

The SAGA continues – searching for the source of gut-derived inflammation

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Introduction

Every ruminant nutritionist worth his/her salt over the past 40 years has been taught about ruminal acidosis – its causes, consequences, and strategies to avoid what can be an extremely detrimental condition. First, the focus was on the acute, clinical form of acidosis, where lactic acid-producing bacteria proliferate and a rapid increase in lactic acid concentrations in the rumen can drive pH below 5 and kill most ruminal microbes. The feedlot industry certainly benefitted from understanding this condition, and strategies derived from this research continue to influence nutrition of finishing cattle.

In the dairy industry, the need to support adequate milk fat yield means that greater amounts of fiber are fed, and it is rare to find evidence of lactic acidosis. However, dairy nutritionists began to refer to sub-acute ruminal acidosis (**SARA**) to describe a condition where pH does not spiral out of control, but where the ruminal microbiota experiences extended hours each day at pH < 5.8. This scenario was proposed to be associated with impaired ruminal digestion (especially of fiber), increased risk of some clinical diseases, and a general impairment of health and productivity. Substantial research has been carried out under the SARA framework, much of which has improved our ability to formulate and deliver optimal dairy cattle diets.

However, a third paradigm has emerged in the last 10 years, focused more on the potential for hind-gut acidosis and disrupted barrier function to induce systemic inflammatory responses. In recognition of the potential for both foregut and hindgut acidosis and dysbiosis to create health challenges, some have proposed that we shift the focus away from a narrow emphasis on SARA to considering sub-acute gastrointestinal acidosis (**SAGA**). In this paper I'll review why this concept has emerged, what recent research has taught us about SAGA, and consider the implications for feeding management of dairy cattle.

Mechanisms for whole-body responses to SARA

It is easy enough to understand why a dramatic (or even modest) decline in ruminal pH could have detrimental effects on gut microbes. Many in vitro microbiology experiments have demonstrated that controlled acidification of culture media can harm or kill bacteria and protozoa, particularly the fiber-degrading species. In turn, the impact on nutrient digestion and absorption of products from microbial metabolism is logical enough. For example, a very large meta-analysis suggests that for every 1% increase in dietary starch concentration, total-tract NDF digestibility declines by about 0.5 units (Ferraretto et al., 2013), presumably due at least partially to decreased ruminal pH.

It is less obvious, though, why systemic effects of SARA should be observed. The abomasum can clearly handle an extremely low pH without ill effect, although not all

regions of the gastrointestinal tract have the same epithelial structure. It is really the case that mildly acidic rumen contents can harm the cow?

A critical insight into this question was provided more than 40 years ago, with the demonstration that rumen fluid had high endotoxin activity, particularly from cattle fed predominantly grain (Nagaraja et al., 1978). Although endotoxin is always found in gut microbial ecosystems, situations that favor the growth of Gram-negative bacteria and those that trigger rapid death of these microbes (and release of their cell wall constituent lipopolysaccharides) could plausibly harm intestinal epithelium. Damage to the ruminal epithelium could theoretically generate a direct inflammatory response to impact whole-animal physiology, or epithelial barrier function could be disrupted, resulting in systemic delivery of not only microbial toxins but even intact pathogens such as *Fusobacterium necrophorum* (Garcia et al., 2017).

There continues to a variety of opinions regarding whether loss of ruminal epithelium barrier integrity is a common occurrence or not. For starters, the ruminal epithelium is vastly better fortified than the intestinal epithelium, with 4 layers of protection vs. 1 in the intestines (**Fig. 1**).

