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ORIGINAL ARTICLE

In vitro rumen fermentation of soluble and non-soluble polymeric carbohydrates in relation to ruminal acidosis

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Abstract The end-products of dietary carbohydrate fermentation catalysed by rumen microflora can serve as the primary source of energy for ruminants. However, ruminants provided with continuous carbohydrate-containing feed can develop a metabolic disorder called "acidosis". We have evaluated the fermentation pattern of both soluble monomeric and non-soluble polymeric carbohydrates in the rumen in in vitro fermentation trials. We found that acidosis could occur within 6 h of incubation in the rumen culture fermenting sugars and starch. The formation of lactic acid and acetic acid, either alone or in mixture with ethanol, accounted for high build-up of acid in the rumen. Acidosis resulted even when only 20% of a normal daily feed load for all soluble and non-soluble carbohydrates was provided. DNA-based microbial analysis revealed that *Prevotella* was the dominant microbial species present in the rumen fluid.

Keywords Ruminal acidosis · Carbohydrate · Fermentation

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Introduction

Rumen fermentation is a complex process carried out by different types of microorganisms, among which rumen bacteria are considered to be the most important (Belanche et al. 2012). The type of carbohydrates consumed by ruminants can modify the microbial population (Fernando et al. 2010). Several types of bacteria are responsible for carbohydrate fermentation in the rumen, including *Selenomonas ruminantium* and *Streptococcus bovis*; these latter bacteria are considered to be lactate producers (Belanche et al. 2012).

As the end-products of carbohydrate fermentation in the rumen can significantly affect host health (Li et al. 2009), evaluation of the effects of different types of carbohydrates consumed on metabolic changes in the rumen could be important. The survival of microorganisms in the rumen environment is highly influenced by a number of critical components, including pH and the amounts of volatile fatty acids (VFAs) and lactic acid (Russell and Wilson 1996; Qadis et al. 2014). A decrease in the pH in the rumen due to the consumption of feed containing large amounts of fermentable carbohydrates may lead to acidosis (Slyter 1976; Plaizier et al. 2008; Danscher et al. 2015).

Elam (1976) reported that lactic acid build-up occurred in the rumen when ruminants were fed starch-based diets such as wheat, barley and corn. An in vitro study conducted by Cullen et al. (1986) showed that rumen fermentation of soluble carbohydrates, including monosaccharides and disaccharides, produced more lactic acid than did starch fermentation. An in vivo study carried out by Khezri et al. (2009) found that adding sucrose to ruminant diets may decrease rumen pH together with an increase of 70% proton concentration. However, Oba (2011) found that supplementing sugars to dairy cow diets may enhance butyrate production. Sutton (1968) mentioned that some of the inconsistent results

reported in studies on rumen fermentation of soluble carbohydrates could be because (1) sugars were not used as the main diet for ruminants, and (2) the rumen microbes were not adapted to fermenting the particular sugars being fed to the ruminants; therefore, the different microorganism populations present may yield different products.

As fermentation of different types of carbohydrates could generate different outcomes, studies on carbohydrate fermentation in the rumen should define the sources of starch or sugar used (Malestein et al. 1982; Cullen et al. 1986). This is important because different types of carbohydrates may affect different fermentation patterns in the rumen, which would adequately explain rumen acidosis caused by feeding carbohydrates (Malestein et al. 1982; Cullen et al. 1986). The aim of the study reported here was to investigate in vitro rumen fermentation of various soluble and non-soluble polymeric carbohydrates at different concentrations, in relation to rumen acidosis.

Materials and methods

Rumen sample

Rumen fluid was collected from a 3-year-old cow which had been fitted previously with a surgically created rumen fistula and fed on irrigated Kikuyu grass pasture at the School of Veterinary and Life Science, Murdoch University, Perth, Western Australia. All procedures on rumen fluid collection were reviewed and approved by the animal ethics committee at Murdoch University.

Substrates

The substrates used in this study were sugar and starch. The sugars used were D(+)-glucose anhydrous (BDH Prolabo Chemicals, VWR International, Radnor, PA), D(+)-maltose monohydrate (BDH Prolabo Chemicals, VWR International) and sucrose (Sigma-Aldrich, St. Louis, MO). Corn, wheat and rice flour were used as sources of starch. The corn flour was a commercial sweet corn flour product [90.2% total solid (TS)] that was manufactured by the Corn Products Refining Company under the trade name of Seagull & Sun Corn Flour. It consisted of untreated starch with no preservative and was sold by the KonJee Trading Company (Singapore). The rice flour was a commercial rice flour product (88% TS) manufactured by the Rice Product Refining Company under the trade name of Erawan Rice Flour. It consisted of untreated starch with no preservative and was sold by Erawan Marketing Co., Ltd. (Bangkok, Thailand). The wheat flour was a commercial product (87% TS) manufactured by the Wheat Product Refining Company under the trade name of Black and Gold Plain Flour. It consisted of untreated starch with no preservative and was sold by Australian Asia/Pacific, Wholesalers Pty Ltd. (Macquarie Park, NSW, Australia).

