Use of Live Yeast Products to Stabilize Rumen pH

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Abstract

One of the most common dietary afflictions in dairy cattle is rumen acidosis, especially in the subacute form that occurs when rumen pH is between 5.2 and 5.6. Rations that are low in fiber and high in rapidly fermentable carbohydrates have a greater tendency to be acidogenic. Rumen acidosis has been associated with low milk fat, erratic feed intake, diarrhea, laminitis, and liver abscesses, although the indistinguishable symptom of subacute rumen acidosis (SARA) is low rumen pH. A minimum content of total fiber and physically effective fiber has been recommended to prevent SARA. However, providing large particle size feeds may not always ensure a high milk fat content and/or prevention of SARA, especially if cows are able to sort out long feed particles. Using prebiotics and probiotics that modulate gastrointestinal flora to create a population that is most beneficial to the host is an alternative to controlling rumen pH. There are three main groups of probiotics: bacteria, fungi and yeast. Lactic-acid producing bacteria have been proposed as a means of ensuring a basal and constant production of lactic acid in the rumen; precipitating a stable mass of lactic acid-utilizing bacteria in the rumen. The most common fungus used as a probiotic is *Aspergillus oryzae*, which has been reported to improve fiber digestion in the rumen, but positive effects on modulating rumen pH have not been found in the literature. The most common active dry yeast used in ruminant nutrition is *Saccharomyces cerevisiae*. Live yeast supplementation to the diet has been associated with stabilization of rumen pH by promoting the use of lactic acid, fostering microbial growth and competing for rapidly fermentable carbohydrates with bacteria in the rumen.

Introduction

Rumen acidosis is thought to be the most common digestive upset in dairy cattle. Garrett et al. (1997) surveyed 15 dairy farms in Wisconsin and revealed the presence of rumen acidosis in 19% of early-lactation cows and 26% of mid-lactation cows. In another survey of 14 dairy farms in Wisconsin, Oetzel et al. (1999) reported incidence of rumen acidosis of 20.1% in early- or peak-lactation cows. Rumen acidosis is a function of fermentation end-product accumulation in the rumen, mainly volatile fatty acids (**VFA**) and lactic acid. Accumulation of end products is due to excessive fermentation that produces large amounts of VFA, or an inadequate removal of these VFA from the rumen via absorption through the rumen wall, wash out from the rumen via passage rate, or neutralization with buffers or alkalinizers. Rumen acidosis is commonly classified as chronic when average rumen pH is about 5.6; acute when average rumen pH is about 5.2; and subacute (**SARA**) when average rumen pH is between 5.2 and 5.6. In addition to potential health issues that SARA may cause, SARA can also impact ruminal digestion of fiber (Beauchemin, 2000) and protein (Bach et al., 2005) and may facilitate erratic feed intake and alter milk composition (Nocek, 1997).

The objective of this review is to address the most common symptoms and causes of SARA and the role of probiotics in its prevention and control. Emphasis will be placed on recent research addressing the use of live yeast to stabilize pH in the rumen.

Symptoms and causes of rumen acidosis

In an attempt to provide sufficient energy to high-producing cows, the proportion of nonfiber carbohydrates (**NFC)** in the diet is increased at the expense of fiber or forage content. As a result, rations that ferment rapidly in the rumen and have low fiber or forage content are considered potentially acidogenic. These rations are effective in promoting a vigorous fermentation in the rumen and have potential to sustain high levels of milk production. Using purine derivatives in the urine as microbial markers, Moorby et al. (2006) demonstrated that the flow of microbial mass leaving the rumen increased as the proportion of forages in the ration decreased.

Rumen acidosis has been associated with low milk fat content, erratic feed intake, diarrhea, laminitis and liver abscesses. However, the primary pathognomonic symptom of SARA is low rumen pH. Because rumen pH is difficult to measure under practical conditions, rumenocentesis can be effectively used to assess the rumen pH status of a herd (Kleen et al., 2004).

