Influence of proportion of wheat in a pasture-based diet on milk yield, methane emissions, methane yield, and ruminal protozoa of dairy cows

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ABSTRACT

Wheat is the most common concentrate fed to grazing dairy cows in Australia, but no studies have examined the effects of wheat proportion in a pasture-based diet on milk production and methane emissions. In this 47-d experiment, 32 Holstein dairy cows were offered 1 of 4 diets during d 1 to 36. Cows in each of the dietary treatment groups were individually offered no wheat (W0) or wheat at 3 kg of dry matter (DM)/d (W3), 6 kg of DM/d (W6), or 9 kg of DM/d (W9). The remainder of the diet was 2.2 kg of DM of concentrate mix and freshly harvested perennial ryegrass (*Lolium perenne*) such that all individual cows were offered a total diet of approximately 20.2 kg of DM/d. From d 37 to 47 the diets of cows receiving treatments W0 and W3 remained unchanged, but cows in treatments W6 and W9 received the W3 diet. Individual cow feed intakes, milk yields, milk compositions, and methane emissions were measured for d 31 to 35 (period 1) and d 45 to 47 (period 2). During period 1, the mean intakes of cows offered the W0, W3, W6, and W9 diets were 19.2, 20.4, 20.2, and 19.8 kg of DM/d. Diet caused differences in energy-corrected milk, and means for W0, W3, W6, and W9 were 29.5, 32.4, 33.0, and 32.9 kg/d, respectively. Milk fat percentage differed with respective means of 3.93, 3.94, 3.69, and 3.17. Diets also caused differences in methane emissions, with means for W0, W3, W6, and W9 of 440, 431, 414, and 319 g/d. During period 1, the cows fed the W9 diet produced less methane and had lower methane yields (g/kg of DMI) and intensities (g/kg of energy-corrected milk) than cows fed the W3 diet. However, in period 2 when the wheat intake of cows in the W9 treatment was reduced to the same level as in the W3 treatment, their methane emissions, yields, and intensities were similar to those offered the

W3 treatment, yet protozoa numbers in ruminal fluid were still much lower than those in cows offered the W3 treatment. Our research shows that for diets based on perennial ryegrass and crushed wheat, only the diet containing more than 30% crushed wheat resulted in substantially depressed milk fat concentration and reduced methane emissions, methane yield, and methane intensity. Thus, although feeding a diet with a high proportion of wheat can cause substantial methane mitigation, it can come at the cost of depression in milk fat concentration.

Key words: greenhouse gas, dose response

INTRODUCTION

Emissions of greenhouse gases from livestock account for 14.5% of global anthropogenic emissions (Gerber et al., 2013). Clear imperatives exist for developing practical strategies that will lead to a reduction in these emissions, and the inclusion of concentrates in the diets of dairy cows could play a role in reducing emissions of methane, a potent greenhouse gas (Beauchemin et al., 2008).

When cows were fed a diet of alfalfa hay with high proportions (>30% on a DM basis) of wheat grain (*Triticum aestivum*), production responses have usually manifested as an increase in milk volume; no change in concentration of milk protein; a reduction in concentration of milk fat; an increase in yield of milk protein; and either a decrease, no change, or a slight increase in yield of milk fat (Moate et al., 2017b, 2018, 2019). Several studies have measured milk production responses to cereal grain supplements fed to grazing dairy cows (Robaina et al., 1998; Walker et al., 2001; Leddin et al., 2010) or when cows fed a basal diet of harvested pasture were also fed some cereal grains (Williams et al., 2013). However, as far as we can ascertain, no studies have been published that has reported milk production responses when cows on a basal diet of perennial ryegrass were fed different amounts of wheat as a partial substitute for the pasture. The ME concentration of wheat grain and spring-harvested perennial ryegrass

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are generally approximately 12 to 13 and 10 to 12 MJ/kg of DM, respectively (Williams et al., 2013; Rugoho et al., 2017). Thus, substituting the pasture with grain of higher energy concentration should theoretically increase milk production. However, given previous findings when wheat was fed in an alfalfa-based diet (Moate et al., 2017b, 2018), the likely effects on milk fat concentration are less certain.

Methane yield (g/kg of DMI) has been reported to be lower for cows fed an alfalfa-based diet containing wheat grain than for cows fed a similar diet containing corn (Moate et al., 2017b, 2018). However, the effect has not been confirmed in lactating cows fed a perennial ryegrass-based diet.

Several dietary interventions have been shown to result in decreased methane emissions and methane yields, but the antimethanogenic effect may be transitory (Guan et al., 2006; Klop et al., 2017; Moate et al., 2018). In a frequently cited study, Weimer et al. (2010) swapped the total ruminal contents of 2 individual cows and found that rumen microbial populations took between 14 and 63 d to fully return to populations that resembled the pre-swap populations. The findings of Weimer et al. (2010) with respect to ruminal microbial populations and the findings of Guan et al. (2006), Klop et al. (2017), and Moate et al. (2017b) suggest that ruminal microbial populations in general and methanogen populations in particular do not respond instantaneously to dietary interventions. Therefore, we propose that a substantial carryover effect could exist against methanogenesis after cessation of feeding a diet containing a high proportion of a wheat-based concentrate. The magnitude and duration of a wheat-induced, carryover inhibitory effect against methanogenesis has not been previously researched.

Ruminal protozoa are known to harbor methanogens, and nutritional options to decrease ruminal populations of protozoa have been proposed as a strategy to mitigate enteric methane emissions (Hegarty, 1999). Previous research has reported that when cows were fed diets containing wheat instead of corn, numbers of protozoa in ruminal fluid are substantially reduced and methane emissions and yield are lower, but no evidence exists that the reduced methane emissions in wheat-fed cows are due to low numbers of protozoa in ruminal fluid. (Moate et al., 2017b, 2019).

The aim of this research was to determine the response of lactating dairy cows to increasing proportions of wheat in a perennial ryegrass-based diet. We hypothesized that (1) the yield of ECM would be positively related to the proportion of wheat in the diet; (2) methane emissions and methane yield would decrease with increasing proportion of wheat in the diet; (3) if

the proportion of wheat were suddenly reduced from high to low, a carryover effect would exist on methane yield; and (4) across treatments and periods, the number of protozoa in ruminal fluid would be positively associated with methane yield.

MATERIALS AND METHODS

The experiment was conducted at Agriculture Victoria Research, Ellinbank, Victoria, Australia (38°14'S, 145°56'E) and was conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, 2004). Animal use was approved by the Animal Ethics Committee of the Department of Jobs, Precincts and Regions, Victoria.

