Incorporating mixed rations and formulated grain mixes into the diet of grazing cows: Effects on milk composition and coagulation properties, and the yield and quality of Cheddar cheese

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ABSTRACT

Effects of different strategies for feeding supplements to grazing dairy cows on the composition and coagulation properties of milk and the subsequent yield and quality of Cheddar cheese were measured. The experiment used milk from 72 Holstein-Friesian cows, averaging 45 d in milk, fed according to 1 of 3 feeding strategies: (1) cows grazed a restricted allowance of perennial ryegrass pasture [approximately 14 kg of dry matter (DM)/cow per day, to ground level] supplemented with milled wheat grain fed in the milking parlor and alfalfa hay offered in the paddock (control); (2) same pasture and allowance as control, supplemented with a formulated grain mix containing wheat grain, corn grain, and canola meal fed in the parlor and alfalfa hay fed in the paddock (FGM); or (3) same pasture and allowance as control, supplemented with a partial mixed ration comprising the same formulated grain mix but mixed with alfalfa hay and presented on a feed pad after each milking (PMR). For all strategies, supplements provided the same metabolizable energy and grain:forage ratio (78:22, DM basis). Within each feeding strategy, milk was sampled from cows receiving either 8 or 16 kg (DM) of supplement/cow per day. There were 2 replicated groups of 6 cows per supplement amount per dietary strategy; approximately 250 L of milk was sampled from each for analyses of composition and coagulation properties and the manufacture of Cheddar cheese. The experiment had a 14-d adaptation period and a 14-d measurement period. For cows fed according to the control strategy, those fed 16 kg/cow per day produced milk with lower concentrations of milk fat than cows fed 8 kg/cow per day. This effect was not observed for cows fed according to the FGM and

PMR strategies. Milk from cows fed 16 kg of DM/cow per day according to the control strategy yielded less Cheddar cheese than milk from cows fed according to the PMR strategy, with cheese yields from FGM cows being intermediate. Amount of supplement offered had minor effects on percentages of some fatty acids. We observed few other effects of feeding strategy on milk composition, types of milk protein, milk coagulation properties, or the composition and quality of the resultant Cheddar cheese. These data show that, compared with the traditional control strategy, feeding PMR or FGM may increase milk fat concentration and the subsequent yield of Cheddar cheese without compromising cheese composition or quality.

Key words: ration, grazing cow, milk fat, milk protein, Cheddar cheese

INTRODUCTION

Grazed pasture and conserved homegrown forage have traditionally been major sources of nutrients for dairy cattle in many parts of the world because of their low cost (Doyle et al., 2000; Bargo et al., 2003; Doyle and Stockdale, 2011). Pasture is commonly supplemented with cereal grain or pelleted concentrates fed in the parlor at milking times to allow increased stocking rates and milk production per cow. In southeast Australia in recent years, the trend has been for heavier reliance on bought-in forage and concentrates to meet the nutritional requirements of the milking herd, due largely to below-average rainfall and reduced pasture production (Wales et al., 2013).

When grain supplements are fed in the parlor, milk production often increases (Walker et al., 2001; Leddin et al., 2009) but the amount of extra milk produced per kilogram of grain usually decreases as the amount of grain increases (Stockdale et al., 1987; Kellaway and Harrington, 2004). These diminishing returns are probably partly due to increases in the variation of ruminal fluid pH and the time per day that the pH is below

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6.0 (Wales and Doyle, 2003). This can be associated with impaired rumen fermentation and NDF digestion (Mould et al., 1983; Dixon and Stockdale, 1999; Wales et al., 2000) and reduced DMI and milk production when high amounts of grain are ingested over a short period (Auldist et al., 2013, 2014, 2016).

Recent research has shown that a more efficient way of feeding high amounts of supplements to grazing dairy cows may be to feed them as a mixed ration on a feed pad, in between bouts of grazing (Bargo et al., 2002). Under some circumstances, supplementing the diet of grazing dairy cows with a so-called partial mixed ration (**PMR**) containing corn (Auldist et al., 2013) and canola meal (Auldist et al., 2014; Golder et al., 2014) has resulted in greater production of ECM than obtained by feeding equivalent amounts of energy via the traditional systems detailed above. These responses were associated with higher and less variable ruminal pH and increased DMI of both pasture and supplement. Further, a companion paper to the current experiment (Auldist et al., 2016), showed that most of the milk production benefits of PMR feeding strategies could be obtained by feeding the grain components of the PMR (i.e., cereal grain, corn grain, and canola meal) in the parlor using the existing automated feeding infrastructure. Such feeding strategies would be relevant to a larger proportion of producers in the pasture-based dairying regions of Australia, the majority of whom do not own mixer wagons or feed pads.

