

Subacute Ruminal Acidosis (SARA) in dairy cattle: new developments in diagnostic aspects and feeding management

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1. Introduction

Over the last few decades, the productivity of dairy cows has increased greatly. As a result, high energy density diets, which are high in grain starch but low in forage, are often fed to the dairy cows in order to meet their nutritional requirements. The most nutritionally challenging time for dairy cows is the early lactation period, during which their feed intake still not fully developed and milk production increases quickly. Dairy farmers, then, tend to push the grain content in the diets to an even higher level in order to meet the dairy cow's productivity potential.

Ruminants are designed to eat fibrous grasses, plants, and shrubs, which are digested slowly in rumen. In contrast, grains are high in rapidly fermentable carbohydrates that are rapidly broken down by ruminal microorganisms, and lead to the accumulation of acids in the rumen and a lower rumen pH. When rumen pH drops too much, the growth of many ruminal bacteria is inhibited, and concentration of toxic compounds in rumen digesta increases. This can lead to impaired animal health, including decreased feed intake and milk fat production, lowered body condition, inflammation, liver abscesses, and laminitis related claw lesions. These signs indicate that dairy cows suffer from Subacute Ruminal Acidosis (SARA).

2. Definition of SARA

SARA is defined as periods of rumen pH depression below physiological range. Rumen pH fluctuates diurnally between nearly neutral before morning feeding and acidic after feeding. When cows are fed by high forage diets, rumen pH can be maintained between 6 and 7, which is considered to be the optimum for cellulolytic bacteria (Mould et al., 1983). Ruminal pH may decline periodically below 6 when dietary grain content increases. Generally, SARA occurs when ruminal pH stays in the range of 5.2 and 6 for a prolonged period.

It is challenging to set up a specific threshold of rumen pH for defining SARA, since rumen pH varies among different sites inside of the rumen. The use of different techniques to collect rumen fluid for pH determination introduces further variation. The highest rumen pH usually observed in the cranial dorsal sac, followed by the cranial ventral, caudal ventral, and the caudal dorsal sac. Rumen pH in the ventral sac and the center of rumen solid mat is the lowest (Duffield et al., 2004, Shen et al., 2012). When rumen fluid is collected using an oral-stomach tube, the specific collection site is unknown but the sample will often be collected from the cranial dorsal sac and it could be contaminated by saliva. In contrast, rumen fluid collected via rumenocentesis is from the ventral sac. Duffield et al. (2004) observed that the pH of rumen fluid samples collected by a stomach tube was on average 0.35 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. Gozho et al. (2006) proposed defining SARA as rumen pH between 5.2 and 5.6 for at least 3 h/day measured by an indwelling pH probe, as this leads to a reduction in feed intake and an inflammatory response of dairy cows during an experimentally grain induced SARA.

Rumen pH below 5.2 or less appears to restrict the growth of cellulolytic bacteria (Slyter et al., 1970). It is suggested that once rumen pH falls to below 5.2 the type of rumen fermentation gradually shifts to lactate acid fermentation (Enemark et al., 2004), leading to accumulation of lactate acid. This eventually leads to a fast drop in ruminal pH and results in acute ruminal acidosis (Nocek, 1997).

3. Prevalence

A few surveys have been conducted to determine the prevalence of SARA in dairy herds. 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). In Ireland, O'Grady et al. (2008) reported that 11% of cows were classified as affected with SARA (pH = < 5.5), 42% were marginal (pH 5.6–5.8). In Australia, about 10% of cows sampled were at a high risk of acidosis (Bramley et al., 2005). In Netherlands, Kleen et al. (2009) reported an overall prevalence of SARA was 13.8%, and a prevalence of SARA on individual farms ranging from 0% to 38%. In Italy, SARA was observed in more than a third of dairy cows in three out of ten farms (Morgante et al., 2007).

4. Which cows are at risk of SARA

4.1 Cows in the early lactation

During early lactation, especially the post partum transition period, dairy cows experience the most challenging time both nutritionally and physiologically. The diet of these cows is switched from a dry cow diet containing high forage, to a lactating diet rich in grains. The feed intake of dairy cows increases gradually but cannot keep up with the increase of nutrition requirements for milk production. Furthermore, the capacity of absorption of ruminal VFAs is limited as the rumen papilla takes time to develop fully. In practice, high energy density diets are formulated for early lactation cows in order achieve their milk production potential, which puts the cows at a great risk of SARA.