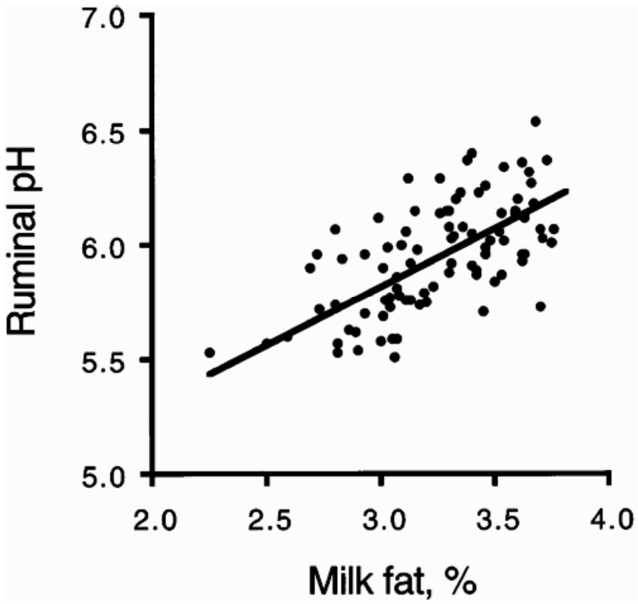
Various signs of SARA can be observed at the slaughterhouse that can be useful in diagnosing the herd, but too late for the cows already affected. For example, SARA induces a hardened rumen wall, a consequence of reduced blood flow to the rumen epithelium which has been described by Huntington and Britton (1979). Also, at the slaughter house, herds with SARA depict an increase in incidences of liver abscesses. These abscesses are usually caused by *Fusobacterium necrophorum* that reach the portal blood through a damaged rumen wall. Presence of liver abscesses can impair milk production, because the liver is a key organ involved in the synthesis of glucose and protein precursors for milk lactose and protein synthesis.

Another common sign of SARA is cyclic intake (Nocek, 1997). One of the reasons for cycling intake can be found in the osmolality receptors of the rumeno-reticular wall that will depress intake as rumen osmolality increases (Carter and Grovum, 1990). Rumen osmolality increases as rumen pH decreases due to an accumulation of VFA, and also an accumulation of bacterial cell contents resulting from bacterial lysis and subsequent death due to low pH. Once the animal ceases eating, the rumen environment progressively returns to its normal conditions, osmolality decreases and appetite resumes. However, this symptom of SARA is not easily observed in commercial operations with loose-housed animals because the average intake for an entire herd or pen may not cycle. For example, when one animal is in the decreasing cycle of intake, another animal may be in the ascending cycle.

Laminitis has been traditionally associated with SARA and is a condition in the cow that not only causes pain, but also compromises milk production (Warnick et al., 2001; Green et al., 2002; Bach et al., 2007). When rumen pH decreases there is an accumulation of lipopolysaccharides (**LPS**) in the rumen (Nagaraja et al., 1978; Andersen et al., 1994; Gozho et al., 2005) due to lysis of Gram negative bacteria that are sensitive to low pH. Lipopolysaccharides are absorbed into the bloodstream of the animal, and are effectively and rapidly cleared from the blood by the liver. Inflammatory response cascade is stimulated by an increase in acute phase proteins, such as serum amyloid A and haptoglobin (Gozho et al., 2005; Gozho et al., 2006; Gozho et al., 2007). However, the increase in LPS in the rumen can also be a consequence of cell lysis due to excessive autolytic enzymes that facilitate growth during the rapid bacterial growth phase (Wells and Russell, 1996). Increases in concentrations of serum amyloid A and haptoglobin have also been observed in cows with mastitis and other inflammatory processes (Nielsen et al., 2004). Although, the inflammation mechanism could play a role in laminitis, it is more likely that other mechanisms are involved in which SARA specifically causes laminitis. An example would be hypoperfusion caused by vasoactive components produced as a response to rumen acidosis, resulting in ischemia in the digit (Nocek, 1997). In addition, a recently identified acid-resistant ruminal bacterium, *Allisonnella histaminoformans*, has been implicated in laminitis development because it produces histamine, a sole metabolite from histidine decarboxylation (Garner et al. 2004).

