Ruminal Acidosis: Beyond pH and Rumen

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Introduction

Studies show that acidosis is a very significant disorder of cattle. Studies in Wisconsin found that 20.1 and 23% of cows had subacute acidosis as defined by rumen pH <5.5 (Oetzel et al., 1999; Oetzel, 2004) and others in Ireland had 11% (O'Grady et al., 2008). A large Australian study found that 10% of dairy cows <100 days in milk had acidosis, as defined by assessment of ruminal VFA, ammonia, lactic acid, and pH (Bramley et al., 2008). Therefore, it is likely that many cows will experience some level of acidosis during lactation and, indeed, some may be affected many times. It can be estimated that if the prevalence of sub-acute acidosis is 10% (Bramley et al., 2008) and the duration of a case is 2 days based on data by Golder et al. (2014b), then there would be an incidence of approximately 1500 cases over a 300 d lactation per 100 cows. Understanding and controlling acidosis is therefore critical to ensuring animal well-being and production.

There is now considerable debate about the definition of acidosis with papers providing varying definitions, many based on ruminal pH, others referring to conditions not solely based on ruminal changes (Plaizier et al., 2018) and some based on a series of different rumen measures (Bramley et al., 2008; Golder et al., 2014; Lean et al., 2013; Morgante et al., 2007). Providing thoroughly defensible definitions of the condition is critical to management of acidosis, because a failure to properly define the condition in scientific experiments can lead to a failure to adequately control the condition. In this paper, we discuss definitions of acidosis, provide some suggestions for definitions and examine recent findings on rumen function that may help prevent acidosis. Lastly, we evaluate evidence that there is cross-talk between the mammal and bacteria and these interactions influence the outcomes of rumen function.

What is acidosis?

Researchers, primarily based in the EU, state that ‘The classification of and terminology used in relation to dietary-induced disorders of the ruminant digestive system are confused and not fit for purpose. The problem is most apparent in relation to the condition referred to as sub-acute rumen acidosis (**SARA**), for which there are no adequate, accepted criteria for definition. Sub-acute is a poorly defined descriptor of the time-course of a disease and is often misinterpreted to refer to either subclinical disease or disease in which clinical signs are mild.’ We agree with their synopsis and provide the following supported thoughts to provide definitions of these conditions that may help with diagnosis and prevention of the disorder.

Acidosis is a continuum of conditions of varying severity that reflect the challenge of safely sequestering hydrogen that accumulates from carbohydrate fermentation. Safe pools to 'hide hydrogen' include starch engulfment by protozoa, bacterial glycogen formation, growth of bacteria, methane, and weak organic acids (VFA). Less safe pools include lactic acid, because that acid is 10 times stronger than the VFA. Decreasing the hydrogen supply by increasing the more slowly fermenting fiber content of the diet and enhancing rumination can reduce the risk of acidosis. It is important to recognize that the effects, and possibly even pathogenesis of acidosis may not be solely ruminal and other parts of the gastro-intestinal tract play a role.

Acute Acidosis

Acute acidosis is defined by the generation of significant amounts of lactic acid in the rumen. Nagaraja and Titgemeyer (2007) characterize acute acidosis as being present when rumen pH is <5.0, there is >50 mM lactic acid and ruminal VFA are less than 100 mM. Other studies support these criteria (Golder et al., 2014a; Golder et al., 2014b). There is a general consistency of definition and understanding of this condition in the literature. Acute acidosis is caused by the sudden access of cattle to rapidly fermentable carbohydrates (**RFCHO**) or changed processing of the same RFCHO. Fructose appears to have greater potential to cause acute acidosis than starches (Golder et al., 2012b; Golder et al., 2014b) and glucose has been used to create lactic acidosis (Nagaraja et al., 1981). Acute acidosis is characterized by fatal or serious disorder.

Definition

Acute acidosis is a serious condition of cattle characterized by death, dehydration, ruminal distension, diarrhea (often with grain in the feces and a sickly, sweet smell), abdominal pain, tachycardia, tachypnea, staggering, recumbency, coma, a marked decline in milk yield, and sequalae including ruminitis, liver abscess, pulmonary infections, epistaxis, and poor production that arises subsequent to the ingestion of large amounts of RFCHO. The rumen fluid can be milky white often containing grain and has a pH of <5.0, >50 mM lactic acid, and VFA <100 mM.

Acidosis

The definition 'sub-acute' does not sit easily in definitions that apply to metabolic diseases. It is simpler and more correct to ignore the term 'sub-acute'. Lean et al. (2009) provided a series of conditions that define metabolic disease based on the postulates of Evans (1976). It is clear that increasing dietary starch (Li et al., 2012), sugars (Nagaraja et al., 1981; Golder et al., 2012b), changing the forage fed (Khafipour et al., 2009), and changing the particle size of the feed (Zebeli et al., 2012), can create acidosis and meet the postulates proposed (Lean et al., 2009). However, there is very considerable variation in the responses of individual cattle to the increase in RFCHO and rumen pH is not the most consistent and easily measured change in rumen outcomes.

