Increasing amounts of crushed wheat fed with pasture hay reduced dietary fiber digestibility in lactating dairy cows

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ABSTRACT

Sixteen cows in mid-lactation (milk yield of 23.8 ± 2.3 kg/d) were individually fed diets consisting of chopped perennial ryegrass hay, offered at 3 kg of dry matter (DM)/100 kg of body weight (BW), fed either alone or supplemented with amounts of crushed wheat ranging from 0.4 to 1.6 kg of DM/100 kg of BW (increasing at nominal intervals of 0.4 kg of DM/100 kg of BW; 5 nominal treatments in total). Three cows were allocated to each treatment except the mid-range wheat treatment, which had 4 cows. Results were analyzed by regression because the intake of the wheat by cows within treatments varied. The hay was used to reflect the characteristics of summer pastures in southeastern Australia. Feed intake and fecal output were measured to determine digestion coefficients, feeds were incubated in nylon bags in the rumen, and rumen variables were monitored. Estimates of metabolizable energy (ME) of the hay from in vivo or in vitro digestibility were also compared. The digestibility of neutral detergent fiber (NDF) was depressed linearly as the amount of crushed wheat consumed increased to 36% of DM intake. The extent to which negative associative effects on NDF digestion were associated with the hay could not be determined, as it was not possible to distinguish between the NDF from hay and that from wheat. However, acid detergent fiber (ADF) digestion also declined, suggesting that most of the response lay with the hay because ADF was negligible in the wheat. Most data indicated that effects of proportion of wheat in the diet on the utilization of consumed nutrients were small. Despite substitution of wheat for hay reducing the forage intake of cows, there was a positive linear effect on marginal milk responses (1.3 kg of energy-corrected milk/kg of DM wheat). Mean rumen fluid pH declined as the proportion of wheat in the diet increased. The lowest pH for any individual cow during a 24-h period was 5.4, and the amount of time that rumen fluid pH was

<6.0 ranged from 0 to 14 h depending on the amount of wheat consumed. It was concluded that these perturbations of the rumen environment were probably sufficient to result in negative associative effects. In addition, estimates of the ME content of the hay were higher when calculated from in vitro compared with in vivo digestibility, which has implications when estimating the amount of feed required for production.

Key words: associative effect, digestion and rumen fermentation, dairy cow, pasture and grain

INTRODUCTION

Dairy farmers in southeastern Australia commonly feed cereal grain supplements to their cows when the availability of ME from pasture is insufficient to support target levels of milk production. These supplements may result in a reduction in pasture intake through substitution (Stockdale, 2000) or there may be associative effects between feeds, whereby the digestion of NDF or starch changes as the proportion of grain in the diet increases (Huhtanen, 1991; Doyle et al., 2005). Negative associative effects occur when rumen fluid pH declines to levels below about pH 6.0 to 6.2 (Mould et al., 1983; Pitt et al., 1996) at which point cellulolysis is impaired and ruminal NDF digestion is reduced. In recent studies using tightly controlled dual-flow continuous culture systems, the magnitude of the reduction in NDF digestibility depended on the degree of reduction of pH below 6.2 and the amount of time for which it remained below that level (e.g., Wales et al., 2004; Cerrato-Sánchez et al., 2007a, 2008). In addition, Wales et al. (2009) reported that maximum OM digestibility occurred when grain constituted 24% of a pasture and concentrate diet and the culture pH was 6.0.

Associative effects between feeds are difficult to measure and therefore generally ignored by nutritionists formulating diets (Weiss, 1999). Even when well-formulated TMR are fed, where selection for particular ingredients is minimized and rumen fluid pH is relatively stable and maintained at a level for efficient fermentation, the amount of energy available to the cow is generally overestimated (Weiss, 1998). Negative

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associative effects between feeds is probably a greater issue in the nutritional management of dairy cows in pasture-based systems than with TMR, because concentrates are generally only fed when cows are milked, resulting in greater variation in ruminal pH (Bargo et al., 2002).

The effects of grain supplementation of dairy cows and associated variations in ruminal pH on NDF digestion *in vivo* have not been quantified for the types of pasture that are grazed in dairy systems in southeastern Australia. Typical pastures range from ryegrass (*Lolium* spp.)- and clover (*Trifolium* spp.)-dominant swards in spring with estimated ME values of around 12 MJ/kg of DM to irrigated paspalum (*Paspalum dilatatum*)-dominant swards in summer with an estimated ME of about 9 MJ/kg of DM or less (Stockdale et al., 2001). In the experiment reported here, lactating cows were fed a basal hay diet approximating to the energy value of an irrigated summer pasture and were supplemented with crushed wheat. Although it is recognized that hay and fresh summer pasture will vary to some degree in their nutritive characteristics, with both of them exhibiting relatively poor nutritive values, we suggest that results from one would give a reasonably good guide of what to expect when feeding the other. It was hypothesized that the digestibility of NDF would decrease in a curvilinear manner as the amount of crushed wheat consumed increased. There was an expectation that the point of inflection would occur when the conditions for rumen fermentation became suboptimal, estimated to be when wheat comprised approximately 25% of the total DM intake. A further objective was to examine differences in estimated ME content of the hay when calculated from *in vitro* DM digestibility compared with *in vivo* DM digestibility in lactating cows.