4.2 Primiparous cows

Primiparous cows are often believed to be a more healthy group in which there is less concern of metabolic diseases compared to older cows. This may not be true regarding SARA. Penner et al. (2007) reported that primiparous cows are particularly susceptible to developing acidosis after parturition. A survey conducted by Enemark (2004) indicated that primiparous cows were generally more prone to low ruminal pH , higher ruminal concentrations of volatile fatty acids and possibly to metabolic acidosis, than multiparous cows. A survey conducted by Krause and Oetzel (2006) also showed that higher prevalence of SARA in primiparous cows than in multiparous cows

4.3 Cows grazing or fed with rapidly fermentable low fiber grass

Although SARA is most likely caused by excessive grain feeding, it may still occur in grass-based systems. High occurrence of lameness of dairy cattle fed predominantly on pasture raised the concern of the occurrence of SARA, as the pasture was characterized by low levels of fiber and, especially, physically effective fiber, rapid rates of fiber degradation, high water content, and high concentrations of rumen degradable protein (Westwood et al., 2003). However, causes of lameness in pasture-based systems are complex and the causal relationship between low ruminal pH and clinical signs such as claw problems remains to be investigated. To address similar concern to Irish dairy cattle, O'Grady et al. (2008) conducted a survey and found that cows on grazing cows fed predominantly rye grass-based pasture were potentially at risk of developing SARA. The supplement with grains or other feeds containing significant amounts of starch to these cows may increase the risk of SARA.

5 Diagnosis

5.1 rumen pH determination

The diagnosis of SARA has previously been based solely on pH measurements of rumen content. Recent studies show that only some types of SARA resulted in general inflammation, despite similar levels of pH in the rumen content (Khafipour et al., 2009a; Khafipour et al., 2009b). This strongly indicates that the problems observed in SARA are not a result of low pH alone. However, so far the most used diagnostic tool for diagnosing SARA is still based on rumen pH determination. By far, four methods are available to measure rumen pH.

1) Rumenocentesis technique: Rumen fluid samples are collected from the caudal ventral part of rumen using this technique. The disadvantage associated with this method is that it is quite invasive, and can result in abscesses at the site of puncture (Hollberg, 1984; Aceto et al., 2000).

2) Oral - stomach tube technique: Collecting rumen fluid with a oral-stomach tube presents very low risk to the cow. When rumen fluid is collected using an oral-stomach tube, the collection site is unknown. Most likely, the sample may be collected from the cranial dorsal sac or reticulum and it could be contaminated by saliva. Therefore, the ruminal pH determined with oral-stomach tube technique is significantly higher than with rumenocentesis and indwelling pH data loggers (Nordlund and Garrett, 1994; Oetzel and Nordlund, 1998). This may be overcome by using a properly designed stomach tube that can reach further towards the center of rumen (for adult lactating Holstein cow, it is about two meters from the mouth to the rumen fluid sampling site inside of the rumen) (Shen et al., 2012). Although this method is less invasive than rumenocentesis, none of these methods can be recommended for repeated sampling.

3) Rumen cannula method: Most experimental SARA trials have been conducted using rumen fistulated cows. Collecting rumen fluid via a rumen cannula is easy and the sample site inside of the rumen can be precisely defined. However, the disadvantage associated with this method is that it is impractical to cannulate a subgroup of cows in a dairy herd for monitoring rumen fermentation.

4) Indwelling pH data logger method: The best way to evaluate rumen pH fluctuation is to insert a pH probe directly into rumen digesta and record its pH at real time (Dado and Allen, 1993). Indwelling rumen pH device are commercially available and comes with a built-in data logger as well as the wireless communication technology (Penner et al., 2006). However, in field trials on non-rumen fistulated animals the rumen pH data logger is not retrievable, the expected lifespan of the pH sensors is relatively short. Therefore, the cost and benefit analysis is still not encouraging for using indwelling pH data loggers in field. Another point worth mentioning is the location where an indwelling pH data logger sits in the rumen is critical. When a data logger is delivered via the mouth of the cow and does not have a mechanism to retain it in the ventral sac of the rumen, it most likely end up into the reticulum due to the movement of rumen content driven by the contraction of the rumen. Compared to the rumen pH in the ventral sac, the rumen pH in reticulum is relative stable and high which may result from dilution due to salivation (Figure 1). Therefore, it is less sensitive for diagnosing SARA when an indwelling pH data logger sits in the reticulum than in the ventral sac of the rumen.