One of the most common symptoms of SARA in lactating dairy cows is a decrease in milk fat content (Figure 1). There are three main hypotheses that attempt to explain this relationship. One hypothesis is the “glycogenic theory”, which postulates that a decrease in acetate to propionate ratio observed during SARA stimulates secretion of insulin that reduces availability of fat (long chain fatty acids) to the mammary gland. Another theory maintains that SARA-inducing rations decrease the total amount of acetate produced in the rumen, resulting in the mammary gland receiving less short-chain fatty acids to produce milk fat. The third and most supported theory states that the decrease in milk fat is due to the inhibitory effect of the intermediate metabolite of rumen biohydrogenation of unsaturated fatty acids; *trans*-10, *cis*-12 conjugated linoleic acid (**CLA**). There are only a few ruminal microorganisms identified that are capable of forming *trans*-10, *cis*-12 CLA, including several *Propionibacteria spp*. and a *Lactobacilli spp*., organisms that can proliferate on a high-grain diet. *Propionibacteria spp*. can also account for increases in ruminal propionate concentration that are typically observed with milk fat depression.

Figure 1. Relationship between milk fat content and rumen pH of dairy cattle. Adapted from Allen (1997).

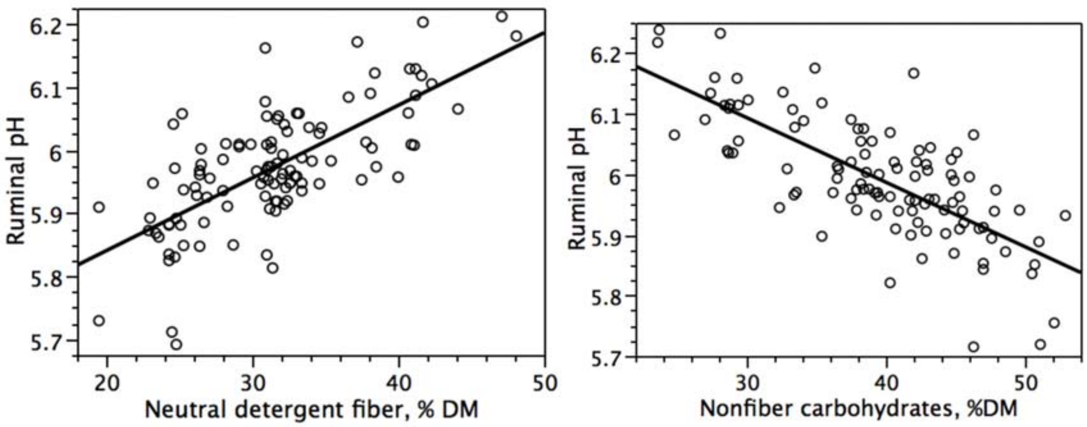


Prevention and control of rumen acidosis

To prevent rumen acidosis, it is commonly recommended to provide a minimum of total fiber and physically effective fiber in the ration. However, the proportion of neutral detergent fiber (**NDF**) in the ration accounts only for about 50% of the variation observed in average rumen pH (Figure 2).

Figure 2 also shows that the dietary content of NFC explains about 50% of the variation in rumen pH, although there is a negative relationship. One of the causes of SARA is an accumulation of VFA derived from fermentation of rapidly fermentable carbohydrates (**CHO**). Therefore, as the proportion of NDF in the ration increases, the proportion of NFC should decrease. It has been suggested that differences in physical form of NDF improve the prediction of rumen pH, leading to the concept of physically effective fiber (**peNDF**). Physically effective fiber is defined as the proportion of dietary NDF contained in particles above 1.8 mm according to Mertens (1997) and above 8 and 19 mm according to Lammers et al. (1996). It is still unclear which of these two approaches best predicts chewing times, saliva production and ultimately rumen buffering capacity (Einarson et al., 2004). Numerous studies have been conducted to evaluate the effects of peNDF, but results have not been conclusive. Several authors (Soita et al., 2000; Krause et al., 2002; Beauchemin et al., 2003; Calberry et al., 2003) reported a relationship between peNDF and rumen pH, whereas others (Yang et al, 2001; Knonoff et al., 2003; Kononoff and Heinrichs, 2003; Einarson et al., 2004) showed no relationship.