Plaizier et al. (2018) highlight a large number of studies that estimate the prevalence of low rumen pH, but cows with low pH did not have significantly different clinical outcomes to other cows, apart from low body condition score. By way of contrast, Bramley et al. (2008) who used both rumenocentesis and stomach tube measures of ruminal pH, but also ruminal VFA and ammonia concentrations found that the rumen pH measures were not highly predictive for a group of cows that were characterized by being in herds where dietary NFC were higher, NDF lower, and that had a markedly (>100%) higher incidence of lameness (Bramley et al., 2013) than other herds. The best predictors for these cows that also had a low milk fat to milk protein content and ratio, was a combination of rumen VFA concentrations, particularly valerate and propionate and rumen ammonia. The least predictive, albeit significant, variables for classifying cows as acidotic were rumen pH and lactic acid. In this paper, we explore the implications of these findings and support for them. Further, it is important to recognize that there is the potential for hindgut changes to influence outcomes of a RFCHO challenge (Gressley et al., 2011).

We consider that the following factors, some of which we explore in this paper are likely to influence the expression of acidosis i) production of toxic substances and clearance of these from the rumen. The generation of toxins and clearance of toxins will be influenced by ruminal populations of micro-organisms; ii) compromised epithelia, through chemical action, conditions such as pestivirus that damage epithelial integrity and ability to appropriately process toxins, iii) rate of passage and differential clearance and exposure of different parts of the gastrointestinal tract. All of the above functions may be influenced by genetics and understanding the interactions of these with the metabolome (physiological responses) and metataxome (the population of rumen microbes) is an important new frontier. Consequently, we propose the following definition.

Definition

Acidosis is a serious condition of cattle characterized by cyclic inappetence, increased risk of lameness, diarrhea (often with grain and/or gas in the feces), increased risk of low milk fat percentage, and sequalae including ruminitis, liver abscess, pulmonary infections, abomasal displacement, epistaxis, and poor production that arises subsequent to the ingestion of large or moderate amounts of rapidly fermentable carbohydrates.

On a herd basis, findings include: variable individual production, high prevalence (>40%) of lameness (Bramley et al., 2013), high prevalence of milk fat to milk protein ratio <1.02, and diets that are high in NFC >40%, but low in NDF <31%. Findings based on Bramley et al. (2008) include rumen fluid that is high in total VFA > 100 mM, of moderately low pH (<5.8 rumenocentesis or 6.2 stomach tube), with concentrations of propionate >30 mM and low ammonia <3 mM.

Other observations likely to be pertinent to increasing the risk of acidosis include evidence of sorting of diets, overstocking of corrals, mixing of heifers and cows and mixing of new cattle (Lean et al., 2014).

Limitations of pH as a diagnostic measure

The series of changes caused by the increase in RFCHO extends well beyond a decrease in pH and includes changes in a vast number of metabolic pathways involved in acidosis including the generation of potentially toxic metabolites (Ametaj et al., 2010a; Zhang et al., 2017). Zhang et al. (2017) found ruminal increases in amino acids, bacterial degradation products including amines, and sugars with increased concentrates fed. There is considerable speculation in regards to the agents that might be implicated in causing some of the clinical signs of acidosis and Lean et al. (2013b) summarized some of the evidence supporting potential roles for histamine, endotoxin, and lactic acid to cause laminitis (Table 1). Given, the known agents capable of causing inflammation and clinical signs, and that less well-known metabolites may be involved in clinical signs of acidosis, it is unsurprising that rumen pH *per se* is largely unrelated to the clinical signs of acidosis. Given the large number of potential toxins, often produced simultaneously in the rumen (Ametaj et al., 2010), a singular focus on any particular toxin is not appropriate.

Table 1. Summary of the evidence supporting the potential for histamine, endotoxin and lactic acid to cause laminitis on diets rich in rapidly fermentable carbohydrates. Sourced from Lean et al. (2013b)

	Histamine	Endotoxin	Lactic acid
Generated in the rumen	√	√	√
Absorbed by healthy rumen	√	√[a]	√
Absorbed by damaged rumen	√	?	?[b]
Induced laminitis when injected	√	√	√

[a]Evidence is inconsistent [b]Appears to be probable

More critically perhaps, in terms of diagnostic potential, a highly accurate measurement of rumen pH is nearly impossible. Simply, the rumen is dynamic and not homogenous and any measure whether continuous and indwelling, or static, regional and singular has limitations. Similar observations can be made in regard to most rumen measures, as rumen function varies within the rumen mat, liquid phase, and near the rumen wall and papillae (Penner, 2014). Table 2 from Golder (2014) shows the differences and correlations between different measures of rumen pH. Figure 1 derived from Bramley et al. (2008) shows the correlations between rumen samples drawn by stomach tube and rumenocentesis in 660 cows (R^2 = 0.2). Table 3 shows the value of different tests for acidosis and highlights that rumen pH provided very similar results whether obtained by stomach tube or rumenocentesis. An extensive series of studies in the United Kingdom with indwelling pH meters demonstrated that these could detect changes in the diet of cattle, but variability in individuals in their baseline pH and responses to diet did not provide adequate diagnostic outcomes for predicting differences among individual cattle without careful use of complex statistics (Denwood et al., 2018). It is, however, this large variability among cattle that provides the most interesting directions for research and prevention of acidosis in the future.