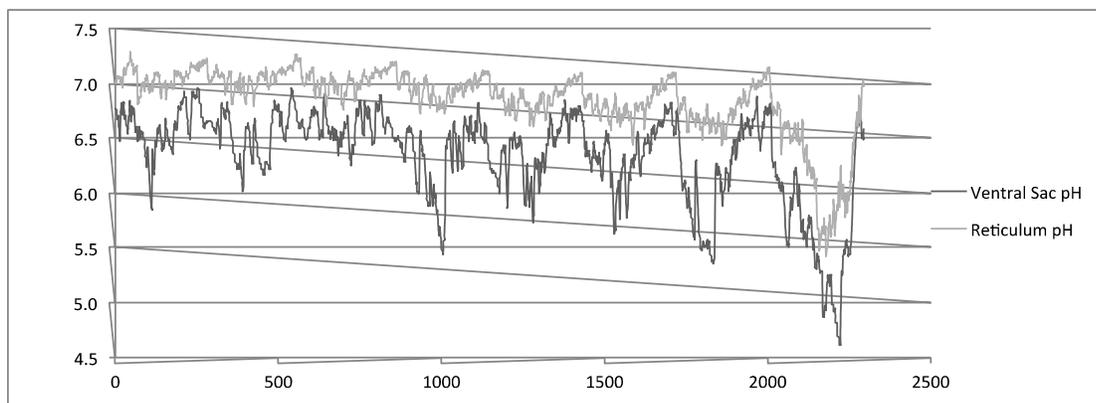


Figure 1. pH in reticulum and rumen ventral sac recorded with indwelling pH data loggers. The inclusion rate of wheat-barley pellet in the TMR diet was increase gradually from d4 to d8. (Danscher and Li, 2013, unpublished data)

5.2 Feeding behavior

Feeding behaviour changes in accordance with physical and chemical properties of diets and animal's health condition. It has been reported that feeding behaviour can be used as a diagnostic tool in dairy cows (Quimby et al., 2001, Urton et al., 2005). Li et al. (2011) investigated the effects of two types of SARA challenges on the feeding behaviour of lactating dairy cows. Feeding behaviour of individual cows was monitored by continuously weighing the feed in the mangers (Growsafe Systems Ltd., Airdire, AB). A grain-pellet SARA challenge (GPSC) was conducted by replacing 21% of DM of the basal diet with wheat-barley pellets. An alfalfa-pellet SARA challenge (APSC) was conducted by replacing alfalfa hay in the control diet with alfalfa pellets. Both GPSC and APSC reduced the duration of the first meal after feed delivery (Li et al., 2011). In another study conducted by the same authors, they found that both SARA challenges reduced the meal frequency, but only the GBSC reduced the daily eating time, and tended to decrease the duration of the first meal after feed delivery (Li et al., 2012a). DeVries et al. (2009) studied the effects of 1-d grain-based SARA challenges during three subsequent periods. These authors observed that the effects of this SARA challenge depended on the period, as the challenge reduced meal frequency in the first period, tended to increase meal frequency in the second period, and only tended to increase meal duration in the third period (DeVries et al., 2009). A comparison among these studies shows that SARA challenges affects feeding behaviour, but more research is needed to assess the potential of using feeding behavior to diagnose SARA.

5.3 Manure evaluation

The increase in grain content in diets, which may induce SARA, can also result in more dietary nutrients bypassing the rumen and reaching the hindgut. Excessive hindgut fermentation then changes the consistency and appearance of the manure. Hence, manure observation may be used as a diagnostic tool to evaluate rumen functionality (Hall, 2007). If the rumen functions normally, only few recognizable feed particles should be observed in manure and the size of the particles should be no longer than half an inch (Hall, 2007). Watery and foamy manure indicates the abnormal fermentation in the hind gut, and mucin casts in manure suggests the damage of gut epithelium (Hall, 2007).