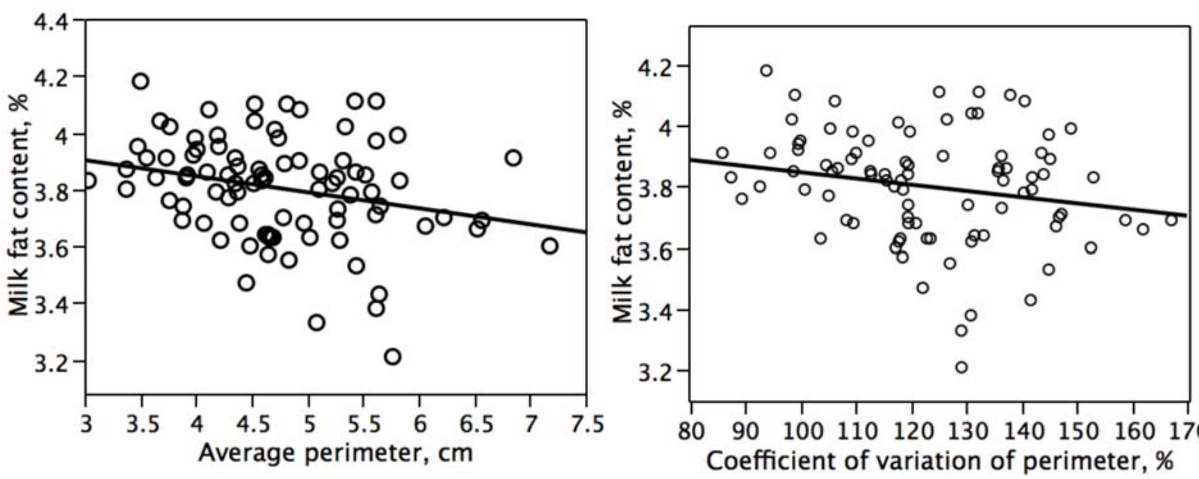
Figure 2. Relationship between neutral detergent fiber (R^2 = 0.49; $P < 0.05$) or nonfiber carbohydrate (R^2 = 0.54; $P < 0.05$) dietary content and ruminal pH of dairy cattle. Data from published literature (adjusted for study effect).



Because of interactions between concentrate inclusion, rate of fermentation, forage to concentrate ratio, total dry matter intake, etc.; it is difficult to recommend a unique peNDF value for preventing and controlling SARA. For example, Beauchemin and Yang (2005) proposed that a combination of peNDF and dietary fermentable OM be used to predict rumen pH and control SARA. Zebeli et al. (2006) presented a model that accounted for peNDF (calculated as > 1.8 mm) and the amount of fermentable OM from forages and rumen degradable starch in the ration. This model improved the prediction of rumen pH compared with using only estimated peNDF. Recently, a study by Rustomo et al. (2006) evaluated the effect of acidogenic value and forage particle length on rumen pH, using four cows in a Latin Square design. They concluded that as the acidogenic value of the concentrate fed increased, rumen pH and time spent below 5.6 decreased. The effect of forage particle size on rumen pH was only evident when the long forage particle size was offered in conjunction with a highly acidogenic concentrate. Effects on rumen pH demonstrated an increase in the maximum pH, but did not affect the mean of the minimum pH.