MATERIALS AND METHODS

Animals, Treatments, and Experimental Schedule

All procedures were conducted with protocols approved by the Northern Victorian Animal Ethics Committee (Kyabram/Rutherglen, Australia). Sixteen rumen-fistulated Holstein-Friesian cows that had calved in spring at the Victorian Department of Primary Industries' Kyabram Centre (36°20'S, 145°04'E), Australia, were selected for the experiment. Before the experiment, the cows had an average ($\pm$ SD) BW of 595 kg ($\pm$ 41), were 152 ($\pm$ 18) d into lactation, and were producing 23.8 ($\pm$ 2.3) kg of milk/d. The experimental schedule is shown in Figure 1. No protein, vitamin, or mineral supplements were added to the diet. Cows were adapted to a diet of chopped (20- to 25-mm chop length) perennial ryegrass hay and crushed wheat (that

included 1% wt/wt sodium bicarbonate) between d -12 (January 25, 2005) and d -6. The sodium bicarbonate was added to reflect farmer practice. Each animal was offered 12.1 kg DM of hay on d -12 and -11, 14.2 kg DM on d -10 and -9, and 16.2 kg DM on d -8, -7, and -6. Wheat was offered at 0.9 kg of DM/100 kg of BW each day for this period. Body weight, BCS, milk production, milk composition, and DMI during this period were used to allocate cows to nominal treatments. These were established by increasing the amount of supplement at intervals of about 0.4 kg of DM/100 kg of BW to create a spread of grain intakes to facilitate analysis of results by regression. Between d -5 and -1, cows were introduced to dietary treatments consisting of chopped hay offered to all cows at 3 kg of DM/100 kg of BW, which was fed alone or supplemented with amounts of crushed wheat ranging from 0.4 to 1.6 kg of DM/100 kg of BW (increasing at nominal intervals of about 0.4 kg of DM/100 kg of BW; 5 nominal treatments in total). Three cows were allocated to each treatment except the mid-range (0.8 kg of DM/100 kg of BW) wheat treatment, which had 4 cows. Results were analyzed by regression because the intake of the wheat by cows within treatments varied. For cows at the 2 lowest grain quantities, the amount of grain offered was reduced to the required amount on d -5, whereas at the 2 highest grain quantities, the amount of grain offered was increased by 0.13 kg of DM/100 kg of BW until the designated amount was achieved.

Between d -12 and d 8, half the daily quantity of grain was given after each milking (cows milked twice daily). On each occasion, the cows were allowed to consume the grain for 15 min. The hay was offered 4 times per d at 0630, 1130, 1645, and 2200 h and the animals had access to the feed for 1 h. When not feeding, cows were kept as a group on rice hulls in a loafing area. No consumption of rice hulls was observed. On d 9, the cows were housed in individual metabolism stalls for digestibility and rumen fermentation measurements and were fitted with fecal harnesses and urine separators. For the 10 d in the stalls, grain was offered immediately after each milking for 15 min, and each cow was fed her hay at 0615, 1130, 1630, and 2200 h. Grain residues were removed immediately after the allocated time elapsed. Hay residues were only removed immediately before the next feeding time.

Measurements and Analytical Methods

Cows were weighed daily from d -13 to d 9 and on d 20 and d 21 following the completion of fecal collection. Body condition scores (Earle, 1976) were assessed by 2 observers on d -6. Cows were milked at 0530 and 1600 h, and milk yields were recorded throughout. On

Figure 1. Experimental timelines for the adaptation of cows to the feeding of hay and wheat supplements and of key measurements during the experiment.

4 consecutive days commencing at the p.m. milking on d 15, a representative subsample of the milk collected from each cow at each milking was added to containers containing 0.25 mL of milk preservative (bronopol) and stored immediately at 3°C. Milk samples were collected using standard herd-testing equipment (HI sample meter, Tru Test Pty. Ltd., Auckland, New Zealand), which sampled 2.2% volume continuously throughout the milking. Aliquot samples of milk from each cow were analyzed for protein, fat, and lactose within 2 d of collection by an infrared milk analyzer (MilkoScan, Foss Electric, Hillerød, Denmark). Energy-corrected milk, as defined by Tyrrell and Reid (1965), was calculated thus: $\text{ECM} = \text{kg of milk} \times (383 \times \text{fat}\% + 242 \times \text{protein}\% + 783)/3,140$.

