

Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences

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Abstract

During subacute ruminal acidosis (SARA) rumen pH is depressed for several hours per day due to accumulation of volatile fatty acids and insufficient rumen buffering. Surveys suggested an incidence of SARA of between 19% and 26% in early and mid-lactation dairy cows. Causes of SARA include feeding excessive amounts of non-structural carbohydrates and highly fermentable forages, and insufficient dietary coarse fiber. Consequences of SARA include feed intake depression, reduced fiber digestion, milk fat depression, diarrhea, laminitis, liver abscesses, increased production of bacterial endotoxin and inflammation characterized by increases in acute phase proteins. The increase in endotoxin is similar among methods for SARA induction, but depends on the diet fed before induction. Increases in acute phase proteins vary among methods of SARA induction, even when the methods result in similar rumen pH depressions. This suggests that the inflammatory response might not be solely due to bacterial endotoxin in the rumen.

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Introduction

Milk yields of dairy cows in North America and Western Europe have increased substantially during recent years (Agriculture and Agri-Food Canada, 2005). As a result, the nutrient density of the diets fed to these cows has had to be increased. This increase has been primarily achieved by feeding more concentrates and less forages.

Feeding nutrient dense diets can result in a build up of organic acids in the rumen and reduced rumen buffering (Kleen et al., 2003; Stone, 2004; Rustomo et al., 2006a,b,c). The combination of these changes can lead to a depression of the rumen pH. When rumen pH is depressed for prolonged periods each day, e.g. <5.6 for >3 h/day, subacute ruminal acidosis (SARA) occurs (Kleen et al., 2003; Stone, 2004; Gozho et al., 2005). This disease affects feed

intake, milk production, rumen microflora, rumen digestion, and can cause diarrhea, rumen mucosal damage, laminitis, inflammation, and liver abscesses in dairy cows (Nocek, 1997; Kleen et al., 2003; Stone, 2004; Alzahal et al., 2007). Several excellent reviews on SARA have been written in recent years (Kleen et al., 2003; Oetzel, 2003; Stone, 2004). This review will describe common causes and the incidence of SARA. Emphasis will be given on the effects of SARA on rumen microorganisms, bacterial immunogens and inflammation.

Definition of SARA

Current definitions of SARA are based on the pH of rumen fluid (Kleen et al., 2003; Oetzel, 2003; Stone, 2004; Duffield et al., 2004). This pH can be measured after the collection of rumen fluid, either with a stomach tube or by rumenocentesis, or by placing in-dwelling pH probes in the rumen of rumen-fistulated cows (Duffield et al.,

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2004). Continuous monitoring of rumen pH is advantageous due to its high diurnal variation (Keunen et al., 2002; Duffield et al., 2004). The technique used to measure rumen pH affects the pH values.

There is disagreement as to a precise definition of SARA and there is also no agreement on which rumen pH depressions are detrimental to the health and production of dairy cows. Duffield et al. (2004) observed that the pH of rumen fluid samples using a stomach tube (oro-ruminal probe) and collected from the ventral sac of the rumen through a cannula were on average 0.35 and 0.33 pH units higher than the pH of rumen fluid samples collected by rumenocentesis. Those authors therefore, proposed that thresholds for abnormal pH indicating SARA should be 5.5, 5.8 and 5.9 when rumen fluid samples are collected by rumenocentesis, through a rumen cannula from the ventral sac, and using an oral probe, respectively. Garrett et al. (1999) used a threshold pH of 5.5 when rumen fluid samples were collected by rumenocentesis. Plaizier (2004) used a SARA threshold pH of 6.0 when rumen fluid samples were collected with a stomach tube at approximately 4 h after feeding, as a rumen pH below 6.0 reduced the growth of fibrolytic bacteria (Shi and Weimer, 2002). As rumen pH varies considerably throughout the day (Keunen et al., 2002), the timing of rumen fluid sampling also affects its pH. Hence, diagnosis of SARA requires standardization of the timing of rumen fluid collection and the threshold for SARA needs to reflect the sampling time.

Gozho et al. (2005) used a threshold of a rumen pH depression between pH 5.2 and 5.6 for at least 3 h/day, and feed intake was only reduced and inflammation only occurred at equal or greater rumen pH depressions. Cooper et al. (1999) also used a threshold of rumen pH between pH 5.2 and 5.6, but did not suggest a specific duration of this pH depression. Beauchemin et al. (2003) used rumen pH depression <5.8 as a threshold for SARA. The various definitions used for SARA combined with variability in

diagnostic techniques used for the diagnosis of this disease, have contributed to different interpretations of this disease.

