



## The effects on ruminal pH and serum haptoglobin after feeding a grain-based supplement to grazing dairy cows as a partial mixed ration or during milking

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### ABSTRACT

Ruminal pH and serum concentrations of haptoglobin (Hp) were measured in order to assess the risk of subacute ruminal acidosis (SARA) in grazing cows offered rolled wheat grain twice daily in the dairy at milking (Control group;  $n = 64$ ), or as a partial mixed ration (PMR group;  $n = 64$ ) on a feedpad. Cows were allocated various levels of the supplement (8, 10, 12 or 14 kg dry matter/day). Ruminal pH was measured in 16 rumen-fistulated cows (eight PMR and eight Control group cows), using indwelling pH meters, recording every 10 min for 14 days. Serum Hp was analysed in samples collected from 125 cows. No differences in ruminal pH or serum Hp concentration were found between treatment groups, or levels of feeding. It was concluded that, using ruminal pH patterns and Hp as markers of SARA at the feeding levels used in this study, there were no differences between grazing cows fed the supplement either as grain in the dairy or as a PMR fed on a feedpad.

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### Introduction

Traditionally, in grazing-based dairy systems, pasture has been the main source of nutrients for dairy cows, with limited supplementation with grain or concentrates in the dairy and/or conserved forage in the paddock. In Australian dairying regions, highly variable rainfall since 2000 has led to an increase by farmers in the use of grain-based supplements in milking herds, from an average of 1.4 tonnes per cow in 2008 to 1.74 tonnes per cow in 2012 (Dairy Australia, 2012).

One of the risks to the health and welfare of grazed dairy cattle fed high amounts of supplementary cereal grain or pelleted concentrates is subacute ruminal acidosis (SARA), particularly if they are offered the supplement in the dairy during milking as a 'slug' of kilograms of grain at a time (Westwood et al., 2003). The fermentation of the grain can lead to the accumulation of volatile fatty acids and lactic acid in the rumen, resulting in a drop in the pH of the ruminal fluid to below the optimal level for digestion (Leddin et al., 2010) or even below the threshold for SARA (usually defined as pH 5.5 to 5.8; Garrett et al., 1999; Gozho et al., 2005; Bramley

et al., 2008; Beauchemin and Penner, 2009). The potential effects of sub-optimal ruminal pH on cow health and welfare include changes in the balance of the rumen microflora and increased epithelial permeability (Owens et al., 1998), ruminitis, with subsequent inflammatory-mediated liver changes (Gozho et al., 2005; Trevisi et al., 2010) and coriosis or degeneration of the corium of the hoof (Nocek, 1997).

Northern hemisphere studies have estimated the prevalence of SARA to range from 11% to 29% in cows in early lactation (Tajik and Nazifi, 2011). Very few studies have recorded the prevalence of SARA in dairy cattle under pasture-based conditions in Australia; Bramley et al. (2008) classified cows into three categories based on measurement at a single timepoint of rumen volatile fatty acids, ammonia, and rumenocentesis pH. They reported that 10.2% of cows were acidotic; 29.9% had suboptimal rumen function, and 59.9% were non-acidotic.

Nutritional experiments (Wales and Doyle, 2003; Wales et al., 2004) have shown a typical diurnal variation in ruminal pH in grazing cows fed supplementary grain. Wales et al. (2004) proposed that to optimise digestion and milk production, strategies should be employed that limit the amount of time that the ruminal pH is below 5.6, and achieve more time with a ruminal pH > 6. One of these strategies may be to avoid large fluctuations in pH by offering the supplement as a partial mixed ration (PMR), where the grain offered

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to the cows is mixed with a source of fibre. This means that rather than the grain being ingested as a 'slug' as is the case when it is fed in the bail at milking, it is offered in a more slowly digestible form (with the addition of the maize) with the aim of reducing the speed of lactic acid production. This could minimise ruminal pH fluctuations and improve milk production (Beever and Doyle, 2007). In the experiment of Auldist et al. (2013), cows fed 12 kg of a maize-based PMR were found to have less milk fat depression and a higher mean rumen pH (measured periodically over a 24-h period) than cows fed a barley-based supplement at the same rate per day in the dairy at milking.