Experimental design and procedures

Fermentation of soluble carbohydrates in rumen culture

The study comprised a series of batch cultivation trials with different substrates. The batch tests were conducted in a thermostatic water bath for 4 days to ensure complete adaptation of the rumen bacteria to each substrate added to the culture and to the environment. Each batch digest was filled with 80 mL of fresh rumen fluid. In the first trial we tested the effect of sugar on rumen fermentation by adding 100 g/L of glucose, maltose and sucrose to the first, second and third digester, respectively; the control reactor was filled with rumen fluid only. The temperature was maintained at 39 ± 0.5 °C, and the working volume of rumen (80 mL) was anaerobically stirred using a magnetic stirrer at 100 rpm. Prior to the start of the fermentation process, each reactor was flushed with 100% N_2 gas to remove oxygen contamination. All of the fermentation parameters, including temperature, mixing speed and the working volume of rumen, used in this test were also applied to all the subsequent experiments unless otherwise mentioned. Repeat tests were carried out using 50 and 10 g/L of sugar, respectively.

Fermentation of non-soluble polymeric carbohydrates in rumen culture

All procedures used in these trials were the same as those used for the fermentation of soluble carbohydrates, with the exception that corn, wheat and rice flour were added to the respective batch digester and not sugar. The test was repeated three times using different concentrations (100, 50 and 10 g/L) of substrates.

Analytical methods

Fermentation end-product analysis

For the fermentation end-product analysis, the collected samples were immediately centrifuged at 4000 rpm for 5 min, following which the supernatants were filtered through a Millex-GP syringe filter with 0.22-μm PES membrane (EMD-Millipore, Merck Millipore, Burlington, MA) at 150 psi (10 bars) of the housing limit. The filtrates were transferred into 1.5-mL Eppendorf tubes and stored at 4 °C prior to analysis (Ye et al. 1996; Milinovich et al. 2008; Feria-Gervasio et al. 2014; Bao et al. 2015). The composition of the fermentation products, including VFAs (acetate, propionate, butyrate), acetone, ethanol, butanol and lactic acid, was analysed by gas chromatography. In a vial test tube with screw

cap, 480 μL of periodic acid (100 mM) and 300 μL of formic acid (10%) were added to 720 μL of standard/sample. The tube with mixture was then closed and heated in a water bath at 100 °C for 60 min. After heating, the test tube was cooled, first at room temperature for 5 min and then in the fridge for 20 min, following which the mixture in the closed test tube was mixed using a vortex mixer for 30 s. The mixture was then transferred to a 1.5-ml gas chromatography vial for injection into the gas chromatograph (Agilent model 7820A gas chromatograph equipped with auto-sampler and flame-ionisation detector; Agilent Technologies, Santa Clara, CA). The injection block was installed with an Agilent 11-mm rubber septum and inlet liner with a standard split (Agilent Technologies model 5190-2295), with the following specifications: low pressure drop, ultra-inert with glass wool and deactivation and a volume of 870 μL. The capillary column was an ECONO-CAP™ GC fused silica capillary column (Alltech Corp., Deerfield, IL) (length 30 m, inside diameter 0.25 mm, film thickness 0.25 μm). Nitrogen gas was used as the carrier gas at a flow rate of 1.2 mL/min, and the sample was split at the inlet 10:1. The injection volume was set at 0.4 μL. The run time was programmed at 11.667 min. The oven temperature was set as follows: initial temperature 50 °C; hold for 2 min; temperature ramp 75 °C/min to 130 °C; hold for 5.0 min; temperature ramp 75 °C/min to 250 °C; hold for 2 min.

Titration analysis

The titration analysis was conducted to evaluate the buffer capacity of the carbohydrates, both sugars (glucose, maltose and sucrose) and starch materials (corn, wheat and rice starch), used in the rumen fermentation trials. The titration was performed at room temperature with a properly calibrated pH meter (LabChem Inc., Zelienople, PA). All procedures were based on the respective standard method (American Public Health Association 2012). The analyte (sample for titration) was placed in a beaker with a magnetic bar and continuously mixed at 50 rpm. Acid titrations were conducted using a burette filled with hydrochloric acid (10 mM) as titrant. The titration process was started after ensuring that there were no air bubbles and leaks in the burette. Samples of sugar and starch feeds (50 g/L) were titrated with 10 mmol/L HCl. The volume of sample used was 25 mL. Thymol blue (0.04% w/v) was used as pH indicator (Kahlert et al. 2016).