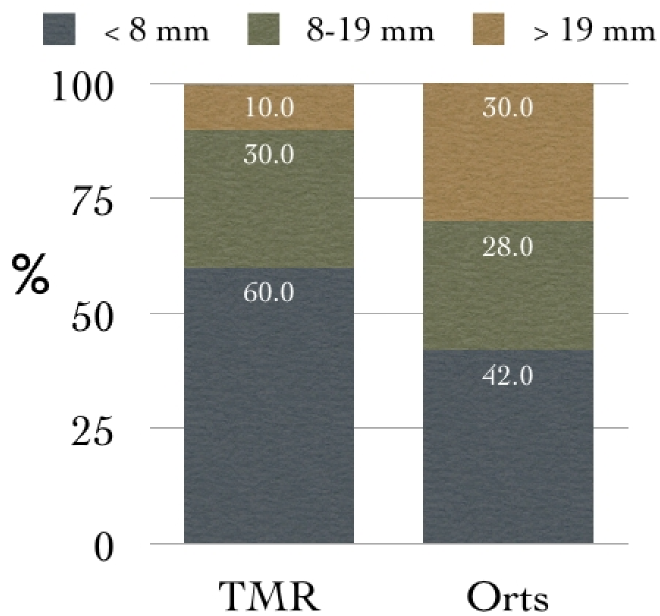
In a survey conducted in northern Spain involving 60 dairy herds, Bach et al. (2003) found that the average perimeter (**PE**) of particles from TMR was negatively correlated ($r = -0.38$; $P < 0.001$) with milk fat test. These results indicate that the smaller the PE, the greater the milk fat content (Figure 3). This observation is discordant with current theories proposing that large mean particle size is important to attain high milk fat percentages. A plausible explanation for the negative relationship between the proportion of large particles and milk fat percentage could be related to the capacity of the dairy cows to sort and separate long particles from small ones. In fact, the negative relationship between average PE of particles in the TMR and milk fat percentage could be explained by the positive relationship ($r = 0.84$, $P < 0.001$) between average PE and coefficient of variation of particles in the TMR, and the negative relationship ($r = -0.34$, $P < 0.002$) between level of dispersion (coefficient of variation) of the particles PE and milk fat percentage (Figure 3). A similar, and also unexpected, negative trend between average particle size and milk fat was described by Allen (1997), who found that the proportion of long hay (particle size was not reported) was negatively correlated with milk fat percentage. Clark and Armentano (2002) reported significant linear increases in chewing and rumination times as the particle size of alfalfa hay in the TMR increased, however, there were no differences in milk fat percentage across particles sizes. Similarly, Beauchemin et al. (2003) reported a good correlation between effective NDF in the TMR and ruminal pH and chewing activity, but no significant effects were found on milk fat percentages. Feeding forages of small particle size has been correlated with decreased chewing activity and saliva secretion, low rumen pH, and low milk fat percentages (Cassida and Stokes 1986; Norgaard 1993). Conversely, feeding forage with excessively long particle size may limit intake, and could decrease milk production and artificially improve milk fat content.

Figure 3. Relationship between average perimeter of particles in a TMR ($R^2 = 0.11$; $P < 0.05$) or the coefficient of variation of the perimeter among the particles of a TMR ($R^2 = 0.10$; $P < 0.05$) and bulk milk fat content. Data were taken from 60 dairy herds in northern Spain.



Calberry et al. (2003) conducted a study where dairy cows were fed a TMR with 30% corn silage and either 10% alfalfa hay, or alfalfa silage, or a mixture of both. Despite the relatively high content of forage in the TMR (40%), milk fat content was low at about 2.8%. A possible explanation for the low milk fat content could be found in the particle size distribution of feed particles in the TMR. Between 38 and 44% of the TMR particles were greater than 8 mm, which may have allowed the cows to select against these large feed particles (Figure 4). Consistent with these observations, Einarson et al. (2004) compared two high-concentrate (58%) rations that contained alfalfa hay finely ground at 10 mm vs alfalfa hay ground at 19 mm. They reported a reduction in milk fat that coincided with selection against large feed particles when alfalfa hay was ground at 19 mm compared with alfalfa hay ground at 10 mm. However, when high forage rations (58%) were compared, no differences were found in particle selection or milk fat content. Kononoff et al. (2003) showed an increase in milk fat content when comparing a TMR with short particle sizes, and reported an increase in selection against large feed particles when feeding large particle sizes in a high concentrate diet.