Attempts to monitor diurnal ruminal pH variation in grazing cows is limited by the difficulty in taking regular pH measurements in cows that spend most of the day at pasture. The development of indwelling ruminal pH boluses (Enemark et al., 2003; Penner et al., 2006; Kaur et al., 2010) has enabled the constant monitoring of ruminal pH to be more practical, and less labour-intensive, and most importantly, allows grazing cows to feed and behave as they normally would throughout the day while their ruminal pH is recorded.

Although a low ruminal pH may be the initial step in the pathogenesis of SARA and its sequelae, a useful marker of inflammation resulting from SARA may be the concentration in the circulation of acute phase proteins (APPs), such as haptoglobin (Hp). Several authors have reported elevated Hp concentrations associated with infectious conditions, leading to an acute phase inflammatory response. For example, Eckersall et al. (2001) reported significantly elevated Hp in cows with mild or moderate mastitis, compared with healthy cows. In steers in which SARA was experimentally induced (rumen pH <5.6 for greater than 3 h/day), Gozho et al. (2005) found that steers with SARA had elevated serum Hp, whereas the non-affected steers did not. The authors noted that Hp levels in the SARA-affected steers were less than those reported in cattle with acute-phase responses associated with infectious conditions. To date, Hp levels in grazing cows fed various levels of grain supplements have not been examined.

The aim of the present experiment was to investigate whether there was a difference in markers associated with rumen health and SARA if grazed cows were fed grain supplement in the bail during milking (Control group) or as a PMR on a feedpad following milking (PMR group), using values and diurnal fluctuations of ruminal pH, plus concentrations of APPs in the blood.

## Materials and methods

The experiment was conducted at Department of Environment and Primary Industries (DEPI) – Victoria, Ellinbank Centre (38°14'S, 145°56'E) with the approval of the DEPI Agricultural Research and Extension Animal Ethics Committee (approval number 2009-17; date of approval, 1 August 2009).

### Cows and management

The experiment was conducted as part of a larger project aimed at measuring milk production responses of grazing cows to supplements offered in different ways (Auldist et al., 2014). Here we used 128 multiparous seasonally-calving Holstein-Friesian cows, including 16 rumen-fistulated cows of mean age  $4.8 \pm 1.5$  (SD) years, producing  $562 \pm 55$  kg milk over  $49 \pm 16$  days in milk (DIM). Cows were milked twice daily at approximately 0700 and 1500 h.

The experiment was conducted over 31 days. This included a 14-day pre-experimental period during which the amounts of feed were increased from approximately 8 kg of dry matter (DM) total supplement/cow/day to the final amount of supplement. Following the pre-experimental period, there was a 14-day measurement period, where ruminal pH was measured. Serum haptoglobin was measured in blood samples collected on day 31 of the experiment.

All cows grazed perennial ryegrass (*Lolium perenne*) pasture at an allowance of approximately 14 kg DM/cow/day (measured to ground level). Cows were divided into 16 groups of eight cows, with groups balanced for DIM, age, bodyweight and prevailing production of milk, milk protein and milk fat, according to the method of Baird (1994). Each group included a rumen-fistulated cow. Any cows displaying signs of illness or disease during the experiment were assessed by a veterinarian and details of the illness were recorded.

### Treatments

One of two dietary treatments was then assigned at random to each of the 16 groups (eight groups per treatment). The treatments were: (1) Control; cows were fed rolled wheat grain twice daily in the milking parlour, with pasture silage provided in the paddock (ratio of supplemented grain to supplemented forage 0.72:0.28 on DM basis). This treatment was assigned to eight groups. (2) PMR; cows were offered a PMR, comprising 39% rolled wheat grain, 20% rolled maize grain, 32% maize silage and 9% lucerne hay (DM basis), which was mixed and chopped in a feed wagon (Model K160, Keenan) before being presented on a concrete feedpad immediately after each milking (half the daily ration was fed following each milking). Water was added to the PMR such that the final DM content of the ration approximated 50%. The formulation of the PMR was designed to provide the same estimated ME intake as the supplements offered to the Control cows, and had the same ratio of grain to forage (0.72:0.28 DM basis).