Cows, Diets, Feeding, and Management

Four dietary treatments were randomized to 32 lactating, multiparous Holstein-Friesian cows. Their BW was 528 ± 35.9 kg (mean $\pm$ SD), DIM was 40 ± 12.4 , and age was 4.0 ± 1.20 yr. Treatment groups were balanced for cow age, BW, and DIM using GenStat 18 software (VSN International, 2016), Procedure COVDESIGN according to the method of Harville (1974). The 4 dietary treatments, each of which was applied individually to 8 cows, were as follows: **W0** = 0.0 kg of DM/d of crushed wheat grain and ~18 kg of DM/d of pasture; **W3** = 3.0 kg of DM/d of crushed wheat grain and ~15 kg of DM/d of pasture; **W6** = 6.0 kg of DM/d of crushed wheat grain and ~12 kg of DM/d of pasture; **W9** = 9.0 kg of DM/d of crushed wheat grain and ~9 kg of DM/d of pasture. Pasture was predominantly perennial ryegrass (*Lolium perenne*). The pasture was harvested twice daily (0600 and 1400 h) at a preflowering stage and a pasture mass of approximately 2,500 kg/ha using a front-mount mower (Novacat 356F; Pöttinger, Grieskirchen, Austria), a tractor (Arion 530; Claas, Harsewinkel, Germany), and a loader wagon (Quantum 3500P; Claas). All diets were further supplemented with 2.2 kg of DM/cow per day of a concentrate mix comprising 455 g/kg cold-pressed canola meal, 455 g/kg cracked corn grain, and 90 g/kg minerals. The canola meal was included in the concentrate mix to ensure that the diets met the cows' dietary requirements for protein (NRC 2001). Cows were milked twice daily at ~0600 and ~1500 h.

During the diet adjustment phase (d 1 to 21), cows offered the W3, W6, and W9 treatments were individually fed half their daily wheat allocation after each milking. The cows in the W0 group waited on a loafing pad while the other cows were eating their grain.

When cows had finished eating their grain, all cows were moved to a paddock to graze pasture *ad libitum* in their respective treatment groups.

During the intensive measurement phase of the experiment (d 22 to 47), all cows were moved to the animal house after each milking, where they were individually fed half their daily grain ration first and then half their daily allocation of harvested pasture. The grain-concentrate was individually offered to cows before offering the harvested pasture. This was to mimic the pattern of feeding of supplemental concentrates that generally occurs in grazing-based dairy systems in which cows are fed concentrates during milking and then they are given access to pasture. Cows were offered their pasture once they had finished eating their wheat or after 30 min, whichever occurred first. Cows were allowed a total of 4 h of access to feed in the morning (0700 to 1100 h) and 4 h in the afternoon (1600 to 2000 h), after which time cows were released from the animal house onto a wood-chip loafing pad. From d 36 onward, all cows received 2.2 kg of DM/cow per day of a concentrate mix, and cows on the W3, W6, and W9 treatments were offered 3.0 kg of DM/d of crushed wheat and ~15 kg of DM/d of pasture.

Measurements

Samples of concentrates offered and refused were collected daily for DM determination by drying at 105°C for 24 h. During the intensive measurement phase of the experiment, samples of pasture offered and refused each day were collected and DM concentration was determined each day.

Representative samples of crushed wheat, cracked corn grain, and cold-pressed canola offered were collected at each feeding, frozen, and bulked over the duration of the experiment. Representative daily samples of harvested pasture from period 1 (d 31 to 35) and from period 2 (d 45 to 47) were collected, frozen, and bulked over the duration of each period. Frozen samples were subsequently freeze-dried, ground to pass through a 0.5-mm sieve, and then analyzed by Dairy One Forage Laboratory (Ithaca, NY) using their methods (Dairy One, 2019). Calculation were for CP (AOAC International, 2000; method 990.06), soluble protein (as % of CP; Roe and Sniffen, 1990), ADF and NDF (Van Soest et al., 1991; without sodium sulfite, using an Ankom Fiber Analyzer, Ankom Technology, Fairport, NY, and with 100 μ L/0.50 g of sample heat-stable α -amylase, no. A3306, Sigma Chemical Co., St. Louis, MO), lignin (AOAC International, 2000; method 973.18), starch (Smith, 1969), ash (AOAC International, 2000; method

942.05), crude fat (ether extract; AOAC International, 2000; method 2003.05), and minerals (Na, K, Ca, Mg, P, Cu, and S; Sirois et al., 1994) and Cl (Cantliffe et al., 1970), with NFC, TDN, and DCAD calculated according to the procedures of Dairy One (2019). Gross energy (GE; MJ/kg of DM) was calculated based on the proportions of ash (*A*) and fat (*F*) in the feeds using an equation $[GE \text{ (MJ)} = 38.5 \times F + 19.3 \times (1 - A - F)]$ we derived from data presented by Klop et al. (2017), and ME was calculated according to the NRC (2001).

Milk yields of individual cows were measured morning and afternoon for the duration of the experiment using a DeLaval ALPRO milk metering system (MM25; DeLaval International, Tumba, Sweden). During period 1, milk samples for composition analysis were collected from both the morning and afternoon milkings on every second day, and during period 2, milk samples were collected daily. Fat, protein, and lactose in milk were measured by a near-infrared milk analyzer (model 2000, Bentley Instruments, Chaska, MN). Energy-corrected milk, standardized to 4.0% fat and 3.3% protein, was calculated using the following equation (Tyrrell and Reid, 1965):

$$\text{ECM (kg/d)} = [\text{milk yield (kg)} \\ \times (376 \times \text{fat \%} + 209 \times \text{protein \%} + 948)]/3,138.$$

Somatic cells were counted by a Fossomatic SC300 cell counter (Foss, Hillerød, Denmark). Milk fatty acids were determined by first extracting milk fat from fresh samples using a method based on the Rose-Gottlieb gravimetric method (International Dairy Federation, 1987). Samples of extracted milk fat were stored at -18°C until they were analyzed for fatty acid composition. Following methylation with sodium methoxide (Slover and Lanza, 1979), the concentrations of fatty acid methyl esters in milk fat were determined by GC by the method described by Agnew et al. (2019). Peak identification was by retention time comparisons with fatty acid standards (189–19, 37 FAME 4:0–24:0; Sigma Chemical Co.) included every 15 samples.