Causes of rumen pH depression

Rumen pH will fall when organic acids, such as volatile fatty acids (VFA) and lactic acid, accumulate in the rumen, and if rumen buffering cannot keep pace with the accumulation of these acids. Feeding more grains and less forages will increase the production of VFA in the rumen, as grains are generally more rumen digestible than forages (National Research Council, 2001). A recent survey in Ireland (O'Grady et al., 2006) suggested that SARA can also occur in pasture fed dairy cows and might be caused by the high rumen digestibility of these pastures. Feeding more grains and less fiber, as well as reducing forage particle size, also reduces the amount of time spent chewing (Mertens, 1997; Yang et al., 2001; Maekawa et al., 2002; Fairfield et al., 2007; Beauchemin et al., 2003; Yang and Beauchemin, 2006). It has been assumed that the increased time spent chewing, i.e. eating and ruminating, enhances saliva production (Church, 1988). Saliva contains inorganic buffers, such as sodium bicarbonate, that contribute to the neutralization of the organic acids produced during fermentation in the rumen (Church, 1988).

There are differences between sources of fiber including differences between sources of forage fiber in their capacity to stimulate chewing, and this capacity is affected by various physical and chemical characteristics of the feed (Mertens, 1997). To overcome this problem, the concept of physically effective fiber (peNDF) has been developed. This measure reflects the ability of a feed to stimulate chewing and saliva buffering in the rumen (Mertens, 1997). Many studies have been conducted on the effect of forage particle size and dietary peNDF on rumen pH. Various measures of peNDF content of feeds and diets have been used. Yang

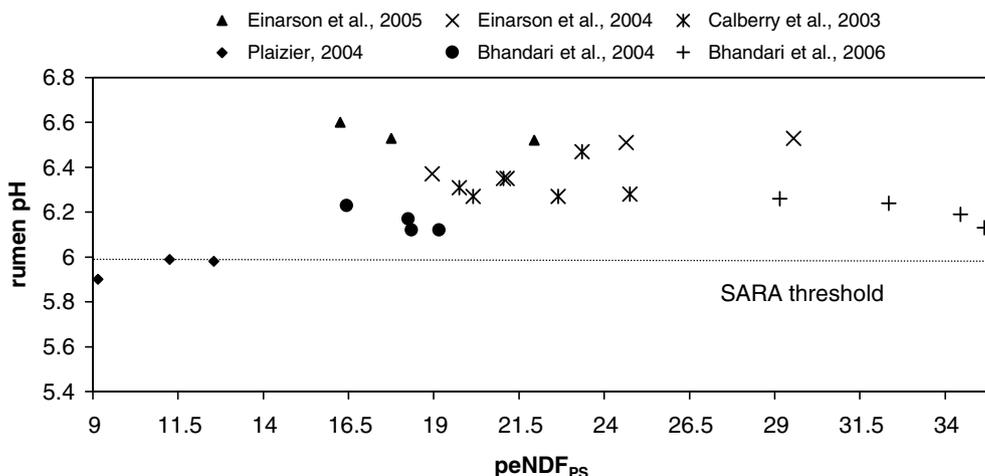


Fig. 1. Results of several studies on the effects of physical effective fiber as determined by the dietary NDF content multiplied by the proportion of the diet retained by 8 and 19 mm screens (peNDF_{ps}) and the pH of rumen fluid collected by stomach tube at 4 h after feeding.

et al. (2001) determined peNDF_{PS} as the proportion of DM retained by the 8 and 19 mm screens of the Penn Sate Particle Separator, multiplied by total dietary (neutral detergent fiber) NDF content. The results of several studies on the effect of peNDF_{PS} on the pH of rumen fluid collected by stomach tube at 4 h after feeding are summarized in Fig. 1.

The studies by Bhandari et al. (2004, 2006) and Rustomo et al. (2006c) varied peNDF_{PS} by varying forage chop length. In the work reported by Einarson et al. (2004), Plaizier (2004) and Einarson et al. (2005), peNDF_{PS} was varied by replacing coarse alfalfa silage by chopped hay, whereas in the study of Calberry et al. (2003) peNDF_{PS} was varied by replacing silage with chopped hay and by changing the concentrate inclusion in the diet. All of these studies used rolled barley as their grain source, but used a variety of forages. Fig. 1 demonstrates the absence of an effect of variation in peNDF_{PS} on rumen pH when this measure is >16.5% DM. However, feeding diets with a peNDF_{PS} of 12.5% DM or lower resulted in rumen pH indicative of SARA (Plaizier, 2004). These findings agree with Mertens (1997) who observed a curvilinear relationship between rumen pH and peNDF and the lack of an effect of dietary peNDF content on rumen pH in coarser diets.

The limited relationship between peNDF_{PS} and rumen pH is also due to the multitude of animal and dietary factors other than peNDF_{PS} that affect rumen pH. Prominent among these factors are forage source, concentrate source, acidogenic value of the feed, feeding frequency, and the inclusion of inorganic buffers (Mertens, 1997; Oetzel, 2003; Kleen et al., 2003; Stone, 2004; Rustomo et al., 2006c). Due to saliva contamination and diurnal variation in rumen pH, the monitoring of rumen pH by spot sampling is less accurate than continuous rumen pH measurement (Duffield et al., 2004; Alzahal et al., 2007). However, studies using continuous measurement of rumen pH have also not demonstrated significant effects of various measures of peNDF and forage particle size on rumen pH, despite increased chewing when peNDF was increased (Yang et al., 2001; Beauchemin et al., 2003; Bhandari et al., 2006; Rustomo et al., 2006; Yang and Beauchemin, 2006). Hence, peNDF might be a better indicator of chewing activity than of rumen pH (Yang and Beauchemin, 2006) and of acidogenic value of the feed (Rustomo et al., 2006a,b,c). This might be explained by the observation from Maekawa et al. (2002) that increased chewing did not increase daily saliva production, as increased chewing during eating and ruminating subsequently reduced saliva production during time periods when chewing activity was absent.