Table 2. Difference and relationship between ruminal pH measurements in ruminal fluid collected using stomach tubing, rumenocentesis, and rumen fistula methods in cattle

Methods compared	No. of cows sampled	Difference in ruminal pH values between methods[1]	Relationship between methods (r^2)	Reference
Stomach tube and rumenocentesis				
	6	+0.04		Shen et al. (2012)
	58	+0.76	0.11	Enemark et al. (2004)
	5	+1.1		Nordlund et al. (1995)
	660	+0.54	0.20	Bramley unpublished
	16	+0.35	0.25	Duffield et al. (2004)
Rumenocentesis and fistula				
	30	+0.28	0.52	Garrett et al. (1999)
	16	+0.33	0.42	Duffield et al. (2004)
	30	+0.34	0.73	Garrett et al. (1995)
Stomach tube and fistula				
	16	+0.34	0.58	Duffield et al. (2004)
Continuous ruminal pH measurement system and fistula			Correlation coefficient (r)	
Mean over 1 min	14	Mean of 1 and 5 min	0.98	Penner et al. (2006)
		-0.03		
Mean over 5 min	14		0.97	Penner et al. (2006)
	4	-0.04	0.99	Sato et al. (2012)
	4	+0.39	0.93	Phillips et al. (2010)
	12	+0.11	0.85	Dado and Allen (1993)
	6		0.65	Graf et al. (2005)
	1	-0.07	0.88	AlZahal et al. (2007)
	16	cranial-ventral site	0.68	Duffield et al. (2004)
	16	caudal-ventral site	0.61	Duffield et al. (2004)
	16	central site	0.35	Duffield et al. (2004)
	16	cranial-dorsal site	0.50	Duffield et al. (2004)
Continuous ruminal pH measurement system and stomach tube				
	16	First sample	0.15	Duffield et al. (2004)
	16	Second sample	0.31	Duffield et al. (2004)
Continuous ruminal pH measurement system and rumenocentesis				
	16		0.43	Duffield et al. (2004)
	6		0.56	Marchesini et al. (2013)

[1]Difference in ruminal pH values were calculated by subtracting the mean ruminal pH value for the second named ruminal collection method from the first named collection method ie Mean ruminal pH of stomach tube ruminal sample - Mean ruminal pH of rumenocentesis ruminal sample.

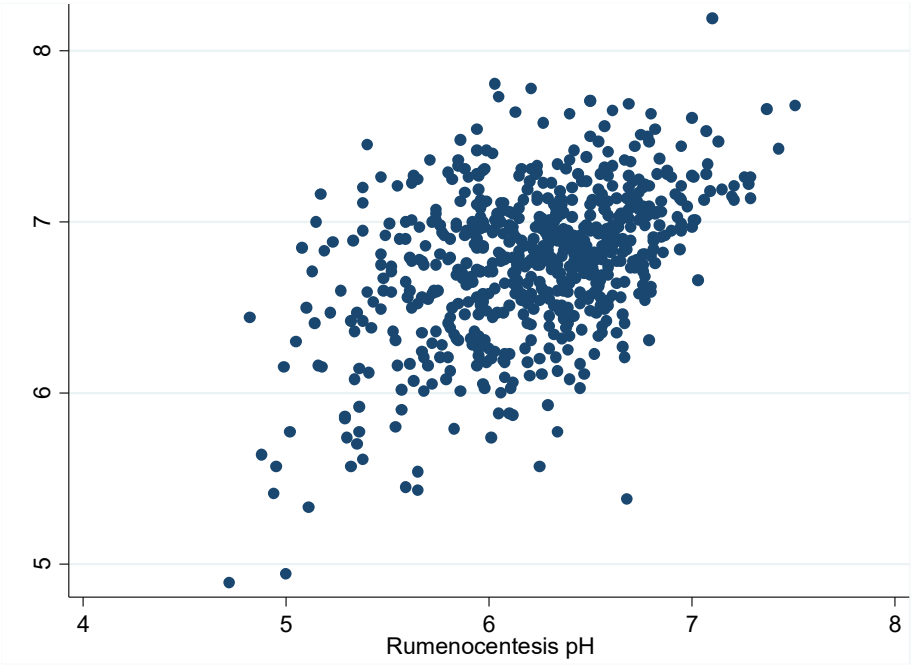

Figure 1. Scatter plot comparing rumen pH measured by rumenocentesis vs. stomach tube (R^2 = 0.20) Sourced from Bramley et al. (2008)

Table 3. Sensitivity, specificity, area under the curve, and cut-off points from receiver operator curves for the acidosis diagnostic value of rumen and milk measure from samples obtained by Bramley et al. (2008). Sourced from Golder et al. (2012a)

Measure	Sensitivity	Specificity	Area under the curve	Cut-points
Acetate (mM)	0.94	0.27	0.627	36.7
Butyrate (mM)	0.94	0.20	0.530	5.28
Propionate (mM)	0.93	0.87	0.955	23.10
Valerate (mM)	0.90	0.90	0.954	1.62
pH (Stomach tube)	0.68	0.84	0.801	6.54
pH (Rumenocentesis)	0.74	0.79	0.822	5.96
Milk Fat:Protein	0.54	0.81	0.716	1.02

Is there a good test for acidosis?

