value
No. of cows	8	8	8	8	—	—
C4:0	3.38 ^b	2.68 ^a	3.50 ^b	3.73 ^b	0.191	<0.001
C6:0	2.21 ^b	1.78 ^a	2.33 ^b	2.47 ^b	0.135	<0.001
C8:0	1.40 ^b	1.22 ^a	1.47 ^b	1.52 ^b	0.082	0.005
C10:0	3.32	3.36	3.61	3.63	0.204	0.314
C10:1	0.31	0.27	0.32	0.32	0.027	0.162
C12:0	3.85	4.36	4.22	4.12	0.230	0.175
C14:0	11.8 ^a	13.2 ^b	13.1 ^b	12.7 ^b	0.40	0.010
<i>iso</i> C15	0.18	0.19	0.17	0.19	0.011	0.326
<i>anteiso</i> C15	0.42	0.59	0.39	0.42	0.083	0.092
<i>iso</i> C14	0.10 ^b	0.07 ^a	0.07 ^a	0.08 ^{ab}	0.012	0.049
C14:1	0.85	1.26	1.05	0.82	0.206	0.140
C15:0	1.09 ^a	1.86 ^b	1.24 ^a	1.00 ^a	0.154	<0.001
<i>iso</i> C16	0.22	0.30	0.26	0.25	0.028	0.068
C16:0	28.1 ^a	28.1 ^a	34.4 ^b	33.3 ^b	1.45	<0.001
C16:1	1.17 ^a	1.71 ^b	1.36 ^a	1.25 ^a	0.151	0.006
<i>iso</i> C17	0.49 ^a	0.67 ^b	0.46 ^a	0.48 ^a	0.051	<0.001
<i>anteiso</i> C17	0.33 ^a	0.58 ^b	0.49 ^{ab}	0.47 ^{ab}	0.080	0.041
C17:0	0.47 ^a	0.85 ^b	0.57 ^a	0.54 ^a	0.071	<0.001
C17:1	0.17 ^a	0.37 ^b	0.20 ^a	0.18 ^a	0.035	<0.001
C18:0	10.59 ^d	5.02 ^a	7.25 ^b	9.15 ^c	0.062	<0.001
C18:1 <i>trans</i> -9	0.27 ^c	0.04 ^a	0.19 ^b	0.17 ^b	0.025	<0.001
C18:1 <i>trans</i> -10	0.45 ^a	6.20 ^b	0.43 ^a	0.27 ^a	0.948	<0.001
C18:1 <i>trans</i> -11	1.10 ^c	0.26 ^a	0.89 ^{bc}	0.68 ^b	0.182	<0.001
C18:1 <i>cis</i> -9	19.55 ^b	14.39 ^a	14.69 ^a	15.53 ^a	0.846	<0.001
C18:1 <i>cis</i> -11	0.90 ^a	1.41 ^b	0.79 ^a	0.68 ^a	0.134	<0.001
C18:2n-6	2.01 ^{bc}	2.48 ^c	1.91 ^{ab}	1.48 ^a	0.244	0.004
C20:0	0.17 ^b	0.12 ^a	0.16 ^b	0.19 ^c	0.008	<0.001
C18:3n-3	0.57 ^a	0.94 ^b	0.64 ^a	0.57 ^a	0.082	<0.001
CLA <i>cis</i> -9, <i>trans</i> -11	0.57 ^b	0.64 ^b	0.51 ^{ab}	0.36 ^a	0.098	0.046
CLA <i>trans</i> -10, <i>cis</i> -12 (mg/kg)	42.5 ^a	106.3 ^b	37.5 ^a	43.8 ^a	17.9	<0.001
Total C18	35.4 ^c	30.7 ^b	26.8 ^a	28.6 ^{ab}	1.47	<0.001
Total SFA	68.1 ^a	64.9 ^a	73.6 ^b	74.2 ^b	1.91	<0.001
Total MUFA	24.8 ^b	25.9 ^b	19.9 ^a	19.9 ^a	1.42	<0.001
Total PUFA	3.14 ^a	4.07 ^b	3.06 ^a	2.42 ^a	0.366	0.001
Total DNFA ³	43.1 ^a	45.0 ^a	48.8 ^b	47.8 ^{ab}	1.29	<0.001
Total OBCFA ⁴	3.47 ^b	5.46 ^b	3.84 ^a	3.62 ^a	0.310	<0.001
Total odd i+a ⁵	0.89 ^b	0.74 ^a	0.73 ^a	0.77 ^a	0.055	0.029
Estimated MMP (°C)	37.84 ^a	38.81 ^{ab}	39.66 ^b	39.38 ^b	0.495	0.005