Individual feed intakes were recorded daily throughout the experiment. Grain and hay residues were collected after each feed and bulked across the day for each cow. Subsamples of feeds offered and refused were taken each day for DM determination, and during the indoor fecal collection period, additional samples were collected and stored at -20°C for processing and analysis. Feed and residue samples for analysis of chemical characteristics and nutritive value (in vitro digestibility) were dried to constant weight at 60°C and ground through a 1-mm screen before analysis. Feces and urine excreted by each cow were collected daily in separate

bins and processed on the morning of d 13 to 19. Urine was weighed and discarded. Feces were weighed and thoroughly mixed and samples taken for analysis. Fecal samples were stored at -20°C until analyzed. Apparent digestibility of DM, OM, CP, and gross energy and digestibility of NDF, ADF, and starch were calculated from feed consumed and feces excreted.

Over 3 d, commencing at 0700 h on d 15, 12 samples of rumen fluid were collected for determination of pH, and ammonia-N and VFA concentrations. The timing of samples was staggered so that samples were collected every 2 h through a 24-h cycle. Rumen fluid pH was measured immediately after collection using a pH meter fitted with a glass electrode. Other samples were frozen immediately for later analysis. Samples for ammonia-N analysis were acidified with 0.1 M hydrochloric acid to inhibit microbial activity and minimize volatilization of ammonia. Samples for VFA analysis had 1 mL of metaphosphoric acid added to 4 mL of rumen fluid to deproteinize the sample. Rumen fluid samples were analyzed for VFA concentrations by GLC (Erwin et al., 1961; Fussell and McCalley 1987) and ammonia-N using the method described by Chaney and Marbach (1962).

Samples (5 g of DM) of wheat (ground through a 2-mm screen) and hay (ground through a 4-mm screen) were incubated in the rumen of each cow using an in

sacco technique (Ørskov and Mehrez, 1977; AFRC, 1992). Bags were made from a woven polyester cloth (Ankom Technologies, Macedon, NY) and had heat-sealed edges, a mean pore size of 50 μm , and dimensions of 10 $\times$ 24 cm. Incubation times to describe the disappearance of wheat were 0, 1, 2, 4, 8, 12, 18, 24, 36, and 48 h, and for hay were 0, 4, 8, 12, 24, 36, 48, 72, and 96 h. The bags containing wheat or hay were incubated separately (see Figure 1) to prevent possible bag-to-bag interactions through entanglement in the rumen and to ensure there were sufficient bags at each incubation time for the required laboratory analyses. The incubation of wheat commenced on d 11, with bags inserted into the rumen at about 1100 h and removed as required over the next 48 h. Hay was incubated during d 15 to 19, with bags inserted as required over the incubation period and all bags removed around 0700 h on d 19. Bags containing wheat and hay that were not incubated in the rumen ($t = 0$) were kept aside for later estimation of the immediate solubility of these feeds. Bags removed from the rumen were immediately put into ice water to stop the microbial action, and then rinsed to remove digesta before being frozen. They were subsequently thawed and washed in a washing machine for 45 min followed by a slow spin (500 rpm) for 5 min. Bags for estimating immediate solubility were also included in the wash. All bags were refrozen, and then freeze-dried and weighed to determine DM loss. Residual wheat samples for all incubation times were transferred to plastic vials after being crushed using a glass rod, whereas residual hay samples were ground through a 1-mm screen before analysis.

Commencing at 0945 h on d 8, the rumen contents of half of the cows were removed by hand into tared bins to obtain a direct estimate of rumen pool sizes. On d 9 at the same time, the rumen contents were removed from the remaining cows. After weighing the bins, the contents were mixed thoroughly, and subsamples of digesta were collected for DM determination, chemical analysis, and particle size distribution. The rumen contents were then returned to each cow, with the entire process taking no more than 30 min.

Subsequent to the experimental period, the proportion of large and small particles entering the rumen when cows were fed the hay was estimated by particle size analysis of swallowed boli. Cows were fasted for 5 h before the procedure, which commenced at 1330 h. The reticulo-rumen was partially emptied to provide access to the cardia. Approximately 5 kg (fresh weight) of hay was offered to each of 4 cows. As the cows consumed the hay, boli were collected at the cardia by hand. After approximately 20 boli were collected from each cow, the rumen contents were returned to the rumen. Collected material was weighed, lightly rinsed in gauze,

and gently squeezed to remove saliva. Samples of feed eaten and collected boli were stored at -20°C for later analysis of DM, nutritive characteristics, and particle size distribution.