Methane Emissions

Methane emissions (g/d) from each cow were sampled on d 31 to 35 (period 1) and on d 45 to 47 (period 2) using a sulfur hexafluoride (SF_6) tracer technique similar to those described by Berndt et al. (2014) and Deighton et al. (2014). In this experiment, the permeation tubes were manufactured in November 2011 by the National Institute of Water and Atmospheric Research, New Zealand, and were filled with about 2.4

g of SF₆. The release rate of SF₆ was determined over the 4-wk period before use by storing the tubes in a dry incubator set at 39°C and weighing each permeation tube twice each week. The release rate of SF₆ was 7.2 ± 0.41 mg/d (average ± SD) and ranged from 6.6 to 8.0 mg/d. The permeation tubes containing SF₆ were placed in the rumen of the cows per os 1 wk before the first measurements of CH₄ were performed. A feature of the SF₆ technique used in this experiment is that we used orifice plate air flow regulators that collect a gas sample at a constant rate over 24 h, and therefore, the collected gas sample reflects the daily methane emissions of each animal. This was to ensure that we did not introduce a bias into the technique, as may be the case if we had used capillary flow regulators (Deighton et al., 2014).

Stainless steel canisters with an 800-mL capacity and a sampling rate of ~0.2 mL/min were used to continuously sample eructated gases. Sentinel canisters (Williams et al., 2011) were used to sample background gases indoors (~0.8 mL/min), 4 canisters were used with one on each fence of the loafing area to sample background gases outdoors (~0.3 mL/min), and orifice plates (Lenox Laser, Glen Arm, MD) were used to control gas sampling rate (Deighton et al., 2014). Canisters were exchanged once per day. Overall background concentrations for individual cows were the time-weighted average of their individual indoor and the group outdoor concentrations (Berndt et al., 2014). Residual vacuum in all used canisters was recorded before addition of sufficient N₂ (999.99 g/kg) to bring the remaining vacuum to ~10 kPa. Cows spent approximately 8 h/d in a well-ventilated (open on 3 sides) animal house as described by Williams et al. (2011) and approximately 16 h/d outdoors, on a wood-chip loafing pad.

Analysis of collected gas samples was done by GC (Williams et al., 2011). After analysis, the dilution by N₂ was reversed mathematically to account for the physical dilution of the samples with N₂ gas before analysis (Equation 1):

$$[G_S] = [(101 - \tau_f)/(\tau_e - \tau_s)] \times [G_A], \quad [1]$$

where $[G_S]$ is the calculated concentration (ppm for CH₄, ppt for SF₆) of the gas as sampled, 101 is the average atmospheric pressure (kPa), τ_f (kPa) is the final vacuum in the canister after the addition of nitrogen, τ_s (kPa) is the vacuum in the canister after the sample is collected, τ_e (kPa) is the vacuum in the evacuated canister before use, and $[G_A]$ (ppm for CH₄, ppt for SF₆) is the gas concentration in the sample presented to the GC. Methane emissions for each cow on each day were calculated using equation 2 of Williams et al. (2011).

Ruminal Fermentation and Protozoa

A single sample of ruminal fluid (~400 mL) was collected from each cow per os at 4 h after the start of feeding, on the last day of period 1 (d 33), and again on the last day of period 2 (d 47). This 4-h delay from the start of feeding was chosen to coincide with the expected nadir in ruminal pH (Greenwood et al., 2014). An oro-ruminal sampling probe, similar to the one described by Geishauser (1993), and a vacuum pump were used to collect samples (Moate et al., 2014). The pH of the ruminal fluid was immediately measured using a Mettler-Toledo FG2 pH meter (Schwerzenbach, Switzerland).

Ciliate protozoa were counted from a 0.5-mL subsample of ruminal fluid transferred to a 12-mL plastic vial; diluted with 4.5 mL of a stain solution (Ogimoto and Imai, 1981) containing 10% formalin, 0.8% saline, and 0.06% methyl green; and then stored at ambient conditions. Counting was done in a Mod-Fuchs-Rosenthal counting chamber. Protozoa in the family *Ophryoscolecidae* were counted in the separate genera of *Entodinium* and of *Epidinium*, and protozoa from the family *Isotrichidae* were counted in the separate genera of *Isotricha* and *Dasytricha*.

Volatile fatty acids in ruminal fluid were measured in a 5-mL sample of ruminal fluid to which 2 drops of concentrated H₂SO₄ were added immediately before storage at -18°C. Analysis was by capillary GC as described in Supelco Bulletin 749D (Supelco Inc., 1975). The D-lactate in ruminal fluid was measured by a Boehringer Mannheim kit (Cat No. 11 112 821 035; Mannheim, Germany) on an Olympus AU400 Autoanalyzer (Tokyo, Japan) after deproteinization with perchloric acid. Ammonia-N concentrations were determined in 5-mL samples of acidified ruminal fluid (Moate et al., 2017b).

Statistical Analyses

Where data were repeated measurements, they were initially averaged within each period for each cow. These, and all other data, were analyzed using mixed models (REML) in GenStat 18 software (VSN International, Hemel Hempstead, UK). For variables (DMI, milk production and composition, and methane) measured in period 1 (d 31 to 35) only, the fixed effects included a factor for wheat proportion, and the random effects were simply the experimental unit, cow. For variables with data measured in both periods 1 and 2 (d 45 to 47), the mixed model included factorial fixed effects for wheat proportion by period, and the random effects were cow and period-within-cow. Note that the protozoal data were from single spot samples

Table 1. Composition of dietary ingredients (g/kg of DM unless otherwise noted)

Parameter	Crushed wheat	Concentrate mix ¹	Pasture period 1	Pasture period 2
DM (g/kg)	930	934	151 ± 25.4 (SD)	179 ± 20.7 (SD)
CP	118	242	184	167
Soluble protein (% CP)	31	31	43	43
ADF	49	163	289	296
NDF	117	227	522	526
Lignin	13	39	33	30
NFC	749	412	170	186
Starch	658	316	2	2
Ash	20	126	100	98
TDN	845	830	710	710
GE ² (MJ/kg of DM)	19.3	18.2	18.4	18.3
ME (MJ/kg of DM)	14.1	14.6	10.7	10.2
Potassium	3.9	8.2	30.4	34.4
Calcium		3.3	4.2	4.6
Magnesium	1.3	3.4	2.8	2.6
Phosphorus	3.6	6.8	3.9	3.6
Sulfur	1.3	3.9	2.5	2.2
Crude fat	20	71	53	51
Fatty acids (as % of total fatty acids)				
C14:0	0.0	0.1	1.0	—
C16:0	19.3	7.4	20.3	—
C16:1	0.0	0.4	0.3	—
C18:0	1.0	1.8	1.7	—
C18:1 <i>cis</i> -9	15.2	51.8	1.9	—
C18:1 <i>cis</i> -11	1.0	4.8	0.7	—
C18:2n-6	59.9	28.1	11.2	—
C18:3n-3	3.6	5.5	62.4	—
Other	0.0	0	0.5	—

¹The concentrate mix comprised (on a DM basis) 45.5% cold-pressed canola meal, 45.5% cracked corn grain, and 9.0% minerals.