DNA analysis

DNA was extracted from 1 mL of rumen fluid in a laminar flow hood in an amplicon-free laboratory, using the PowerSoil DNA Isolation kit (Mo Bio Laboratories, Carlsbad, CA) according to the manufacturer's instructions. Samples used for DNA extraction and sequencing analysis were collected from the fresh rumen fluid in order to analyse microbial community present in the rumen fermentation. The DNA was stored in the freezer (−20 °C) until further analysis. DNA was extracted and quantified using a Qubit fluorometer, and 1-ng samples were amplified using the 16S ribosomal ribonucleic acid (rRNA) gene V4/5 primers (515F: GTGCCAGCMGCCGCGGTAA and 806R: GGACTACHVGGGTWTCTAAT) (Caporaso et al. 2010).

Specifically, a mixture of gene-specific primers and gene-specific primers tagged with Ion Torrent-specific sequencing adaptors and bar codes was used in this analysis. The tagged and untagged primers were mixed at a ratio of 90:10. The amplification of all samples was achieved using 18–20 cycles, which minimised primer–dimer formation and allowed streamlined downstream purification. Amplification was confirmed by agarose gel electrophoresis, and product formation was quantified by fluorimetry. Up to 100 amplicons were diluted to equal concentrations and adjusted to a final concentration of 60 pM. Template-positive Ion Shere™ Particles (ISP) were generated and loaded onto sequencing chips using the Ion Chef system (Thermo Fisher Scientific, Waltham, MA) and sequenced on a PGM semiconductor sequencer (Thermo Fisher Scientific) for 650 cycles using a 400-bp sequencing kit, yielding a modal read length of 309 bp. Data collection and read trimming/filtering were performed using Torrent Suite 5.0 (Caporaso et al. 2010). This method has been tested on commercial mock community DNA samples and the results found to have good concordance with the expected results (data not shown) (Nagel et al. 2016). The sequencing data obtained included the levels of kingdom, phyla, class, order, family and genus. Data on the composition of the microbial community in this study were presented at the genus level. Family-level data with a high percentage were included and presented as an unclassified category at the family level.

Results and discussion

Fermentation of soluble carbohydrates in rumen culture

To evaluate whether the type of soluble carbohydrate could influence the behaviour of rumen microbial activities, we added three types of sugars, namely glucose, maltose and sucrose, to separate rumen cultures. As shown in Electronic Supplementary Material (ESM) Fig. S1, relative to the control culture, the addition of 100 g/L sugar caused more severe acidosis, with a permanent pH depression to less than 4.0, which would be classified as an acute ruminal acidosis (Owens et al. 1998). In all cases, lactic acid was the key metabolite, accumulating to up to 200 mM. In contrast, the acetate concentration never reached more than 100 mM in any of the trials involving the fermentation of soluble carbohydrates (Table 1). It is interesting to note that only the addition of

Table 1 Rumen fermentation results according to the different substrates

Type of substrate	Concentration of substrate added (g/L)	pH of feed sample (after 48 h of incubation)	Main end-products
Glucose	100	3.87	Lactic acid
	50	3.6	Lactic acid
	10	5.04	Lactic acid
Maltose	100	3.89	Lactic acid
	50	3.59	Lactic acid, acetate
	10	4.93	Lactic acid
Sucrose	100	3.79	Lactic acid
	50	3.57	Acetate
	10	4.88	Lactic acid
Corn starch	100	4.01	Lactic acid
	50	4.06	Lactic acid, acetate
	10	4.74	Lactic acid, acetate
Wheat starch	100	3.83	Lactic acid
	50	4.07	Lactic acid
	10	4.92	Lactic acid
Rice starch	100	4.3	Lactic acid
	50	3.96	Lactic acid
	10	4.73	Lactic acid

glucose led to significant ethanol formation, of about 183.7 mM (ESM Fig. S1A).