In these experiments (Auldist et al., 2013, 2014, 2016), the ECM production responses were associated with changes in the concentrations of milk fat and protein. Because concentrations of fat and protein in milk, and their ratios relative to one another, are known to influence milk coagulation properties and the yield and quality of dairy products (Guinee et al., 1997, 2006, 2007), information is needed about the effects of these alternative feeding strategies on the suitability of milk for manufacturing before they can be widely recommended to dairy producers. The Australian dairy industry is heavily reliant on exporting high-quality dairy products, especially Cheddar cheese. Thus, the current experiment had the aim of determining the effects of 3 different strategies for feeding supplements to grazing dairy cows on the composition and coagulation properties of milk and the subsequent yield and quality of Cheddar cheese. The hypotheses tested were that (1) cows fed large amounts of corn and canola-based supplements would produce milk that yielded more Cheddar cheese than cows fed similar amounts of ME as wheat grain in the parlor and lucerne hay in the paddock; and (2) supplementary feeding strategy would not affect the composition and quality of the cheese.

MATERIALS AND METHODS

Experiment Details

The experiment was undertaken at the Department of Economic Development, Jobs, Transport and Resources (DEDJTR), Ellinbank, Victoria, Australia (38°14'S, 145°56'E). All procedures were conducted according to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and were approved by the DEDJTR Agricultural Research and Extension Animal Ethics Committee.

The feeding experiment was conducted in spring using 144 multiparous, seasonally calving Holstein-Friesian dairy cows and has been described in full by Auldist et al. (2016). All cows had calved in late winter and early spring, were between 4 and 7 yr old and an average of 45 DIM, and were milked twice daily at approximately 0700 and 1500 h. The experiment comprised a 14-d adjustment period followed by a 14-d measurement period during which milk and cheese measurements were made.

Supplement Feeding Strategies

Cows were allocated into 6 groups of 24 cows balanced for DIM, age, BW, and production of milk, milk protein, and milk fat in the previous lactation. One of the following 3 feeding strategies was then randomly assigned to 2 of the 6 groups (Table 1):

- (1) Control: Cows grazed perennial ryegrass (*Lolium perenne* L.) pasture supplemented with milled wheat grain fed twice daily in the milking parlor and alfalfa hay provided in the paddock. The ratio of grain to hay was 78:22 (DM basis).
- (2) Formulated grain mix (**FGM**): Cows grazed perennial ryegrass pasture at the same allowance as the control cows. They were also fed a FGM comprising milled wheat grain, crushed corn grain and canola meal in the parlor during milking, and alfalfa hay in the paddock. The composition of the total supplement (DM basis) was milled wheat grain (38%), crushed corned grain (18%), canola meal (22%), and alfalfa hay (22%). The ration in this treatment provided the same estimated ME intake as the supplements offered to the control cows, and had the same ratio of grain:forage (78:22 DM basis).
- (3) PMR: Cows grazed perennial ryegrass pasture twice daily at the same allowance as the control and FGM cows. They were also offered a PMR comprising milled wheat grain, crushed corn

Table 1. Diagrammatic representation of the experimental design, showing grouping of 144 cows into 4 amounts of supplement offered (either 8, 12, 14, or 16 kg of DM of total supplement/cow per day) for each of 3 replicated strategies for feeding supplements (control, FGM, and PMR)¹

Item	Control (n = 48)								FGM (n = 48)								PMR (n = 48)							
	A (n = 24)				B (n = 24)				A (n = 24)				B (n = 24)				A (n = 24)				B (n = 24)			
Amount of supplement (kg of DM supplement/cow per day)	8	12	14	16	8	12	14	16	8	12	14	16	8	12	14	16	8	12	14	16	8	12	14	16
No. of cows	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6

¹For the current experiment, milk was sampled only from cows offered either 8 or 16 kg of DM/cow per day of supplement according to the control, FGM, and PMR strategies: control = cows grazed a restricted allowance of perennial ryegrass pasture (approximately 14 kg of DM/cow per d, to ground level) supplemented with milled wheat grain fed in the milking parlor and alfalfa hay offered in the paddock; FGM = same pasture and allowance as control, supplemented with a formulated grain mix containing wheat grain, corn grain, and canola meal fed in the parlor and alfalfa hay fed in the paddock; PMR = same pasture and allowance as control, supplemented with a partial mixed ration comprising the same formulated grain mix but mixed with alfalfa hay and presented on a feed pad after each milking.

grain, canola meal and alfalfa hay. This ration was assembled by mixing the FGM described above with alfalfa hay in a feed wagon (model K160; Richard Keenan and Co. Ltd., Co. Carlow, Ireland). These cows were also hand-fed a small amount of the FGM in the dairy at each milking (between 1.6 and 2.8 kg of DM/cow per day depending on the total supplement intake targeted). Water was mixed with the PMR such that the final DM content approximated 50%, before it was presented on a concrete feed pad after each milking (half the daily ration was fed after each milking). The PMR therefore had the same components as the FGM, the same estimated ME content, and the same grain:forage ratio.

Amounts of Supplement

Each of the 6 groups of 24 cows was further divided into 4 groups of 6 cows, 1 of which was randomly assigned to receive 1 of 4 amounts of supplement. These were 8, 12, 14, or 16 kg of DM total supplement/cow per day. Thus, there were 2 replicate groups of 6 cows per amount of supplement per feeding strategy (making a total of 24 groups of 6 cows; see Table 1).