²GE = gross energy.

measured on the last day of period 1 (d 35) and the last day of period 2 (d 47). Linear and quadratic orthogonal polynomial contrasts for wheat proportion were applied to the estimated cow-by-period means and tested by *t*-tests derived from the variance-covariance matrix following the mixed model analysis. Similarly, pairwise comparisons between means, as well as interactions of polynomial contrasts with periods, were computed using the estimated means and their variance-covariance matrix. *F*-tests for differences between diet treatments within each period were derived by including additive fixed effects for period, wheat proportion in period 1, and for wheat proportion in period 2, and by dropping each of the wheat proportion factors in turn from the full model. The 2 factors for wheat proportion within period were defined with 5 levels: the 4 proportion treatments and a level for the other period.

All analyses included covariates DMI, age, and BW, which were used initially to balance treatment groups, and these were always the first fixed effects to be entered into the model. Distributional assumptions of normality and constant variance were examined graphically using histograms and normal quantile plots of residuals, and plots of residuals against fitted values. Somatic cell counts were transformed as $\log_{10}(\text{SCC})$,

and protozoa counts were transformed as $\log_{10}(\text{count/mL})$. Total protozoa count means (geometric means) were back-transformed from this log-transformed scale.

RESULTS

Feed Composition and Feed Intake

The DM concentration of harvested pasture ranged from 93 to 208 g of DM/kg. This variation in DM was mainly due to day-to-day differences in weather, with instances of rain leading to decreases in DM concentration of harvested pasture. The chemical compositions of the dietary ingredients are shown in Table 1.

Cows generally ate all of the concentrate offered and at least 95% of the pasture offered. The mean DMI of pasture, crushed wheat, and concentrate for each of the dietary treatments during period 1 are shown in Table 2. Targeted total mean DMI for cows in all treatments was 20.2 kg of DM/d. However, day-to-day variation in the DM concentration of harvested pastures resulted in pasture offered at each feed not always matching the targeted quantities. Also, some cows in W6 and W9 dietary treatments did not consume all of the offered wheat. Mean total DMI for cows in all treatments

Table 2. Influence of diet on feed intake, milk production, and milk composition during period 1

Item	Diet ¹				SEM	Contrast (<i>P</i> -value)		
	W0	W3	W6	W9		Treatment	Linear	Quadratic
Number of cows	8	8	8	8				
Feed intake (kg of DM/d)								
Pasture	16.9	15.4	12.2	8.8	—	—	—	—
Crushed wheat	0	3.0	6.0	9.0	—	—	—	—
Concentrate mix ²	2.1	2.1	2.1	2.1	—	—	—	—
Total DMI	19.0	20.5	20.3	19.9	0.35	<0.001	0.042	<0.001
Total CP	3.60	3.68	3.43	3.17	0.065	<0.001	<0.001	<0.001
Total NDF	9.29	8.85	7.51	6.10	0.18	<0.001	<0.001	<0.001
Total starch	0.66	2.70	4.62	6.59	0.013	<0.001	<0.001	0.003
Total fat	1.04	1.02	0.91	0.79	0.019	<0.001	<0.001	<0.001
Gross energy intake (MJ/d)	348	379	377	373	6.6	<0.001	0.002	<0.001
ME intake (MJ/d)	210	237	244	251	3.8	<0.001	<0.001	<0.001
Wheat (%)	0	15.1	29.8	45.6	0.45	<0.001	<0.001	0.207
Production (kg/d)								
Milk	30.4	32.8	34.5	36.7	0.95	<0.001	<0.001	0.959
ECM yield	29.5	32.4	33.0	32.9	0.82	0.015	0.005	0.101
Fat	1.18	1.30	1.27	1.17	0.045	0.168	0.686	0.033
Protein	0.91	1.04	1.12	1.19	0.025	<0.001	<0.001	0.369
Lactose	1.53	1.67	1.79	1.96	0.046	<0.001	<0.001	0.699
FP ³	2.09	2.34	2.38	2.36	0.058	0.005	0.002	0.041
Milk composition								
Fat (%)	3.93	3.94	3.69	3.17	0.141	0.001	<0.001	0.076
Protein (%)	3.01	3.16	3.24	3.24	0.041	0.001	<0.001	0.088
Lactose (%)	5.05	5.08	5.17	5.31	0.055	0.006	<0.001	0.354
Log ₁₀ SCC (cells/μL)	1.55	1.84	1.67	1.45	0.204	0.645	0.579	0.288

¹W0 = no wheat; W3 = 3 kg of DM of wheat per day; W6 = 6 kg of DM of wheat per day; and W9 = 9 kg of DM of wheat per day. All diets included 2.2 kg of DM/d of concentrate mix and freshly harvested perennial ryegrass (*Lolium perenne*) such that all individual cows were offered a total diet of approximately 20.2 kg of DM/d.

²The concentrate mix comprised (on a DM basis) 45.5% cold-pressed canola meal, 45.5% cracked corn grain, and 9.0% minerals.

³FP = yield of milk fat plus milk protein.

were similar, ranging from 18.9 to 20.5 kg/d. Intakes of total CP and of NDF decreased as pasture intake decreased, whereas intake of starch was directly related to the intake of wheat. The average dietary concentrations of CP were 190, 180, 170, and 160 g/kg of DMI; NDF concentrations were 492, 432, 372, and 308 g/kg of DMI; starch concentrations were 35, 132, 229, and 333 g/kg of DMI; and fat concentrations were 55, 50, 45, and 40 g/kg of DMI for the W0, W3, W6, and W9 diets, respectively.

Milk Yield and Composition (Period 1)

During period 1, cows consuming the W3, W6, and W9 diets all produced substantially more milk than cows consuming the W0 diet (Table 2) and a positive, linear ($P < 0.001$) relationship existed between dietary wheat proportion and milk yield. The ECM was positively, linearly ($P = 0.005$) related to wheat proportion, and a trend ($P = 0.101$) existed for a quadratic relationship between ECM and wheat proportion (Table 2). Yield of milk fat and wheat proportion had a quadratic relationship ($P = 0.033$). Yields of milk

protein and milk lactose were positively, linearly ($P < 0.001$) related to wheat proportion. Milk fat concentration was negatively, linearly ($P < 0.001$) related to wheat proportion, whereas concentrations of protein and lactose in milk were both positively, linearly ($P < 0.001$) related to wheat proportion.

Mean concentrations of milk fatty acids are shown in Table 3. Of the 25 major individual fatty acids, dietary wheat proportion and concentrations of 16 individual fatty acids had linear ($P < 0.05$) relationships, and dietary wheat proportion and concentrations of 14 individual milk fatty acids had quadratic ($P < 0.05$) relationships. Quadratic ($P < 0.01$) relationships existed between dietary wheat proportion and the concentrations of total SFA, total UFA, and total MUFA.