Even when diets have been formulated to contain sufficient fiber and physically effective fiber, the feed eaten by the cows might not result in sufficient rumen buffering capacity due to sorting against long particles in favor of shorter particles (Calberry et al., 2003; Leonardi and Armentano, 2003). Furthermore, errors in mixing the diet, differences between the assumed and the real dry matter

contents of the forages and excess mixing of the diet resulting in chopping up the coarse feed particles can result in diets with a lower than expected peNDF contents (Kleen et al., 2003; Oetzel, 2003; Stone, 2004).

The clearance of acids from the rumen is affected by the size and density of the rumen papillae, as these determine how fast these acids can be absorbed (Van Soest, 1994). A reduction in absorption, e.g. by inflammation or parakeratosis of the rumen wall due to low rumen pH, puts cows at increased risk of SARA. Freshly calved cows are also at a higher risk of SARA compared to cows in mid and late lactation, as the ruminal absorption capacity for acids due to a reduction in the length and density of rumen papillae can decrease by 50% during the dry period (Dirksen et al., 1985). It will take several weeks for this capacity to be restored after high concentrate diets are reintroduced, possibly due to the increased absorption of VFA, especially butyrate (Dirksen et al., 1985). Reynolds et al. (2004) also found that the rumen papillae mass was greater at 10 and 22 days after calving compared to 7 and 21 days before calving, but observed that this mass did not differ between 10 and 22 days after calving. This increase in rumen papillae mass might explain why rumen pH can increase during the first 3 weeks after calving despite increased feed intake (Fairfield et al., 2007).

Prevalence and consequences of SARA

Due to the difficulties in diagnosing SARA, only limited information on the prevalence of this disease is currently available. A survey of 15 dairy farms in Wisconsin revealed a prevalence of SARA in 19% of early lactation cows and 26% of mid-lactation cows (Garrett et al., 1997). Another survey on 14 dairy farms in Wisconsin detected SARA in 20.1% of early and peak lactation cows (Oetzel et al., 1999). The impact of SARA was demonstrated by a field study on a large dairy farm in New York State that found that SARA reduced milk yield by 2.7 kg/day, milk fat production by 0.3% points and milk protein production by 0.12% points (Stone, 1999). The percentage reduction of milk fat and milk protein applied to an entire lactation using these reductions can amount to a financial loss of as much as \$400¹ per cow per lactation. These costs exclude costs due to increased culling and veterinary treatments.

Dairy cows require sufficient coarse fiber in their diet to provide adequate rumination, saliva production and rumen buffering (Mertens, 1997). It has, therefore, been recommended that at least 40% of feed particles of dietary ingredients in total mixed rations (TMR) for dairy cows be longer than 8 mm (Heinrichs and Kononoff, 2002). A survey on Manitoba dairy farms revealed that total mixed rations on at least 25% of farms were finer than this recom-

¹ US \$1 = approx. £0.50, €0.67 as at February 2008.

mendation, and had a $\text{peNDF}_{\text{PS}} < 16.5\%$ DM, which would put the cows on these farms at risk of SARA (Heinrichs and Kononoff, 2002; Plaizier, 2004; Plaizier et al., 2004).

Feed intake depression

Decreased dry matter intake (DMI) has been used as a clinical sign to diagnose SARA (Kleen et al., 2003; Oetzel, 2003). Grain-induced SARA reduced DMI in steers and lactating dairy cows (Gozho et al., 2005, 2006; Fairfield et al., 2007). However, a similar rumen pH depression obtained by replacing alfalfa hay with alfalfa pellets did not reduce feed intake (Khafipoor et al., 2007). Reasons for the feed intake depression can include reduced fiber digestibility and increases in volatile fatty acids, especially propionate, and in the osmolarity in the rumen (Allen, 2000). Grain induced SARA reduced *in situ* fiber digestibility in the rumen due to increased rumen acidity (Plaizier et al., 2001; Krajcarski-Hunt et al., 2002). Also, grain induced SARA increased VFA and osmolarity in the rumen of dairy cows (Gozho et al., 2006; Khafipoor et al., 2006, 2007). However, SARA induced by feeding alfalfa pellets resulted in similar rumen pH, rumen VFA and osmolarity as grain induced SARA, without reducing DMI. The disparity between the effects of grain induced SARA and SARA induced using alfalfa pellets on DMI suggests that factors other than fiber digestion, rumen VFA and osmolarity must play a role in the feed intake depression.