Cows fed PMR received their supplement on a feed pad, with groups of 6 cows separated by electric tapes. Control and FGM cows were offered their grain supplements in the milking parlor during each milking, while alfalfa hay was offered by placing the allocation for each group of 6 cows adjacent to an electric wire in their grazing area each day.

Grazing

Daily pasture allowance was approximately 14 kg of DM/cow per day (to ground level) and was available as a plot of approximately 0.02 ha of pasture per day.

Herbage mass during the experiment was generally in the order of 4,000 kg of DM/ha. Control and FGM cows had access to pasture immediately after each milking. Cows fed according to the PMR strategy had access to pasture after they had consumed their ration on the feed pad following each milking. Groups of 6 cows grazed on adjacent areas separated from the other groups by electric tapes.

Pre- and postgrazing pasture mass was estimated every day for each group using a rising plate meter. This information was used to calculate average pasture DMI for each group (Auldist et al., 2016). The plate meter was calibrated for each set of paddocks the cows entered.

Milk Collection and Sampling

Milk was collected for cheese making on 3 occasions from 12 of the 24 groups of 6 cows in the main experiment. These groups were the 8 and 16 kg of DM supplement groups from the control, FGM, and PMR groups (2 replicate groups from each; Table 1). Cheese making was staggered such that 4 vats of cheese were made on each of the 3 collection occasions.

On each collection occasion, milk from the 4 groups of 6 cows was bulked from 4 consecutive milkings (pm + am + pm + am). Milk was cooled with a ripple cooler (Commonwealth Scientific and Industrial Research Organisation, Werribee, Australia; in-house design and manufacture) immediately after each milking, and bulked milk from each group was stored separately at 4°C in large plastic containers until the 4 milkings were complete.

At the conclusion of each sampling occasion, milk was sampled from the bulked milk using a ladle after thorough but gentle agitation of the whole. These samples were then appropriately subsampled for subsequent analyses of milk composition, sizes of fat globules and casein micelles, and coagulation properties.

Cheese Manufacture

At the completion of each 2-d milk collection, the 4 containers of milk were transported to the experimental cheese-making facility at the Commonwealth Scientific and Industrial Research Organisation (Werribee, VIC, Australia). Upon arrival, milk was standardized to a protein:fat ratio of approximately 0.85 to obtain a fat-in-DM concentration of approximately 52% in the finished cheese. The cheese milk was then pasteurized (approximately 73°C/15 s) using a plate heat exchanger (model VT20FC, GEA Westfalia Separator Pty Ltd., Thomastown, VIC, Australia), cooled to approximately 32°C, and weighed into one of four 250-L cheese vats (Tieman Industries Pty Ltd., Keon Park, VIC, Australia). Freeze-dried mesophilic starter (strain RA 22 from Danisco Australia Pty, Moorabbin, VIC, Australia) for Cheddar cheese making was added at a rate of 7.5 units/100 L of milk, and the whole was ripened for 30 min at 32°C before addition of rennet. A fermentation-produced chymosin (Chy Max Plus from Chr. Hansen, Bayswater, VIC, Australia) was used as the rennet and added at a rate of 20 g/100 L of milk.

The coagulum was cut into 13-mm cubes 5 min after the curd was at the minimum firmness for cutting, as assessed by the cheese maker. The curds and whey were then stirred and heated from 32 to 38°C over 40 min and held at 38°C for a further 80 min. The pH of the whey was recorded at this stage of cheese making.

The whey was then separated from the curd and the curd cheddared to a curd pH of approximately 5.45. The curd was then weighed and milled into chips of approximately 10 × 10 × 100 mm. Salt was added to the milled curd at a rate of 2.5 g/100 g of curd. The salted curd was mellowed for 30 min before being transferred into a 20-kg rectangular cheese mold and pressed at approximately 100 kPa overnight (18 h) in a 14°C room.

The cheeses were then removed from the molds and weighed before being vacuum-packed in barrier bags for ripening at 8°C. After approximately 4 mo of ripening, cheeses were sampled for analysis of moisture, fat, protein, and salt concentrations and pH. Cheeses were also graded at this time.

Analyses

Milk Fat, Protein, and SCC. Milk samples were tested for concentrations of protein and fat using an infrared milk analyzer (model 2000, Bentley Instruments, Chaska, MN). Samples were tested for SCC using a Bentley Somacount 300 automated cell counter (Bentley Instruments, Chaska, MN).

Milk Fatty Acid Proportions. Milk samples were stored at −4°C until analyses, at which time they were

allowed to thaw at room temperature. A 50-mL aliquot was transferred to a centrifuge tube and centrifuged at $1,800 \times g$ for 30 min. The separated fat layer was placed in a 2-mL tube and spun at $25,000 \times g$ for 15 min, and then the sample was heated to 55°C to release the fat, which was transferred for storage. A 30-μL aliquot was subsequently dissolved in hexane (Fisher Scientific, Fair Lawn, NJ) and methylated with sodium methoxide (0.5 M in methanol; Fisher Scientific), and 1 μL of the hexane phase was injected onto a GC with flame-ionization detector with a Restek Rtx 2550 column (105 m × 0.25 mm i.d. × 0.2 μm film thickness; Restek Corporation, Bellefonte, PA). Fatty acids were identified by matching retention time with mixtures of known composition and single-component solutions. Results are reported as percent fatty acid (g of fatty acid per 100 g of fat) after correction for detector response and FAME to fatty acid conversion.