Figure 4. Particle size distribution of feed particles in a TMR and its orts. Adapted from Calberry et al. (2003).



The role of probiotics or direct-fed-microbials

Increasing concern in the use of antibiotics as feed additives has led to an attempt to use naturally-occurring substances that have an antibiotic effect, but are considered to be safer than conventional antibiotics. These substances include plant extracts and essential oils, and act in a similar way to antibiotics. Therefore, they could potentially lead to similar negative consequences. An alternative to using antibiotics or similar substances is the use of prebiotics and probiotics that aim to modulate gastrointestinal flora creating a population that is most beneficial to the host. There are three main groups of probiotics; bacteria, fungi and yeast. Probiotics are defined as a "live microbial feed that beneficially affects the host animal by improving its intestinal microbial balance" (Fuller, 1989). A stricter and more modern definition was presented by Schrezenmeir and de Vrese (2001) who defined probiotics as "a preparation of or a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects in this host". With this definition in mind, many of the microorganisms traditionally classified as probiotics for ruminants would not fit this description. For example, supplemented yeasts are unable to survive in the rumen which is why they need to be supplied daily with the feed, thus the colonization or implantation of the microflora is rather weak. Despite these limitations, the terms probiotics or direct-fed microbials are used commonly and indistinctly in the field and in the scientific literature when referring to yeast and fungi as feed additives.

Bacteria

Supplementation of lactic-acid producing bacteria to the ruminant ration has been proposed as a means of ensuring a basal and constant production of lactic acid in the rumen. These bacteria would maintain a stable mass of lactic acid-utilizing bacteria in the rumen, such as *Megasphaera elsdenii* which is found at relatively low numbers and increases significantly only when lactic acid becomes a major product of fermentation (Counotte et al., 1981). Under conditions when fermentation leads to an increase in production of lactic acid, increasing the risk of SARA, the lactic acid-utilizing population would potentially consume the lactic acid and avoid or diminish SARA. In fact, supplementation of specific *Enterococcus* strains raised daily average pH and average minimum pH in the rumen of dairy cattle (Nocek et al., 2002). In addition, *Enterococcus faecium* has been shown to also improve DMI and subsequently milk production of pre-partum and early lactation dairy cows (Nocek et al., 2003; Nocek and Kautz, 2006).

Fungi

The most common fungus used as a probiotic is *Aspergillus oryzae.* This fungus has been reported to improve fiber digestion in the rumen (Judkins and Stobart, 1988; Fondevilla et al., 1990), but with no effects on rumen VFA or ammonia N (Gomez-Alarcon et al., 1990). Some strains of *A. oryzae* have been reported to stimulate the growth of *M. elsdenii* (Waldrip and Martin, 1993) and *Selenomonas ruminantium* (Nisbet and Martin, 1993), which both actively metabolize lactic acid into propionic acid, thus reducing the risk and severity of acidosis. However, positive effects of *A. oryzae* on modulating rumen pH have not been found in the literature.

Yeast

The most common active dry yeast (**ADY**) used in ruminant nutrition is *Saccharomyces cerevisiae* with high concentration of viable cells (more than 10 billion CFU/g), with these cells mixed or not mixed with their fermentation medium. Other yeast species used in ruminant nutrition include *Trichosporon sericeum*, which has been reported to reduce methane production in the rumen when fed in conjunction with galacto-oligosaccharides (Mwenya et al., 2004), but has shown no effect on rumen pH (Mwenya et al., 2005). Supplementation of live yeast has been associated with an increase in DMI (Stella et al., 2007), improved fiber digestion (Chaucheyras et al., 1995), and improved animal performance (Sniffen et al., 2004). However, results in the literature are variable and frequently conflicting. Variable results are most likely due to large differences that exist between different strains of *S. cerevisiae*.

