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# Effects of partial mixed rations and supplement amounts on milk production and composition, ruminal fermentation, bacterial communities, and ruminal acidosis

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## ABSTRACT

Late-lactation Holstein cows (n = 144) that were offered 15 kg dry matter (DM)/cow per day of perennial ryegrass to graze were randomized into 24 groups of 6. Each group contained a fistulated cow and groups were allocated to 1 of 3 feeding strategies: (1) control (10 groups): cows were fed crushed wheat grain twice daily in the milking parlor and ryegrass silage at pasture; (2) partial mixed ration (PMR; 10 groups): PMR that was isoenergetic to the control diet and fed twice daily on a feed pad; (3) PMR+canola (4 groups): a proportion of wheat in the PMR was replaced with canola meal to produce more estimated metabolizable protein than other groups. Supplements were fed to the control and PMR cows at 8, 10, 12, 14, or 16 kg of DM/d, and to the PMR+canola cows at 14 or 16 kg of DM/d. The PMR-fed cows had a lower incidence of ruminal acidosis compared with controls, and ruminal acidosis increased linearly and quadratically with supplement fed. Yield of milk fat was highest in the PMR+canola cows fed 14 or 16 kg of total supplement DM/d, followed by the PMR-fed cows, and was lowest in controls fed at these amounts; a similar trend was observed for milk fat percentage. Milk protein yield was higher in the PMR+canola cows fed 14 or 16 kg of total supplement DM/d. Milk yield and milk protein percentage were not affected by feeding strategy. Milk, energy-corrected milk, and milk protein yields increased linearly with supplement fed, whereas milk fat percentage decreased. Ruminal butyrate and D-lactate concentrations, acetate-to-propionate ratio, (acetate + butyrate)/propionate, and pH increased in PMR-fed cows compared with controls for all supplement amounts, whereas propionate and valerate concentrations decreased. Ruminal acetate, butyrate, and ammonia concentrations, acetate-to-propionate ratio, (acetate + butyrate)/propionate, and pH linearly decreased with amounts of supplement fed. Ruminal propionate concentration linearly increased and valerate concentration linearly and quadratically increased with supplement feeding amount. The *Bacteroidetes* and *Firmicutes* were the dominant bacterial phyla identified. The *Prevotellaceae*, *Ruminococcaceae*, and *Lachnospiraceae* were the dominant bacterial families, regardless of feeding group, and were influenced by feeding strategy, supplement feeding amount, or both. The *Veillonellaceae* family decreased in relative abundance in PMR-fed cows compared with controls, and the *Streptococcaeae* and *Lactobacillaceae* families were present in only minor relative abundances, regardless of feeding group. Despite large among- and within-group variation in bacterial community composition, distinct bacterial communities occurred among feeding strategies, supplement amounts, and sample times and were associated with ruminal fermentation measures. Control cows fed 16 kg of DM of total supplement per day had the most distinct ruminal bacterial community composition. Bacterial community composition was most significantly associated with supplement feeding amount and ammonia, butyrate, valerate, and propionate concentrations. Feeding supplements in a PMR reduced the incidence of ruminal acidosis and altered ruminal bacterial communities, regardless of supplement feeding amount, but did not result in increased milk measures compared with isoenergetic control diets component-fed to late-lactation cows.

**Key words:** bacterial community composition, partial mixed ration, protein, ruminal acidosis, supplements

## INTRODUCTION

A TMR fed to cattle on a feed pad between grazing periods is termed a partial mixed ration (**PMR**) (Bargo

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et al., 2002b; Auldist et al., 2013). This feeding strategy increases milk yield and milk fat and protein percentage (Bargo et al., 2002a) and improves marginal milk responses and increased yields of milk fat (Auldist et al., 2013) over those of pasture-fed cows supplemented with grain in the milking parlor and conserved forage fed on the pasture.

Auldist et al. (2013) proposed that a well-formulated PMR that is consumed over a longer period could lead to more stable ruminal fermentation compared with grain fed in the parlor. The risk of ruminal acidosis may then be reduced, a hypothesis that is supported by a higher ruminal pH in PMR-fed compared with control cows fed grain in the parlor, silage, and fresh-cut pasture (Greenwood et al., 2014).