Milk Protein Types. Casein and whey proteins were separated and characterized according to a reversed-phase HPLC method adapted from Bobe et al. (1998) and Bordin et al. (2001). Skim milk samples were analyzed for milk proteins using a BioRad Hi-Pore Reverse phase C18 column (RP-318, 250 × 4.6 mm, Bio-Rad Laboratories Pty. Ltd., Auckland, New Zealand). Samples were prepared by mixing 200 μL of the skim milk with 600 μL of 6 M guanidine hydrochloride, 0.1 M Bis-Tris, and 5.37 mM sodium citrate, pH 7, and standing for 1 h. An aliquot of this mixture (500 μL) was then added to 490 μL of 4.5 M guanidine hydrochloride and 10 μL of 2-mercaptoethanol, and filtered through a 0.45-μm filter. Individual milk proteins were separated on the column using a flow rate of 1 mL/min and a gradient from approximately 25 to 50% acetonitrile in water containing 0.1% trifluoroacetic acid. Compounds in the column eluent were detected by absorbance at 210 or 280 nm.

Casein Micelle Size. Measurement of casein micelle size was conducted by dynamic light scattering using a Malvern Zetasizer Nano ZS analyzer (Malvern, Worcestershire, UK). Skim milk samples were diluted 1:100 in deionized water. Measurements were made using a scattering angle of 173° and laser wavelength of 633 nm. A cumulate analysis correlation function was used to obtain the intensity mean diameter (z-average diameter) of each sample by using a viscosity of 1 mPa/s and a refractive index of $n = 1.343$. At least 2 cumulate z-average measurements were obtained for each sample and at least duplicate samples were measured for each milk.

Coagulation Properties. Rennet setting time, curd firming rate and rennet gel strength were assessed using low amplitude strain oscillation rheometry according to the method of Auld et al. (2001).

Table 2. Composition and coagulation properties of cheese milks, and composition and yield of Cheddar cheese made from milk from groups of cows fed supplements according to the control, FGM, and PMR feeding strategies at 1 of 2 different amounts (8 or 16 kg of DM of supplement/cow per d)¹

	Control		FGM		PMR		SED ²	P-value		
	8 kg	16 kg	8 kg	16 kg	8 kg	16 kg		Strategy (S)	Amount (A)	S × A
Milk composition										
Fat (%)	3.63 ^b	2.45 ^a	3.81 ^b	3.22 ^{ab}	3.51 ^b	3.49 ^b	0.264	0.195	0.030	0.115
Protein (%)	3.02	3.01	3.14	3.25	3.09	3.37	0.096	0.214	0.104	0.240
Casein (mg/mL)	27.5	26.0	26.8	25.6	25.1	28.6	2.29	0.984	0.862	0.358
Whey protein (mg/mL)	4.86 ^{ab}	4.92 ^{ab}	4.75 ^{ab}	5.03 ^{ab}	4.49 ^a	5.25 ^b	0.097	0.997	0.007	0.030
SCC (log ₁₀)	1.44 ^a	2.21 ^{bc}	2.58 ^c	1.46 ^{ab}	1.99 ^{abc}	1.63 ^{abc}	0.170	0.762	0.093	0.010

^{a-c}Within rows, values with different superscripts differ significantly ($P < 0.05$).

¹Data are means of 2 replicate groups of 6 cows per amount per feeding strategy. Control = cows grazed a restricted allowance of perennial ryegrass pasture (approximately 14 kg of DM/cow per d, to ground level) supplemented with milled wheat grain fed in the milking parlor and alfalfa hay offered in the paddock; FGM = same pasture and allowance as control, supplemented with a formulated grain mix containing wheat grain, corn grain, and canola meal fed in the parlor and alfalfa hay fed in the paddock; PMR = same pasture and allowance as control, supplemented with a partial mixed ration comprising the same formulated grain mix but mixed with alfalfa hay and presented on a feed pad after each milking.

²SED = standard error of the difference between means for groups receiving different amounts of supplement fed according to the same dietary strategy.

Cheese Composition. Concentrations of moisture, salt, fat, and protein, and cheese pH, were measured using standard methods in a commercial laboratory (Dairy Technical Services, Kensington, VIC, Australia).

Cheese Grades. All cheeses were graded by a professional cheese grader from a commercial Cheddar cheese manufacturer in Victoria (Warrnambool Cheese and Butter Factory Company Holdings Ltd.). Cheese was graded according to a 100-point scale, with scores restricted to between 86 and 94. Cheeses scoring 93 to 94 do not have any defects and are considered choice grade. Half or full points are deducted for defects, depending on the number and severity of the defects. Cheeses scoring 90 to 92.5 are considered first grade and 86 to 89.5 second grade. Cheeses below second grade are considered unfit for retail sale.

