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## Importance of yeast viability for reducing the effects of ruminal acidosis in beef heifers during and following an imposed acidosis challenge



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#### ABSTRACT

The study was aimed at determining the importance of yeast (Saccharomyces cerevisiae) viability for reducing the severity of ruminal acidosis in cattle during and following an imposed acidosis challenge. Six ruminally cannulated beef heifers were used in a replicated  $3 \times 3$  Latin square design and fed a diet consisting of 400 g/kg barley silage, 100 g/kgchopped grass hay, and 500 g/kg barley grain based concentrate (dry matter basis). Treatments were: (1) control (no yeast); (2) active dried yeast (ADY; 4 g providing 10<sup>10</sup> colony forming units/g; AB Vista, UK); and (3) killed dried yeast (KDY; 4 g autoclaved ADY). The periods consisted of 2 weeks of adaptation (day 1-14), week 3 of baseline measurements (day 15-21) and week 4 of acidosis challenge (day 22-28). The challenge model involved restricting consumption of the TMR to 0.5 of *ad libitum* intake for 24h (day -1; prechallenge) followed by adding barley grain (amount equivalent to 0.25 of DMI) directly to the rumen prior to feeding the TMR (day 0; challenge day). Data were collected from 0 to 24 h on the challenge day, 25-48 h post grain challenge (day 1 postchallenge) and during a 5-day recovery period. No treatment effects were observed on mean (P=0.40), nadir (P=0.37) and maximum (P=0.29) ruminal pH on the challenge day. Similarly, no treatment differences were observed for ruminal lactate (P=0.46) and total VFA concentrations (P=0.15) on the challenge day. However, proportion of ruminal propionate was increased (P=0.01) while caproate reduced (P=0.01) with ADY. The duration of time that pH < 5.8 (P=0.26) and 5.6 (P=0.32) was similar for all treatments. No treatment effects were observed on DMI on the challenge day (P=0.95); and day 1 postchallenge (P=0.30). In conclusion, contrary to our hypothesis, yeast supplementation did not influence ruminal pH during a severe acidosis challenge as the efficacy of both viable and killed yeast was reduced at low ruminal pH. Lack of significant effects observed in the present study might also be due to variability associated with the animal responses to an acute acidosis challenge.

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*Abbreviations:* ADY, active dried yeast; ADF, acid detergent fiber expressed inclusive of residual ash; AUC, area under curve; NH<sub>3</sub>–N, ammonia nitrogen; CFU, colony forming unit; CP, crude protein; DFM, direct fed microbial; DM, dry matter; DMI, dry matter intake; KDY, killed dried yeast; aNDF, neutral detergent fiber assayed with heat stable amylase and expressed inclusive of residual ash; MGA, melengesterol acetate; SARA, sub-acute ruminal acidosis; TMR, total mixed ration; VFA, volatile fatty acids.

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#### 1. Introduction

Sub-acute ruminal acidosis (SARA) is considered a prevalent nutritional disorder in feedlot cattle characterized by low ruminal pH (pH < 5.6) due to an accumulation of ruminal volatile fatty acids (VFA) and lactic acid. Low ruminal pH is not only detrimental to production performance but also it has negative impacts on health of the animals (Nagaraja and Titgemeyer, 2007). Preventative measures for acidosis and maintenance of healthy rumen function includes the use direct-fed microbials (DFM).

Among DFM, *Saccharomyces cerevisiae* is of interest because of its proposed effects of stabilizing ruminal pH and reducing the incidence of SARA (Chaucheyras-Durand et al., 2008). Although the mechanism by which yeast supplementation stabilizes ruminal pH has not been clearly established, it is thought to reduce the accumulation of ruminal lactate by stimulating growth of lactic acid utilizing bacteria (Nisbet and Martin, 1991; Williams et al., 1991). Previous studies observed greater efficacy of live yeast as compared to autoclaved yeast emphasizing the importance of viability in stimulating ruminal fermentation (Chaucheyras et al., 1995; Koul et al., 1998). Conversely, recent *in vitro* studies have observed stimulatory effects of inactivated cells of *S. cerevisiae* by providing nutrients contained within the cells to autochthonous microbiota (Oeztuerk et al., 2005; Oeztuerk, 2009; Opsi et al., 2012). Similarly, irrespective of its viability, yeast supplementation elevated ruminal pH in beef heifers not experiencing acute acidosis (Vyas et al., 2014). Therefore, the extent to which viable yeast cells are necessary to exert their effects on ruminal fermentation is not known. In addition, it is not known whether yeast supplementation can improve ruminal fermentation when ruminants encounter severe acidosis. Hence, the present study was undertaken to investigate the importance of yeast viability with the hypothesis that viable yeast would be more effective in stabilizing ruminal pH during and after a severe acidosis challenge in beef heifers.