An in vitro study conducted by Cullen et al. (1986) showed that lactic acid accumulated in the rumen cultures of fermenting sugars within 12 h of incubation, leading to a drop in the pH of the rumen culture to 4.3, which is considered to be an acute acidosis (Olson 1997; Garrett et al. 1999). In the present study, the onset of lactic acid accumulation in the rumen cultures of fermenting sugars occurred within 2 h of incubation and resulted in a severe lactic acidosis in which the pH dropped from 5.7 to 3.5. Lactic acid build-up in our fermentation trials was more rapid than that reported by Cullen et al. (1986). Possible explanations for this difference may be that Cullen et al. (1986) used only a quarter of the sugar concentration that we did in our study, and they also added 30% ruminant saliva buffer and urea (0.8 g/L) at the beginning of fermentation process to prevent the sudden accumulation of lactic acid.

The 50 g/L feed addition corresponds approximately to the daily feed load of a ruminant of 50 g/L per day (Oba and Allen 2003; Department of Primary Industries NSW 2016; Kashongwe et al. 2017). In all cases, a dramatic pH drop to less than 3.5 was obtained (ESM Fig. S2). However, this drop was not exclusively due to lactic acid formation. The presence of acetic acid as a main metabolite was noted in the sucrose sample (Fig. S2C), and acetic acid and ethanol as main metabolites were noted in the glucose sample (ESM Fig. S2A). This result suggests that ruminal acidosis was also initiated with acetic acid build-up, which in our study resulted in a drop in the pH in the rumen, and that once the pH was low the lactic acid build-up occurred (ESM Fig. S2A, B, C). In an in vivo study by Mitsumori and Sun (2008), low pH was also found to inhibit hydrogen consumption by methanogens and thereby generate hydrogen accumulation, also possibly leading to the build-up of acid in the rumen.

The results of our study are in agreement with those of an in vitro study by Sutton (1968), who found that the addition of sucrose to rumen culture produced acetate as the main metabolite. This author found that the sucrose fermented in the rumen produced more acetate than did glucose fermentation. This result is quite different from that of the present study in which analysis revealed that acetate and ethanol were the major fermentation end-products of glucose fermentation. These different results could be attributed to a much lower sugar concentration (12.5 g/L) used in the earlier study.

Sugar added at a lower concentration to the rumen culture could also generate a lower concentration of acid compared to the addition of the high sugar concentration, and thereby could affect the change in microbial community in the rumen. Furthermore, the use of a buffer in the rumen culture fermenting sucrose could be the reason why acetic acid was produced as the main metabolite in the study of Sutton (1968); this buffer enabled the rumen pH to be maintained between 5.9 and 6.3, which is considered to be the optimum pH for VFA production in the rumen (Kolver and De Veth 2002). However, in our study buffer was not added, and high concentrations of sugars were used. Thus, a dramatic drop in the pH of the rumen culture could stimulate the production of ethanol and lactic acid.

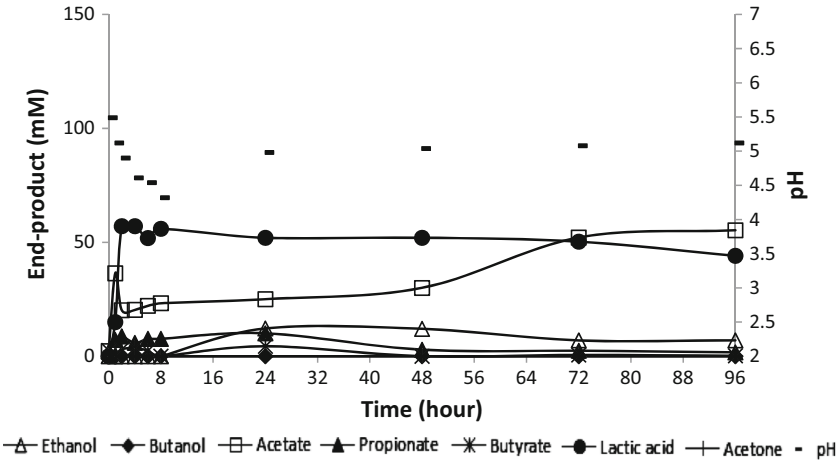

Fig. 1 Profile of end-products and pH from rumen culture fermenting a soluble substrate (glucose 10 g/L)

In some studies lactic acid was the main metabolite once soluble carbohydrates, including glucose, maltose and sucrose, were added to the rumen culture (Slyter 1976; Cullen et al. 1986). The results from our study showed that lactic acid was gradually formed once the pH in the rumen dropped from 6 to 4. It is interesting to note that at an extremely low pH (< 4.0), fewer fermentation end-products were made in the rumen culture, possibly because an acidic condition may slow down the activity of rumen microbes (Franzolin and Dehority 2010; Oliveira et al. 2011).