The ability of ADY to control lactic acid concentrations in the rumen has been reported in rumen cannulated dairy cows (Williams et al, 1991) and in incubations of mixed ruminal microorganisms *in vitro* (Lila et al., 2004; Lynch and Martin, 2002). This ability to control ruminal lactic acid concentrations could be attributed to the fact that one strain of *S. cerevisiae* has been shown to exceed *S. bovis* in utilization of sugars, consequently limiting the amount of lactic acid produced (Chaucheyras et al., 1996). Similar to observations made with *Enterococcus* strains and *A. oryzae*, some species of yeast have been found to stimulate the growth of specific lactic acid-utilizing bacteria in the rumen such as *Selenomonas ruminantium* (Nisbet and Martin, 1991; Rossi et al., 2004), by supplying different growth factors such as amino acids, peptides, vitamins and organic acids.

Under most practical conditions lactate accumulates in the rumen only at low levels (Goad et al., 1998), and the fermentation pattern and decrease in rumen pH is driven by high total VFA concentrations in the rumen (Brossard et al., 2004). In a recent experiment at the University Of Minnesota, Thrune et al. () detected a trend ($P < 0.10$) towards decreasing total ruminal VFA (m*M*) in lactating dairy cows (Table 1) when supplementing active dry yeast (*S. cerevisae)*. The only observed difference ($P < 0.05$) in VFA between treatments in this study was in ruminal butyrate concentration, which was greater with active dry yeast supplementation. Krause and Oetzel (2005) attempted to induce rumen acidosis in dairy cows by feeding high amounts of wheat grain after one day of restricted feeding. This feeding regimen resulted in lactate concentrations greater than 40 m*M* in less than 30% of the cases, with the majority of the animals having lactate concentrations around 10 m*M*. The impact of ADY under this situation of SARA has not been well studied, but it is likely that the ability of live yeast to utilize starch and soluble sugars plays an important role in lowering the rate of acid production in the rumen.

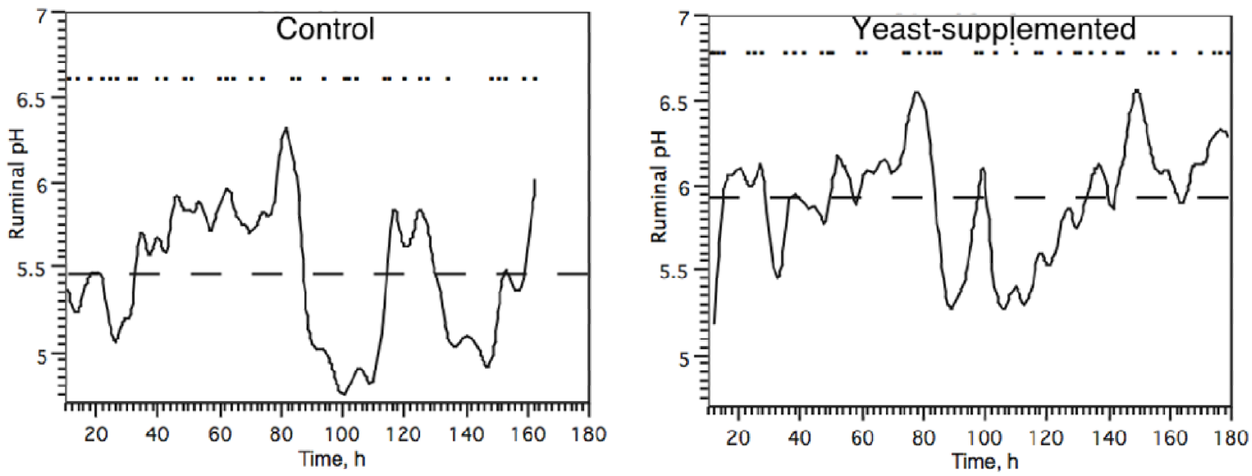
Table 1. Effects of active dry yeast (*S. cerevisae)* supplementation on VFA concentrations in the rumen of dairy cows[1]

VFA	Control	Yeast-supplemented	SE	*P*-value
Total (m*M*)	122.4	107.3	6.4	0.10
Individual (mol/100mol)				
Acetate	67.5	67.0	0.8	0.53
Propionate	18.2	18.1	0.4	0.70
Butyrate	9.7	10.4	0.4	0.02
BCVFA (mol/100mol)	4.8	4.7	0.6	0.85
A:P ratio	3.7	3.8	0.1	0.90

[1] Thrune et al. (unpublished data).