For a test to be effective, it needs to be able to be both sensitive ie detect true cases of the condition and be specific, that is have few false positive detections and be applicable across a wide range of conditions. Bramley et al. (2008) conducted their study on a wide range of herds that fed only pasture, through to different levels of grain and supplement feeding including total mixed ration herds. Herd was not a significant factor in the study in the prediction of acidosis. Subsequent, tightly controlled challenge studies using 1.2% of BW fed as grain, showed that propionate, ammonia, and valerate concentrations were the most sensitive indicators of the potential for different grains to cause acidosis (Lean et al., 2013a), and that the Bramley model was sensitive to ruminal change consistent with acidosis.

Further, a study performed using gradated steps of 2 kg of additional supplement, primarily wheat grain, but also canola meal demonstrated that as supplement increased, so did acidosis as measured using the Bramley model and that at 16 kg of supplement all cattle were acidotic most of the day (Figure 2). The cattle with acidosis had decreased milk production and milk fat percentage; however, feeding the supplement as a part of a mixed ration or substituting some of the wheat for canola deceased the prevalence of acidosis. There were very few cattle with acidosis in the low supplement groups and a high prevalence in the high supplement groups. It appears that the model for evaluating acidosis is fit for purpose, but requires a method to simply apply in the field. While it is likely that this model will be refined, the critical value in the model is that it demonstrates that acidosis is much more than pH and that performance of cattle is much more closely aligned to a model that considers more than pH.

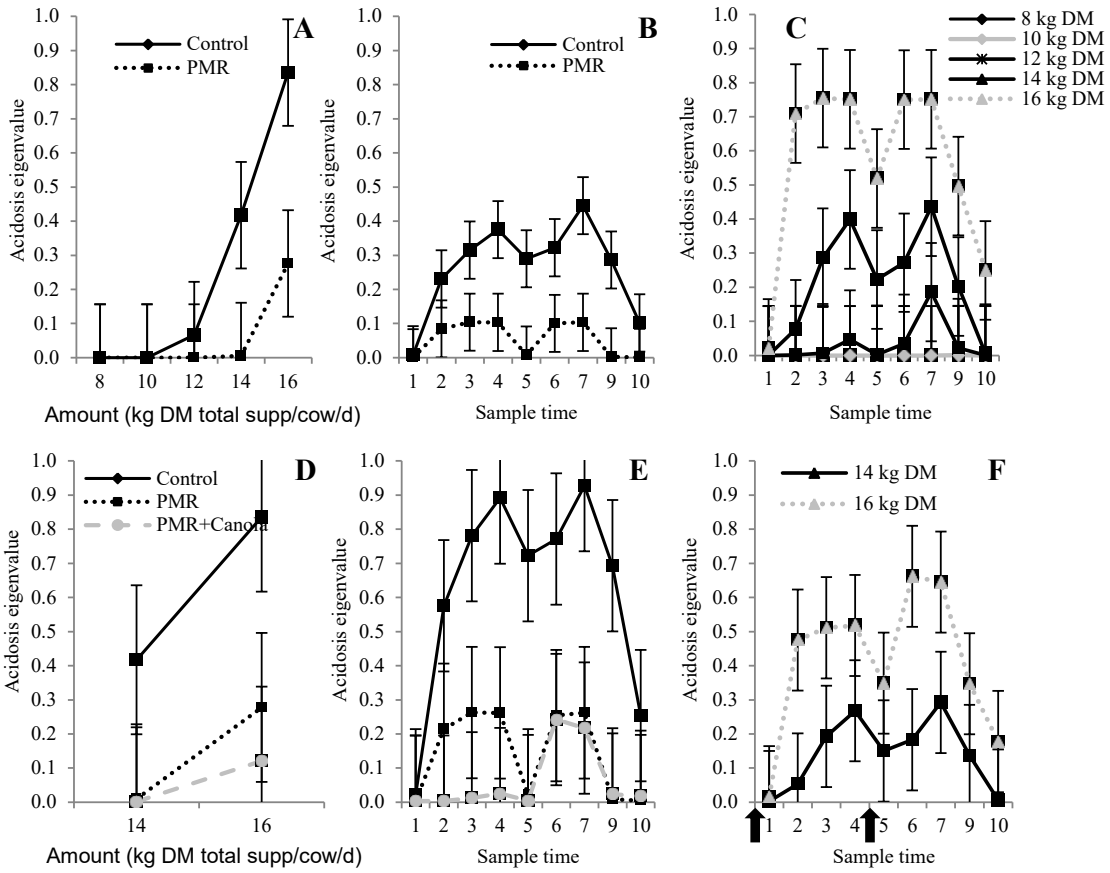

Figure 2. Mean (±SEM) acidosis eigenvalues for dairy cows from all feeding groups showing interactions between (A) feeding strategy and supplement feeding amount, (B) feeding strategy and sample time, and (C) supplement feeding amount and sample time. Mean (±SEM) acidosis eigenvalues for dairy cows from the high supplement feeding amount groups only (14 and 16z kg of DM of total supplement/cow per day) showing interactions between (D) feeding strategy and supplement feeding amount, (E) feeding strategy and sample time, and (F) supplement feeding amount and sample time. An eigenvalue of 0 corresponds to healthy, non-acidotic rumen sample and 1.0 represents an acidotic sample. Sample times were approximately 2.4 h apart over a 24-h period. Sample time 1 was approximately 8:20 h and milking was at 7:00 and 15:00 h (black arrows). PMR = partial mixed ration; PMR+Canola = partial mixed ration + canola meal; Amount = kg of DM of total supplement/cow per day. Sourced from Golder et al. (2014d).

