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The relative roles of the ruminal fluid and epithelium in the aetiology of ruminal acidosis

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ABSTRACT

Acute lactic acidosis (ALA) and subclinical ruminal acidosis (SARA) are economically-important pathologies associated with poor adaptation of the ruminal ecosystem to diets high in soluble carbohydrates. Both pathologies result from an imbalance in the rate of production of fermentation acids relative to removal of these acids by absorption, flow of acids post-ruminally, and buffering of the acids by bicarbonate from the saliva and blood. In ALA, lactic acid accumulates, driving the pH of the ruminal fluid below 5.0, while subclinical acidosis is characterised by the accumulation of short-chain volatile fatty acids and a ruminal pH of 5.0–5.5. These reductions in ruminal fluid pH reflect changes in the profile and activity of the microbial populations, the buffering capacity of the ruminal fluid, and the absorptive capacity of the ruminal epithelium. The relative importance of the ruminal fluid (microbial and chemical properties) and the ruminal epithelium (absorptive capacity) is unknown. A novel ruminal transfaunation model was used to separate these two components of the pathologies. Four groups of sheep were formed by combining two ruminal fluid adaptions (adapted to high-energy density or low-energy density diets) and two ruminal epithelium adaptations (adapted to high-energy density and low-energy density diets). The four combinations of fluid and epithelium were then challenged with an intra-ruminal infusion of glucose to simulate conditions conducive to the development of acidosis. All groups developed acute acidosis in response to the glucose challenge and there were no differences between groups in the pattern of pH decline over a 24-h period. There was no difference between groups in ability to regulate rumen fluid pH, suggesting the major factor contributing to development of acidosis is rapid microbial fermentation of soluble carbohydrates irrespective of adaption of the epithelium or microbial populations. Modifications to the challenge protocol to more closely reflect normal dietary challenges would be a useful modification in future studies using this technique.

1. Introduction

Acute lactic acidosis (ALA) and subclinical ruminal acidosis (SARA) are significant pathologies of ruminal livestock, with estimated economic losses of $500 million to $1000 million per year in the USA alone (Stone (1999) *cit. loc.* Blanch et al. (2009)). They also present a very significant animal welfare problem for beef cattle and sheep in feedlots, and dairy cattle on high-concentrate rations. Both pathologies reflect an imbalance in the production of fermentation acids and removal of these acids by buffering, absorption and onward flow to the omasum. Acute lactic acidosis is characterised by accumulation of lactic acid in addition to the short-chain fatty acids (SCFA), with pH below 5.0 (Nocek, 1997; Owens et al., 1998; Krause and Oetzel, 2006). Sub-acute ruminal acidosis, in contrast, is characterised by accumulation of SCFA along with a fluid pH between 5.0 and 5.5 (Kleen et al., 2003; Krause and Oetzel, 2006; McLaughlin et al., 2009). Rather than being distinct pathologies however, ruminal acidosis is a continuum of pathologies ranging from subclinical to acute (Lean et al., 2014) with some comon features.

Maintenance of a ruminal fluid pH 6.0–6.8 (Mould et al., 1983; Nagaraja and Titgemeyer, 2007) is normally achieved by a combination of mechanisms (Allen, 1997; Aschenbach et al., 2011). Fermentation acids are absorbed either undissociated, which takes the hydrogen ion out of the fluid, or dissociated but in exchange with bicarbonate which neutralises the dissociated proton (Aschenbach et al., 2011). Both methods effectively buffer the ruminal acids. Bicarbonate and other buffers in the saliva also buffer the acids as they are produced, and some acids leave the rumen in digesta flowing through the reticulo-

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omasal orifice (Allen, 1997; Aschenbach et al., 2011).

The effectiveness of these mechanisms depends on the relative rate and type of acid production (microbial activity and soluble carbohydrate supply) and the rate of acid removal and buffering (Nocek, 1997; Owens et al., 1998; Bramley et al., 2008). The former are characteristics of the fluid and the latter are largely characteristics of the epithelium. The relative roles of fluid adaption and epithelial adaption have not been elucidated. Fluid adaption mainly involves adaptation of the ruminal microbes to the new high-soluble carbohydrate regime (Warner, 1962; Nagaraja and Titgemeyer, 2007). Microbes adapted to soluble carbohydrates and lower pH proliferate, changing the composition of the SCFA end products, and producing lactic acid. If the change to high-soluble carbohydrate is too rapid there is insufficient time for lactic acid utilising bacteria to proliferate, so lactate accumulates in the fluid, further reducing rumen fluid pH (Nagaraja and Titgemeyer, 2007). The ruminal papillae respond by increasing their total surface area to maximise absorption of the large quantity of VFA (Sakata and Tamate, 1978; Ahmed, 2007), and possibly other adaptations to the new ruminal fluid environment.

Most discussions of acidosis disorders concentrate on the microbial adaptations as the key predisposing factor. We speculated that adaptations of the ruminal epithelium also plays an important role in the development of acidosis in ruminants. We tested this by exchanging rumen fluid from sheep either 'adapted' or 'not-adapted' to a high-energy density diet, into sheep with ruminal epithelia that was 'adapted' or 'not-adapted' to high-energy density diets. We then used a glucose-challenge to quantify the pH responses of these sheep.