Statistical Analyses

The data were analyzed by ANOVA using blocking structure, herd split for group. The treatment structure was factorial in diet (control, FGM, and PMR) by supplement amount (8 or 16 kg of DM/cow per day). Distributional assumptions of normality and constant variance were checked visually using graphs of residuals against fitted values, and histograms and normal quantile plots of residuals. Somatic cell counts were log-transformed.

RESULTS

Milk Composition

Concentrations of fat, protein, casein, and whey protein and SCC are presented in Table 2 for groups

of cows fed the high and low amounts of supplements according to the 3 different feeding strategies. Groups of cows fed the higher amount of supplement had lower concentrations of milk fat than those fed the lower amount. We detected no overall effect of feeding strategy on fat concentration, but the groups of cows fed 16 kg of supplement/cow per day of the control diet had a lower milk fat concentration than cows fed the same amount of the PMR.

Groups of cows fed the higher amount of supplement had higher concentrations of total whey proteins in milk, due mostly to a large difference in the groups offered the PMR. There were no effects of feeding strategy or amount of supplement for concentrations of protein, casein or SCC.

Milk Fatty Acids

Percentages of individual fatty acids in milk fatty acids are presented in Table 3 for groups of cows fed the high and low amounts of supplements according to the 3 different feeding strategies. Dietary feeding strategy had no significant effect ($P > 0.05$) on percentages of any of the individual fatty acids. The higher rate of supplementary feeding was associated with lower percentages of C4:0, C6:0, C18:0, and C18:1 *cis*-9, but higher percentages of C12:0, C14:0, C15:0, C17:0, C18:1 *trans*-9, and C18:1 *cis*-11 fatty acids compared with the lower rate of supplementary feeding.

Milk Protein Types

Concentrations of the different types of milk proteins in milk from groups of cows offered different amounts of supplement offered according to the 3 different feeding

strategies are presented in Table 4. Cows offered, on average, 8 kg of DM supplement/cow per day of the PMR diet had lower concentrations of κ -casein than cows offered 16 kg of DM/cow per day of the same diet. We detected no other effects of amount of supplement or feeding strategy on any of the milk proteins measured.

Coagulation Properties and Casein Micelle Size

Rennet coagulation time, curd firming rate, curd firmness, and casein micelle size for milk from cows offered the different amounts of supplement according to the 3 feeding strategies are presented in Table 5. Amount of supplement or feeding strategy did not affect any of these parameters.

Cheese Yield, Composition, and Quality

Yields of pressed curd and moisture-adjusted cheese, cheese composition, and cheese grades for Cheddar

cheese manufactured from milk from groups of cows offered low or high amounts of supplement according to the 3 different feeding strategies are presented in Table 6. Milk from groups of cows offered 16 kg of DM/d of supplement according to the FGM and PMR strategies produced more pressed curd per kilogram of milk than milk from groups of cows fed the same amount of supplement according to the control strategy. Similarly, yield of moisture-adjusted cheese from groups of cows fed 16 kg DM/d of supplement according to the PMR strategy was greater than for milk from groups of cows offered the same amount of the control diet.

We found no effects of amount of supplement or feeding strategy on any of the cheese composition parameters examined. Cheese from milk produced by cows fed 16 kg DM/d of supplement according to the FGM strategy scored slightly more points for flavor than cows fed 8 kg DM/d of supplement via the same strategy. There were no other effects of amount of supplement or feeding strategy on cheese grades.

Table 3. Proportions of fatty acids (% of total fatty acids) in milk fat from groups cows fed supplements according to the control, FGM, and PMR feeding strategies at 1 of 2 different amounts (8 or 16 kg of DM supplement/cow per d)¹