It is worth noting that the characteristics of manure may vary remarkably during SARA. For instance, including more grain in the diet usually increases the feed intake, which lowers rumen pH to the range where SARA occurs. The manure in this period may appear to be pasty, or watery. Once daily rumen pH reduction reaches a critical level, the feed intake during the following days may be drastically reduced. As a result of low feed intake, the manure can appear to be stiff. By reducing feed intake, rumen fermentation is then limited and rumen pH gradually recovers to a physiologically normal condition. Feed intake will gradually recover back to normal as well. Subsequently, the appearance of manure also becomes normal again. This cycle will take several days. However, a recovered feed intake may lead to another cycle (bout) of SARA. Overall, the result of manure score or fecal evaluation during SARA should be interpreted with caution.

A commercially available tool, Nasco Digestion Analyzer, is useful for feces analysis in field. Nasco Digestion Analyzer is comprised of three metal screens with pore size of 4.76mm, 2.38mm and 1.59mm respectively. The recommendation from the inventor indicates that after analysis, retained material on the top screen should be less than 10%, middle screen less than 20% and bottom screen more than 50% for the manure sampled from healthy dairy cows (Cotanch and Darrah, 2013). More than 10 % of manure retaining on the top screen may indicate the risk of ruminal acidosis because of decreased digestion efficiency.

5.4 Fecal LPS

Feeding high-grain diets to induce subacute ruminal acidosis (SARA) in dairy cows has been associated with the increase in the concentration of lipopolysaccharide (LPS) endotoxin originating from gram-negative bacteria in feces (Li et al., 2012a, Li et al., 2012b). A survey was conducted on 300 lactating dairy cows on 10 dairy farms to determine how risk factors for and symptoms of excessive grain feeding and subacute ruminal acidosis are associated with the concentrations of endotoxins in feces (Li et al., 2010). The results indicated that fecal LPS concentration varies dramatically among the dairy farms, and is correlated with dietary NDF content and days in milk (Li et al., 2010). These results indicated that testing LPS in feces of dairy cow is helpful for diagnosing SARA. The advantage of using feces as a testing target is that feces is easy to collect. However, the method used for LPS test is pricy and complicated.

5.5 Blood gas analysis

Given that SARA is characterized as an acid overload in the rumen, it is logical to speculate that SARA may cause an acid-base imbalance in blood. Hence, blood gas analysis may be helpful for diagnosing SARA. A survey on dairy farms indicated that cows at high risk of SARA had relative high $p\text{CO}_2$, low $p\text{O}_2$, and low blood pH (Gianesella et al., 2010). In the laboratory, a significant increase $p\text{CO}_2$ and unchanged $p\text{O}_2$ was observed in SARA-challenged cows (Li et al., 2012a). However, Danscher et al. (2013a) conducted two experimental SARA challenge studies, in which a tendency of increase $p\text{CO}_2$ was only observed in one study and $p\text{O}_2$ was not affected by SARA challenges (Danscher et al., 2013a, Danscher et al., 2013b). The discrepancy may be due to differences in the severity of SARA among the studies. Gianesella et al. (2010) observed a significant decrease of blood pH on the cows at high risk of SARA, whereas it was not observed by Li et al. (2012a) and Danscher et al. (2013a, b). Another explanation of this discrepancy may be due to the blood sampling site. Gianesella et al. (2010), Danscher et al. (2013a, b) collected blood samples from jugular vein, whereas Li et al. (2012a) collected blood samples from the tail vein. Blood samples collected from the tail vein can be contaminated by arterial blood. Arterial blood contains higher O_2 and lower CO_2 compare to venous blood (Tvedten et al., 2000). To sum up, blood gas analysis might be helpful for diagnosing SARA, although results should be interpreted with utmost caution. Draining blood from the jugular vein may be the better option for blood sampling although the tail vein is easier to access and sampling from it puts less stress on the cows.

