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RESEARCH ARTICLE

In vitro investigation of the effect of dairy propionibacteria on rumen pH, lactic acid and volatile fatty acids

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Abstract

Ruminal acidosis is a prevalent disorder in ruminants such as dairy cows and feedlot beef cattle, caused primarily by the inclusion of a high percentage of readily fermentable concentrates in the diet. The disorder presents as an accumulation of lactic acid, a decrease of pH in the rumen and a subsequent imbalance of the rumen fermentation process with detrimental impacts on the animal's health and productivity. Dairy propionibacteria, a group of bacteria characterised by utilization of lactic acid as the favoured carbon source, with propionic acid produced as a by-product, were evaluated in this study as potential direct-fed microbials for use in controlling ruminal acidosis. Acidosis was simulated by introduction of high concentrations of lactic acid into rumen fluid samples and a multi-strain *in vitro* analysis was conducted, whereby changes in pH and lactic acid metabolism were compared in identical acidified rumen samples, following inoculation with various propionibacteria. This was followed by a study to evaluate the effect of bacterial inoculation dosage on acid metabolism. The results indicated that lactic acid levels in the rumen fluid were significantly reduced, and propionic acid and acetic acid concentrations both significantly increased, following addition of propionibacteria. Significant 'between strains' differences were observed, with *Propionibacterium acidopropionici* 341, *Propionibacterium freudenreichii* CSCC 2207, *Propionibacterium jensenii* NCFB 572 and *P. jensenii* 702 each producing more rapid reduction of lactic acid concentration than *P. freudenreichii* CSCC 2206, *P. acidopropionici* ATCC 25562 and *Propionibacterium thoenii* ATCC 4874. Furthermore, the efficacy of this application was dosage related, with the rates of reduction in lactic acid levels and production of propionic acid, both significantly greater for the higher (10^{10} cfu mL^{-1}) compared with lower (10^{5} cfu mL^{-1}) dosage inoculation. The results confirmed that the introduction of propionibacteria could promote more rapid reduction of lactic acid levels than would occur without their addition, demonstrating their potential in controlling ruminal acidosis.

Keywords: probiotics, ruminal acidosis, lactic acid, propionic acid, dairy *Propionibacterium*

1. Introduction

Lactic acid accumulation in the rumen is characteristic of ruminal acidosis in lactating cows and intensively reared beef cattle, as well as in other ruminants such as sheep. The main reason for the accumulation of lactic acid in the rumen is feeding on highly fermentable carbohydrates

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(such as a high grain diet). The available carbohydrates act as stimulation agents for the rapid growth of lactic acid bacteria such as *Streptococcus bovis* in the rumen (Elghandour *et al.* 2015). These bacteria are usually present as part of the normal rumen micro-flora, but during rapid growth they can produce large quantities of lactic acid which may imbalance the buffering capacity of the rumen, resulting in acidosis in healthy adult cattle (Owens *et al.* 1998; Parrot *et al.* 2001; Nagaraja and Titgemeyer 2007; Hutton *et al.* 2012). In healthy adult cattle, the lactic acid concentration in the rumen does not typically exceed 5 mmol L^{-1}, but it may increase as much as 40 mmol L^{-1} during acute acidosis (Dawson *et al.* 1997). The rapid accumulation of lactic acid can result in a significant decrease in pH within the rumen. When ruminal pH drops to a level between 5.0 and 5.5, fermentation within the rumen is disturbed, upsetting the animal's capacity to digest food, leading to a reduction in feed intake, energy absorption, and reduced meat and milk production (Dirksen 1985; Enemark *et al.* 2004; Plaizier *et al.* 2008). The continued production of lactic acid in the rumen can lead to further reductions in pH, which can be fatal to the animal if treatment is delayed.

There are a number of established strategies for treatment and prevention of ruminal acidosis, such as neutralization using chemicals like bicarbonate soda, feed management (improved fibre content) and antibiotic treatment (Enemark 2008). Although these methods can be effective in controlling and preventing ruminal acidosis, there are shortcomings and limitations to their application, such as development of resistant strains of bacteria through the long term use of antibiotics. Therefore the use of direct-fed microbials (DFM) for the treatment of ruminal acidosis has been investigated (Kung and Hession 1995; Martin and Streeter 1995; Nocek *et al.* 2002; Elghandour *et al.* 2015). Several lactobacilli including *Lactobacillus acidophilus* and *Lactobacillus casei*, as well as *Enterococcus diacetylactis* and *Bacillus subtilis,* are commonly available as DFM products for ruminants. These microorganisms may aid in providing of a constant lactic acid supply, adapting of overall rumen microbiota to the lactic acid accumulation, stimulation of lactate utilizing bacteria and stabilizing of ruminal pH (Seo *et al.* 2010). Compared with the prevention methods outlined above, the DFM approach does not encourage proliferation of antibiotic resistant strains; avoids the potential problem of overdosing of buffering chemicals and may have better cost-efficiency than strict feed management. However, acid metabolism patterns can vary significantly between bacterial species or strains, therefore appropriate selection of DFM candidates as a treatment for ruminal acidosis is critical. For the treatment and prevention of ruminal acidosis, the capacity for lactic acid consumption is the primary selection criteria. It is also important that the selected organisms do not produce any by-products during the application that might be harmful to either the recipient animal or the environment.

Lactic acid metabolism by propionibacteria results in production of acetic and propionic acid which can be readily absorbed through the ruminal wall as energy sources for the animal (Huntington *et al.* 1983; Peters *et al.* 1990; Pascal 1999). Propionibacteria such as *Propionibacterium acnes* belong to the indigenous micro-flora of the rumen but are generally present in low numbers under normal conditions (Mackie and White 1990; Koniarova 1993), possibly due to their less competitive nature. There are no reports to indicate that substantial increases in the number of propionibacteria occur in the rumen in response to rising lactic acid concentrations during ruminal acidosis. However, several previous studies have assessed the effects of direct supplementation with dairy propionibacteria on rumen fermentation processes in cattle. Lehloenya *et al.* (2008) reported that feeding *Propionibacterium* strain P169 and yeast culture to Angus×Hereford steers for 21 days increased the molar proportion of propionate by 9.7% compared with a control group fed a diet without supplementation of propionibacteria or yeast. Previous studies have also shown that the introduction of certain strains of *Propionibacterium* helped to improve calf weight gain (Adams *et al.* 2008) and milk quality (Francisco *et al.* 2002; Stein *et al.* 2006) without any adverse effect on the animal, and that direct feeding with *Propionibacterium* strain P169 was responsible for higher production of ruminal propionate in Holstein cows (Stein 2006). Another advantage in using dairy propionibacterium as DFM is their natural ability to survive in low pH conditions (Cousin *et al.* 2011). Certain propionibacteria strains have demonstrated better survival in acidic conditions around pH 3–4 (Huang and Adams 2004; Ranadheera *et al.* 2012). Theoretically, the application of DFM such as dairy propionibacteria in ruminal acidosis is based on a potential ability to consume and reduce the lactic acid accumulated in the rumen and redirect the imbalance of disturbed fermentation back to normal. To comprehend how these bacteria behave and metabolize in rumen fluid is important for their potential application in cattle. Not only would it provide detailed evidence to support the theory of their application in treating ruminal acidosis, but would also provide a valuable guideline for future research.

The objective of the present study was to investigate the feasibility and efficacy of using dairy propionibacteria as DFM to treat ruminal acidosis, by examining changes in acid levels in rumen fluid following introduction of propionibacteria, under simulated acidosis conditions.