While the addition of 50 g/L of sugar is within the normal level found in ruminant feed, the sudden shock of adding a soluble sugar to the feed could represent a larger acidosis risk than that typically obtained during animal feeding. To examine this possibility, we tested a one-fifth dose for its acidosis potentia and found that the addition of 10 g/L sugar caused a rapid lactic acid build-up (60 mM) within 6 h (Fig. 1; ESM Fig. S3). We also found that the ruminal pH dropped from its original value of 5.7 to lower than 5, subsequently stabilising within 48 h at around pH 5. Overall, acidosis was caused by all sugar additions. Further, these tests showed that even the addition of soluble sugar (sucrose, maltose, glucose) at only 20% of a normal daily feed load (50 g/L per day) would initiate acidosis.

Fermentation of non-soluble polymeric carbohydrates in rumen culture

The trials involving soluble carbohydrates (ESM Figs. S1–S3) showed that acidosis occurred during rumen fermentation of all three sugars tested (glucose, maltose, sucrose). In order to understand whether the addition of a starch source to the rumen could cause similar effects as sugar feeding with respect to the initiation of ruminal acidosis, we conducted in vitro trials of rumen fermentation of non-soluble polymeric carbohydrates. Compared with the trials involving soluble carbohydrates (ESM Fig. S1), the main difference observed was that much higher concentrations of the key metabolites were formed from the polymeric carbohydrates (Fig. 2; ESM Fig. S4; Table 1).

Clearly, the addition of sugars (sucrose, maltose, glucose) had rapidly resulted in some form of inhibition. These results suggested that the low production of end-products of sugar fermentation in the rumen could be due

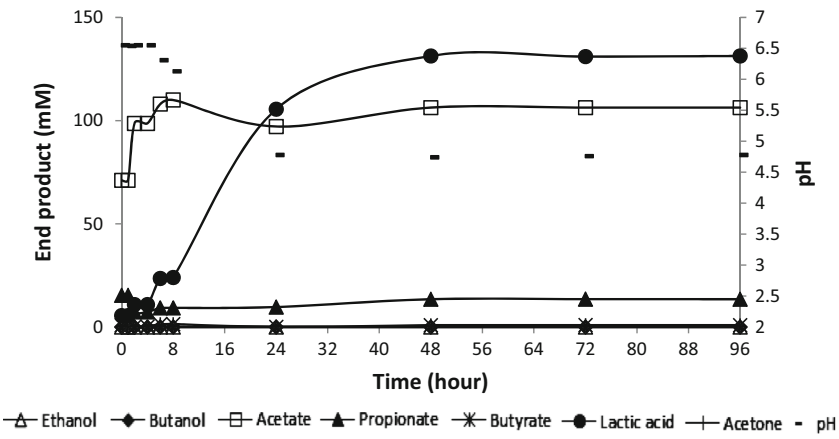

Fig. 2 Profile of end-products and pH from rumen culture fermenting a non-soluble substrate (corn starch 10 g/L)

to the microbial organisms present in the rumen fluid not being adapted to this high sugar level. These results also agree with those from studies conducted by Cullen et al. (1986) and Weisbjerg et al. (1998) who reported that the rate of sugar fermentation and the formation of fermentation end-products in the rumen can be varied depending on whether the rumen fluid has been taken from animals adapted to a high-sugar diet. Some authors also report that the addition of high sugar concentrations could inhibit the production of fermentation end-products in the rumen (Hobson 1965; Hobson and Summers 1967; Hishinuma et al. 1968). These same studies showed that the production of fermentation end-products by ruminal bacteria in a continuous culture system could be increased by maintaining a low concentration of sugar in the rumen culture.

When the concentration of non-soluble polymeric carbohydrates (starch) in the feed was applied based on a daily normal ruminant consumption of 50 g/L, acidosis occurred in all types of starch feed (ESM Fig. S6). In this condition, lactic acid was not the only metabolite formed in the rumen culture fermenting starch; acetate was also produced as the second-most abundant fermentation end-product (150–190 mM) from the corn and rice starch feeds (Figs. S6A, C). Furthermore, compared to the addition of sugar, the addition of starch to the rumen culture resulted in increased organic acid formation (Fig. ESM S2). The pH in the rumen culture with added starch was not as low as the pH from the rumen culture with added sugar, possibly due to the buffering effect of starch whereby starch may have about twice the buffering capacity of sugars (Fig. 3; ESM Fig. S5).

A repeat test with one-fifth of the concentration showed that 10 g/L starch added to the rumen culture still resulted in acidosis (Fig. 2). Acetate was the major end-product produced within 6 h of incubation, while the onset of lactic acid occurred after 8 h of incubation (ESM Fig. S7). These results agree with those of an in vivo study by Crichlow and Chaplin (1985) which revealed that lactic acid accumulation in the rumen fermenting starch occurred within an 8-h fermentation process coincident with a drop in the pH to lower than 5.0.