Methane Emissions and Protozoa (Periods 1 and 2)

During period 1, linear contrasts existed for methane emission, methane yield (g/kg of DMI), and methane intensity (g/kg of ECM), all of which decreased with increasing rates of wheat. Furthermore, for methane emissions a quadratic contrast existed ($P = 0.045$),

Table 3. Means of individual fatty acids (g/100 g of total fatty acids unless otherwise stated) and classes of fatty acids in milk fat from cows fed diets containing different concentrations of wheat during period 1

Item	Diet ²				SEM	Contrast (<i>P</i> -value)		
	W0	W3	W6	W9		Treatment	Linear	Quadratic
Number of cows	8	8	8	8				
Fatty acid ¹								
C4:0	4.08	3.76	3.84	2.95	0.196	0.001	<0.001	0.096
C6:0	2.35	2.42	2.45	1.88	0.115	0.004	0.009	0.008
C8:0	1.30	1.50	1.51	1.21	0.074	0.020	0.439	0.003
C10:0	2.80	3.56	3.61	3.19	0.179	0.017	0.123	0.004
C10:1	0.30	0.28	0.30	0.30	0.021	0.942	0.812	0.896
C12:0	3.01	3.93	4.05	3.92	0.205	0.005	0.003	0.025
C14:0	11.5	12.2	13.0	12.6	0.377	0.044	0.016	0.153
C14:1	0.93	0.76	0.85	1.26	0.089	0.003	0.007	0.004
iso C15:0	0.23	0.23	0.20	0.19	0.010	0.019	0.003	0.924
anteiso C15:0	0.51	0.51	0.45	0.47	0.031	0.353	0.176	0.588
C15:0	0.94	0.94	1.04	1.66	0.129	<0.001	<0.001	0.020
C16:0	29.5	29.5	29.2	29.1	1.03	0.987	0.726	0.986
C16:1	1.39	1.41	1.54	2.03	0.081	<0.001	<0.001	0.132
anteiso C17:0	0.45	0.48	0.46	0.49	0.027	0.783	0.517	0.795
C17:0	0.47	0.46	0.49	0.65	0.034	0.001	<0.001	0.019
C17:1	0.22	0.21	0.24	0.36	0.018	<0.001	<0.001	0.001
C18:0	11.1	10.8	9.6	7.0	0.59	<0.001	<0.001	0.066
C18:1 <i>trans</i> -9	0.27	0.28	0.24	0.25	0.014	0.199	0.118	0.816
C18:1 <i>trans</i> -10	0.21	0.23	0.29	0.70	0.057	<0.001	<0.001	0.002
C18:1 <i>cis</i> -9	19.3	17.7	18.0	20.3	0.716	0.060	0.277	0.013
C18:1 <i>cis</i> -11	0.35	0.40	0.49	0.76	0.040	<0.001	<0.001	0.009
C18:2n-6 <i>cis</i>	0.86	0.95	1.14	1.53	0.062	<0.001	<0.001	0.022
C18:2 <i>cis</i> -9, <i>trans</i> -11	0.87	0.82	0.73	0.60	0.113	0.327	0.073	0.751
C18:3n-3	0.56	0.49	0.41	0.38	0.029	<0.001	<0.001	0.474
C20:0	0.11	0.12	0.11	0.08	0.005	<0.001	<0.001	0.003
Total C18	35.8	33.8	32.7	32.6	1.15	0.177	0.040	0.428
Total de novo ³	42.7	44.8	45.9	44.2	0.948	0.134	0.181	0.055
Total SFA	68.4	70.3	70.0	65.4	1.07	0.011	0.047	0.005
Total UFA	31.6	29.7	30.0	34.6	1.07	0.011	0.047	0.005
Total MUFA	25.2	23.5	23.8	27.1	0.86	0.024	0.104	0.008
Total PUFA	2.29	2.26	2.28	2.51	0.150	0.570	0.290	0.375

¹Expressed as number of carbon atoms.²W0 = no wheat; W3 = 3 kg of DM of wheat per day; W6 = 6 kg of DM of wheat per day; and W9 = 9 kg of DM of wheat per day. All diets included 2.2 kg of DM/d of concentrate mix and freshly harvested perennial ryegrass (*Lolium perenne*) such that all individual cows were offered a total diet of approximately 20.2 kg of DM/d.³Total de novo = sum(C4:0 to C15:0) + 0.5 × (C16:0).

with no difference in methane emission for W0, W3, and W6, but methane emissions for W9 were less than those for the other treatments. For each of the methane variables, both linear and quadratic contrasts interacted with period ($P < 0.01$). For each methane variable, the rate of decline was greater in period 1 than in period 2 (Table 4). In particular, between-period differences were substantial ($P < 0.001$) in methane emission, yield, and intensity for cows offered the W9 diet.

During period 1, the total number of protozoa in ruminal fluid and the wheat proportion had a negative linear relationship ($P < 0.001$). This mainly occurred because of the negative relationships between the wheat proportion and the numbers of *Entodinium* spp. and *Dasytricha* spp. During period 2, cows in treatment W9 had smaller ($P = 0.002$) numbers of *Entodinium* spp. and smaller ($P = 0.005$) numbers of total protozoa

than cows offered the other treatments. For cows offered treatment W9, despite substantial ($P < 0.001$) between-period differences in methane emission, yield, and intensity, periods did not differ ($P > 0.05$) in the total count of protozoa, and differences between periods in the counts of *Entodinium* spp. and *Isotricha* spp. were small.

Rumen Fermentation (Period 1)

The pH in ruminal fluid was not influenced by the wheat proportion, but a negative linear relationship ($P < 0.001$) existed between the wheat proportion and the NH_3 concentration in ruminal fluid (Table 5). Wheat proportion did not influence the total concentration of VFA in ruminal fluid, but generally linear and quadratic relationships existed between wheat proportion

and the molar proportions of most of the individual VFA. Wheat proportion and the acetate-to-propionate ratio in ruminal fluid had a negative linear relationship ($P < 0.001$). A positive linear relationship ($P < 0.001$) existed between the proportion of wheat in the diet and the concentration of D-lactate in ruminal fluid.