Brossard et al. (2006) reported that *S. cerevisiae* was able to stabilize ruminal pH by stimulating ciliated Entodiniomorphid protozoa, which are known to rapidly engulf starch granules (Abbou-Akkada and Howard, 1964) and thus compete effectively with amylolytic bacteria for their substrate. In addition, starch is fermented at a slower rate by ciliated protozoa than by amylolytic bacteria. Entodiniomorphs are also able to take up some lactate and possibly prevent lactate accumulation (Newbold et al., 1987).

Figure 5. Rumen pH and eating bouts (represented as dots) of loose-housed dairy cows as affected by active dry yeast (*S. cerevisae)* supplementation. Dashed line depicts average rumen pH. Adapted from Bach et al., 2006.



One of the most consistent results observed with live yeast supplementation in ruminants is an increase in the number of rumen bacterial cells. Wallace and Newbold (1993) and Newbold et al. (1996) reported significant increases in viable bacteria that could be recovered from the rumen of animals fed *S. cerevisiae*. As the bacterial population increases, requirements for rumen available N also increases. Assuming that sufficient available N is present in the medium, it is logical to expect an increase in the proportion of available carbon skeletons being diverted towards microbial protein synthesis rather than being fermented to VFA as end-products. Therefore, an increase in viable microbial cell numbers in the rumen promoted by live yeast supplementation, can actually minimize the increase in ruminal concentration of VFA, negating a decrease in ruminal pH.

In a recent study by Bach et al. (2006), supplementation of active dry yeast (*S. cerevisiae*) increased average ruminal pH and average maximum pH by 0.5 units, and average minimum pH by 0.3 units of loose-housed lactating cows (Figure 5). Furthermore, the authors described a significant change in the eating behavior of the animals. Cows supplemented with active dry yeast had a shorter inter-meal interval (3.32 h) than non-supplemented cows (4.32 h). It has been suggested that this change in feeding behavior could also be responsible for changes in rumen pH. In a similar study, Thrune et al. (unpublished data) reported an increase in average rumen pH of 0.2 units when comparing active dry yeast (*S. cerevisae)* supplemented cows with non-supplemented cows kept in tie-stalls (Table 2).

Table 2. Rumen pH of dairy cows kept in tie-stalls as affected by active dry yeast (*S. cerevisae)* supplementation[1]

Item	Control	Yeast-supplemented	SE	*P*-value
Average pH	6.32	6.53	0.07	< 0.05
Average minimum pH	5.69	5.97	0.09	< 0.05
Average maximum pH	6.80	7.01	0.09	< 0.05

[1] Thrune et al. (unpublished data).

Conclusions

Low ruminal pH can impair fiber digestion, reduce feed intake, cause health disorders to dairy cattle and decrease animal performance. Improving the balance between fiber and nonfiber carbohydrates in the ration and avoiding selection against long particles in high-concentrate rations are pivotal in avoiding subacute rumen acidosis. The use of probiotics, especially some lactic acid-producing bacteria, and live yeast such as *Saccharomyces cerevisiae* may help in stabilizing rumen pH. Supplementation of lactic acid-producing bacteria will foster their establishment in the rumen and maintain a mass of lactic acid-utilizing bacteria that may prevent rises in rumen pH. In contrast, yeast may modulate rumen pH by promoting the use of lactic acid, fostering microbial growth, and competing for rapidly fermentable carbohydrates with bacteria in the rumen.

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