Ruminal Ecology and Risk

The rumen is central to our understandings of cattle nutrition but is still largely unexplored, which is not too surprising given the large number of organisms present. Only a minority of the bacteria, archaea, viruses, fungi, and protozoa present in the rumen have been named or are able to be cultured, let alone their functions fully characterized. However, this field is rapidly changing with rapid sequencing of the DNA and rRNA or

rumen organisms, termed metataxomics, allowing investigations of the rumen environment to become more detailed (Jami et al., 2013). Recently, the effects of perturbing the rumen have been evaluated (Weimer et al., 2010; Golder et al., 2014b; Plaizier et al., 2017). Goldansaz et al. (2017) reviewed the opportunity for metabolomics, that is analytical techniques that can quantify small molecular weight products of metabolism, to be utilized in the investigation of production disease and examples of this include Loor et al. (2007) and Hailemariam et al. (2014). Metabolomics may be particularly powerful when used to evaluate responses to rumen perturbation (Ametaj et al., 2010b; Zhang et al., 2017). These new techniques are offering insights to the function and control of the rumen.

The *Bovidae,* including cattle, are among the most widely disseminated of the mammals. An important perspective can be obtained from a paper on the metataxome of the feces of mammals (Ley et al., 2008). This paper metatexamined similarities and differences in the fecal biota of a very diverse selection of mammals in the context of co-evolution of meta-taxomic communities. A key finding was that bacteria appear to be fairly promiscuous between hosts, a factor the authors speculated could account for the spectacular success of herbivores in general. The observations of Ley et al. (2008) are important to consider in the context of the way in which a species manages risk. In the case of cattle, times of abundance, for example lush legumes or abundant sugars or starches, or even toxic plants pose a risk to the animal and even a herd. This leads to a key understanding of the concept of a core rumen microbiome and a group of non-core organisms (Jami and Mizrahi, 2012; Lettat and Benchaar, 2013; Firkins and Yu, 2015). The core organisms appear to be common to most cattle in a group; however, there is very considerable diversity in the non-core (Zue et al., 2018). Perhaps the best example to consider is the protozoa that cattle maintain despite a high cost of predation of bacteria, leading to loss of approximately 20% in microbial protein outflow and lower average daily gain than defaunated cattle. However, these physiological responses are less for cattle on concentrate diets, suggesting an important role for protozoa in slowing the rate of starch degradation (Eugène et al., 2004) and a potentially valuable role in reducing the risk of acidosis. The adaptive responses of the rumen to severe dietary challenge; therefore, might be an expected variable, based on the concept that maintaining populations of organisms that may be less efficient but vital for survival, under particular challenge conditions, is a function of managing risk in a population.

Genome, meta-taxome, and function

Recent findings highlight the potential for further targeted manipulation of the rumen and the likelihood that acidosis is much more than a ruminal condition. These differences have been explained by different host genetics and interactions with the rumen meta-taxome (Weimer et al., 2010; King et al., 2011; Hernandez-Sanabria et al., 2013). Weimer et al. (2010) showed the ability of the rumen to revert to pre-exchange VFA concentration and rumen pH and nearly return to pre-exchange bacterial community composition within 24-hours of a 95% exchange of ruminal content with a cow on a similar diet. A second cow took a longer period to revert indicating the potential for variability in this response (Weimer et al., 2010).

Golder et al. (2018) demonstrated with limited numbers of cattle that there are strong links between the mammalian genome, the meta-taxome, and rumen function. There were several putative quantitative trait loci (**QTL**) identified for different metabolites. Five putative QTL were identified for the acetate to propionate ratio on chromosomes 1, 3, 5, 6, and 8. Eight putative QTL regions were identified for total lactate concentrations on chromosomes 1, 4, 6, 11, 22, and 24. Three putative QTL regions were identified for D-lactate concentrations on chromosomes 2, 8, and 26 and six putative QTL were identified for L-lactate concentrations on chromosomes 1, 4, 8, 17 and 24. One QTL was identified for the acidosis eigenvalue (this measure is obtained using the data from Bramley et al., 2008 and predicts how well the cows fits with an acidosis classification) on chromosome 19. Further, a large number of putative QTL were identified for bacterial phyla (Golder et al., 2018). Xue et al. (2018) evaluated the relationships between the meta-taxome and phenotypes for rumen function and production in over 300 cattle fed the same diet. They (Xue et al., 2018) found 6 phyla that represented over 45% of bacterial genotypes and included Firmicutes (21.67%), Bacteroidetes (20.68%), Proteobacteria (0.52%), Spirochaetes (1.35%), Fibrobacteres (0.86%), and Tenericutes (0.44%). There was marked animal variation in the prevalence of these taxa; however, relationships were identified among the bacteria, VFA, and production outcomes. These studies provide new insights that may allow better targeted nutrition and genetic selection in the future and provide a further basis to understand responses to perturbation of the rumen.