The DM contents of samples of feed, swallowed feed boli, rumen digesta, and feces were determined by oven drying at 100°C to constant weight. Samples of feeds offered and refused, rumen digesta, nylon bag residues, and feces for chemical analysis were freeze-dried and ground through a 1-mm screen. Samples for starch analysis were ground through a 0.2-mm screen. Organic matter content was determined by ashing in a muffle furnace for 3 h at 520°C . Total N was determined by combustion in a Leco instrument (model FP-428, Leco Corporation, St. Joseph, MI). Crude protein was calculated as $\text{N} \times 6.25$. Neutral detergent fiber, ADF, and lignin were measured using methods described by Van Soest et al. (1991). Because of the high starch content, α -amylase solution was added to the wheat samples during the digestion stage in the NDF and ADF analysis, but sodium sulfite was not used in the NDF analysis. Feed, rumen digesta, and fecal samples were analyzed for starch using an enzymatic method that converts starch to glucose in the presence of amyloglucosidase using a commercial starch analysis kit (Megazyme, Megazyme International Ireland Ltd., Bray, Co. Wicklow, Ireland; McCleary et al., 1994). The *in vitro* DM digestibilities of grain and hay were obtained using a rumen fluid-pepsin method (Tilley and Terry, 1963) and a 2-stage pepsin-cellulase technique (Clarke et al., 1982) for grain and hay, respectively. Estimated ME (MJ/kg of DM) was calculated from *in vitro* or *in vivo* DM digestibility (DMD) data using $0.17 \times \text{DMD} (\%) - 2.0$ (Standing Committee on Agriculture, 1990) for hay and $0.134 \times \text{DMD} + 0.235 \times \text{ether extract} + 1.23$ (CSIRO, 2007) for grain. The gross energy contents of feeds and feces were measured using an automated adiabatic bomb calorimeter (Parr 1281, Parr Instrument Co., Moline, IL).

Particle size distributions of hay, swallowed feed boli, and rumen digesta samples were determined after frozen samples were defrosted at 3°C and mixed well. A subsample was dried at 100°C for 24 h to determine DM content, and at least 3 subsamples containing about 10 g of DM were weighed for sieving. The sieving device used was similar to that described by Poppi et al. (1980) with 5 mesh sizes: 4, 2, 1, 0.5, and 0.25 mm. The bank of sieves was suspended in water and oscillated through a vertical distance of 6.5 cm at a frequency of 40 strokes/min for 15 min. Clumps of material on the top sieve were dispersed regularly by gentle agitation using tweezers or a spray of water to prevent entrapment of smaller particles. Particles retained on each sieve were transferred to tared filter paper and dried at

Figure 2. Daily intakes of hay (open symbols) and wheat (closed symbols) for cows fed hay alone (3 cows; $\nabla, \blacktriangledown$), and for those consuming 10 to 20% (5 cows; $\diamond, \blacklozenge$), 20 to 30% (6 cows; $\triangle, \blacktriangle$), and more than 30% (2 cows; $\square, \blacksquare$) of their diet as crushed wheat.

100°C to constant weight for feed and digesta samples, and at 60°C for boli samples. The DM of these samples was determined.

Statistical Analysis

The relationship between a (dependent) digestion variable Y (digestibility coefficients, rumen fluid and digesta measurements, nylon bag rates of degradation) and the (independent variable) percentage wheat intake X was modeled by regression analysis using GenStat 9 statistical software (2006; Lawes Agricultural Trust, Rothamsted, UK). We fitted and retained a second-order polynomial regression model $Y = b_0 + b_1 X + b_{11} X^2$, if the coefficient b_{11} was significant at 5% level of significance, because in that case both b_0 and b_1 must be retained to avoid bias in the estimate of b_{11} (Draper and Smith 1998). If b_{11} was not significant, this was dropped from the model and the first-order linear regression model $Y = b_0 + b_1 X$ was fitted to describe the relationship between Y and X . The relationship was inferred as significant if the coefficient b_1 (in case of the first-order model) or at least the coefficient b_{11} (in case of the second-order model) was significant at 5% level of significance. The adequacy of a fitted model to describe the underlying relationship was assessed by the coefficient of determination R^2 , which quantified the percentage variation in the dependent variable Y explained by the fitted model. Milk production variables and DMI were regressed against amount of wheat consumed instead of percentage wheat in the diet so

that marginal milk response and substitution of grain for hay could be obtained. In the case of ECM, previous production (collected before treatments were imposed) was included as a covariate to account for between-cow variability. Because cows were fed individually and daily intakes of both feeds were recorded, each cow was considered to be an experimental unit. Because 16 cows cannot be evenly divided into 5 categories, the extra cow was included in the middle of the grain-feeding range to strengthen the likelihood of detecting a curvilinear relationship if one existed. Residual diagnostics performed after each analysis showed that the data reasonably met the regression analysis assumptions of constant variance and normality.

An exponential model, using the FITNONLINEAR procedure in GenStat 9, was fitted to describe the degradation of DM and NDF in nylon bags for hay and wheat. The degradation rates for hay and wheat were compared using a t -test. The curves that were generated were used to calculate degradation coefficients, as outlined by Ørskov and McDonald (1979).