Several studies have shown that grain-induced SARA causes an increase in acute phase proteins in blood, which is an indicator of inflammation (Gozho et al., 2005, 2006, 2007). It has been shown that inflammation of various organs of the cow reduces feed intake (Weingarten, 1996; Andersen et al., 2000). Hence, the inflammation resulting from grain induced SARA could contribute to the feed intake depression. This assumption is strengthened by the observation that induction of SARA by replacing alfalfa hay with alfalfa pellets did not result in inflammation, and also did not result in feed intake depression (Khafipoor et al., 2007).

Reduced fiber digestion

Subacute ruminal acidosis induced by adding grain pellets to the total mixed ration (TMR) of lactating dairy cows reduced the 24 and 48 h *in situ* NDF degradability of forages by an average of 20.5% and 24.8%, respectively (Krajcarski-Hunt et al., 2002). Plaizier et al. (2001) also found that grain-induced SARA reduced the 24 and 48 h *in situ* NDF degradability of mixed hay by 19.6% and 21.8%, respectively. The reduction in fiber digestion that occurs during SARA is most likely the result of the acid sensitivity of the fibrolytic rumen bacteria. These bacteria generally cannot tolerate a rumen pH below 6.0, which will reduce their numbers in the rumen and, subsequently reduce fiber digestion (Shi and Weimer, 2002). The reduced fiber digest-

ibility caused by SARA reduces the net energy content of the diet and might impact feed intake (Allen, 2000).

Altered milk composition

It has long been assumed that SARA causes milk fat depression (Nocek, 1997; Kleen et al., 2003; Oetzel, 2003; Stone, 2004). A field study on a large dairy farm in New York State found that SARA reduced milk yield, milk fat production and milk protein production by 2.7 kg/day, 0.3% points and 0.12% points, respectively (Stone, 1999). Experimentally induced SARA, either by adding grain pellets to the diet or by replacing alfalfa hay with alfalfa pellets, reduced milk fat percentage but increased milk protein percentage (Fairfield et al., 2007; Khafipoor et al., 2007). However, Keunen et al. (2002) and Gozho et al. (2007) did not observe changes in milk fat content due to grain-induced SARA. It has also been suggested that the inconsistent response in milk fat in experimentally-induced SARA may be related to the duration of the bout(s) of SARA with short bouts unlikely to have an effect on milk fat content (Krause and Oetzel, 2005). Furthermore, the milk fat content of cows prior to the induction of SARA could affect the severity of depression observed (Gozho et al., 2007).

Milk fat depression has been associated with a reduction in the acetate to propionate ratio and increased insulin (Byers and Schelling, 1988; Bauman and Griinari, 2003), and the production of *trans*-octadecenoic acids in the rumen (Griinari et al., 1998; Bauman and Griinari, 2003). Induction of SARA by adding grain pellets or by adding alfalfa pellets to the diet reduced both milk fat and the acetate to propionate ratio in the rumen fluid, but the decrease in this ration was due to an increase in propionate and not due to a decrease in acetate (Gozho et al., 2006; Fairfield et al., 2007; Khafipoor et al., 2007). Griinari et al. (1997) found that a hyperinsulinemic–euglycemic clamp did not depress milk fat. An increase in insulin decreases lipolysis (Bauman and Griinari, 2003). This might explain why stage of lactation and energy balance could affect the milk fat depression, as the contribution that body fat makes to milk fat is much greater in cows in a negative energy balance compared to cows in a positive energy balance. The results obtained by Griinari et al. (1998) support the theory that a low rumen pH caused by feeding a low fiber diet results in incomplete biohydrogenation of fatty acids and increases in *trans*-octadecenoic acids, and especially the *trans*-10 isomer of *trans*-octadecenoic acid, that cause milk fat depression. It is not yet understood why experimentally induced SARA increases milk protein, but an increase in rumen digestible organic matter, which increases microbial protein synthesis in the rumen (National Research Council, 2001) might play a role.

Diarrhea

Diarrhea has been associated with SARA in dairy herds (Nocek, 1997; Kleen et al., 2003; Oetzel, 2003). Ireland-

Perry and Stallings (1993) concluded that cows consuming low fiber diets that were expected to cause a low rumen pH had feces that appeared to be more liquid, but actually had greater DM content, than cows on high forage diet. Feces from cows with SARA may appear brighter and more yellowish than the feces of cows without SARA (Kleen et al., 2003). Foamy feces and diarrhea suggest extensive hindgut fermentation which has also been associated with SARA (Nordlund et al., 2004). Fermentation in the hindgut produces VFA and gases such as carbon dioxide. Whereas the VFA can be absorbed, microbial protein is excreted in feces and the gas produced appears as bubbles in feces giving them the 'foamy' appearance.

Hindgut fermentation also results in increased acidity of hindgut contents and feces. The increased acidity in the hindgut that results from SARA leads to sloughing of the epithelial cells in the large intestine (Hall, 2002). The animal protects itself from further damage by secreting mucous or fibrin to protect the injured tissue (Argenzio et al., 1988). Mucin or fibrin casts found in the feces often have the tubular form of the gut which is evident that intestinal damage has occurred (Hall, 2002). Extensive hindgut fermentation may contribute to diarrhea. This is because fecal consistency is determined by movement of water into the digestive tract when digesta are hypertonic compared to blood plasma as a result of SARA (Huber, 1976).