Perturbing the rumen

The primary methods used to perturb the rumen are feeding or administering single or multiple doses of RFCHO in the form of starches, sugars, or their combinations. Studies have noted considerable variation in responses among cattle fed a common diet designed to induce ruminal acidosis (Brown et al., 2000; Bevans et al., 2005; Penner et al., 2009; Golder et al., 2014b; Xue et al., 2018). Perturbation differences appear to be affected by genetic (Golder et al., 2018) and environmental factors (Xue et al., 2018) and likely their interactions (Golder et al., 2018). Substrate and other factors such as length of challenge and prior exposure to RFCHO etc affect rumen perturbation. Golder et al. (2012) fed non-pregnant Holstein heifers no grain or combinations of grain (1.2% of BW), fructose (0.4% of BW with 0.8% of BW grain), and histidine (6 g) in a single challenge feeding. It was evident that the rumen altered in response to the different substrates and substrate combinations. Heifers that had fructose included in their challenge ration had bacterial populations associated with increased total lactic acid and butyrate concentrations and decreased pH, while those that were not fed fructose had bacterial populations associated with the amount of grain consumed and ruminal ammonia, valerate, and histamine concentrations (Figure 3; Golder et al., 2014c).

In a longer-term challenge study, rumen perturbation increased with an increase in the amount of supplementary feeding and when isoenergetic diets included grain supplements fed in the milking parlor as opposed to supplements primarily fed in a mixed ration as shown by acidosis eigenvalues in Figure 2 for late lactation dairy cattle (Golder

et al., 2014d). Differences in associations between microbial populations and rumen metabolites between different groups of cattle fed differing amounts of supplement with these different feeding strategies are shown in Figure 4. Importantly, these findings show that substrate types (Figure 3) and amounts (Figure 4) determine the rumen populations and functions.

Further, Golder et al (2014b) fed pregnant heifers with a target DMI of 1.0% and 0.2% of BW of wheat and fructose, respectively with a non-fiber carbohydrate (**NFC**) content of 76.3% if 100% of the ration was consumed. These heifers had a 20-day exposure to total mixed rations including 10-days with an NFC content of 47.7% and a NFC of 46.1% prior to challenge. In contrast with the shorter challenge study with very similar amounts of grain and/or fructose (Golder et al., 2012) there were very large intra-group differences in rumen metabolites on the challenge day. Similarly, Firkins and Yu (2015) note that differences in the meta-taxome among animals within the same diet group often exceeds those among different diet groups.