RESULTS

Feed Intake and Digestibility

The nutritive characteristics of the feeds on offer are given in Table 1. Through the experimental period, day-to-day intakes of hay and wheat were relatively consistent as shown in Figure 2. During the fecal collection period, the cows not offered any wheat ate 17.0 kg of DM/d of hay, and as wheat intake increased up to 7.8 kg/d (36% of total intake), total intake increased and hay intake declined (Figure 3). Mean substitution rate of wheat for hay was 0.30 kg of DM/kg of DM.

In vivo digestibilities of DM, OM, and gross energy increased as the proportion of wheat in the diet increased (Figure 4). Starch digestibility in all cows fed wheat was high, ranging between 97.2 and 98.7%. There

Table 1. Nutritive characteristics of the hay and crushed wheat fed to lactating dairy cows throughout the experiment

Item	Chopped hay	Crushed wheat
OM (% of DM)	85.7	98.1
In vitro digestibility (% of DM)	73.6	92.0
NDF (% of DM)	52.2	12.0
ADF (% of DM)	29.3	2.8
Lignin (% of DM)	2.4	0.7
CP (% of DM)	19.5	15.6
Starch (% of DM)	1.8	54.4
Gross energy (MJ/kg of DM)	15.5	17.1
Estimated ME ¹ (MJ/kg of DM)	10.5	14.0

¹Calculated from in vitro DM digestibility (DMD) using $0.17 \times \text{DMD} (\%) - 2.0$ (Standing Committee on Agriculture, 1990) for hay and $0.134 \times \text{DMD} + 0.235 \times \text{EE} + 1.23$ (CSIRO, 2007) for crushed wheat, with EE (ether extract) being assumed to be 2%.

Figure 3. Total (■) and hay (◇) DMI in relation to the DMI of wheat, and described by the following regression equations: Total DMI = $17.3 (\pm 0.50) + 0.70 (\pm 0.116) \times \text{wheat intake}$; $R^2 = 0.72$, $P < 0.001$, root mean square error (RMSE) = 1.09; hay DMI = $17.3 (\pm 0.50) - 0.30 (\pm 0.116) \times \text{wheat intake}$; $R^2 = 0.32$, $P = 0.022$, RMSE = 1.09.

was no relationship between the amount of wheat consumed and starch digestibility. As the proportion of wheat in the diet increased, digestibility of NDF and ADF declined (Figure 4), but there was no effect on apparent N digestibility:

$$\begin{aligned} \text{CP digestibility} &= 72.6 (\pm 0.76) \\ &- 0.07 (\pm 0.037) \times \% \text{ wheat}; \end{aligned}$$

$$R^2 = 0.20; P = 0.079; \text{RMSE} = 1.60; n = 16.$$

Nylon Bag Incubation

The rates of degradation of wheat in nylon bags incubated in the rumen were greater than those of hay for both DM (mean $\pm$ SE of 29.9 ± 2.23 vs. $12.6 \pm 0.63\%/h$) and NDF (mean $\pm$ SE of 13.9 ± 0.93 vs. $10.4 \pm 0.50\%/h$). The proportion of wheat in the diet did not affect these rates for wheat (curves of best fit showed, for the slope b_1 , $P = 0.360$ for DM and $P = 0.612$ for NDF) or hay [curves of best fit showed, for the slope b_1 , $P = 0.061$ for DM (declining at high amounts of wheat) and $P = 0.140$ for NDF] because of the high between-cow variation.

Rumen Fermentation

Mean rumen fluid pH and ammonia-N concentration declined as the proportion of wheat in the diet increased, whereas total VFA, acetate, propionate, and butyrate concentrations all increased as the proportion of wheat in the diet increased (Figure 5). Ruminal pH was above 6.0 throughout the day in cows consuming hay only, but it declined to lower values and fluctuated more as the proportion of wheat (% wheat) in the diet increased (Figure 6). The lowest pH for any individual cow at any stage during a 24-h period was 5.4 and there was a negative linear relationship between lowest ruminal pH and the proportion of wheat in the diet:

$$\begin{aligned} \text{Lowest pH} &= 6.00 (\pm 0.076) \\ &- 0.017 (\pm 0.0037) \times \% \text{ wheat}; \end{aligned}$$

$$R^2 = 0.60; P < 0.001; \text{RMSE} = 0.160; n = 16.$$

The amount of time that rumen fluid pH was below 6.0 ranged from 0 to 14 h, and there was a positive linear relationship with proportion of wheat in the diet:

$$\begin{aligned} \text{Hours below pH 6.0} &= 0.24 (\pm 1.460) \\ &+ 0.36 (\pm 0.071) \times \% \text{ wheat}; \end{aligned}$$

$$R^2 = 0.65; P < 0.001; \text{RMSE} = 3.07; n = 16.$$

The nonglucogenic to glucogenic VFA ratio (acetate + butyrate/propionate) was not affected by the proportion of crushed wheat in the diet and averaged ($\pm$ SD) $4.1 (\pm 0.30)$.