DISCUSSION

The dairy industries in Australia, Chile, Ireland, and New Zealand are based on the grazing of perennial ryegrass pastures. To date, much research has been published on strategies for methane mitigation from dairy cows fed TMR diets, but much less research has targeted methane mitigation in dairy systems based on grazing of pastures, especially perennial ryegrass (Martin et al., 2010; Knapp et al., 2014). The current research focused on milk production and methane responses when dairy cows were offered a perennial ryegrass-dominant diet substituted with different amounts of crushed wheat. This area of research is particularly relevant to dairy farmers in Australia, because Australia is one of the

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Table 4. Influence of diet on feed intake and CH₄ emissions of cows during the last 4 d of period 1 and during the last 2 d of period 2

Item	Diet ¹				SEM	Contrast (<i>P</i> -value)		
	W0	W3	W6	W9		Treatment	Linear	Quadratic
Period 1								
Number of cows ²	8	7	8	8				
Feed intake (kg/d)								
Pasture	16.9	15.6	12.2	8.8				
Crushed wheat	0	2.9	6.0	9.0				
Concentrate mix ³	2.1	2.1	2.1	2.1				
Total DMI	19.0	20.6	20.3	19.9				
CH ₄ (g/d)	440	431	414	319	20.3	<0.001	<0.001	0.045
CH ₄ (g/kg of DMI)	23.6	21.3	21.1	16.4	0.91	<0.001	<0.001	0.196
CH ₄ (g/kg of ECM)	15.3	13.5	12.5	9.0	0.99	<0.001	<0.001	0.308
CH ₄ (% of GEI ⁴)	7.03	6.28	6.13	4.82	0.27	<0.001	<0.001	0.201
Protozoa ⁵ [log ₁₀ (count/mL)]								
<i>Entodinium</i> spp.	5.79	5.73	5.22	4.64	0.264	0.011	0.002	0.337
<i>Epidinium</i> spp.	4.37	4.08	4.11	3.75	0.143	0.053	0.011	0.794
<i>Isotricha</i> spp.	4.09	4.04	4.10	3.89	0.104	0.373	0.206	0.416
<i>Dasytricha</i> spp.	4.50	4.46	4.09	3.87	0.130	0.002	<0.001	0.496
Other	3.96	3.88	3.80	3.84	0.058	0.260	0.103	0.286
Total	5.86	5.81	5.31	4.70	0.245	0.005	<0.001	0.268
Total (count/μL)	710	644	196	45	—	—	—	—
Period 2								
Feed intake (kg/d)								
Pasture	17.0	15.8	14.9	14.5				
Crushed wheat	0	3.0	3.0	3.0				
Concentrate mix	2.1	2.1	2.1	2.1				
Total DMI	19.1	20.9	20.0	19.6				
CH ₄ (g/d)	462	442	442	422	20.3	0.578	0.191	0.996
CH ₄ (g/kg of DMI)	25.0	21.2	22.3	21.7	0.91	0.042	0.039	0.100
CH ₄ (g/kg of ECM)	17.0	14.9	14.5	13.4	0.99	0.023	0.004	0.550
CH ₄ (% of GEI)	7.37	6.40	6.70	6.51	0.27	0.040	0.036	0.121
Protozoa ⁶ [log ₁₀ (count/mL)]								
<i>Entodinium</i> spp.	5.85	5.43	5.08	4.32	0.264	0.002	<0.001	0.522
<i>Epidinium</i> spp.	4.19	3.72	3.92	3.78	0.143	0.187	0.141	0.298
<i>Isotricha</i> spp.	3.82	4.08	3.97	4.12	0.094	0.174	0.078	0.614
<i>Dasytricha</i> spp.	4.53	4.17	4.13	4.04	0.130	0.056	0.013	0.309
Other	3.79	3.78	3.78	3.75	0.058	0.952	0.619	0.850
Total	5.89	5.46	5.13	4.60	0.245	0.005	<0.001	0.862
Total (count/μL)	778	279	129	34	—	—	—	—

¹W0 = no wheat; W3 = 3 kg of DM of wheat per day; W6 = 6 kg of DM of wheat per day; and W9 = 9 kg of DM of wheat per day. All diets included 2.2 kg of DM/d of concentrate mix and freshly harvested perennial ryegrass (*Lolium perenne*) such that all individual cows were offered a total diet of approximately 20.2 kg of DM/d. In period 2, treatments W3, W6, and W9 all received 3 kg of DM of wheat per day.

²Equipment failure prevented methane measurements on one cow in treatment W3, and this is why average DMI for this treatment group differs from that shown in Table 2.

³The concentrate mix comprised (on a DM basis) 45.5% cold-pressed canola meal, 45.5% cracked corn grain, and 9.0% minerals.

⁴GEI = gross energy intake.

⁵Ruminal fluid was collected 4 h after the start of feeding on the last day of period 1.

⁶Ruminal fluid was collected 4 h after the start of feeding on the last day of period 2.

Table 5. Rumen pH¹ and fermentation characteristics in dairy cows fed diets based on perennial ryegrass with different proportions of wheat during period 1

Item	Diet ²				SEM	Contrast (<i>P</i> -value)		
	W0	W3	W6	W9		Treatment	Linear	Quadratic
Number of cows	8	8	8	8				
pH	6.54	6.51	6.66	6.30	0.089	0.051	0.156	0.068
NH ₃ (mg/L)	198	137	123	55	13.5	<0.001	<0.001	0.564
Total VFA (mM)	94	91	91	100	6.5	0.677	0.493	0.318
Individual VFA (mol/100 mol)								
Acetic acid (A)	64.5	64.6	62.8	54.6	0.99	<0.001	<0.001	<0.001
Propionic acid (P)	20.9	20.3	21.9	32.9	1.38	<0.001	<0.001	<0.001
<i>iso</i> -Butyric acid	0.91	0.91	0.89	0.71	0.043	0.005	0.002	0.048
<i>n</i> -Butyric acid	11.2	11.7	11.5	8.2	0.644	0.001	0.002	0.006
<i>iso</i> -Valeric acid	1.39	1.51	1.54	1.23	0.091	0.083	0.254	0.024
<i>n</i> -Valeric acid	1.05	0.95	1.26	2.00	0.127	<0.001	<0.001	0.003
Caproic acid	0.07	0.02	0.09	0.35	0.046	<0.001	<0.001	0.003
A:P ratio	3.15	3.13	2.99	1.73	0.194	<0.001	<0.001	0.003
D-Lactate (μM)	11.9	26.1	27.4	38.6	4.83	0.004	<0.001	0.932

¹Ruminal fluid was collected 4 h after the start of feeding on the last day of period 1.

²W0 = no wheat; W3 = 3 kg of DM of wheat per day; W6 = 6 kg of DM of wheat per day; and W9 = 9 kg of DM of wheat per day. All diets included 2.2 kg of DM/d of concentrate mix and freshly harvested perennial ryegrass (*Lolium perenne*) such that all individual cows were offered a total diet of approximately 20.2 kg of DM/d.

few countries where the dairy industry is based on grazing of perennial ryegrass-dominant pastures with the addition of substantial amounts of grain, principally wheat, fed either as a supplement or a substitute for pasture (Moate et al., 2016). Wheat is generally the supplement of choice because it economically provides ME, the nutrient which is often limiting in pasture-based diets (Kolver and Muller, 1998).

