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Efficacy and mode of action of selected non-ionophore antibiotics and direct-fed microbials in relation to *Megasphaera elsdenii* NCIMB 41125 during *in vitro* fermentation of an acidosis-causing substrate

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ABSTRACT

The efficacy of prominent in-feed antibiotics and direct-fed microbials (DFM) to prevent or mitigate ruminal acidosis and lactate accumulation, in addition to whether their presence will enhance or inhibit *Megasphaera elsdenii* strain NCIMB 41125 (Me) were studied *in vitro*. The antibiotics studied were aureomycin+sulfamethazine as AS-700 (AS), terramycin as TM-200 (TM), zinc bacitracin (ZB), flavomycin (FM) and tylosin (TS). The DFM were Bovamine (BM) which contains a propionic bacterium and a lactobacillus, Levucell (LC) which contains a strain of the yeast *Saccharomyces cerevisiae* and Progut (PG) which contains a hydrolysate of *S. cerevisiae*. The antibiotics and DFM were introduced alone or in the presence of Me to an *in vitro* system with fermentation vessels containing a medium that promoted rapid gas production and lactate development. Dose sizes of the antibiotics were chosen to inhibit fermentation by 10–20% or 30–40% and for DFM dose sizes were according to the manufacturers. For Me the dose size was 100 μl/40 ml containing 2.5×10^5 colony forming units per ml. Me on average reduced lactate from 20.0 mM to 4.89 mM, increased VFA production and shifted VFA proportions to more butyrate and valerate (respectively from 5.80 to 16.0 mM/100 mM and from 0.51 to 4.71 mM/100 mM). The antibiotics moderately reduced lactate (26.7–16.8 mM), and AS, ZB and TS enhanced a VFA proportional shift towards propionate (from 22.6 to 28.7 mM/100 mM). In the presence of Me lactate was reduced to levels of Me alone and the ratio butyrate to propionate was reduced. None of the antibiotics inhibited the action of Me; on the contrary the interaction was additive. In contrast to the antibiotics and PG, the DFM BM and LC did not affect fermentation resulting in no response with respect to any of the variables measured. PG in the presence of Me apparently enhanced the action of Me, as noticed by an additional increase in butyrate and valerate proportions.

1. Introduction

Ruminal or lactate acidosis is characterised by rapid accumulation of lactic acid and volatile fatty acids (VFA),

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resulting in sharp declines in ruminal pH to the detriment of fermentation and physiological function. Gradual adaptation of the concentrate-fed ruminant and the inclusion of buffers in the diet have been only partially successful, resulting in wide-spread investigations into the usage of an array of antibiotic and direct-fed microbial (DFM) products. The efficacy and probable mode of action of these products have recently been reviewed (Brown and Nagaraja, 2009; Krehbiel et al., 2003; Meissner et al., 2010).

Mitigation of digestive disorders such as lactate acidosis has been done with antibiotics such as ionophores (Mutsvangwa et al., 2002; Nagaraja et al., 1987). Less is known of the mode of action of non-ionophore antibiotics. Antibiotics have been banned as feed additives for livestock production in some parts of the world (Cogliani et al., 2011) and their use limited or reconsidered in other parts (Wileman et al., 2009), which prompted many investigations to find alternatives (Allen et al., 2013). Non-ionophore antibiotics nevertheless are still used in certain countries, thereby justifying investigations into their efficacy in mitigating or controlling digestive orders.

The in-feed non-ionophore antibiotic tylosin reduces lactic acid production *in vitro* (Nagaraja et al., 1987) and is effective against *Fusobacterium necrophorum* (Lechtenberg et al., 1998) which is the primary aetiologic agent of liver abscesses (Nagaraja and Chengappa, 1998), and which is known to proliferate in lactate-challenged steers. This specialised action is one reason why tylosin and monensin are often included together in feedlot diets (Harvey et al., 2009). Flavomycin also inhibits *F. necrophorum* and other bacterial species, and it reduces VFA concentrations at low *in vitro* pH (Edwards et al., 2005). In general, there is a lack of data on the control of lactate acidosis by non-ionophore antibiotics and their interactions with other rumen modifiers (Australian Veterinary Association – RAGFAR, 2007), one reason being that most of these products were registered many years ago and for other reasons.