Rumen Contents

Mean ($\pm$ SD) total, DM, and fluid amounts in the rumen were $107 (\pm 13.5)$, $13 (\pm 1.8)$, and $94 (\pm 12.2)$ kg, respectively. The proportion of wheat in the diet did not influence these amounts (for the slope b_1 , total, $P = 0.458$; DM, $P = 0.203$; fluid, $P = 0.476$). Mean ($\pm$ SD) digesta DM and NDF concentrations were $12.4 (\pm 1.12)$ and $54.4 (\pm 2.42)\%$ and again, these variables were not related to proportion of wheat in the diet [for the slope b_1 , DM, $P = 0.086$ (a positive trend); NDF, $P = 0.114$]. There was, however, a significant positive relationship between proportion of wheat in the diet and digesta starch concentration (for the slope b_{11} , curve of best fit: $R^2 = 0.78$, $P < 0.001$, CV = 25.8%). Mean ($\pm$ SD) proportions of hay before consumption in the 3 particle size categories, large (retained on 2-mm sieve and above), small (retained on 0.25- and 1-mm sieves),

Figure 4. Relationships between in vivo digestibilities (D) of the total diet and the proportion of wheat in the diet (% wheat) of lactating cows fed pasture hay as their basal forage (◆ = DMD; ■ = OMD; ▲ = NDFD; ▼ = ADFD; □ = gross energy digestibility, GED). DMD = $64.7 (\pm 0.68) + 0.17 (\pm 0.033) \times \% \text{ wheat}$; $R^2 = 0.65$, $P < 0.001$, root mean square error (RMSE) = 1.42; OMD = $70.8 (\pm 0.67) + 0.11 (\pm 0.033) \times \% \text{ wheat}$; $R^2 = 0.46$, $P = 0.004$, RMSE = 1.40; NDFD = $71.0 (\pm 0.70) - 0.12 (\pm 0.034) \times \% \text{ wheat}$; $R^2 = 0.49$, $P = 0.003$, RMSE = 1.47; ADFD = $54.9 (\pm 1.55) + 0.27 (\pm 0.145) \% \text{ wheat} - 0.013 (\pm 0.0057) \times \% \text{ wheat}^2$; $R^2 = 0.34$, $P = 0.026$, RMSE = 2.77; GED = $59.9 (\pm 0.86) + 0.38 (\pm 0.108) \% \text{ wheat} - 0.006 (\pm 0.0031) \times \% \text{ wheat}^2$; $R^2 = 0.73$, $P < 0.001$, RMSE = 1.53.

Figure 5. Relationships between mean rumen fluid pH (□), and ammonia-N (◇), total VFA (◆), acetate (■), propionate (▲), and butyrate (▼) concentrations and the proportion of wheat in the diet (% wheat) of lactating cows fed hay as their basal forage. pH = $6.3 (\pm 0.04) - 0.012 (\pm 0.0019) \times \% \text{ wheat}$; $R^2 = 0.73$, $P < 0.001$, root mean square error (RMSE) = 0.081; ammonia-N = $4.3 (\pm 0.10) - 0.024 (\pm 0.0049) \times \% \text{ wheat}$; $R^2 = 0.63$, $P < 0.001$, RMSE = 0.213; total VFA = $110.9 (\pm 2.87) + 0.49 (\pm 0.140) \times \% \text{ wheat}$; $R^2 = 0.47$, $P = 0.004$, RMSE = 6.05; acetate = $74.4 (\pm 2.02) + 0.27 (\pm 0.099) \times \% \text{ wheat}$; $R^2 = 0.35$, $P = 0.016$, RMSE = 4.25; propionate = $20.8 (\pm 1.01) + 0.11 (\pm 0.049) \times \% \text{ wheat}$; $R^2 = 0.25$, $P = 0.048$, RMSE = 2.11; butyrate = $11.8 (\pm 0.69) + 0.11 (\pm 0.033) \times \% \text{ wheat}$; $R^2 = 0.45$, $P = 0.004$, RMSE = 1.44.

Figure 6. Changes in ruminal pH throughout a 24-h period for cows fed hay alone (3 cows; ■), and for those consuming 10 to 20% (5 cows; □), 20 to 30% (6 cows; ▲), and more than 30% (2 cows; ◇) of their diet as crushed wheat. The arrows indicate when hay was fed, and W indicates when wheat was also fed. The dashed line defines the ruminal pH below which NDF digestion theoretically declines.

and fines (passing through all sieves), were $71 (\pm 1.6)$, $4 (\pm 4.2)$, and $25 (\pm 5.8)\%$. Chewing in the process of eating reduced particle size in boli to $47 (\pm 3.5)$, $19 (\pm 3.0)$, and $34 (\pm 3.1)\%$ for large, small, and fines, respectively, and this was further reduced 3 to 4 h after the first morning feed to $21 (\pm 4.2)$, $28 (\pm 5.8)$, and $51 (\pm 9.0)\%$ in rumen digesta. Particle size distribution of rumen digesta was not influenced by proportion of wheat in the diet (for the slope b_1 , large, $P = 0.440$; small, $P = 0.458$; fines, $P = 0.369$).