#### 2. Materials and methods

The Lethbridge Research Centre Animal Care Committee approved the protocol before the experiment began and heifers were cared for according to the guidelines of Canadian Council on Animal Care (Ottawa, Ontario, Canada).

#### 2.1. Animal, diets and experimental design

Six ruminally cannulated crossbred beef heifers ( $680 \pm 50$  kg) were used in a replicated  $3 \times 3$  Latin square design. Animals were randomly assigned to (1) control (no yeast), (2) active dried yeast (ADY; 4 g/day), or (3) killed dried yeast (KDY; 4 g/day). Experimental animals were fed basal diet composed of 400 g/kg barley silage, 100 g/kg chopped grass hay, and 500 g/kg barley grain based concentrate on dry matter (DM) basis (Table 1). Melengestrol acetate (MGA) supplement was top-dressed on the TMR to provide 0.4 mg of MGA per animal per day for estrus suppression. Yeast strain used was *S. cerevisiae* (AB Vista, Marlborough, Wiltshire, UK) and the viability of the preparation was checked prior to starting the experiment. Treatments were dosed via the rumen cannula daily at the time of feeding using a gelatin capsule (Torpac Inc., Fairfield, NJ) to ensure each animal received the full amount. Control animals received an empty capsule. The procedure for inactivation of ADY is described earlier (Vyas et al., 2014). Briefly, yeast cells were ground in a Knifetec 1095 sample mill (Foss Tecator, Höganäs, Sweden) for 20 s, followed by standard autoclaving for 20 min at 121 °C and 15 psi (103.4 kPa; BetaStar Corporation, Telford, PA). The efficacy of the method used was tested by incubating 10 mg of autoclaved yeast in yeast extract/peptone/dextrose liquid medium in a shaking incubator at 30 °C followed by plating 100 µL of inoculate from the liquid medium onto yeast extract/pepton/dextrose agar, and incubating for 3 days at 30 °C (Oeztuerk et al., 2005). The mean number of yeast colonies detected on agar were  $3.48 \pm 0.88 \times 10^{10}$  colony forming units (CFU)/g for ADY and  $2.86 \pm 4.36 \times 10^2$  CFU/g for KDY.

#### 2.2. The acidosis challenge model

The experimental period consisted of 28 days. After 2 weeks of adaptation to the treatments, 7 days were used for baseline measurements in which the heifers had *ad libitum* access to TMR. Data from baseline measurements were presented earlier (Vyas et al., 2014). The baseline measurement week was followed by restricted feed delivery on the day before the challenge (day -1; prechallenge day). Feed was restricted to 0.5 of *ad libitum* intake for 24 h. On the challenge day (day 0), barley grain, ground through a 4.5 mm screen (standard model 4, Arthur Thomas Co., Philadelphia, PA), equivalent to 0.25 of dry matter intake (DMI) (measured on 10 previous days) of each heifer was administered directly in the rumen, before offering the TMR for *ad libitum* consumption. During the remaining days including day 1 postchallenge and day 2 to day 6 recovery days, heifers received TMR *ad libitum*. If ruminal pH declined below 4.8, heifers were provided with an intraruminal dose of 250 g of sodium bicarbonate. This intervention was required for two of the six heifers in the first two periods while no intervention was required in the third period. Data from these heifers were included in the data analysis. Each period was followed by a 7-day washout period to minimize carryover effects in the next treatment period.

#### 2.3. Data and sample collection

Daily intakes and refusals of the TMR for individual heifers were recorded. Samples of the TMR and ingredients were collected weekly. Samples were composited and stored frozen until analyzed for DM, organic matter (OM), crude protein

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