^{a-d}Means within a row with different superscripts differ significantly ($P < 0.05$).

¹CRN = single-rolled corn; WHT = single-rolled wheat; SRB = single-rolled barley; DRB = double-rolled barley.

²SED = standard error of the difference.

³DNFA = de novo fatty acids.

⁴OBCFA = odd- and branched-chain fatty acids.

⁵i+a = *iso* and *anteiso* fatty acids.

Table 4. Influence of corn-, wheat-, and barley-based diets¹ on methane emissions, yields, and intensities of dairy cows

Item	CRN	WHT	SRB	DRB	SED ²	P-value
No. of cows	8	8	8	8		
CH ₄ (g/d)	446 ^b	300 ^a	518 ^b	533 ^b	43.6	<0.001
CH ₄ (g/kg of DMI)	20.3 ^b	14.3 ^a	22.9 ^b	23.4 ^b	1.80	<0.001
CH ₄ (g/kg of ECM)	14.8 ^b	10.8 ^a	16.6 ^{bc}	17.8 ^c	1.43	<0.001
CH ₄ (% of GEI ³)	6.12 ^b	4.28 ^a	6.98 ^b	7.15 ^b	0.55	<0.001
Estimated MEI ⁴ (MJ/d)	259	254	253	256	NA ⁵	NA
Predicted CH ₄ ⁶ (g/d)	390	384	383	386	NA	NA

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

¹CRN = single-rolled corn; WHT = single-rolled wheat; SRB = single-rolled barley; DRB = double-rolled barley.

²SED = standard error of the difference.

³GEI = gross energy intake.

⁴ME intake estimated using the ingredient data from Table 1, diet compositions and cow data shown in Table 2, and CPM-Dairy computer software (Boston et al., 2000).

⁵NA = not applicable.

⁶Predicted daily methane emissions based on Equation 1d from Ellis et al. (2007) and assuming an energy density of 55.5 MJ/kg of methane.

DISCUSSION

The type of grain had no effect on milk yield even though cows fed the WHT diet ate less than other cows. However, contrary to our first hypothesis, the WHT diet reduced ECM, driven by a reduction in the concentration of milk fat. In this experiment, the concentrate percentages in the diets were similar in all 4 diets. Consequently, any differences between dietary treatments with respect to milk production, ECM, milk composition, or methane emissions can

be attributed either to differences in total DMI or to differences between wheat, corn, and barley. Including wheat in the diet of dairy cows has previously been reported to reduce the concentration of milk fat compared with when the same cows were fed corn (Moate et al., 2012). In the present experiment, there was no difference in the concentrations of milk fat for cows fed the corn and barley diets. This finding is in agreement with the research of Khorasani et al. (2001), Kargar et al. (2014), and Chibisa et al. (2015). Tommervik and Waldern (1969) also conducted 2 experiments in which

Table 5. Influence of corn-, wheat-, and barley-based diets¹ on rumen fermentation parameters after feeding