Within each dietary treatment, groups were assigned to different amounts of supplement. For both Control and PMR cows (of which there were eight groups of eight cows each), two groups were assigned to receive 8, 10, 12 or 14 kg DM total supplement/cow/day. After measurement of refusals, the actual DM intakes of supplement per day were 7.7, 9.6, 11.6 and 13.4 for Control group cows and 8.8, 10.8, 12.9 and 14.8 kg for PMR group cows.

Cows on the PMR treatment received their supplements on the feedpad, with the groups separated by electric tapes. Cows on the Control diet were fed their grain in the milking parlour at each milking, and their pasture silage was fed by placing the allocation for each group in their grazing area each day. Cows receiving the highest amounts of supplement (12 and 14 kg DM/cow/day) were introduced gradually to the diet, reaching their full amount of ration 5 days after the commencement of the experiment, during the 14-day adaptation period. The detailed chemical composition of the diets is provided in Auldist et al. (2014).

All cows had several opportunities each day to access water ad libitum, from troughs located in and adjacent to the milking parlour, and in laneways adjacent to the paddocks used for grazing. Control cows had access to pasture immediately after each milking. Cows on the PMR treatment were given access to pasture after they had consumed their ration on the feedpad following each milking, and grazed in groups of eight cows on adjacent areas separated from the other groups by electric tapes. Cows were prevented from re-grazing areas that had been grazed on previous days.

### Measurements

Ruminal pH was measured for the 16 fistulated cows. A pH measurement bolus (KB5, Kahne) was calibrated and then inserted into the rumen of 16 rumen-fistulated cows (eight from the Control group and eight from the PMR group) at the commencement of the 31-day feeding period. The ruminal pH measured by the boluses was logged every 10 min and the data automatically stored in the bolus memory. These boluses were an improved version of those previously used by Kaur et al. (2010), and had been checked for measurement drift in vitro and in vivo before the experiment, and calibrated at the time of deployment in the rumen of the fistulated cows.

Serum Hp concentration was measured on 125/128 cows in the experiment. A 2-mL sample of blood was withdrawn from the coccygeal vein of each cow on the final day of the 31-day feeding period. There were 63 PMR and 62 Control blood samples available. The blood was analysed for concentrations of Hp according to the method described by Jones and Mould (1984). The assay was run against a standard (2.9 mg/mL) obtained from ovine blood that had an inflammatory response induced as a result of fly-strike (Slocombe and Colditz, 2012). Readings of assays were maintained with a CV of <10%. Any assays above this level were repeated. The values were corrected for haemoglobin interference using the formula derived by Slocombe and Colditz (2012). When this correction resulted in a value less than the lowest detectable concentration in serum (0.009 mg/mL), the sample was assigned a value of 0.0045 mg/mL (half the lowest detectable concentration).

### Statistical analyses

One of the pH boluses had been incorrectly calibrated prior to the 14-day measurement period. The data for this PMR (10 kg) group cow were excluded from the analyses of ruminal pH.

The ruminal pH data for each of the 15 cows during the 14-day period following the 2-week adaptation period comprised 2016 data points. Each data point specified the cow identity, bolus identity, time, date and ruminal pH. These were summarised using a script written in Matlab Release 2012b (Mathworks). The script generated for each cow included: (1) the daily average pH; (2) percentage of time (per day) that the ruminal pH was <5.6; (3) percentage of time (per day) that the ruminal pH was <6; (4) the integration of time  $\times$  pH when ruminal pH was <5.6, and (5) the integration of time  $\times$  pH when ruminal pH was <6.0.

The ruminal pH data were analysed using ANOVA for the effects of method of supplementation (2 levels) and linear effect of rate (4 levels), plus their interaction. The interaction was not significant for each of the pH variables (all  $P > 0.10$ ) and was therefore deleted from the ANOVA. The values for serum concentrations of Hp were  $\log_{10}$  transformed. The interaction was not significant ( $P = 0.85$ ) and was deleted from the ANOVA. The analysis of data was performed using SPSS Version 20.0 (IBM).

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