2. Materials and methods

2.1. Animals, treatments, and experimental design

Sixteen, 12 month old Merino castrate males were housed in a temperature-controlled building and acclimatised for three weeks in individual pens. All sheep were fed a 'low-energy' diet of oaten hay/lucerne chaff (50/50) as indicated in Table 1, with a mineral supplement (Supplemins MidMag, Lienert, South Australia, Australia) at 20 kg/tonne. Sheep were then divided into two groups by stratified randomisation method on bodyweight. One group was transitioned over three weeks to a high-energy density diet (Table 1), while the remaining group remained on the low-energy density diet. This transition to the high-energy density diet was based on an extension of a two-week step up diet from Holst and White (2007) (Fig. 1). Throughout the experiment all sheep were fed at 1.5 x their maintenance metabolisable energy (ME) requirement (National Research Council (US) 2007). The diets were fed for an eight week period before surgery to allow for adaptation of the rumen fluid and epithelium (Warner, 1962; Dirksen et al., 1985).

Four experimental groups, with four sheep in each group, were created as described in Fig. 2 by total rumen fluid transfaunation. The experimental group size was calculated based on rumen fluid pH. An alpha error of 0.05, beta error of 0.2, with the standard deviation of the rumen fluid pH calculated from the standard error of the mean using previous sheep studies of 0.2–0.3 was used (Lopez et al., 2003; Commun et al., 2009). These calculations generated an effective experimental group size of four sheep. These groups were created to investigate the impact of the rumen fluid and rumen epithelium on pH regulation.

Table 1
Nutritional analysis of the high-energy and low-energy density diets. The nutritional analysis was completed by Feedtest, Agrifood technology, Werribee, Victoria, Australia using standard wet chemistry methods (Australian Fodder Industry Association Limited, 2009).

	High-energy diet (80% feedlot pellet/20% low-energy diet)	Low-energy diet (Oaten/lucerne chaff)
Crude protein (%)	25.5	12.0
Metabolisable energy (MJ/kg dry matter)[1]	11.4	7.2
Dry matter (%)	88.9	86.5
Organic matter Digestibility (%)	74.2	50.3
Fat (% of dry matter)	5.0	2.5
Acid detergent fibre (%)	22.1	37.9
Neutral detergent fibre (%)	35.5	58.3

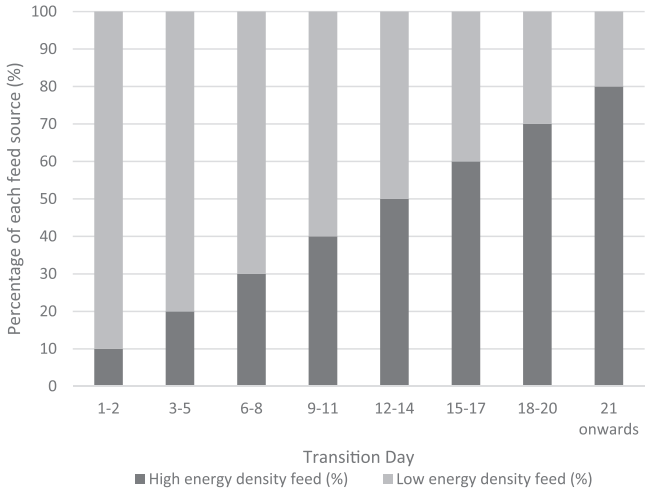

Fig. 1. Diet transitioning regime adapted from Holst and White (2007). The percentage of each feed on a wet weight basis making up the ration of each sheep on the high-energy density diet during the transition period.

2.2. Surgical procedures and transfaunation

Feed and water were not withheld prior to surgery. The sheep were then fistulated and cannulated, with rumen fluid transferred between sheep to create the four experimental groups as described in Section 2.1. Following surgery, sheep were recovered in their pens and only had access to water until the glucose challenge experiment the following day.

2.3. Samples taken during surgery

During surgery a biopsy of the rumen wall was taken from the ventral sac; and a full thickness rumen wall biopsy was taken from the dorsal sac from the fistula site. Ruminal pH and temperature loggers (T9, Dascor Inc, California, United States of America), set to record the pH and temperature at one minute intervals were inserted through the cannula into the ruminal lumen. These loggers provide an accurate measurement of rumen fluid pH (Penner et al., 2009).

The setup and assemble procedures followed as *per* manufacturer instructions. Calibration and adjustment of loggers for sensor drift was made as *per* manufacturer recommendations and previous studies (Penner et al., 2006; Castillo-Lopez et al., 2014).

2.4. Processing and analysis of ruminal biopsy samples

Histological slides were prepared as *per* Kiernan (1999). Histological sections (5 μm) were cut on a Thermo Scientific Microm HM340E microtome (Thermo Fisher Scientific Australia Pty Ltd, Victoria, Australia) and floated onto Starfrost glass slides (ProSciTech, Queensland, Australia). Sections were then stained with Lillie-Mayer's haematoxylin (product number MHS16, Sigma Aldrich, New South Wales, Australia) and then counterstained with eosin yellow (product number HT11031, Sigma Aldrich, New South Wales, Australia).

Measurements and assessments were undertaken using 10X