Item	CRN	WHT	SRB	DRB	SED ²	P-value
No. of cows	8	8	8	8		
Mean pH	6.3 ^{ab}	6.1 ^a	6.8 ^b	6.7 ^b	0.22	0.013
NH ₃ (mg/L)	121 ^c	48 ^a	57 ^a	92 ^b	12.4	<0.001
Total VFA (mM)	107	114	93	103	8.2	0.095
Individual VFA (molar %)						
Acetic	61.9 ^b	58.0 ^a	63.8 ^b	64.1 ^b	1.68	0.004
Propionic	25.6 ^b	30.2 ^c	20.7 ^a	19.3 ^a	2.23	<0.001
Isobutyric	0.9 ^b	0.7 ^a	0.9 ^b	1.0 ^b	0.09	0.005
n-Butyric	9.2 ^a	7.4 ^a	11.8 ^b	12.6 ^b	0.91	<0.001
Isovaleric	0.9 ^b	0.5 ^a	1.0 ^b	1.1 ^b	0.15	0.004
n-Valeric	1.4 ^a	2.1 ^b	1.7 ^a	1.6 ^a	0.16	<0.001
Caproic	2.4 ^a	0.5 ^b	1.7 ^a	1.7 ^a	0.36	<0.001
Acetate:propionate ratio	2.5 ^a	2.0 ^a	3.2 ^b	3.4 ^b	0.30	<0.001
D-Lactate (mM)	0.07 ^a	0.30 ^b	0.08 ^a	0.06 ^a	0.035	<0.001
L-Lactate (mM)	0.11 ^a	0.29 ^b	0.13 ^a	0.11 ^a	0.052	0.004
Protozoa (log ₁₀ count/mL + 1,000)						
Entodinia	4.59 ^b	3.60 ^a	4.85 ^b	5.39 ^b	0.40	<0.001
Epidinia	3.78 ^b	3.29 ^a	4.58 ^b	4.48 ^b	0.29	<0.001
Other	2.22	2.00	2.48	2.00	0.27	0.262
Total	4.73 ^b	3.83 ^a	5.18 ^b	5.46 ^b	0.317	<0.001

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

¹CRN = single-rolled corn; WHT = single-rolled wheat; SRB = single-rolled barley; DRB = double-rolled barley.

²SED = standard error of the difference.

they investigated the effects on milk production and composition when diets contained either (1) wheat or (2) corn or barley. In their first experiment, in which each of the 3 diets contained approximately 47% (DM basis) of the particular grain, FCM yields and mean milk fat concentrations were all similar (~ 39 g/kg). In the second experiment by Tommervik and Waldern (1969), in which the grains composed approximately 65% of the dietary DMI, FCM yields and milk fat concentrations were again similar between diets, but the milk fat concentrations from all diets were less than 29 g/kg. Contrary to these findings, Granzin (2004) and Auldist et al. (2013) reported that for diets with high proportions of concentrate, diets containing barley produced milk with lower milk fat concentrations than diets containing corn. Thus, although it is clear that a high proportion of grain in the diet will predispose cows to low milk fat concentrations, the relative effects of different grain types is still an issue for research.

Consistent with our second hypothesis, the mean methane emissions (g/d) of cows fed the WHT diet were substantially less than emissions from cows fed the other diets. Nevertheless, the magnitudes of these methane emissions are within the range of 237 to 623 g/d previously reported for Holstein cows (Charmley et al., 2016). The absolute reductions (g/d) and percentage reductions in methane emissions due specifically to feeding WHT in place of CRN (146, 33%), SRB

(218, 42%), and DRB (233, 44%) are substantial and greater in magnitude than what has been achieved by most other nutritional interventions (e.g., Martin et al., 2010; Grainger and Beauchemin, 2011). The cows fed the WHT diet ate approximately 1.0 kg of DM less than cows fed the CRN diet and approximately 1.6 kg of DM less than cows fed the diets containing barley. Taking into account the methane yield of cows fed the WHT diet, it can be calculated that the reduced DMI of cows fed the WHT diet accounts for only approximately 10% of the observed reduction in methane emissions from the cows fed the WHT diet. Notwithstanding the effects of wheat feeding on milk fat concentration, the magnitude of the reduction in methane emissions in cows fed the WHT diet indicates that the feeding of wheat to dairy cows has the potential to play an important role in the abatement of methane from dairy production.