The milk production and methane responses of cows offered basal diets of alfalfa hay and a grain component of either crushed corn grain or crushed wheat grain have been reported previously (Moate et al., 2017b, 2018, 2019). The grain portion of the diet in those experiments always constituted between 43.5 and 47.6% of DMI. Our experiment differs from those of Moate et al. (2017b, 2018, 2019) in that the basal diet was composed of freshly harvested perennial ryegrass, and the wheat component of the diet was offered at 0, 15, 30, or 45% of total DMI. Indeed, as far as we can ascertain, this is the first experiment to document milk production and methane responses to a pasture-based diet containing different proportions of wheat.

Yield of ECM increased linearly with increasing proportion of wheat in the diet of dairy cows, leading us to accept our first hypothesis. However, compared with the W0 diet, the W9 diet depressed milk fat concentration. This negative effect on milk fat was offset by increases in protein and lactose concentrations and an increase in milk volume, resulting in net increases in ECM. Depression of milk fat concentration has also been observed when cows grazing at pasture were fed a supplement of wheat (Stockdale, 2004) and when

wheat and corn feeding were compared (Moate et al., 2017b, 2018, 2019). Increases in ECM with increasing proportions of wheat in the diet have been reported for dairy cows fed a basal diet of Persian clover (Leddin et al., 2010) or ryegrass (Robaina et al., 1998; Wales et al., 2009; Auldist et al., 2013). Production of fat plus protein appeared to plateau at 6 kg of DM of wheat per day, and this is close to the average value of 5.7 kg of DM/d of concentrate fed to grazing dairy cows in Australia (Dharma et al., 2012).

The magnitude of milk yield responses from grazing dairy cows fed a concentrate feed supplement may not always reflect the ME supplied by the grain because the cows usually eat less pasture when they are offered grain (pasture substitution), and excreting the excess nitrogen often associated with pasture diets has an energy cost. The milk responses from our cows given the W9 diet were 0.7 kg of milk/kg of DM of wheat and 0.4 kg of ECM/kg of DM of wheat. This response is similar to the 0.75 kg of milk/kg of DM of concentrate reported for grazing cows given 5 kg of DM of concentrate per day (Robaina et al., 1998). This is expected given our substitution rate of 1 and the calculated substitution rate of 0.98 in the grazing experiment of Robaina et al. (1998). The energy cost to excrete excess urea produced by cows on pasture-only diets is 30 kJ of ME/g of nitrogen. This is associated with the high CP intakes from pasture-only diets or pasture diets supplemented with a low amount of cereal grain (Tyrrell et al., 1970). We note that the overall dietary CP concentrations in our W0 and W3 diets were 190 and 180 g/kg of DM, and these concentrations are above requirements rec-

ommended by the NRC (2001). Thus, milk production of the cows fed the W0 and W3 diets was affected by the energy cost of excreting excess nitrogen, whereas those fed the W9 diet required less energy to excrete nitrogen due to the substitution of pasture by grain.

Differences between the concentrations of individual fatty acids in the milk of cows offered the W0 and W9 diets were not large. This is despite 16 individual milk fatty acids being linearly related to the proportion of wheat in the diet in period 1. The concentrations of all the individual fatty acids measured in this experiment were within previously reported ranges (Stockdale et al., 2003; Moate et al., 2007). Furthermore, our findings on the trends in individual milk fatty acids with increasing proportions of wheat in the diet of dairy cows are similar to the findings of Wales et al. (2009), who measured milk fatty acids from cows that grazed perennial ryegrass pasture and fed 0, 2.6, or 4.9 kg of DM/d of a concentrate supplement composed of 65% rolled barley grain (*Hordeum vulgare*), 35% steam-flaked corn (*Zea mays*), and 5% molasses. The perennial ryegrass used in our experiment had a greater concentration of crude fat than did wheat, with linolenic acid (C18:3), an n-3 fatty acid regarded as being beneficial for health (Haug et al., 2007), constituting the majority of the fatty acids in the perennial ryegrass. Furthermore, C18:3 in milk fat is a preformed fatty acid that originates from the diet (Moate et al., 2007). Thus, in our experiment the proportion of C18:3 in milk fat was linearly, positively related to the proportion of grass in the diet. This relationship was also reported by Couvreur et al. (2006). Total SFA in milk were positively related to the proportion of grass in the diet in our work, but Couvreur et al. (2006) reported the opposite relationship. Both C15:0 and C17:0 were negatively, linearly related to the proportion of grass in the diet in our experiment, but again Couvreur et al. (2006) reported the opposite. This contrast in results may be due to differences in the diets used. We used combinations of grass and crushed wheat, whereas Couvreur et al. (2006) fed their cows combinations of grass and corn silage. Regardless of the reason for these differences, the fact that the direction of the correlations between the proportion of grass in the diet and the concentrations of either C15:0 or C17:0 was different in our experiment compared with that in the experiment of Couvreur et al. (2006) suggests that the concentrations of C15:0 and C17:0 in milk could not be used as reliable predictors of the proportion of grass in a diet. Overall, our findings in milk fatty acids from cows fed pasture supplemented with wheat are similar to previously reported findings when cows were fed alfalfa hay and wheat (Moate et al., 2017b, 2018, 2019).

The concentration of C18:1 *trans*-10 in milk fat was negatively associated with the concentration of milk fat and with methane production and methane yield. This reflects previously reported results (Moate et al., 2017b, 2018, 2019). Concentrations of C18:1 *trans*-10, along with C15:0 and C17:0, have been identified as being indicators of SARA (Fievez et al., 2012). All 3 of these fatty acids were linearly related to the proportion of wheat in the diet, suggesting that our cows may have experienced SARA for some of the time. However, this was not reflected in the measured values of ruminal fluid pH measured at the expected time of pH nadir.

Methane emission and yield decreased with increasing proportion of wheat in the diet, and we therefore accept our second hypothesis. The effect of wheat on methane emission was quadratic with no difference between W3 and W6 but with substantially less methane for the W9 treatment. This finding is similar to the finding of Sauvant et al. (2011), who found that methane emissions only declined when concentrates constituted more than approximately 50% of the diet. The methane yields for cows consuming the W0, W3, and W6 diets were all slightly greater than the 20.5 g/kg of DM reported by Charmley et al. (2016) for beef and dairy cows consuming diets containing less than 30% concentrate. However, during period 1, the methane emissions, methane yield, and methane intensity of cows fed the W9 diet were similar in magnitude to those in previous experiments when cows were offered diets with a similar proportion of wheat (Moate et al., 2017b, 2018, 2019). The methane emissions and yields from cows fed the W3 and W6 diets were not statistically different from each other nor from those of cows given the W0 diet. However, methane emission declined linearly and quadratically and methane yield declined linearly with increasing proportions of wheat in the diet. In contrast, Sauvant et al. (2011) reported a quadratic relationship between the proportion of concentrate in the diet and methane yield expressed as g of CH₄/kg of digestible OM, with a maximum when concentrate (mainly corn grain) constituted 23% of DMI. The difference in responses observed in our research and that of Sauvant et al. (2011) is likely due to the different basal diets (freshly harvested perennial ryegrass vs. alfalfa hay), different grain types (wheat grain vs. corn grain), and different range of concentrate inclusion (11 to 52% vs. 0 to 90%).