The substitution of some of the wheat for canola meal in a PMR also increased ECM and was associated with a higher concentration and yield of milk fat, and higher pasture DMI (Auldist et al., 2014). Other protein supplements, such as canola meal, have also increased milk yield (Oldham, 1984; Huhtanen et al., 2011; Martineau et al., 2013). Allen et al. (2006) proposed that high protein feeds have a buffering capacity in the rumen, which is consistent with the report of Auldist et al. (2014), in which substituting wheat for canola meal in a PMR decreased the amount of time pH was under 6.0 and increased mean daily ruminal pH, despite having no significant influence on ruminal VFA (Auldist et al., 2014).

Understanding the complex and dynamic ruminal microbial ecosystem (Fernando et al., 2010) is essential to the development of feed management practices that promote optimal production efficiency (de Menezes et al., 2011). Ruminal acidosis is an important example of an interaction between ruminal microbial metabolism and diet that can impair health and production (Tajima et al., 2000; Khafipour et al., 2009). Weimer et al. (2010) suggested that the ruminal microbiome is reasonably resistant to dietary changes, and a change in the microbiome is not always related to the severity of ruminal acidosis (Mohammed et al., 2012). Early understandings of rumen microbiology and bacteria believed to be involved with ruminal acidosis were based on bacterial cultures. More recently, molecular techniques, which are rapidly improving and becoming less expensive, have been adopted in evaluations of rumen microbiology. Integration of knowledge obtained from classical culture-based microbiology and modern molecular techniques is rapidly increasing our understanding of the rumen microbiome and its functions in general (Pers-Kamczyc et al., 2011). This knowledge will potentially facilitate optimal dietary management and reduce the incidence of nutritional disorders such as ruminal acidosis, among other benefits. Despite constant evolution of methods for studying the rumen ecosystem, only about 10% of the rumen microbiome is known (Pers-Kamczyc et al., 2011). This limited knowledge impedes understanding of the importance of changes in ruminal microbial populations observed during rumen perturbation and ruminal acidosis.

Recent work suggests that the rumen has a core bacterial microbiome that consists primarily of bacteria from the *Firmicutes* and *Bacteroidetes* phyla, which appear to change in cattle fed various ruminal acidosis induction diets or increasing amounts of grain (Khafipour et al., 2009; Callaway et al., 2010; Fernando et al., 2010; de Menezes et al., 2011). Bacteria from the *Proteobacteria* appear to be the third most dominant in the rumen in several (Khafipour et al., 2009; Jami and Mizrahi, 2012), but not all, ruminant studies (Golder et al., 2014b). Increases in *Proteobacteria* have been reported in TMR-fed compared with pasture-fed cattle (de Menezes et al., 2011) and in cattle with grain-induced compared with alfalfa pellet-induced subacute acidosis (Khafipour et al., 2009). Increases in *Streptococcus* and *Lactobacillus* were associated with ruminal acidosis in early work (Hungate et al., 1952; Hungate, 1966); however, as only a small portion of the rumen microbiome is known, other rumen bacteria may have prominent roles in acidosis.

The objectives of this study were to examine relationships between milk measures, ruminal fermentation measures, ruminal acidosis, and ruminal bacterial community composition (**BCC**) in lactating cows offered a restricted pasture allowance and fed supplements using different feeding strategies and at linearly increasing supplement amounts. The aim was to identify feed management systems that improve milk measures, promote optimal conditions in the rumen for digestion, and reduce ruminal acidosis.

The hypotheses tested were (1) that cows fed supplements as a PMR, with or without inclusion of canola meal, would have increased milk measures, altered ruminal measures, distinct ruminal BCC, and decreased ruminal acidosis compared with control cows fed isoenergetic diets as grain in the parlor and forage fed on the pasture; (2) that a linear increase in the amount of supplement fed (or increase when 14 and 16 kg of supplement DM are fed) would alter milk and ruminal fermentation measures, create a distinct ruminal BCC, and increase ruminal acidosis.

## MATERIALS AND METHODS

The experiment was conducted in late fall at the Department of Environment and Primary Industries (DEPI), Ellinbank Centre, Victoria (**VIC**), Australia (38°14′S, 145°56′E). All experimental procedures were