The methane yields of cows fed the SRB and DRB diets were not ($P > 0.05$) significantly different, suggesting that the third hypothesis can be rejected. However, the mean particle sizes for the single-rolled barley and double-rolled barley were similar, and it could be argued that in this experiment the difference in particle size due to processing was simply not sufficient to cause a difference in methane yields. Methane yields of 20.3, 22.9, and 23.4 g/kg of DM for cows fed the CRN, SRB, and DRB diets, respectively, are similar in magnitude to the recently reported universal methane yield of 20.7

Figure 1. Diurnal pH of Holstein cows fed the wheat ($n = 3$, ●), corn ($n = 3$, ■), double-rolled barley ($n = 3$, △), or single-rolled barley ($n = 3$, ◇) diets. The SEM shown is the mean for all treatments.

Figure 2. Relationships between methane yield (MY) and (A) mean pH, (B) minimum pH, (C) maximum pH, (D) pH range, (E) duration below pH 6, and (F) area below pH 6 of 12 individual cows (3/diet) fed the wheat (●), corn (■), double-rolled barley (Δ), or single-rolled barley (◇) diets.

± 0.28 for Australian beef and dairy cattle consuming diets containing more than 70% forage (Charmley et al., 2016). In comparison, feeding the WHT diet to dairy cows reduced their methane emissions by 33%, methane yield by 30%, and ECM methane intensity by 27% relative to feeding CRN. This result supports findings from previous similar experiments with wheat feeding in which even greater reductions in methane emissions, methane yield, and methane intensities were reported (Moate et al., 2012, 2016).

Many articles have documented the *in vivo* effects on enteric methane emissions of diets containing concentrates (see articles cited by Kebreab et al., 2003; Yan et al., 2010). Limited research illustrates the potent inhibitory effects on enteric methane emissions of wheat in comparison with other cereal grains. Many of the published studies have included complex mixtures of different concentrates or contained only small quantities of wheat, and most experiments appear not to have been designed to compare the effects of different

grains on methane emissions (e.g., van Zijderveld et al., 2011). Many of the published studies in this area involved TMR, whereas in the current experiment the concentrate portion of the diet was fed separately from the forage portion of the diet. Therefore, an important research question is whether the methane inhibitory effect observed in this experiment would occur when a high proportion of wheat is included in a TMR.

The methane yield of beef heifers fed corn has been shown to be lower than that of those fed barley when grain composed 80% of the diet but not when grain composed 20% of the diet (Beauchemin and McGinn, 2005). Sauvant et al. (2011) reported that the inclusion of grain in the diets of dairy cows has limited effect on methane yield until the proportion of concentrate in the diet is greater than 30%. In our current experiment, cereal grains constituted approximately 47% of the diet, so it would be expected that any inherent difference in fermentative properties between corn and barley should have been evident in methane metrics. However, in this experiment, contrary to our second hypothesis, there were no differences between the corn and the barley diets with respect to methane emissions or methane yields. In contrast to our findings, Fellner et al. (2008) conducted an in vitro study and reported that when substrates containing 48% of either corn or barley were fermented in continuous fermenters, the corn substrates yielded less methane. Moate et al. (2013) have previously identified instances where in vivo studies on the methane abatement potential of algal meal, saponin containing *Yucca schidigera*, and monensin have produced findings that were at variance with findings from in vitro investigations. The current experiment is further evidence that in vivo findings do not always agree with published in vitro findings, indicating the need for caution when interpreting the results from in vitro studies. In our experiment, the mean methane intensity of cows fed the CRN diet was less than that of cows fed the DRB diet, but we can offer no explanation for this observation.

In this experiment some differences were observed in the total amount of starch consumed for each diet, but there does not appear to be any simple relationship between the total starch intake and the methane emissions. The cows fed the diets containing barley consumed substantially more NDF than the cows fed the CRN or WHT diets, and the cows fed the diets containing barley had significantly greater methane emissions than the cows fed the WHT diet and numerically greater methane emissions than the cows fed the CRN diet. This finding supports the findings of Ellis et al. (2007) that fiber or NDF consumption is an important determinant of methane emissions. In addition,

the fat concentrations in the CRN, WHT, SRB, and DRB diets were 38.7, 31.8, 35.0, and 36.1 g of fat/kg of DMI. Methane yield has been shown to decrease by 3.5% for each increase of 10 g/kg of DM in dietary fat concentration (Moate et al., 2011). Thus, the observed differences in methane emissions from the different diets in the current research cannot be attributed to differences in dietary fat concentrations.