Fatty acid	Control		FGM		PMR		SED ²	P-value		
	8 kg	16 kg	8 kg	16 kg	8 kg	16 kg		Strategy (S)	Amount (A)	S × A
C4:0	2.5 ^{bc}	2.0 ^a	2.5 ^{abc}	2.0 ^{ab}	2.8 ^c	2.2 ^{ab}	0.19	0.339	0.006	0.766
C6:0	1.6	1.4	1.7	1.4	1.7	1.5	0.16	0.555	0.041	0.872
C8:0	1.0	1.0	1.1	1.0	1.1	1.1	0.12	0.671	0.357	0.905
C10:0	2.5	2.9	2.8	3.1	2.7	3.0	0.34	0.783	0.087	0.870
C12:0	3.3	4.1	3.6	4.2	3.3	4.1	0.36	0.719	0.007	0.868
C14:0	12.2 ^{ab}	13.1 ^b	12.3 ^{ab}	13.13 ^b	11.6 ^a	13.2 ^b	0.40	0.669	0.008	0.287
C14:1	1.0	1.3	1.0	1.4	0.9	1.1	0.24	0.667	0.055	0.796
C15:0	1.4 ^a	2.5 ^b	1.1 ^a	1.8 ^{ab}	1.0 ^a	1.5 ^{ab}	0.38	0.206	0.024	0.441
C16:0	32.2 ^b	31.4 ^b	31.0 ^b	31.5 ^b	27.6 ^a	30.6 ^b	0.88	0.076	0.081	0.048
C16:1	2.3	2.6	2.0	2.2	2.2	2.0	0.47	0.581	0.782	0.701
C17:0	0.85	1.02	0.73	0.92	0.76	0.83	0.102	0.261	0.092	0.702
C18:0	8.9 ^{ab}	5.8 ^a	9.7 ^{ab}	6.4 ^{ab}	10.6 ^b	8.5 ^{ab}	1.42	0.381	0.002	0.305
C18:1 <i>trans</i> -9	0.19 ^a	0.37 ^b	0.24 ^{ab}	0.38 ^b	0.27 ^{ab}	0.28 ^{ab}	0.047	0.301	0.030	0.152
C18:1 <i>trans</i> -10	0.37	2.24	0.35	1.46	0.34	0.44	0.651	0.286	0.071	0.293
C18:1 <i>trans</i> -11	1.9	1.5	1.8	2.0	2.0	1.7	0.23	0.456	0.311	0.288
C18:1 <i>cis</i> -9	18.8 ^{ab}	15.2 ^a	19.1 ^{ab}	15.8 ^a	22.3 ^b	18.4 ^a	1.25	0.076	0.011	0.953
C18:1 <i>cis</i> -11	0.7 ^a	1.0 ^c	0.7 ^{ab}	0.9 ^{bc}	0.8 ^{abc}	1.1 ^c	0.09	0.055	0.014	0.718
C18:2n-6	1.3 ^a	2.1 ^c	1.4 ^{ab}	2.1 ^c	1.5 ^{ab}	1.7 ^b	0.11	0.345	0.003	0.060
C20:0	0.09	0.04	0.09	0.06	0.10	0.09	0.027	0.495	0.077	0.411
C18:3n-3	0.48 ^{ab}	0.41 ^a	0.49 ^{ab}	0.49 ^{ab}	0.55 ^b	0.44 ^a	0.030	0.210	0.019	0.089
CLA	0.70	0.69	0.61	0.76	0.67	0.66	0.137	0.929	0.630	0.690
Other ³	5.9 ^{ab}	7.6 ^b	5.7 ^{ab}	7.0 ^{ab}	5.2 ^a	5.6 ^{ab}	0.23	0.287	0.003	0.060
Total SFA ⁴	66.5	65.1	66.6	65.6	63.3	66.5	1.06	0.726	0.685	0.091

^{a-c}Within rows, values with different superscripts differ significantly ($P < 0.05$).

¹Data are means of 2 replicate groups of 6 cows per amount per feeding strategy: control = cows grazed a restricted allowance of perennial ryegrass pasture (approximately 14 kg of DM/cow per d, to ground level) supplemented with milled wheat grain fed in the milking parlor and alfalfa hay offered in the paddock; FGM = same pasture and allowance as control, supplemented with a formulated grain mix containing wheat grain, corn grain, and canola meal fed in the parlor and alfalfa hay fed in the paddock; PMR = same pasture and allowance as control, supplemented with a partial mixed ration comprising the same formulated grain mix but mixed with alfalfa hay and presented on a feed pad after each milking.

²SED = standard error of the difference between means for groups receiving different amounts of supplement fed according to the same dietary strategy.

³Minor and identified fatty acids.

⁴As a percentage of total fatty acids.

Table 4. Concentration of different proteins (mg/mL in milk from groups of cows fed supplements according to the control, FGM, and PMR feeding strategies at 1 of 2 different amounts (8 or 16 kg of DM supplement/cow per d)¹

Milk protein	Control		FGM		PMR		SED ²	P-value		
	8 kg	16 kg	8 kg	16 kg	8 kg	16 kg		Strategy (S)	Amount (A)	S × A
κ-CN	4.10 ^{ab}	3.88 ^{ab}	3.58 ^{ab}	3.98 ^{ab}	3.52 ^a	4.16 ^b	0.623	0.942	0.085	0.096
α _{S2} -CN	1.94	1.75	1.83	1.93	1.78	1.86	0.140	0.881	0.937	0.236
α _{S1} -CN	8.76	8.35	8.93	8.13	8.06	9.35	1.526	0.991	0.965	0.397
β-CN	12.68	12.03	12.50	11.55	11.75	13.22	1.920	0.964	0.952	0.397
α-LA	1.15	1.25	1.13	1.28	1.13	1.30	0.114	0.983	0.039	0.760
β-LG B	1.50	2.01	1.85	1.66	1.82	1.72	0.513	0.999	0.791	0.572
β-LG A	2.22	1.66	1.78	2.08	1.55	2.23	0.497	0.989	0.646	0.328

^{a,b}Within rows, values with different superscripts differ significantly ($P < 0.05$).
¹Data are means of 2 replicate groups of 6 cows per amount per feeding strategy: control = cows grazed a restricted allowance of perennial ryegrass pasture (approximately 14 kg of DM/cow per d, to ground level) supplemented with milled wheat grain fed in the milking parlor and alfalfa hay offered in the paddock; FGM = same pasture and allowance as control, supplemented with a formulated grain mix containing wheat grain, corn grain, and canola meal fed in the parlor and alfalfa hay fed in the paddock; PMR = same pasture and allowance as control, supplemented with a partial mixed ration comprising the same formulated grain mix but mixed with alfalfa hay and presented on a feed pad after each milking.
²SED = standard error of the difference between means for groups receiving different amounts of supplement fed according to the same dietary strategy.