Direct fed microbial products of the bacterial category are for example lactate-producing lactobacilli fed alone or in combination with lactate-utilising bacteria (*e.g. Propionibacterium*). They have shown some indication of reduced lactate acidosis through higher ruminal pH, positive responses to systemic acid base variables (Ghorbani et al., 2002; Nocek and Kautz, 2006) and maintenance of an active lactate-utilizing population (Jouany and Morgavi, 2007; Nocek and Kautz, 2006) such as *Megasphaera elsdenii*. It is conceivable that this may provide a competitive advantage to *M. elsdenii*. However, the main advantage of lactobacillus species appears to be their probiotic action with primary benefit in the lower digestive tract (Brown and Nagaraja, 2009). *Propionibacterium* as lactate-utiliser converts lactic acid to propionic acid which supports gluconeogenesis in the liver and therefore the combination with lactobacilli should promote animal production responses (Jouany and Morgavi, 2007; Krehbiel et al., 2004). However, a major impact on control of lactate acidosis is not expected.

Direct fed microbial products of the yeast category support ruminal bacterial growth and may alter fermentation products (Chaucheyras et al., 1996; Mutsvangwa et al., 1992). The yeast *Saccharomyces cerevisiae* stimulated growth of *M. elsdenii* by providing essential nutrients (Chaucheyras et al., 1996; Rossi et al., 2004) and increased pH *in vitro*, thereby reducing the risk of lactate acidosis (Jouany and Morgavi, 2007). *S. cerevisiae* hydrolysates are providing soluble bio-active oligo- and polysaccharides and peptides (Provenza and Villalba, 2010; Rossi et al., 2004). Therefore, the primary mode of action of these products in control of lactate acidosis appears to be largely indirect.

Megasphaera elsdenii may also be developed into a useful DFM. It is an effective lactate-utilising bacterial species in the rumen which gives preference to lactic acid as substrate (Marounek et al., 1989; Russell and Baldwin, 1978). Several patented strains prevented lactic acid accumulation and pH decline *in vitro* or in the rumen (Hino et al., 1994; Wiryawan and Brooker, 1995); the robust, fast-growing strain NCIMB 41125 being highly successful in controlling lactate acidosis (Henning et al., 2010; Meissner et al., 2010). Since *M. elsdenii* as a DFM will often have to operate in the presence of ionophores or therapeutic and prophylactic antibiotics, sensitivity or resistance to these products have been evaluated. In general, *M. elsdenii* strains are not sensitive to monensin (Callaway et al., 1999; Marounek et al., 1989) and are either resistant to, or not inhibited by other antibiotic products (Marounek et al., 1989) among them tylosin, bacitracin and oxytetracycline.

The objective of this investigation was to study the efficacy, compared to *M. elsdenii* strain NCIMB 41125, and mode of action of selected non-ionophore antibiotics and DFM when introduced to a substrate resulting in high lactate levels during *in vitro* fermentation. The second objective was to establish whether strain NCIMB 41125 and these products when introduced together into the *in vitro* system have enhancing or inhibiting interactions.

2. Materials and methods

2.1. Treatments

2.1.1. Experiment 1

The effect of *M. elsdenii* strain NCIMB 41125 (hereafter referred to as Me) on *in vitro* fermentation was tested at four levels of inoculation to establish an optimum for inclusion in the studies on the non-ionophore antibiotics and DFM. The four levels were respectively 1, 10, 100 and 1000 μl Me/40 ml, which are equivalent to 0.025, 0.25, 2.5 and 25×10^5 colony forming units (cfu) Me/ml. The results of the four levels were compared with a control (no amendments) (CON) and an autoclaved treatment (aMe; 1000 μl Me/40 ml). The experiment revealed that 100 μl Me/40 ml containing approximately 2.5×10^5 cfu Me/ml was optimum (see Section 3), which then became the inoculum level of Me in Experiments 2 and 3.

2.1.1.1. Origin and cultivation of M. elsdenii strain NCIMB 41125. *M. elsdenii* strains were selected from the rumen population of *M. elsdenii* of dairy cows (Horn et al., 2009). These strains were tested by pH-auxostat enrichment using stringent selection criteria of high growth rate and