No carryover effect existed on methane yield when the amount of wheat was suddenly reduced from high to low; therefore, we reject our third hypothesis. Although the cows offered the W9 treatment were receiving a high proportion of grain, their methane yield was less than that of the cows on the low grain treatments.

However, 12 d after the cows on the W9 treatment had their grain reduced to a level the same as for the W3 treatment, their methane yield was not different from that of those cows on the W3 treatment. Although we were able to find reports describing the transience of methane responses to some dietary methane mitigants, we were unable to find any reports describing the carryover effects once a dietary treatment was removed. We had considered the possibility that feeding high amounts of wheat intermittently instead of continuously might be a cost-effective strategy to reduce methane emissions from dairy cows as a smaller amount of expensive wheat would need to be fed. However, as no carryover effect existed on methane mitigation, an intermittent wheat-feeding strategy is unlikely to be a practical strategy for this purpose.

The number of protozoa in ruminal fluid was not associated with methane yield; therefore, we reject our fourth hypothesis. During period 1, negative, linear associations existed between the dietary wheat proportion and the numbers of *Entodinium* spp., *Dasytricha* spp., and total protozoa in ruminal fluid. Furthermore, for cows offered the W9 diet, the numbers of total protozoa in ruminal fluid were approximately only 5% of the number of total protozoa in the ruminal fluid of cows offered the W0 diet. The effect in period 1 of the W9 (high-wheat) diet on protozoal numbers, and concomitantly methane yield, is consistent with previous reports (Moate et al., 2017b, 2019). Ruminal protozoa have been reported to have a close association with many methanogens (Chagan and Ushida, 2004), with some researchers claiming a causal link between methane emissions and protozoa numbers (Kreuzer et al., 1986; Guan et al., 2006). However, in our experiment methane emissions, methane yield, and methane intensity for cows in treatment W9 were all greater during period 2 compared with period 1, and the numbers of total protozoa in ruminal fluid of cows in the W9 treatment were similar in each period. Similarly, Animut et al. (2008) found large differences between treatments in numbers of ruminal protozoa but no differences in methane yields. This suggests the numbers of protozoa in ruminal fluid and methane parameters are not always linked. We acknowledge that our cows may not have fully adapted to the change in diet before measurements were made in period 2 and that ruminal protozoa were counted on just 2 occasions in a small sample of ruminal fluid. However, our data show that methane emission, yield, and intensity had all reverted to relatively normal levels even if milk production and ruminal parameters had not. Thus, even though our cows may not have fully adapted by the time of mea-

surement in period 2, clearly, no link exists between methane and ruminal protozoa in our experiment. We suggest that future studies of the relationship between methane production rates and the dynamic changes that occur in ruminal protozoal populations collect more frequent samples to allow clearer elucidation of this phenomenon.

The pH of ruminal fluid was not substantially influenced by the different dietary treatments. Furthermore, diet W9 resulted in a ruminal fluid pH of 6.3. This is in contrast to previous research using similar methods and similar diets where ruminal fluid pH values in the range 5.6 to 5.9 were reported (Moate et al., 2018, 2019). In our research, proportion of wheat in the diet and concentration of ammonia in ruminal fluid had a strong negative relationship, and we surmise that this was related to the CP concentration in the diet, which was also negatively related to the amount of wheat in the diet. Many articles in the scientific literature have discussed the influence of forage-to-concentrate ratio of dairy cow diets on ruminal fermentation, with emphasis on pH of ruminal fluid and concentrations of ammonia, total VFA, and individual fatty acids (Lana et al., 1998; Yang et al., 2001). These studies have often focused on identifying appropriate forage-to-concentrate ratios to avoid ruminal acidosis and low milk fat syndrome (Plaizier et al., 2008). However, most of this previous research was not focused on pasture-based diets, or diets containing substantial proportions of wheat, and recommendations regarding minimum dietary NDF requirements have mostly been based on diets containing alfalfa or corn silage as the predominant forage and ground corn as the predominant starch source (NRC, 2001). In our research, even when wheat was included at 30% in a perennial ryegrass-based diet, the dietary NDF concentration was 372 g/kg of DM and milk fat concentrations was not decreased relative to the pasture-based diet containing no wheat.

The concentration of total VFA in ruminal fluid was not affected by the different dietary treatments. However, the proportion of wheat in the diet and the molar proportion of propionate had a positive linear relationship, and the proportion of wheat in the diet and the molar proportion of acetate as well as the acetate-to-propionate ratio had negative relationships. The acetate-to-propionate ratio is generally positively related to the milk fat concentration and to methane yield (Mohammed et al., 2004; Moate et al., 2018). In our research, only the W9 diet was associated with a low acetate-to-propionate ratio and low milk fat concentration. In light of the findings of our research, it is not surprising that in Australia, grazing dairy cows

are fed on average approximately 1.7 t of DM of concentrates per year (i.e., approximately 6 kg of DM/d; Dharma et al., 2012; Moate et al., 2016).

It has been suggested that diets containing a high proportion of grain (wheat or corn) should not be fed to ruminants on ethical grounds because cereals could be fed directly to humans or monogastric animals (Doreau et al., 2014). However, Australian dairy cows generally consume less than 10% of the wheat grown in Australia, and most of this is classified as unsuitable for human consumption due to weather damage, sprouting, or presence of foreign materials (Moate et al., 2016). Furthermore, in terms of producing food for humans, it is more efficient to feed grain to dairy cows and produce milk than it is to use it to produce chicken or pig meats (Wilkinson, 2011). Thus, the feeding of feed-quality wheat to dairy cows is an efficient way to produce high-quality food for humans.

CONCLUSIONS

This experiment has quantified for the first time milk production and methane responses from perennial ryegrass pasture-based diets containing different proportions of crushed wheat. The important finding from this research was that for diets based on perennial ryegrass and crushed wheat, only the diet containing more than 30% crushed wheat resulted in substantially depressed milk fat concentration and reduced methane emissions, methane yield, and methane intensity. Thus, although feeding a diet with a high proportion of wheat can cause substantial methane mitigation, it can come at the cost of depression in milk fat concentration.

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