Laminitis and lameness

Laminitis (pododermatitis aseptica diffusa) is defined as the inflammation of the dermal layers inside the foot (Nocek, 1997). This disorder is prevalent in dairy cows during early lactation and in beef cattle in feedlots (Underwood, 1992). This observation has led to the assumption that feeding high concentrate diets or rapidly increasing dietary concentrate are associated with laminitis (Greenough et al., 1990; Nocek, 1997). The true mechanistic causes of laminitis in dairy cows are poorly understood, and are assumed to be multi-factorial (Nocek, 1997; Ruegg, 2000). Nocek (1997) proposed that rumen pH depression triggers the release of vasoactive substances, such as histamine and lipopolysaccharide endotoxin (LPS) of bacterial origin that damage the capillaries of the lamellae in the foot and causing hemorrhage, inflammation and lameness (Nocek, 1997). However, Gozho et al. (2007) demonstrated that grain-induced SARA increased free LPS in the rumen, but not in peripheral blood, which disagrees with the hypothesis of Nocek (1997) that LPS damages the capillaries of the hoof. Laminitis in horses can be caused by metalloproteinase enzymes that destroy the lamellar detachment (Kyaw-Tanner and Pollitt, 2004). However, it is unclear if metalloproteinases also cause bovine laminitis and what effects nutrition might have on metalloproteinase activity in cows. Feet and leg problems resulting from lameness are a major cause of economic loss to the dairy industry, as feet and leg problems have been reported as the fourth largest reason for disposal of dairy

cows (Canwest, 2004). The causes for lameness are also multi-factorial, but laminitis is one of the major causes of this disorder (Frankena et al., 1992).

Liver abscesses

SARA is associated with liver abscesses (Dirksen et al., 1985; Nocek, 1997; Kleen et al., 2003; Oetzel, 2003). These liver abscesses are caused by translocation of rumen bacteria such as *Fusobacterium necrophorum* and *Arcanobacterium pyogenes* to the portal blood as a result of decreased barrier function of the rumen mucosa (Dirksen et al., 1985; Nocek, 1997; Kleen et al., 2003). These bacteria can spread from the liver to other organs, such as the heart, lungs, and kidneys (Nordlund et al., 1995; Nocek, 1997; Kleen et al., 2003; Oetzel, 2003). The reduced barrier function of the rumen mucosa is associated with rumenitis and lesions in the rumen mucosa due to the low rumen pH that occurs during SARA (Nocek, 1997; Andersen et al., 2000; Kleen et al., 2003).

Inflammation

Animals respond to trauma, tissue injury, or infection by activating the acute phase response (Baumann and Gauldie, 1994). This response includes the production of acute phase proteins, such as serum amyloid A (SAA) and haptoglobin (Hp) in the liver (Baumann and Gauldie, 1994). The function of the acute phase response is to prevent further injury, to isolate and destroy the infective organism, to remove harmful molecules and debris, and to activate the repair processes necessary to return the organism to normal function (Baumann and Gauldie, 1994). As SAA and Hp are among the most reactive acute phase proteins in cattle, their concentrations in blood serum are used as inflammatory markers in cattle (Alsemgeest et al., 1994). Their concentrations in blood serum have been shown to increase as a result of mastitis (Grönlund et al., 2005) and metritis (Hirvonen et al., 1999).

Grain-induced SARA increased the concentrations of both SAA and Hp in peripheral blood of steers, albeit that the increase in Hp was greater in steers that had been adapted to a 60% concentrate diet than in steers that received an all forage diets before the SARA induction (Table 1, Gozho et al., 2005, 2006). Studies by Gozho et al. (2007) and Khafipoor et al. (2006) showed that grain-induced SARA also increased SAA in lactating dairy cows (Table 1). However, the concentrations of SAA and Hp in the control treatment of the study by Gozho et al. (2007) were higher than the SAA and Hp concentrations after SARA induction in the study by Khafipoor et al. (2006), suggested that the cows in the control treatment of Gozho et al. (2007) experienced a mild form of SARA. Furthermore, grain-induced SARA only increased Hp in the study of Khafipoor et al. (2006). The discrepancies between these studies might be explained by the low rumen pH of the control treatment of the former study. The dura-

Table 1
Effects of experimental induction of subacute ruminal acidosis (SARA) on rumen pH, concentrations of free lipopolysaccharide (LPS) in rumen fluid, and concentration of serum amyloid A (SAA) and haptoglobin (Hp) in peripheral blood of cattle