The results of this experiment indicated that sub-ruminal acidosis may occur when VFAs, mainly acetate, accumulate as a result of the addition of a starch carbohydrate to the rumen culture (ESM Fig. S7). Since acetate accumulation also can generate acidity in the rumen culture, this condition may lead to marginal acidosis or the development of full ruminal acidosis (pH <5.7) (Olson 1997; Garrett et al. 1999; Gozho et al. 2007; Danscher et al. 2015). Once marginal acidosis occurs, an acidic condition in the rumen culture may induce lactic acid producers to form lactic acid as the main metabolite, and therefore this condition may disturb completely the rumen microbiota (Lettat et al. 2010).

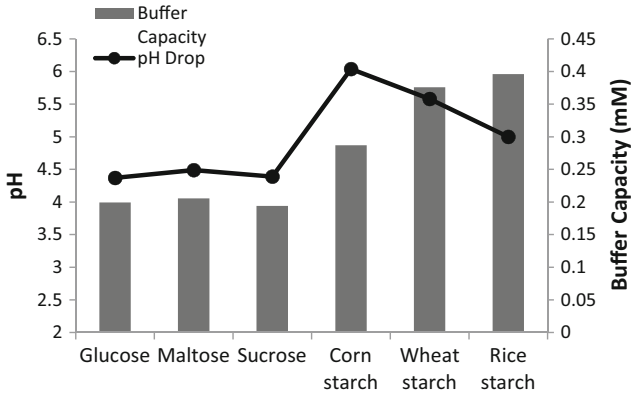

Fig. 3 Relationship between buffer capacity of different substrates and pH drop (after 6 h incubation)

Microbial community in the rumen

The results of our study showed that acidosis tended to occur from the rumen fermentation of soluble and non-soluble carbohydrates. This is possible because some microbial communities present in the rumen consist of lactate producers, such as members of the genera *Prevotella*, *Selenomonas*, *Ruminococcus* and *Streptococcus* (Fig. 4) (Nagaraja and Taylor 1987; Janssen et al. 1995; Somkuti and Steinberg 2003; Guss et al. 2011; Belanche et al. 2012). The species of these genera can utilise carbohydrates as an energy source to form lactic acid as the main metabolite (Guss et al. 2011; Belanche et al. 2012).

The results of the metagenomics sequencing analysis showed that *Prevotella* was the dominant bacterial genus present in the rumen fluid samples (Fig. 4). A study by Hernandez et al. (2008) on the characterisation of lactic acid-producing

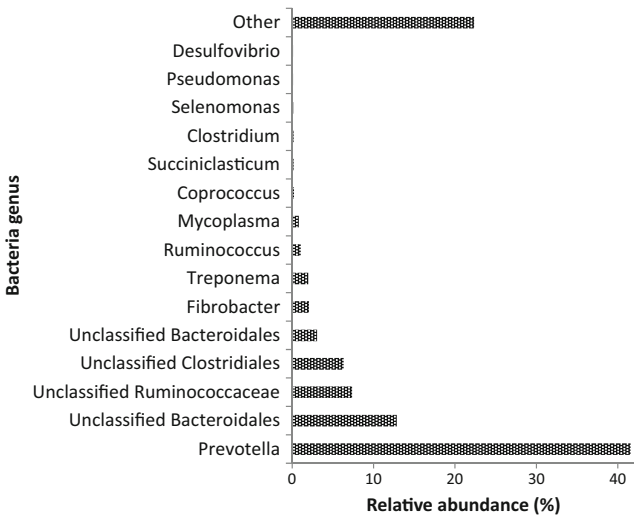

Fig. 4 Relative abundance of microbial community in samples of rumen fluid at the genus level. Microorganisms present at the family level at a high percentage (≥ 3%) were included as unclassified category at the family level. Others are included in the "other" category, which also includes some microbes that could not be classified at the genus level.

bacteria from the rumen of dairy cattle grazing on an improved pasture supplemented with wheat and barley grain found that *Prevotella* was the bacterial genus that produced lactic acid. These authors mentioned that the species of *Prevotella bryantii* was the lactate-producing bacteria present in the rumen and that the abundance of this specie grew rapidly once starch-based feed was given to the ruminants.