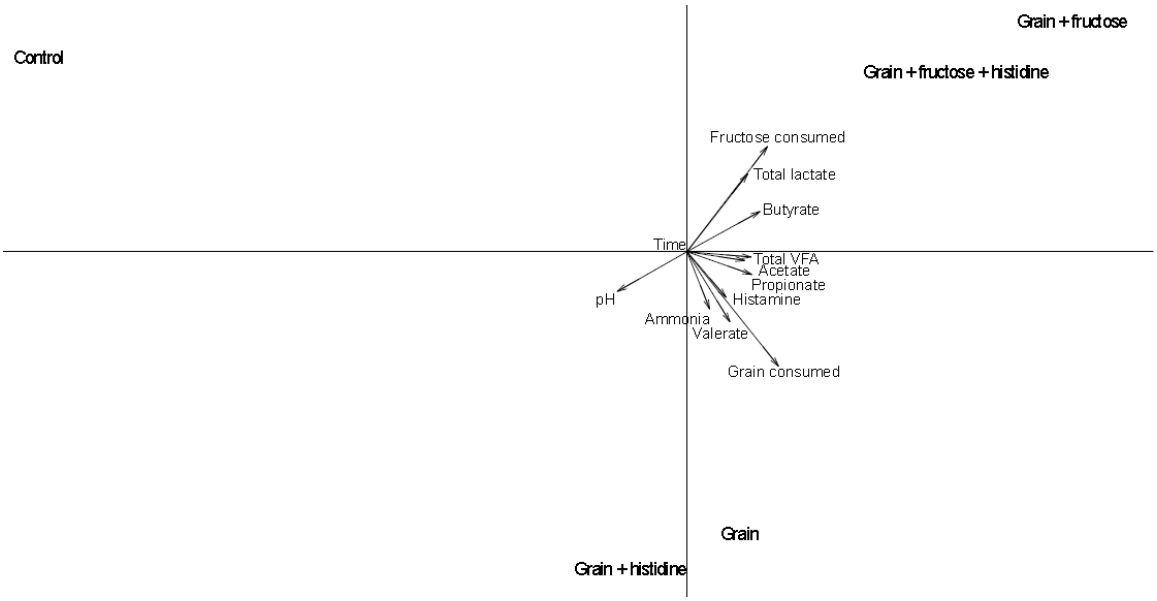

Figure 3. Duality diagram of co-inertia analysis of ruminal bacterial communities from 16S rDNA 454 pyrosequences, measures of ruminal fermentation, and percentages of offered grain and fructose from heifers that consumed the following single challenge rations: (1) control (no grain); (2) grain (1.2% of BW DM); (3) grain (1.2% of BW DM) + histidine (6 g/head); (4) grain (0.8% of BW DM) + fructose (0.4% of BW DM) or; (5) grain (0.8% of BW DM) + fructose (0.4% of BW DM) + histidine (6 g/head) (n of heifers = 6/group). Ruminal fluid was collected over approximately a 3.6-h period after (n of samples = 18/group). On the bi-plot the ruminal fermentation measures are represented as arrows. The direction of the arrow of each ruminal fermentation measure indicates an increasing concentration of that measure. The angle between the arrows indicates their degree of correlation. The magnitude of the arrows indicates the importance of the measure on the bacterial community composition. Measures with long arrows are more strongly correlated with the ordination axes than short arrows and have a greater influence on the pattern of variation (Carberry et al., 2012). Sourced from Golder et al. (2014c)

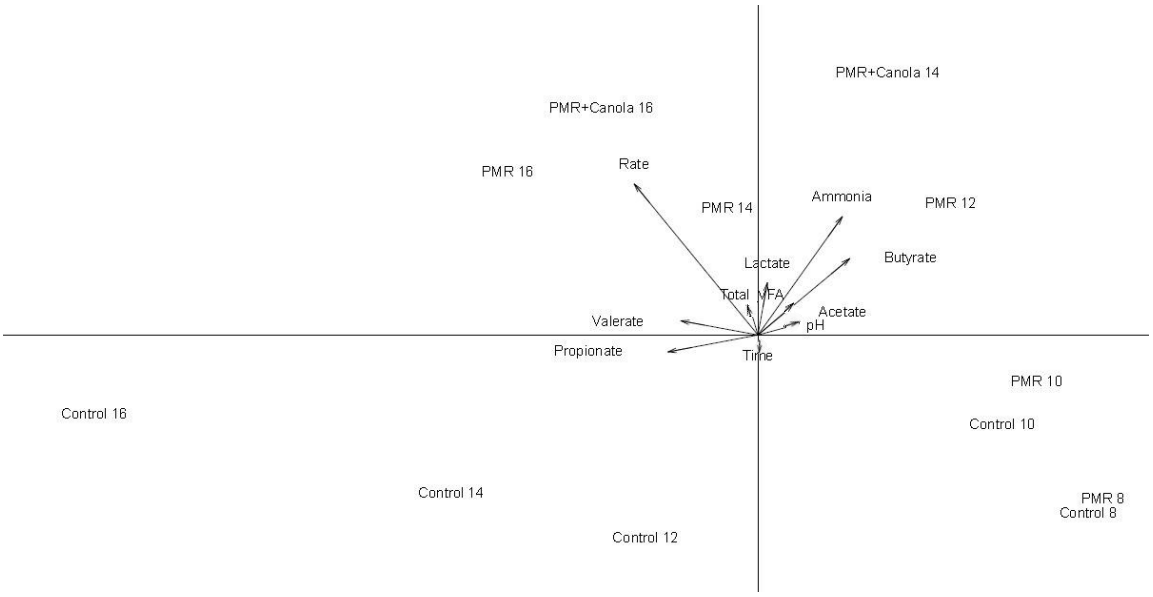

Figure 4. Duality diagram of co-inertia analysis of ruminal bacterial communities from 16S rDNA 454 pyrosequences, measures of ruminal fermentation, sample time, and amount of total supplements fed in dairy cattle fed 1 of 3 feeding strategies: control (n = 10 cows), partial mixed ration (PMR; n = 10 cows), or PMR+Canola (PMR+Canola meal n = 4 cows) at amounts 8, 10, 12, 14, or 16 kg of DM of total supplement/cow per day (2 cows per supplement feeding amount at 3 times from each feeding strategy). On the bi-plot the ruminal fermentation measures are represented as arrows. The direction of the arrow of each ruminal fermentation measure indicates an increasing magnitude of that measure. The angle between the arrows indicates their degree of correlation. The magnitude of the arrows indicates the importance of a measure on bacterial community composition. Measures with long arrows are more strongly correlated with the ordination axes than short arrows and have a greater influence on the pattern of variation (Carberry et al., 2012).

Controlling the Rumen

The studies outlined above, when combined with other literature provide the following clear guidelines to controlling the rumen.

Diet Form, Formulation, and Function

Consistency of supply of feed is important as many studies have withheld feed as part of a protocol to create acidosis (Nagaraja and Titgemeyer, 2007). Providing adequate fiber and particle length (Zebeli et al., 2012) and >30% NDF, based on Bramley et al. (2008) is appropriate for lactating dairy cattle. Diets formulated as partial mixed rations were safer, despite a higher NFC content, than diets that were component-fed (Golder et al., 2014c).

Sugars in the diet should be controlled based on Nagaraja et al. (1981) and (Golder et al., 2012b; Golder et al., 2014b). We suggest the following guidelines for TMR based on Bramley et al. (2008) and Golder et al. (2014d) for a maximum total NFC of 40 to 42%, 22 to 24% of starch, and 8% of sugar based on not exceeding approximately 0.35% of BW for sugars intake. It is very likely that not all sugars will have the same effect on the rumen (Plazier et al., 2018), and it is very evident that not all grains (Lean et al., 2013) or starches have the same effect on rumen function. Further, form of processing the concentrate components in the diet will influence function.