Experiment	Time below pH 5.6 (min/day)		LPS (EU/mL)		SAA (μ g/mL)		Hp (mg/mL)	
	Control	SARA	Control	SARA	Control	SARA	Control	SARA
Steers, abrupt ^c	5 ^b	187 ^a	3714 ^b	12,589 ^a	33.6 ^b	170.7 ^a	0.43 ^a	0.79 ^b
Steers, gradual ^d	0 ^b	219 ^a	6542 ^b	32,275 ^a	36.5 ^b	131.3 ^a	0.45 ^a	2.39 ^b
Cows, grain ^e	187 ^b	309 ^a	21,373 ^b	145,383 ^a	343.5 ^b	498.8 ^a	0.24	0.26
Cows, grain ^f	118 ^b	278 ^a	28,400 ^b	107,000 ^a	77.6 ^b	218.6 ^a	0.0 ^b	0.47 ^a
Cows, pellets ^g	112 ^b	510 ^a	37,153 ^b	162,181 ^a	23.1	6.8	0.06	0.01

^{a,b} Means within the same row not followed by the same letter differ ($P < 0.05$).

^c Steers, abrupt = abrupt induction with grain, control 100% forage diet (Gozho et al., 2005).

^d Steers, gradual = gradual induction with grain, control 100% forage diet (Gozho et al., 2006).

^e Cows, grain = control 60% concentrate diet, induction with grain (Gozho et al., 2007).

^f Cows, grain = control 50% concentrate diet, induction with grain (Khafipoor et al., 2006).

^g Cows, pellets = control 60% concentrate diet, induction with alfalfa pellets (Khafipoor et al., 2007).

tion of the rumen pH < 5.6 of this control treatment was higher than 3 h/day, which has been used and an indicator of SARA (Gozho et al., 2005). As a result, it is probable that the morphology of the rumen mucosa and the populations of rumen microbes prior to the induction of SARA differed between these two studies. Hence, the severity of the inflammatory response caused by SARA most likely depends on conditions in the rumen, such as pH, composition of microbial populations, and existing challenges to the barrier function and inflammation of the rumen mucosa.

The lack of an increase in Hp, despite increased SAA, might be explained by differences in the cytokines involved in initiating the synthesis of these acute phase proteins (Jacobsen et al., 2004). Either interleukin-6 or tumor necrosis factor- α is required for the synthesis of SAA, but both of these cytokines are required for Hp synthesis (Alsemgeest et al., 1996). The cows used by Gozho et al. (2007) had received the control diet for at least 17 weeks prior to the study. As this diet induced a mild form of SARA, these cows might have suffered from chronic SARA, whereas acute SARA was induced in the study from Khafipoor et al. (2006). The acute phase response varies between acute and chronic inflammations (Alsemgeest et al., 1994; Horadagoda et al., 1999), which could explain why the acute phase response different between the study from Gozho et al. (2007) and the study from Khafipoor et al. (2006).

Intravenous administration of LPS causes immune activation, including the production of cytokines and increase in acute phase proteins in the peripheral blood (Werling et al., 1996; Waldron et al., 2003). Hence, the inflammation that accompanies grain-induced SARA could be the result of LPS translocation from the rumen to the liver. However, as SARA can also cause rumenitis (Nocek, 1997; Kleen et al., 2003; Oetzel, 2003), the inflammation could also occur within the rumen. Also, it is unclear if LPS is responsible for rumenitis, as low rumen pH can also lead to rumenitis (Nocek, 1997; Kleen et al., 2003; Oetzel, 2003). Therefore, the inflammation that accompanies grain induced SARA might not be the result of high rumen LPS.

The translocation of LPS from the rumen could be facilitated by increases in free rumen LPS and reduction of the

barrier function of the rumen mucosa due to SARA (Dirksen et al., 1985; Nocek, 1997; Kleen et al., 2003). Dougherty et al. (1975) reported that experimentally-induced ruminal acidosis increased LPS in peripheral blood. However, other studies did not show that experimentally-induced ruminal acidosis increased LPS in peripheral blood (Andersen et al., 1994; Gozho et al., 2007). This does not mean that free LPS does not cause inflammation, as this effect of free LPS might be confined to the liver. Also, as inflammation outside of the rumen gives rise to acute phase proteins, the inflammatory response that occurs during grain-induced SARA does not prove that the inflammation occurs in the rumen. This strengthens the conclusions of Hall (2002) that SARA can cause damage to and inflammation of the epithelium of other components of the gastrointestinal tract of cows, such as the large intestine.

Induction of SARA in lactating dairy cows by gradually replacing 10 kg/day of alfalfa hay with alfalfa pellets reduced rumen pH more than grain-induced SARA and increased free rumen LPS, but did not increase SAA and Hp (Table 1; Gozho et al., 2007; Khafipoor et al., 2006, 2007). This suggests that factors other than low rumen pH and high free rumen LPS might be responsible for the inflammation during grain-induced SARA.