5.6 Acute phase protein in blood

Acute phase response is the core of the innate immune response and a systemic early-defense system activated by infection, stress, and inflammation. Acute phase proteins are an integral part of the acute phase response and have been identified in several common animal species. Measuring acute phase proteins has a great value in human diagnostic medicine. SARA induced by increasing dietary grain content triggers an inflammatory response indicated by an increase in plasma serum amyloid A, lipopolysaccharide binding protein, and occasionally haptoglobin (Gozho et al., 2007, Khafipour et al., 2009, Li et al., 2012b). However, in practice, an increase in serum amyloid A, lipopolysaccharide binding protein, and haptoglobin may not necessarily be related to SARA, as other sources of infection, stress and inflammation can trigger the release of these acute phase proteins. Nonetheless, it is beneficial to monitor these acute phase proteins, as all causes including SARA which trigger acute phase response should be addressed in order to warrant the health of dairy cows.

6 Prevention SARA

6.1 Supplying adequate dietary fiber

The key of preventing SARA is to supply adequate dietary fiber to dairy cows. NRC (2001) listed the recommended minimum concentration of NDF and ADF in lactating diets (Table 1). These recommendations were made based on appropriate forage particle size, good practice of TMR feeding, and using ground corn as the predominant starch source. Therefore, for on-farm application, an adjustment should be considered according to the type and particle size of forage ingredients, starch source as well as feeding regime. Some examples are that legume forages and silages have more buffering capacity than grass forage and corn silage because legume forages have higher mineral content; forage in larger particle size can stimulate more saliva secretion, which buffers ruminal pH (Van Soest, 1994); and the degradation rate of starch source ranks as oats > barley, wheat > corn, sorghum (McAllister et al., 2006). In addition, a “safety margin” should be added in order to protect against variation in the feed intake and nutrient requirements of dairy cows.

Table 1 fiber guidelines for diets of lactating dairy cows

Forage NDF	Dietary NDF	Dietary ADF
19	25	17
18	27	18
17	29	19
16	31	20

(NRC, 2001)

6.2 Reducing sorting

Properly formulated diets do not necessarily guarantee healthy digestion of dairy cows, unless the diets are properly prepared and fed. Animals sort for the most palatable feed components. Dairy cows usually sort for small grain particles and against long forage particles. Depending upon the intensity of the sorting behavior, the composition of feed actually ingested by cows could be substantially different from what is originally formulated and offered. This sorting behavior may lead to unexpected insufficient rumen buffer capacity and, subsequently, to SARA. Another interesting observation is that cows may sort for medium particle size forage and against grain during SARA in order to compensate low rumen pH (Devries et al., 2007). Sorting can be reduced by preparing TMR with an appropriate particle size distribution and moisture content. A guideline of forage and TMR particle size established by using Penn State Particle Separator is available for reference (Table 2) (Heinrichs and Kononoff, 2002).

Table 2 Forage and TMR particle size recommendations

Screen	Pore Size (mm)	Particle Size (mm)	Corn Silage (%)	Haylage (%)	TMR (%)
Upper Sieve	19	8 -19	3 to 8	10 to 20	2 to 8
Middle Sieve	8	1.8 – 8	45 to 65	45 to 75	30 to 50
Lower Sieve	1.25	0.7 - 1.8	30 to 40	20 to 30	30 to 50
Bottom Pan		<0.7	< 5	< 5	< 20

Heinrichs and Kononoff (2002)

Appropriate water content in diets is critical for reducing sorting. Diets that are too dry not only limit feed intake, also prone to excess sorting (Leonardi et al., 2005). A well accepted range of ideal moisture content in TMR is about 40 -50%. Keep in mind that adding water to TMR in order to increase its moisture content is the last resort, as water may cause the feed to be less palatable, resulting in reduced feed intake and increased sorting behaviour (Miller-Cushon and DeVries (2009). The better way of adjusting the water content of diets is to decrease drier forages in favor of wetter forages, or include more high moisture feed ingredients like wet distiller grain soluble, molasses, if available.