Based on the literature values for the rates of degradation of starch and DM for wheat, barley, and corn, we had expected that the WHT diet would have the lowest methane yield, the SRB and DRB diets would have an intermediate yield, and the CRN diet would have the greatest methane yield. Our findings partially support our fourth hypothesis in that the WHT diet was associated with the lowest methane yield. However, the methane yields from the barley-based diets and the CRN diet were not significantly different. As discussed previously, this latter finding may be associated with the greater NDF content in the barley-based diets compared with the CRN diet.

Increasing the degree of processing of cereal grains has been reported to increase their rate of ruminal degradation and to decrease ruminal fluid pH (Herrera-Saldana et al., 1990; Yang et al., 2001). Therefore, we had expected that the DRB diet would result in a smaller ruminal fluid pH and smaller methane yield than the SRB diet due to the increased degree of processing. However, there was no significant difference in particle size between SRB and DRB and, not surprisingly, there were no differences between the SRB and DRB diets in terms of effects on ruminal fluid pH; concentrations of total VFA, acetic, propionic, isobutyric, n-butyric, isovaleric, n-valeric, caproic, or D- and L-lactic acids; or methane metrics. This finding that single or double rolling barley does not substantially affect these parameters suggests that, in this experiment, the differences in these parameters observed between the WHT diet and the other diets were unlikely to be attributable to differences in the degree of processing between the wheat grains and the other grains but rather to differences in the grains per se.

In this experiment, cows fed the WHT diet had a substantially greater ruminal fluid concentration of propionate and smaller concentration of acetate than cows fed the other diets. In the rumen, fermentations that produce acetate liberate hydrogen, whereas fermentations that produce propionate serve as a sink for hydrogen (Beauchemin et al., 2009). Methanogens utilize hydrogen to reduce carbon dioxide to CH₄. Thus, diets that increase production of propionate and decrease production of acetate have been associated with reduced production of CH₄ (Beauchemin et al., 2009).

We speculate that this could be a major mechanism explaining the reduced CH₄ production by the cows fed the WHT diet in this experiment.

For each dietary treatment, the average diurnal patterns of ruminal fluid pH (as measured by intraruminal bolus) demonstrated a W-shaped pattern typical of that reported for cows fed twice daily (Greenwood et al., 2014; Figure 1). Consistent with our fourth hypothesis, ruminal fluid pH parameters were all correlated with methane yield, with the strongest being the minimum daily pH ($R^2 = 0.81$). Previous research has shown that cows fed wheat instead of corn had a greater duration and area of ruminal fluid pH below 6 (Williams et al., 2012) and a lower methane yield (Moate et al., 2012). In our experiment, the daily mean of ruminal fluid pH correlated strongly and positively with methane yield ($R^2 = 0.60$). A similar trend has been reported between daily mean of ruminal fluid pH and methane emission, but only moderately (Hünerberg et al., 2015). There is evidence of a positive relationship between ruminal fluid pH measured 4 h after feeding and daily methane emissions (e.g., Beauchemin and McGinn, 2005; Moate et al., 2014a; Williams et al., 2016).

Volatile fatty acids, lactic acid, and protozoal counts in ruminal fluid reflected the pH measured about 4 h after feeding. The rapid fermentation of the WHT resulted in a buildup of lactic acid in ruminal fluid, and lactic acid is particularly potent at depressing pH in the rumen (Dijkstra et al., 2012). In addition, the decrease in ruminal protozoa could explain the decrease in ruminal pH in the cows fed the WHT diet (Brossard et al., 2004). Moreover, because protozoa engulf starch, they restrict access to the starch by amylolytic bacteria, which slows and delays the fermentation of starch. Rapid fermentation of the starch in the WHT diet would also have led to the rapid production of carbon dioxide in the rumen, and a recent review has highlighted the major contribution that dissolved carbon dioxide makes to reducing ruminal fluid pH (Laporte-Urbe, 2016). We speculate that the low ruminal fluid pH and the buildup of lactic acid in the ruminal fluid of the cows fed the WHT diet may have inhibited the growth of methanogens and fiber-fermenting microbes. Thus, the altered proportions of VFA in the ruminal fluid, decreased ruminal fiber digestion, and decreased methanogens would explain the observed decreased milk fat concentration and decreased production of ECM and methane.