Rumen microorganisms and bacterial immunogens

The rumen pH is a major determinant of the type of digestion that occurs in the rumen, and it itself is significantly influenced by rumen digestion. An increase in readily fermentable carbohydrates in the diet initially leads to increased growth rate of most rumen bacteria (Nocek, 1997). This is due to the increased substrate availability for oxidation by the various groups of bacteria and less competition among them. As fermentation proceeds and the concentration of products of fermentation such as acetate, propionate and butyrate increases, the absorptive capacity of the rumen papillae is reached and the concentration of VFA in the rumen increases leading to a decrease in rumen pH. Within the pH range between 6 and 5.6, the fermentative capacity and growth rates of major lactic

acid-producing bacteria such as *Streptococcus bovis* and lactic acid-utilizing bacteria such as *Megasphaera elsdenii* exist in equilibrium and therefore lactic acid produced is immediately utilized. Thus a decrease in rumen pH within this range is due to increases in total VFA rather lactic acid (Goad et al., 1998; Enemark et al., 2002).

In general, excessive rumen acidity results in a reduction in the number of cellulolytic bacteria and a shift in bacterial population so that Gram-positive cocci and rods predominate even though Gram-negative bacterial numbers also increase under these conditions (Nagaraja et al., 1978; Goad et al., 1998.). There is a paucity of data on changes in ruminal bacterial species that occur when rumen pH changes. One possible reason for this is that the factors influencing rumen bacterial growth in vivo, including nutrient and environmental requirements, such as pH, or the presence or absence of oxygen within the rumen are not well understood. Thus, the diversity in the requirements for various microbial groups does not allow enumeration of different species of bacteria on a single sample in the same medium. A few studies have used a common group of micro-organisms such as total coliform counts to determine changes in microbial populations under different ruminal pH conditions (Slyter and Rumsey, 1991). A microbial model that can be used to determine changes in rumen Gram-negative bacteria populations that encompasses all enteric Gram-negative bacteria is currently lacking.

Some protozoal species such as entodiniomorphid protozoa are important in maintaining rumen pH. This is because these protozoa engulf starch and sequester it from bacteria preventing its rapid fermentation by lactic acid producing bacteria (Bonhomme, 1990). Other species of protozoa are susceptible to rumen acidity. For example, a study by Goad et al. (1998) inducing SARA in two groups of steers that had been adapted to either a high grain or high forage diet, showed that protozoal numbers decreased more in the hay-adapted steers than in grain-adapted steers.

The LPS is a component of the cell wall of Gram-negative bacteria that is released into the rumen, i.e. becomes 'free', when these bacteria die (Andersen et al., 2000). However, rapid growth of these bacteria can also result in the shedding of LPS in the rumen (Wells and Russel, 1996). Nocek (1997) assumed that translocation of LPS into the portal blood stream, and possibly to the peripheral blood stream was one of the causes of laminitis. Grain-induced SARA, resulting in a rumen pH depression below 5.6 for more than 3 h/day, increased free LPS in rumen fluid of steers (Table 1, Gozho et al., 2005, 2006). The increase in free LPS was greater when steers had been adapted to a 60% concentrate diet before SARA induction than when SARA was induced in steers on a 100% forage diet. Grain-induced SARA in dairy cows on a 60% concentrate diet resulted in a rumen pH lower than 5.6 for 309 and 278 min/day and caused larger increases in free LPS, compared to the experiments with steers (Table 1; Gozho et al.,

2007; Khafipoor et al., 2006). The concentrations of free LPS in the rumen of the steers on 100% concentrate diets were also lower than these in cows on a 60% concentrate diet. Induction of SARA in dairy cows by replacing alfalfa hay with alfalfa pellets in a diet containing 60% concentrate, resulted in a larger rumen pH depressions and increases in free rumen LPS, compared to grain induced SARA (Table 1; Khafipoor et al., 2007). Despite a nearly 7-fold increase in free rumen LPS due to grain-induced SARA, no free LPS could be detected in peripheral blood (Gozho et al., 2007). The studies on the effects of SARA on free rumen LPS suggest that the depression in rumen pH increases lysis of Gram-negative bacteria, but that this increase depends on the diet before the induction of SARA, and, therefore, on rumen bacterial populations at the time of this induction.

Although our laboratory has focused on LPS it is not by any means clear that this is the primary cause of the inflammatory response in SARA. LPS is one immunogenic virulence factor in digestive tract inflammation but there are others. For example, *Escherichia coli* spp., as well as other members of the Enterobacteriaceae, produce a range of virulence factors that have the potential to cause inflammation (Hayward et al., 2006). These include, but are not restricted to, fimbrial adhesins, heat-stable and heat-labile toxins, and inflammatory peptides. The interested reader should refer to recent publications on *E. coli* virulence factors to obtain a better understanding of the wide range of genes, and gene products, produced by *E. coli* that potentially cause inflammation in dairy cattle (Gyles, 2007).

In light of the fact that *E. coli* spp. are a major reservoir of virulence factors, dietary factors that result in an increase in their numbers are worth considering. Clearly, high-corn diets, which are normally associated with SARA, result in much higher numbers of *E. coli* in the rumen (Diez-Gonzalez et al., 1998). Higher numbers of *E. coli* are not only a consequence of corn-based diets but seem to be a function of the amount of readily fermentable starch in the rumen (Gilbert et al., 2005; Krause et al., 2003). For *E. coli* or other bacteria to have an effect at the rumen epithelial level, it is essential that they gain access to the underlying epithelial capillaries and lymphatics so that immune functions can be stimulated. Thus, some sort of disruption to the barrier function is a prerequisite in the pathophysiology of rumen epithelial infection.