Milk Production

There was a significant relationship between daily ECM (kg/cow) and daily wheat intake (kg of DM/cow), described by the following regression equation:

$$\text{ECM} = 16.9 (\pm 0.72) + 1.27 (\pm 0.164) \times \text{wheat intake};$$

$$R^2 = 0.85 \ (P < 0.001); \text{RMSE} = 1.50; n = 16.$$

The marginal response in milk production was 1.3 kg of milk per kg DM of wheat consumed. There were no significant effects of wheat in the diet on milk composition with mean ($\pm$ SD) fat, protein, and lactose concentrations being $4.3 (\pm 0.46)$, $3.4 (\pm 0.36)$, and $5.0 (\pm 0.16)\%$, respectively.

DISCUSSION

The results of this experiment did not support our hypothesis that the digestibility of NDF would be depressed in a curvilinear manner as the amount of crushed wheat increased beyond 25% of a pasture-based diet. Digestibility of NDF declined linearly as the amount of crushed wheat increased to 36% of DM intake, and that of ADF declined with more than 25% crushed wheat in the diet. A basal diet of chopped perennial ryegrass hay was used to reflect the nutritive characteristics of summer pastures in northern Victoria, and with an *in vivo* DM digestibility of 64%, which equates to an estimated ME concentration of 8.9 MJ/kg of DM, this objective was achieved (Stockdale et al., 2001). Associative effects occur when more than one feed is introduced into the digestive system and the rumen environment and digestibilities of the feed change, with the available energy of the whole diet rarely being a simple addition of the separate ingredients (e.g., Doyle et al., 2005). The negative associative effects on digestion of NDF were most likely associated with the forage because ADF digestibility also declined, and there was negligible ADF in the wheat. The higher rates of *in sacco* loss of NDF from wheat also seem to indicate that the NDF in crushed wheat was fermented in the rumen to a greater degree than that in the forage. Most data indicated, however, that effects of proportion of wheat in the diet on the utilization of consumed nutrients were small.

The degradation of cell wall carbohydrates by cellulolytic microorganisms is inhibited when rumen pH is low, such as situations where animals are fed both forage and a high-starch concentrate. Cellulolytic microorganisms are less tolerant of low pH than are rumen microorganisms that digest starch or sugars (e.g., Russell and Wilson, 1996); therefore, NDF digestibility will be depressed at low pH. The optimal ruminal pH, below which cellulolysis will be significantly inhibited, is regarded as being around 6.0 (Mould et al., 1983) to 6.2 (Pitt et al., 1996). Cows with more than 25% wheat in their diet in our experiment had mean daily ruminal pH values below 6.0, which would be considered sufficient to adversely affect fiber digestion. However, we recorded a decline in NDF digestion across the range of diets. Recent research with continuous culture systems has shown that the magnitude of the reduction in NDF digestibility depended on the degree of reduction of pH below 6.2 (de Veth and Kolver, 2001a; Cerrato-Sánchez et al., 2008) and the amount of time for which it remained low (de Veth and Kolver, 2001b; Cerrato-Sánchez et al., 2007a,b). The effect of increasing the proportion of wheat in the diet on these factors was less severe or prolonged than expected (e.g., Williams et al., 2005c; Figure 6); hence, depressions in fiber digestion

were not large. The sodium bicarbonate added to the wheat may have influenced rumen fluid pH, but this was likely to have only a short-term effect as cows received grain supplements in meals at milking times only (Dalley et al., 2001). The use of hay and the practice of feeding it 4 times daily probably allowed for enough chewing to buffer the rumen environment sufficiently to prevent major prolonged reductions in ruminal pH (Allen, 1997). Ruminal pH is very responsive to meals and chewing behavior (Williams et al., 2005c), and the decline in pH after a meal is dependent on meal size (Allen, 1997). Saliva is the key buffering agent in the rumen, and total saliva flow is a function of time spent resting, eating, and ruminating. For example, total saliva flow for a lactating cow eating 20 kg of DM/d of a silage-based diet was estimated by Cassida and Stokes (1986) to be 233 L/d, of which about 30% was produced while eating and the balance when resting/ruminating. Moreover, the greatest mean concentration of fermentation acids measured here of about 140 mM/L may not have been high enough to elicit a sufficiently negative effect on ruminal pH and, therefore, digestion (Williams et al., 2005a, b). It is not surprising, on the other hand, that starch digestion was not influenced by variations in ruminal pH because Hoover et al. (1984), Shriver et al. (1986), de Veth and Kolver (2001a), and Wales et al. (2004) have shown NSC to be highly digestible over a wide range in pH (4.5 to 7.5). The general conclusion was that microorganisms participating in NSC digestion are largely insensitive to pH changes. In addition, starch digestion occurring beyond the rumen can often be sufficient to counter any adverse effects in the rumen (Knowlton, 2001).