The concentrations of individual milk fatty acids observed in this experiment were generally within the ranges previously reported in the scientific literature (Moate et al., 2007). In recent years, researchers have focused on the fatty acid profiles of milk because evidence shows that several specific fatty acids or classes

of fatty acids, such as PUFA, have beneficial effects on health (Haug et al., 2007). In this experiment, the milk fat from the cows fed the WHT diet had elevated concentrations of total PUFA compared with the milk fat from cows fed the other diets.

The finding of an elevated concentration of CLA *trans*-10,*cis*-12 in the milk fat from cows fed the WHT diet and the low concentration of total milk fat in these cows supports the theory that CLA *trans*-10,*cis*-12 is a major fatty acid that inhibits production of milk fat in the mammary gland (Bauman and Griinari, 2003). However, it is surprising that, for the cows fed the WHT diet, the mean milk concentration of CLA *trans*-10,*cis*-12 was only 0.01%, a concentration not normally associated with milk fat depression (Bauman and Griinari, 2003; Kadegowda et al., 2008). Thus, we cannot discount the possibility that in cows fed diets containing substantial amounts of wheat, factors other than CLA *trans*-10,*cis*-12 may play a role in milk fat depression. In this experiment, the concentration of C18:1 *trans*-10 in the milk fat of cows fed the WHT diet was more than 13 times greater than that in the milk fat of cows fed the other diets. The elevated C18:1 *trans*-10 concentration and decreased concentration of C18:0 in the milk fat of WHT-fed cows in comparison with that in the milk fat of cows fed the other diets are likely to have arisen because of incomplete biohydrogenation of C18 UFA due to acidic conditions in the ruminal fluid in the cows fed the WHT diet (Bauman and Griinari, 2003). Jurjanz et al. (2004) also reported that the concentration of C18:1 *trans*-10 was elevated in the milk fat of cows fed a wheat-based diet. This particular fatty acid has often been reported to be associated with milk fat depression, but it has never been shown to cause milk fat depression (Kadegowda et al., 2008).

The fatty acid composition of milk fat can influence the estimated MMP of milk fat (Jensen and Patton, 2000). In this experiment, the significantly lower MMP of milk fat from the CRN-fed cows compared with that from the cows fed the barley-based diets suggests that the butter produced from the milk of cows fed corn-based diets may be more spreadable than butter produced from the milk of cows fed barley-based diets.

The profiles of fatty acids in milk were generally related to the methane yields, with cows fed WHT exhibiting a different milk fatty acid profile than the cows fed the other diets. In recent years, several authors have reported both positive and negative relationships between the concentrations of a large number of individual milk fatty acids and methane emissions (Chilliard et al., 2009) or methane yields (Dijkstra et al., 2011). For some fatty acids (e.g., C8:0, C17:1, C18:1 *cis*-11), our findings are consistent with regression slopes reported by Dijkstra et al. (2011). However, in our experiment,

the cows fed the CRN and WHT diets produced markedly different amounts of methane and had markedly different methane yields despite having similar concentrations of C10:0 and C16:0, a finding in disagreement with the prediction equations of both Chilliard et al. (2009) and Dijkstra et al. (2011). In our experiment, the cows fed the WHT diet had the lowest methane emissions but had an intermediate concentration of total C18 fatty acids, a finding at variance with the strong negative relationship between total C18 fatty acids and methane emissions reported by Chilliard et al. (2009). Dijkstra et al. (2011) reported a strong negative relationship between C18:1 *cis*-9 and methane yield, but contrary to the prediction equation of Dijkstra et al. (2011), we found that cows fed the CRN diet had a greater concentration of C18:1 *cis*-9 in their milk fat and a greater methane yield than cows fed the WHT diet. These conflicting findings indicate that caution and perhaps further research will be required before the concentrations of individual fatty acids in milk fat can be reliably used to accurately predict methane emissions and methane yield from dairy cows across all diets.