Conclusions

The results of this study reveal that fermentation of sugars and starch in rumen culture can cause pH depression leading to acidosis. Ruminal acidosis was not always caused by lactic acid accumulation, as acetate and ethanol could also accumulate in the rumen culture fermenting sugars (sucrose and glucose). The low buffering capacity of sugar feeds resulted in greater pH depressions in comparison to starch feeds. Metagenomic analysis showed that *Prevotella* was the dominant species present in the rumen microbiota and the species responsible for the production of lactic acid.

References

American Public Health Association (APHA) (2012) Standard method for the examination of water and wastewater, 22nd edn. APHA, AWWA, WPCF, Washington D.C.

Bao H, Jiang L, Chen C, Yang C, He Z, Feng Y, Wang A (2015) Combination of ultrasound and Fenton treatment for improving the hydrolysis and acidification of waste activated sludge. RSC Adv 5(60):48468–48473

Belanche A, Doreau M, Edwards JE, Moorby JM, Pinloche E, Newbold CJ (2012) Shifts in the rumen microbiota due to the type of carbohydrate and level of protein ingested by dairy cattle are associated with changes in rumen fermentation. J Nut 142(9):1684–1692

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Huttley GA (2010) QIIME allows analysis of high-throughput community sequencing data. NatMeth 7(5):335–336

Crichlow EC, Chaplin RK (1985) Ruminal lactic acidosis: Relationship of forestomach motility to nondissociated volatile fatty acids levels. Am J Vet Res 46(9):1908–1911

Cullen AJ, Harmon DL, Nagaraja TG (1986) In vitro fermentation of sugars, grains, and by-product feeds in relation to initiation of ruminal lactate Production1. J Dairy Sci 69(10):2616–2621

Danscher AM, Li S, Andersen PH, Khafipour E, Kristensen NB, Plaizier JC (2015) Indicators of induced subacute ruminal acidosis (SARA) in Danish Holstein cows. Acta Vet Scand 57(39):1–14

Department of Primary Industries NSW (2016) Animals and livestock, feeding management. http://www.dpi.nsw.gov.au/animals-and-livestock/beef-cattle/feed/lotfeeding/feed-mgt. Accessed 17 Aug 2016

Elam CJ (1976) Acidosis in feedlot cattle: Practical observations. J Anim Sci 43(4):898–901

Feria-Gervasio D, Tottey W, Gaci N, Alric M, Cardot JM, Peyret P, Brugère JF (2014) Three-stage continuous culture system with a self-generated anaerobia to study the regionalized metabolism of the human gut microbiota. J Microbiol Method 96:111–118

Fernando SC, Purvis HT, Najar FZ, Sukharnikov LO, Krehbiel CR, Nagaraja TG, Roe BA, DeSilva U (2010) Rumen microbial population dynamics during adaptation to a high-grain diet. Appl Environ Microbiol 76(22):7482–7490

Franzolin R, Dehority BA (2010) The role of pH on the survival of rumen protozoa in steers. Rev Bras Zootecnia 39(10):2262–2267

Garrett EF, Pereira MN, Nordlund KV, Armentano LE, Goodger WJ, Oetzel GR (1999) Diagnostic methods for the detection of subacute ruminal acidosis in dairy cows. J Dairy Sci 82(6):1170–1178

Gozho GN, Krause DO, Plaizier JC (2007) Ruminal lipopolysaccharide concentration and inflammatory response during grain-induced subacute ruminal acidosis in dairy cows. J Dairy Sci 90(2):856–866

Guss AM, Roeselers G, Newton IL, Young CR, Klepac-Ceraj V, Lory S, Cavanaugh CM (2011) Phylogenetic and metabolic diversity of bacteria associated with cystic fibrosis. ISME J 5(1):20–29

Hernandez JD, Scott PT, Shephard RW, Al Jassim RAM (2008) The characterization of lactic acid producing bacteria from the rumen of dairy cattle grazing on improved pasture supplemented with wheat and barley grain. J Appl Microbiol 104(6):1754–1763

Hishinuma F, Kanegasaki S, Takahashi H (1968) Ruminal fermentation and sugar concentrations: a model experiment with *Selenomonas ruminantium*. Agric Biol Chem 32(11):1325–1330

Hobson PN (1965) Continuous culture of some anaerobic and facultatively anaerobic rumen bacteria. Microbiology 38(2):167–180

Hobson PN, Summers R (1967) The continuous culture of anaerobic bacteria. Microbiology 47(1):53–65

Janssen PH, Evers S, Rainey FA, Weiss N, Ludwig W, Harfoot CG, Schink B (1995) *Lactosphaera* gen. Nov., a new genus of lactic acid bacteria, and transfer of *Ruminococcus pasteurii* Schink 1984 to *Lactosphaera pasteurii* comb. nov. Int J Syst Evol Microbiol 45(3):565–571