6.3 Using feed supplements to stabilize rumen fermentation

Yeast (*Saccharomyces cerevisiae*) supplements have been fed to dairy cows in last few decades. Although there is considerable variation among the effectiveness of different yeast supplements, meta-analysis has shown that feeding yeast supplements to dairy cows may increase feed intake, milk production, and milk fat yield (Poppy et al., 2012). *In vitro* studies suggested that yeast products interact with *S. bovis* and *M. elsdenii* to reduce lactate accumulation (Chaucheyras et al., 1996), which implies that yeast supplement may be beneficial to the cows suffering from SARA. However, the results from *in vivo* studies are controversial (Moya et al., 2009, Li et al., 2012c). For instance, Moya et al. (2009) suggested that yeast culture addition had no significant impact on rumen fermentation; whereas Li et al. (2013) reported that *Saccharomyces cerevisiae* fermentation product (SCFP, Original XPC, Diamond V) stabilizes rumen microbial communities, alleviate milk fat depression and may reduce the production of ruminal LPS during grain-induced SARA.

Exogenous buffers, like sodium bicarbonate and magnesium oxide have been routinely supplemented to dairy cows in order to stabilize ruminal pH. The effect of these inorganic buffers has been broadly studied and its mode of action has been well documented (Erdman, 1988, Russell and Chow, 1993).

7 Summary

SARA is one of the main metabolic diseases in the modern dairy industry. Cows in the early lactation, primiparous cows, as well as cows grazing or fed with rapidly fermentable low fiber grass are in particular risk to develop SARA. Although rumen pH measurement remains the first choice for diagnosing SARA, research has shown that pH measurements cannot stand alone, but have to be combined with other techniques such as monitoring of feeding behavior, blood gas analysis, and detection of acute phase proteins and fecal LPS. The preferred approach to prevent SARA is formulating adequate fiber in the diets, preparing diets with adequate particle size distribution and moisture content to reducing sorting. Feeding supplements such as yeast and exogenous buffer can be considered to stabilize rumen pH.

Reference:

1. Bramley, E., I. J. Lean, W. J. Fulkerson, and N. D. Costa. 2005. Clinical acidosis in a Gippsland dairy herd. *Australian veterinary journal* 83(6):347-352.
2. Chaucheyras, F., G. Fonty, G. Bertin, J. M. Salmon, and P. Gouet. 1996. Effects of a strain of *Saccharomyces cerevisiae* (Levucell(R) SC1), a microbial additive for ruminants, on lactate metabolism in vitro. *Can. J. Microbiol.* 42(9):927-933.
3. Cotanch, K. and J. Darrah. 2013. Fecal fractions of the Nasco digestion analyzer . http://www.whminer.com/fr_12_06_05.html . Accessed 04-2013.
4. Dado, R. G. and M. S. Allen. 1993. Continuous computer acquisition of feed and water intakes, chewing, reticular motility, and ruminal pH of cattle. *J. Dairy Sci.* 76(6):1589-1600.
5. Danscher, A. M., S. C. Li, P. H. Andersen, E. Khafipour, N. B. Kristensen, and J. C. Plaizier. 2013a. Biomarkers for bovine rumen acidosis (abstract). JAM 2013 conference.
6. Danscher, A. M., L. S., A. P.H., K. E., K. N.B., and P. J.C. 2013b. In search of indicators of bovine subacute rumen acidosis (SARA) (abstract). in ICPD 2013. Uppsala, Sweden.
7. DeVries, T. J., K. A. Beauchemin, F. Dohme, and K. S. Schwartzkopf-Genswein. 2009. Repeated ruminal acidosis challenges in lactating dairy cows at high and low risk for developing acidosis: feeding, ruminating, and lying behavior. *J. Dairy Sci.* 92(10):5067-5078.
8. DeVries, T. J., K. A. Beauchemin, and M. A. von Keyserlingk. 2007. Dietary forage concentration affects the feed sorting behavior of lactating dairy cows. *J. Dairy Sci.* 90(12):5572-5579.
9. Duffield, T., J. C. Plaizier, A. Fairfield, R. Bagg, G. Vessie, P. Dick, J. Wilson, J. Aramini, and B. McBride. 2004. Comparison of techniques for measurement of rumen pH in lactating dairy cows. *J. Dairy Sci.* 87(1):59-66.
10. Enemark, J. M. D., R. J. Jorgensen, and N. B. Kristensen. 2004. An evaluation of parameters for the detection of subclinical rumen acidosis in dairy herds. *Vet. Res. Commun.* 28(8):687-709.
11. Erdman, R. A. 1988. Dietary buffering requirements of the lactating dairy cow: A review. *J. Dairy Sci.* 71(12):3246-3266.