Similar rumen pool sizes and particle size distributions of rumen digesta indicated that there was probably an increase in apparent retention time of rumen contents as the proportion of wheat in the diet increased because fiber digestion did not decline to the degree we expected. Total DMI increased, but hay intake declined, and hay was likely to constitute most the rumen contents across the range of diets employed. The quantity of rumen contents was measured at least 4 h after the wheat had been consumed by which time it should have been almost completely degraded. An increase in the time that digesta remains in the rumen allows the microbial population more time in which to digest a greater proportion of all ingested nutrients, and fiber in particular, thereby limiting the decline in fiber digestion.

The milk yield response to supplementation with wheat was linear across the range of diets, and averaged 1.3 kg of ECM/kg DM wheat. This good response is supported by results found by Stockdale (1999) and Penno et al. (2006) who both showed that the lower the

energy concentration of the base herbage consumed by cows, the better the response to concentrates. Stockdale (1999) reported that the average response in FCM was 1.1 kg/kg of DM concentrates in summer compared with 0.5 kg/kg of DM in spring, when mean herbage ME concentrations were 8.6 and 10.7 MJ/kg of DM, respectively. Neither milk protein nor milk fat concentrations were affected. Thus, in support of the digestion and fermentation data, animal production responses suggested that negative associative effects across the range of diets used were small.

Two aspects linked to associative effects, both of which are related to intake, can affect responses to supplementary feeding, and thus profitability. The first concerns the reduction of basal forage intake when a supplement is fed, often referred to as substitution. In grazing cows, substitution of concentrates for pasture is generally between 0 and 1.0 kg DM reduction in pasture intake per kilogram of DM supplement consumed (Stockdale, 2000; Kellaway and Harrington, 2004). The substitution rate was 0.30 kg DM reduction in hay intake per kilogram of DM of wheat eaten in the current experiment. It has been hypothesized that substitution may be caused by negative associative effects in the rumen in which interactions between the digestion of concentrates and herbage may reduce the rate of fiber digestion and consequently herbage intake (Dixon and Stockdale, 1999). In grazing dairy cows, Doyle et al. (2005) suggested that a cow's preference for feeds and the physiological constraints of prehension affect substitution rates, whereas Bargo et al. (2003) proposed that the major effect of supplements on substitution rate was through reductions in grazing time.

The second aspect associated with intake concerns the feeding level of lactating cows in relation to the estimation of ME. The *in vivo* digestibility of standard feeds used in *in vitro* systems is usually measured by feeding mature sheep at maintenance. This has important implications when using estimated ME values to formulate diets for lactating dairy cows where intakes and rates of passage through the digestive tract are high relative to those of nonlactating animals fed at maintenance. We used both *in vitro* and *in vivo* DM digestibilities to estimate dietary ME in the cows fed hay alone, and estimates from *in vitro* data were about 1.5 MJ/kg of DM higher than those estimated from *in vivo* data. The *in vitro* DMD for the hay was 73.6% (about 10.5 MJ/kg of DM) and the *in vivo* DMD was 64.2% (about 8.9 MJ/kg of DM). The decline in ME due to level of feeding equated to about 5% for each unit increase in feeding level above maintenance. This compares with the 4% decline in digestibility of total digestible nutrients reported by Tyrrell and Moe (1975), and the 4.1% decline in energy digestibility found by Gabel

et al. (2003) for each multiple increase in intake above maintenance. Gabel et al. (2003) further reported that the decline in digestibility was greatest for structural carbohydrates as feeding level increased. The practical implication of this result is that using ME values estimated from *in vitro* DMD to formulate diets and to do feed budgets, without the required adjustments for intake, may greatly underestimate the amount of feed that needs to be supplied.

CONCLUSIONS

The digestibility of NDF was depressed as the amount of crushed wheat increased up to 36% when diets based on chopped perennial ryegrass hay were fed to lactating dairy cows, indicating that negative associative effects occurred. Although ruminal pH was below an optimum pH of 6.0 for up to 14 h in individual animals, most other ruminal variables measured were indicative of the efficient operation of the rumen, and milk responses were good even though substitution of wheat for hay reduced the forage intake of the cows. When we used *in vitro* and *in vivo* DM digestibilities to estimate ME content of the hay, estimates from *in vitro* data were about 1.6 MJ/kg of DM higher than those estimated from *in vivo* data.

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