DISCUSSION

This experiment provides the first information about the effects of offering grazing dairy cows different amounts of supplements as either a PMR or a FGM on the detailed composition of milk and its potential for manufacturing Cheddar cheese. Previous research has shown that offering cows supplements as a corn and canola-based PMR on a feed pad can lead to potentially profitable increases in ECM compared with the traditional method of feeding equivalent amounts of estimated ME as cereal grain in the dairy and conserved legume forage in the paddock (Auldish et al., 2013, 2016; Golder et al., 2014). Before such feeding strategies could be recommended to farmers on a wider scale, however, it was necessary to identify potential

beneficial effects and unintended negative consequences of the technology in relation to the yield and quality of one of Australia’s core dairy export products, Cheddar cheese.

In previous experiments, one of the main benefits of PMR feeding systems over traditional methods of feeding was that milk fat concentrations generally remained constant as the amount of supplement offered increased. This finding contrasts to the marked decline in milk fat concentration with increasing supplement intake that generally occurs when cows are fed “control” diets (Auldish et al., 2013, 2014). This phenomenon was also seen in a related experiment (Auldish et al., 2016) and the current experiment, in which cows fed 16 kg DM of the PMR had higher milk fat concentrations than cows offered the same amount of supplement via

Table 5. Rennet coagulation time, curd firming rate, curd firmness, and casein micelle size for milk from groups of cows fed supplements according to the control, FGM, and PMR feeding strategies at 1 of 2 different amounts (8 or 16 kg of DM supplement/cow per day)¹

Item	Control		FGM		PMR		SED ²	P-value		
	8 kg	16 kg	8 kg	16 kg	8 kg	16 kg		Strategy (S)	Amount (A)	S × A
Rennet coagulation time (s)	900	780	840	788	1,013	1,013	85.6	0.077	0.342	0.668
Curd firming rate (Pa/s)	37	39	39	63	39	47	10.6	0.356	0.206	0.506
Curd firmness (Pa)	73	76	77	113	74	93	16.5	0.324	0.145	0.493
Casein micelle size (nm)	164	165	172	160	159	164	7.1	0.777	0.618	0.250

¹Data are means of 2 replicate groups of 6 cows per amount per feeding strategy: control = cows grazed a restricted allowance of perennial ryegrass pasture (approximately 14 kg of DM/cow per d, to ground level) supplemented with milled wheat grain fed in the milking parlor and alfalfa hay offered in the paddock; FGM = same pasture and allowance as control, supplemented with a formulated grain mix containing wheat grain, corn grain, and canola meal fed in the parlor and alfalfa hay fed in the paddock; PMR = same pasture and allowance as control, supplemented with a partial mixed ration comprising the same formulated grain mix but mixed with alfalfa hay and presented on a feed pad after each milking.
²SED = standard error of the difference between means for groups receiving different amounts of supplement fed according to the same dietary strategy.

the control feeding strategy, with the FGM strategy being intermediate.

The mechanisms causing such reductions in milk fat concentration are not fully understood, but diet is a major factor (Bauman and Griinari, 2003; Shingfield et al., 2010). Diets with low fiber and high starch or lipids rich in PUFA are associated with milk fat reduction (Stockdale et al., 1987). These diets are coincident with low ruminal fluid pH, a shift in the ruminal microbial population, and altered rumen lipid metabolism, leading to an increase in specific biohydrogenation intermediaries with antilipogenic effects (Bauman and Griinari, 2003). The main long-chain fatty acid with milk fat depressing effects in the mammary gland is *trans*-10,*cis*-12 CLA (Bauman and Griinari 2003). This fatty acid is normally present in milk fat at very low concentrations (Moate et al., 2007) and was not detected in milk in this experiment. The concentration of C18:1 *trans*-10 in milk is often much greater than the concentration of *trans*-10,*cis*-12 C18:2, and the concentration of C18:1 *trans*-10 is correlated with that of *trans*-10,*cis*-12 C18:2 (Moate et al., 2007). Furthermore, milk fat concentrations have been negatively correlated with the percentage of C18:1 *trans*-10 in milk fatty acids (Moate et al., 2007).

Increased milk protein concentration in response to increased DMI has been observed previously (O'Brien et al., 1997; Auldist et al., 2000). In the current experiment, which measured milk protein concentration in milk from the same cows but in bulked milk, there was no significant difference between the groups, although milk protein concentration was numerically greater for groups of cows fed 16 kg DM supplement/d of the FGM and PMR diets compared with the protein concentration in milk from groups of cows fed 8 kg DM/d of the same diets. This was consistent with the fact that few differences were observed between groups in terms of the type of milk protein present.