12. Giancesella, M., M. Morgante, C. Cannizzo, A. Stefani, P. Dalvit, V. Messina, and E. Giudice. 2010. Subacute ruminal acidosis and evaluation of blood gas analysis in dairy cow. *Veterinary medicine international* 2010.
13. Gozho, G. N., D. O. Krause, and J. C. Plaizier. 2006. Rumen lipopolysaccharide and inflammation during grain adaptation and subacute ruminal acidosis in steers. *J. Dairy Sci.* 89(11):4404-4413.
14. Gozho, G. N., D. O. Krause, and J. C. Plaizier. 2007. Ruminal lipopolysaccharide concentration and inflammatory response during grain-induced subacute ruminal acidosis in dairy cows. *J. Dairy Sci.* 90(2):856-866.
15. Hall, M. 2007. Carbohydrate nutrition and manure scoring. Part II: Tools for monitoring rumen function in dairy cattle. . Pages 81-86. in *Proc. Proceedings of Minnesota Dairy Health Conference*, May 15, 2007, St. Paul, Minnesota.
16. Heinrichs, J. and P. Kononoff. 2002. Penn State Particle Separator. Vol. 2013, <http://extension.psu.edu/animals/dairy/health/nutrition/forages/forage-quality-physical/separator>. Accessed 04-2013
17. Khafipour, E., D. O. Krause, and J. C. Plaizier. 2009a. Alfalfa pellet-induced subacute ruminal acidosis in dairy cows increases bacterial endotoxin in the rumen without causing inflammation. *J. Dairy Sci.* 92(4):1712-1724.
18. Khafipour, E., D. O. Krause, and J. C. Plaizier. 2009b. A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation. *J. Dairy Sci.* 92(3):1060-1070.
19. Kleen, J. L., G. A. Hooijer, J. Rehage, and J. P. T. M. Noordhuizen. 2009. Subacute ruminal acidosis in Dutch dairy herds. *Vet. Rec.* 164(22):681-684.
20. Krause, K. M. and G. R. Oetzel. 2006. Understanding and preventing subacute ruminal acidosis in dairy herds: a review. (Special Issue: Feed and animal health.). *Anim. Feed Sci. Technol.* 126(3/4):215-236.
21. Leonardi, C., F. Giannico, and L. E. Armentano. 2005. Effect of water addition on selective consumption (sorting) of dry diets by dairy cattle. *J. Dairy Sci.* 88(3):1043-1049.
22. Li, S., G. N. Gozho, N. Gakhar, E. Khafipour, D. O. Krause, and J. C. Plaizier. 2012a. Evaluation of diagnostic measures for subacute ruminal acidosis in dairy cows. *Canadian Journal of Animal Science* 92(3):353-364.
23. Li, S., E. Khafipour, D. O. Krause, L. A. Gonzalez, and J. C. Plaizier. 2011. Effects of grain-pellet and alfalfa-pellet subacute ruminal acidosis (SARA) challenges on feeding behaviour of lactating dairy cows. *Canadian Journal of Animal Science* 91(2):323-330.
24. Li, S., E. Khafipour, D. O. Krause, A. Kroeker, J. C. Rodriguez-Lecompte, G. N. Gozho, and J. C. Plaizier. 2012b. Effects of subacute ruminal acidosis challenges on fermentation and endotoxins in the rumen and hindgut of dairy cows. *J. Dairy Sci.* 95(1):294-303.