As the pH of the rumen declines with SARA there is an increase in osmotic pressure and the potential for damage to the rumen epithelium (Nocek, 1997). Osmotic pressure of the rumen is mainly determined by the concentrations of VFA (Bennink et al., 1978), which are produced as a result of fermentation of carbohydrates. The quantity of carbohydrate consumed and its fermentability correlate with increased ruminal osmotic pressure. Sodium is typically absorbed via a Na^+/H^+ exchange mechanism in the luminal membrane (Chien and Stevens, 1972; Martens et al., 1991; Sehested et al., 1996). Sodium transport is stimulated by the luminal presence of VFA (Gäbel et al., 1991;

Sehested et al., 1996), and ammonia is transported via a cation channel in the luminal membrane that in turn stimulates electroneutral Na transport via intracellular release of protons (Abdoun et al., 2003). These activities contribute to a declining barrier function (degradation of gap junctions and tight junctions) of the rumen epithelial tissue which predisposes the ruminal lining to transport of endotoxin and absorption of biogenic amines.

The production of inflammatory peptides, such as polyamines in the rumen has been linked to laminitis, and thus SARA (Nocek, 1997). Polyamines are organic compounds that have two or more primary amino groups and are typically transformed by a series of enzymatic activities to the active form (Liang et al., 2006). For instance, histamine is derived from a decarboxylation reaction of the amino carboxylic acid of histamine (Liang et al., 2006). The accumulation of histamine by anaerobic decarboxylation in the rumen, and its subsequent absorption across the rumen wall may contribute to SARA (Garner et al., 2002).

High levels of histamine are involved in the pathogenesis of bronchial constriction (Vignola et al., 1997), cardiovascular shock (Nakamura et al., 1997), and histamine is an important regulator of feed and water intake in cattle (Lecklin and Tuomisto, 1990; Rossi et al., 1998). One can therefore hypothesize that those conditions in the rumen that lead to increased histamine absorption, like barrier function defects, would lead to systemic effects on ruminant health. High histamine concentrations of certain ruminant feeds, like silage, are well tolerated by the animal (Os et al., 1997). However, these diets did not necessarily result in low levels of ruminal pH, such that osmolarity and the barrier function of the rumen epithelium would be affected. High rumen histamine levels in acidotic animals are associated with increases in blood plasma histamine levels and signs of systemic histaminosis such as laminitis and cardiovascular disturbances (Underwood, 1992). Recent experiments have demonstrated that the permeability of rumen epithelia to histamine is low when ruminal pH is normal, but absorption increases significantly when pH declines (Aschenbach et al., 2000). Thus, declining ruminal pH predisposes animals to increased absorption of histamine.

Garner et al. (2002) decimally diluted rumen fluid from dairy cows fed high-grain diets in medium containing histadine. They observed histamine in the higher dilutions and isolated an ovoid Gram-positive bacterium they classified as *Allisonella histaminiformans*. This organism could not be isolated from cattle on high-forage diets. Subsequently Garner et al. (2004) examined the nutritional requirements of *A. histaminiformans* and found that alfalfa and corn silage water extracts (mostly histadine) produced significant amounts of histamine from histadine but that extracts from non-ensiled alfalfa or corn did not. This was particularly apparent with non-ensiled alfalfa. In view of the fact that we did not observe the typical signs of SARA when alfalfa pellets were fed, determining the exact role ensiling plays remains a fascinating problem.

Conclusions

Subacute ruminal acidosis is a common disease in high yielding dairy cows that receive highly digestible diets, and has a high economic impact. Due to the limited information on the prevalence of this disease and the non-specific nature of many of its manifestations, the importance of SARA is not fully appreciated. This disease not only affects feed intake and milk production, but can also compromise cow health by causing diarrhea, laminitis, liver abscesses, production of bacterial immunogens, and inflammation. Many of the mechanisms by which depression of rumen pH compromises cow health are not well understood. Recent research has suggested that the production of immunogens in the rumen, such as LPS and histamine, reduction of the barrier function of the rumen, and translocation of immunogens from the rumen are part of these mechanisms. It has also been shown that cows with similar depressions in rumen pH differ in their response to the low rumen pH. This implies that cows may differ in their susceptibility to SARA and that assessment of rumen pH alone is not adequate to diagnose and characterize this disease.

Recommendations for the formulation of diets that prevent SARA are available, although more research is needed to optimize diets for physically effective fiber along with acidogenic value. However, even if these recommendations are followed, SARA can occur, as the diet ingested by the cow can differ from the diet that has been formulated.

Conflict of interest statement

None of the authors (J.C. Plaizier, D.O. Krause, G.N. Gozho and B.W. McBride) has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the paper entitled *Subacute ruminal acidosis in dairy cows, the physiological causes, incidence, and consequences*.

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