The higher concentrations of milk fat and numerically greater concentrations of milk protein resulted in higher concentrations of these solids in the milk used for cheesemaking, even after standardizing the milk to a consistent fat:protein ratio (data not presented). This was, in turn, expected to result in an increased yield of cheese (Guinee et al., 1998), because it is primarily casein and fat that are incorporated into the curd during cheesemaking. This was measured as a 1.57-kg increase in pressed curd per 100 kg of milk (17.8%) and a 1.73-kg increase in moisture-adjusted cheese yield per 100 kg of milk (19.8%), when control cows fed 16

Table 6. Yields of pressed curd (kg/100 kg of milk) and moisture-adjusted cheese (kg/100 kg of milk), cheese composition, and cheese grades for Cheddar cheese manufactured from milk from groups of cows offered low or high amounts of supplement according to the 3 different feeding strategies¹

Item	Control		FGM		PMR		SED ²	P-value		
	8 kg	16 kg	8 kg	16 kg	8 kg	16 kg		Strategy (S)	Amount (A)	S × A
Pressed curd	8.81 ^{ab}	8.39 ^a	9.20 ^{ab}	9.65 ^b	8.97 ^{ab}	9.96 ^b	0.357	0.090	0.196	0.143
Moisture-adjusted yield	8.72 ^{ab}	8.23 ^a	9.09 ^{ab}	9.68 ^{ab}	8.85 ^{ab}	9.96 ^b	0.463	0.118	0.226	0.187
Cheese moisture (%)	37.7	38.2	37.8	36.8	37.9	37.0	1.124	0.782	0.566	0.623
Cheese fat (%)	31.3	30.2	30.6	31.8	30.8	31.2	0.85	0.797	0.732	0.283
Cheese protein (%)	25.4	25.7	25.7	25.4	25.5	25.7	0.255	0.958	0.546	0.354
Cheese pH	5.40	5.38	5.43	5.41	5.35	5.52	0.064	0.784	0.342	0.192
Cheese salt (%)	1.70	1.65	1.80	1.70	1.75	1.75	0.076	0.306	0.391	0.740
Salt-in-moisture (%)	4.52	4.32	4.77	4.62	4.62	4.73	0.151	0.130	0.452	0.445
Fat-in-DM (%)	50.1	48.8	49.1	50.3	49.6	49.5	0.51	0.871	0.888	0.080
Cheese grade ³										
Flavor	42.5 ^{ab}	42.5 ^{ab}	42.8 ^b	42.3 ^a	42.5 ^{ab}	42.3 ^{ab}	0.14	0.829	0.058	0.192
Texture	29.5	29.5	29.8	29.5	29.3	29.8	0.48	0.898	0.800	0.631
Color	19.8	19.5	16.0	19.8	20.0	19.5	2.17	0.560	0.483	0.412
Total	91.8	91.5	92.5	91.5	91.8	91.5	0.74	0.732	0.324	0.732

^{a,b}Within rows, values with different superscripts differ significantly ($P < 0.05$).

¹Control = cows grazed a restricted allowance of perennial ryegrass pasture (approximately 14 kg of DM/cow per d, to ground level) supplemented with milled wheat grain fed in the milking parlor and alfalfa hay offered in the paddock; FGM = same pasture and allowance as control, supplemented with a formulated grain mix containing wheat grain, corn grain, and canola meal fed in the parlor and alfalfa hay fed in the paddock; PMR = same pasture and allowance as control, supplemented with a partial mixed ration comprising the same formulated grain mix but mixed with alfalfa hay and presented on a feed pad after each milking.

²SED = standard error of the difference between means for groups receiving different amounts of supplement fed according to the same dietary strategy.

³Cheese was graded using a 100-point scale with scores limited to between 86 and 94. Cheeses scoring 90 to 92.5 were considered first grade and 86 to 89.5 second grade.

kg of DM total supplement were compared with PMR cows fed the same amount. Thus, the first hypothesis is supported.

When the cheese was graded, they all scored a total of between 91.5 and 92.5 points. All cheese was thus first grade and no cheeses displayed meaningful texture or flavor defects of any kind. Furthermore, there was no difference between the cheeses in terms of the compositional parameters measured. Thus, the second hypothesis is supported. This is a positive outcome in that it shows that the cheese yield benefits obtained when feeding 16 kg DM of supplement according to the PMR strategy compared with the control strategy were not associated with a reduction in cheese quality.

In conclusion, this experiment has shown that, compared with feeding equivalent amounts of ME as wheat grain in the dairy and alfalfa hay in the paddock, offering grazing cows supplements as a PMR containing corn grain and canola meal can increase the cheese-yielding capacity of milk. Increased yields of Cheddar cheese of the order of 20% were obtained by altering the form of the energy presented to the cows. This was not accompanied by any changes in the composition or quality of the cheese. These findings should provide dairy producers and the Australian dairy industry with confidence that PMR feeding strategies could be deployed without risk of compromising the core business of producing high-quality dairy products for the export market.

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