Kahlert H, Meyer G, Albrecht A (2016) Colour maps of acid–base titrations with colour indicators: How to choose the appropriate indicator and how to estimate the systematic titration errors. ChemTexts 2(2): 1–28

Kashongwe, OB, Bebe BO, Matofari JW, Huelsebusch CG (2017) Effects of feeding practices on milk yield and composition in peri-urban and rural smallholder dairy cow and pastoral camel herds in Kenya. Trop Anim Health and Prod 49(5);909–914

Khezri A, Rezayazdi K, Mesgaran MD, Moradi-Sharbabk M (2009) Effect of different rumen-degradable carbohydrates on rumen fermentation, nitrogen metabolism and lactation performance of Holstein dairy cows. Asian-Aust J Anim Sci 22(5):651–658

Kolver ES, De Veth MJ (2002) Prediction of ruminal pH from pasture-based diets. J Dairy Sci 85(5):1255–1266

Lettat A, Nozière P, Silberberg M, Morgavi DP, Berger C, Martin C (2010) Experimental feed induction of ruminal lactic, propionic, or butyric acidosis in sheep. J Anim Sci 88(9):3041–3046

Li M, Penner GB, Hernandez-Sanabria E, Oba M, Guan LL (2009) Effects of sampling location and time, and host animal on assessment of bacterial diversity and fermentation parameters in the bovine rumen. J Appl Microbiol 107(6):1924–1934

Malestein AAT, Van't K, Counotte GHM, Prins RA (1982) Concentrate feeding and ruminal fermentation, 2: Influence of concentrate ingredients on pH and on L-lactate concentration in incubations in vitro with rumen fluid. Neth J Agric Sci 30:259

Mitsumori M, Sun W (2008) Control of rumen microbial fermentation for mitigating methane emissions from the rumen. Asian-Aust J Anim Sci 21(1):144–154

Nagaraja TG, Taylor MB (1987) Susceptibility and resistance of ruminal bacteria to antimicrobial feed additives. Appl Environ Microbiol 53(7):1620–1625

Nagel R, Traub RJ, Allcock RJ, Kwan MM, Bielefeldt-Ohmann H (2016) Comparison of faecal microbiota in Blastocystis-positive and Blastocystis-negative irritable bowel syndrome patients. Microbiome 4(1):47

Oba M (2011) Review: Effects of feeding sugars on productivity of lactating dairy cows. Can J Anim Sci 9(1):37–46

Oba M, Allen MS (2003) Effects of corn grain conservation method on feeding behavior and productivity of lactating dairy cows at two dietary starch concentrations. J Dairy Sci 86(1):174–183

Oliveira JSD, Queiroz ACD, Mantovani HC, Melo MRD, Detmann E, Santos EM, Bayão GFV (2011) Effect of propionic and lactic acids on in vitro ruminal bacteria growth. Rev Bras Zootecnia 40(5): 1121–1127

Olson JD (1997) The relationship between nutrition and management to lameness in dairy cattle. Bovine Pract 31:65–68

Owens FN, Secrist DS, Hill WJ, Gill DR (1998) Acidosis in cattle: A review. J Anim Sci 76:275–286

Plaizier JC, Krause DO, Gozho GN, McBride BW (2008) Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. Vet J 176(1):21–31

Qadis AQ, Satoru GOYA, Ikuta K, Yatsu M, Kimura A, Nakanishi S, Shigeru SATO (2014) Effects of a bacteria-based probiotic on ruminal pH, volatile fatty acids and bacterial flora of Holstein calves. J Vet Med Sci 76(6):877

Russell JB, Wilson DB (1996) Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? J Dairy Sci 79(8):1503–1509

Slyter LL (1976) Influence of acidosis on rumen function. J Anim Sci 43(4):910–929

Somkuti GA, Steinberg DH (2003) Pediocin production by recombinant lactic acid bacteria. Biotechnol Lett 25(6):473–477

Sutton JD (1968) The fermentation of soluble carbohydrates in rumen contents of cows fed diets containing a large proportion of hay. Br J Nutr 22(04):689–712

Weisbjerg MR, Hvelplund T, Bibby BM (1998) Hydrolysis and fermentation rate of glucose, sucrose and lactose in the rumen. Acta Agric Scand A Anim Sci 48(1):12–18

Ye JA, Liu JX, Ya J (1996) The effects of ammoniated rice straw diets supplemented with Chinese milk vetch silage on rumen fermentation and microflora in sheep. Livestock Res Rural Dev 8(4):45–52