In this experiment, there were no differences in methane emission, yield, or intensity between cows fed the SRB or DRB diets. This finding with barley in this study differs from findings in other studies, where it has been found that significantly less enteric methane was produced when more extensively processed (high moisture or steam flaked) corn grain was fed to cattle compared with when dry-rolled corn was fed (Archibeque et al., 2006; Hales et al., 2012). Thus, it cannot be ruled out that differences in particle sizes resulting from differential processing may have played some role in causing differences in methane emissions and methane yields from the diets in the current experiment. Nevertheless, the magnitude of the reduction in methane emissions and methane yield of the WHT diet compared with the other diets suggests that this effect might be attributable to inherent differences in the grains.

It is not apparent what attribute of wheat contributes to its greater methane inhibitory effect compared with barley or corn. In recent years, considerable research has related methane production to MEI (Ellis et al., 2007; Yan et al., 2010). However, the estimates of MEI provided by all 4 diets were similar (see Table 4). We estimate that the WHT diet provided 1.4% less MEI than the SRB diet, yet the WHT diet produced 42% less methane. Therefore, it can be concluded that small differences in estimated MEI between the WHT and other diets do not account for the reduced methane production from the WHT diet. All 3 grains had

relatively high concentrations of starch and similar concentrations of some other major nutrients. Moreover, the wheat and barley grains used in this experiment were almost identical in physical appearance. Wheat, barley, and corn differ in their rates of DM degradation in the rumen (Herrera-Saldana et al., 1990; Moate et al., 2016), which in turn can affect ruminal pH (Herrera-Saldana et al., 1990; Yang et al., 2001), a factor known to be related to methane emissions (Russell, 1998; Hünerberg et al., 2015). In this experiment, the cows fed the WHT diet produced at least 3 kg/d less ECM than cows fed the other diets; this equates to approximately 9.4 MJ/d less energy secreted in milk. In addition, the cows fed the WHT diet produced at least 146 g/d less methane than cows fed the other diets; this is equivalent to approximately 8.1 MJ/d of less energy lost as methane. This occurred despite the fact that predicted daily methane emissions were similar for all diets. The fate of the spared milk and methane energy is not clear because a total energy balance was not measured. We speculate that some of the spared energy may have been partitioned to BW gain, as cows fed a diet containing wheat have been reported to gain more BW than cows fed a similar diet containing corn (Tommervik and Waldern, 1969). However, during this short-term experiment, we did not detect any significant differences in BW change associated with dietary treatments.

The implications of this and other research are that if dairy cows are fed diets containing a high proportion of wheat, their methane emissions are likely to be considerably less than those from cows fed diets with more than 70% forage (Charmley et al., 2016) or diets with a high proportion of barley or corn. However, the reduced methane emissions from diets supplemented with wheat come at the potential cost of reduced production of ECM. In Australia, wheat commonly is fed to dairy cows (Moate et al., 2016) because it is readily available as a stock feed and the price of wheat is generally approximately two-thirds the price of corn. However, dairy farmers are paid on the basis of the amounts of milk fat and protein produced and are unlikely to include wheat in diets at proportions that induce milk fat depression. Thus, further research is required to elucidate milk production and methane production responses when different proportions of wheat are included with different forages in dairy cow diets. Martin et al. (2010) pointed out that various nutritional strategies for methane mitigation vary in their persistency. The duration of the current experiment was 5 wk, and additional research will be required to determine whether the methane inhibitory effect from feeding wheat persists in the long term.

CONCLUSIONS

In comparison with feeding dairy cows diets containing a high proportion of corn or barley, feeding a diet containing a high proportion of wheat had a specific inhibitory effect on methane emissions, methane yields, and methane intensities but was associated with reduced milk fat concentration and reduced production of milk fat and ECM. Importantly, the current research identified that the daily minimum pH was the pH metric most strongly related to methane yield.

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