Lastly, observations that acidotic cattle have low rumen concentrations of ammonia (Bramley et al., 2008) and a reduction in the incidence and prevalence of acidosis with increased nitrogen in the diet (Golder et al., 2014d) support the observation that microbial protein is a significant sink for hydrogen in the rumen and that energy spilling ie an inability of bacteria to reproduce, hence produce more VFA, may be an important part of the pathogenesis of acidosis.

Feed additives

Buffers and Neutralizing Agents

These have been well reviewed and a buffer, by definition, reduces the decrease in pH without causing an increase in pH (Staples and Lough, 1989). Questions remain; however, in regard to the function of sodium bicarbonate, potassium carbonate, potassium bicarbonate, sodium sesquicarbonate, and the skeletal remains of the seaweed *Lithothamnium calcareum.* In the case of sodium bicarbonate, there are questions whether the effects are mediated through buffering the accumulated acid or increases in DM and water intakes caused by sodium, facilitated through an increased ruminal fluid dilution rate and reduced starch digestion rate (Russell and Chow, 1993; Valentine et al., 2000). Similarly, potassium-based products including potassium carbonate sesquihydrate, may be contributing to production increases through increased dietary cation anion difference or potassium requirements rather than through buffering actions. There are positive interactions for sodium bicarbonate with magnesium oxide and combination of sodium bicarbonate and magnesium oxide had similar effects as virginiamycin in controlling cyclic eating behaviour in cattle during adaptation to a diet high in grain and containing fructose (Golder et al., 2014b).

Antibiotics: While these are subject to regulatory change, there is strong evidence that some antibiotics can control the risk of acidosis (Lean et al., 2014). Tylosin has been widely used in finishing diets for the US beef industry. Virginiamycin is effective in controlling acidosis and tylosin, in combination with monensin, is also effective. It appears that combinations of monensin and bambermycin are also effective in favourably modifying rumen function. Both the latter are non-human class therapeutical agents.

Ionophores: Ionophores, particularly monensin and lasalocid are widely used in beef and dairy production. There is evidence of more sustained appetite (Lunn et al., 2005) and of increased production of propionate from lactate, which is a ruminal

adaptation that sequesters hydrogen ions in safer ruminal pools, when monensin is fed in diets that may cause acidosis. Monensin appears to be very effective in controlling acidosis risk when fed with tylosin or virginiamycin. Nagaraja et al. (1981) investigated the use of lasalocid to control lactic acidosis induced using finely ground corn or glucose. Use of lasalocid equalled or exceeded the reduction in lactic acid production observed for monensin (Nagaraja et al., 1981). Both monensin and lasalocid prevented acute lactic acidosis in the study of Nagaraja et al. (1981); however, both products were included in the diet at concentrations of 1.30 ppm of diet, and above concentrations recommended. Nagaraja et al. (1982) found that 0.33, 0.65, and 1.30 ppm of lasalocid were effective in reducing lactic acid concentrations and increasing pH compared to control cattle with lactic acidosis induced using glucose at 12.5 g/kg of BW. More studies would be useful to evaluate the effect of lasalocid on rumen acidosis.

Yeasts: There is increasing evidence that yeasts and yeast cultures may have a role in stabilizing rumen function. Actions that have been identified with live yeasts include small increases in rumen pH, reductions in lactic acid, enhanced fiber digestion, alterations in immune function and small increases in VFA production. These actions, are modest in magnitude, but may synergize with other strategies to control the risk of acidosis. Li et al. (2016) found that a *Saccharomyces cerevisiae* fermentation product stabilized rumen pH and Bach et al. (2018) demonstrated changes in immune markers in the epithelium and rumen to a live yeast. Weight gains and average daily gain improvements have been identified in beef receival cattle fed a hydrolyzed yeast (Salinas-Chavira et al., 2018) and reductions in severe liver abscess incidence also noted with an autolysed yeast (Ran et al., 2018). While these findings are encouraging, it is challenging to understand differences in the different yeast-based products and the best application of these in the field.

Probiotics: There is also some evidence that probiotics may provide benefits in terms of acidosis control; however, there are challenges in this area as candidate agents such as *Megasphera elsdenii* has not provided clear and consistent benefit in studies to date. It seems likely that more studies will investigate the roles of other agents in acidosis control in the future.

Conclusions

Acidosis is a much more complex condition than simply reflected in a drop in ruminal pH. Acidosis is increased by diets higher in starch and sugars and lower in fiber and is reflected in increases in propionate and valerate concentrations and reduced ammonia concentrations and rumen pH. While the clinical expression of acidosis may be influenced by the interactions of the gastrointestinal tract and immune system, we consider that prevention will depend on control of substrate and form and delivery of the diet. Better tests for acidosis will help identify, research, and manage the condition. These better tests, resulting in the more accurate identification of cattle with acidosis, will be critical to produce new interventions to assist in the control of acidosis in a higher percentage of the population. Recent developments in evaluating and understanding the

rumen and gastrointestinal tract function will provide new methods for controlling rumen function including selection of more production system adapted genotypes.

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