25. Li, S., E. Khafipour, D. O. Krause, J. C. Rodriguez-Lecompte, and J. C. Plaizier. 2010. Free endotoxins in the feces of lactating dairy cows. *Canadian Journal of Animal Science* 90(4):591-594.
26. Li, S., E. Khafipour, I. Yoon, M. Scott, and J. C. Plaizier. 2013. *Saccharomyces cerevisiae* fermentation product stabilized rumen microbial communities of lactating dairy cows during subacute ruminal acidosis (abstract). in Congress on gastrointestinal function meeting. Chicago, IL.
27. Li, S., E. Tesfaye, H. Khazanehei, M. Scott, I. Yoon, E. Khafipour, and J. C. Plaizier. 2012c. Impact of feeding yeast culture under normal and SARA conditions in lactating dairy cows (abstract). *J. Anim Sci.* 90(Suppl. 3).
28. McAllister, T. A., D. J. Gibb, K. A. Beauchemin, and Y. Wang. 2006. Starch type, structure and ruminal digestion. in Proc. Oklahoma state beef extension: Cattle Grain Processing Symposium
29. Miller-Cushon, E. K. and T. J. DeVries. 2009. Effect of dietary dry matter concentration on the sorting behavior of lactating dairy cows fed a total mixed ration. *J. Dairy Sci.* 92(7):3292-3298.
30. Morgante, M., C. Stelletta, P. Berzaghi, M. Gianesella, and I. Andrighetto. 2007. Subacute rumen acidosis in lactating cows: an investigation in intensive Italian dairy herds. *J Anim Physiol Anim Nutr (Berl)* 91(5-6):226-234.
31. Mould, F. L., E. R. Orskov, and S. O. Mann. 1983. Associative effects of mixed feeds. 1. Effects of type and level of supplementation and the influence of rumen pH on cellulolysis in vivo and dry matter digestion of various roughages. *Anim. Feed Sci. Technol.* 10(1):15-30
32. Moya, D., S. Calsamiglia, A. Ferret, M. Blanch, J. I. Fandino, L. Castillejos, and I. Yoon. 2009. Effects of dietary changes and yeast culture (*Saccharomyces cerevisiae*) on rumen microbial fermentation of Holstein heifers. *J. Anim. Sci.* 87(9):2874-2881.
33. Nocek, J. E. 1997. Bovine acidosis: implications on laminitis. *J. Dairy Sci.* 80(5):1005-1028.
34. NRC. 2001. *Nutrient Requirements of Dairy Cattle: Seventh Revised Edition, 2001.* The National Academies Press.
35. O'Grady, L., M. L. Doherty, and F. J. Mulligan. 2008. Subacute ruminal acidosis (SARA) in grazing Irish dairy cows. (Special Issue: Production diseases of the transition cow.). *Vet. J.* 176(1):44-49.
36. Penner, G. B., K. A. Beauchemin, and T. Mutsvangwa. 2006. An Evaluation of the Accuracy and Precision of a Stand-Alone Submersible Continuous Ruminant pH Measurement System. *J. Dairy Sci.* 89(6):2132-2140.
37. Penner, G. B., K. A. Beauchemin, and T. Mutsvangwa. 2007. Severity of ruminal acidosis in primiparous holstein cows during the periparturient period. *J. Dairy Sci.* 90(1):365-375.
38. Poppy, G. D., A. R. Rabiee, I. J. Lean, W. K. Sanchez, K. L. Dorton, and P. S. Morley. 2012. A meta-analysis of the effects of feeding yeast culture produced by anaerobic fermentation of *Saccharomyces cerevisiae* on milk production of lactating dairy cows. *J. Dairy Sci.* 95(10):6027-6041.
39. Russell, J. B. and J. M. Chow. 1993. Another theory for the action of ruminal buffer salts: decreased starch fermentation and propionate production. *J. Dairy Sci.* 76(3):826-830.

40. Shen, J. S., Z. Chai, L. J. Song, J. X. Liu, and Y. M. Wu. 2012. Insertion depth of oral stomach tubes may affect the fermentation parameters of ruminal fluid collected in dairy cows. *J. Dairy Sci.* 95(10):5978-5984.
41. Slyter, L. L., R. R. Oltjen, D. L. Kern, and F. C. Blank. 1970. Influence of type and level of grain and diethylstilbestrol on the rumen microbial populations of steers fed all-concentrate diets. *J. Anim. Sci.* 31(5):996-1002.
42. Tvedten, H., M. Kopia, and C. Haines. 2000. Mixed venous and arterial blood in bovine coccygeal vessel samples for blood gas analysis. *Veterinary clinical pathology.* 29(1):4-6.
43. Van Soest, P. J. 1994. *Nutritional ecology of the ruminant* (2nd). Cornell University Press, Ithaca, NY.
44. Westwood, C. T., E. Bramley, and I. J. Lean. 2003. Review of the relationship between nutrition and lameness in pasture-fed dairy cattle. *New Zealand veterinary journal* 51(5):208-218.