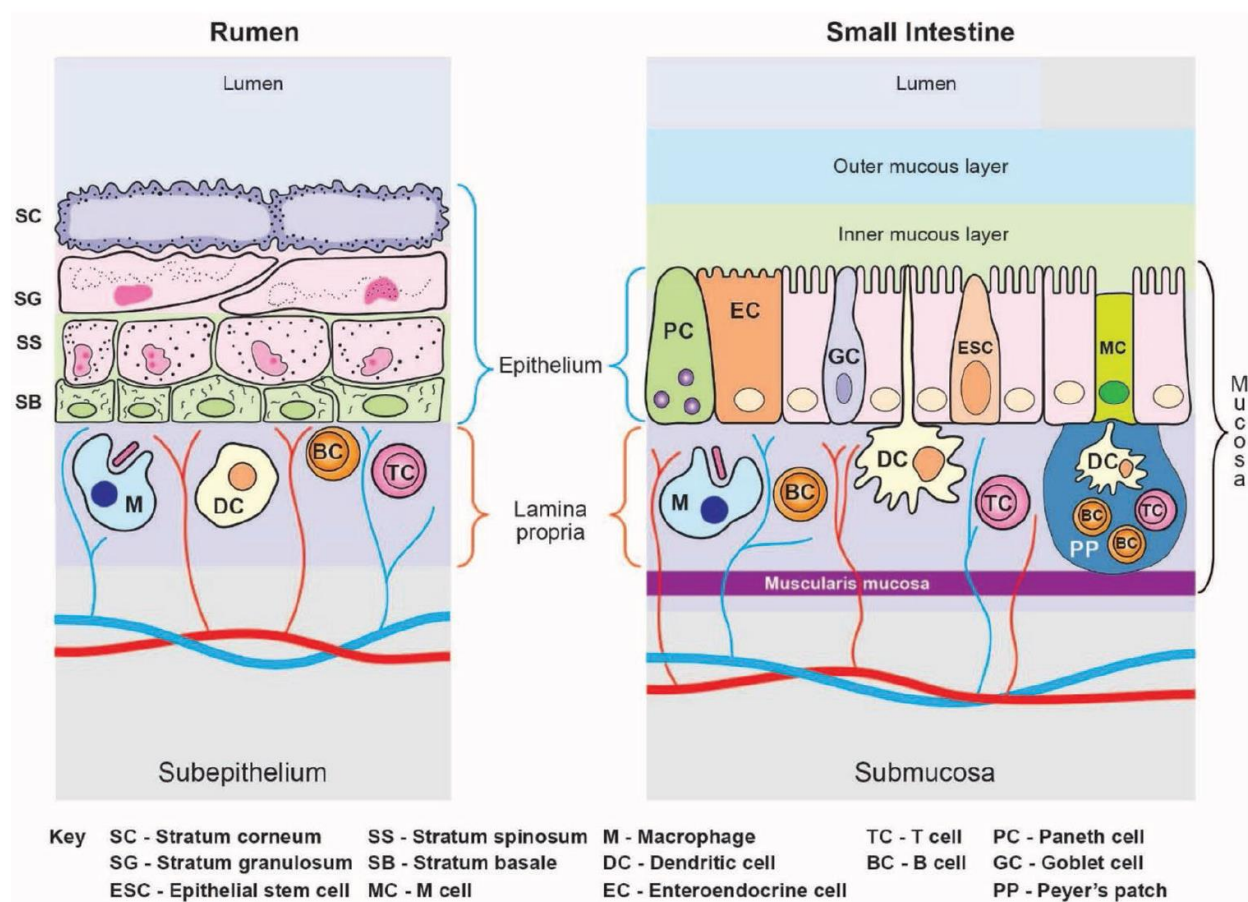


Figure 1. Morphology of the epithelium in the rumen vs. the distal intestine. From Garcia et al. (2017).

Despite the anatomic resilience of the rumen epithelium, commercial abattoir surveys have demonstrated that approximately 10% of dairy cows have active or healed ulcerations of the rumen at slaughter (Rezac et al., 2014). These gross lesions are certainly of sufficient size and severity to enable translocation of microbes and their products. Additionally, a recent report may explain how more microscopic disruption of the epithelium can also contribute to movement of microbes or microbial toxins. Meissner and colleagues (2017) collected ruminal tissue for ex vivo experiments to evaluate barrier integrity against a variety of molecules. Interestingly, maintaining the ruminal tissue at pH 5.1 rather than 6.1 had only minimal effects on electrical conductivity and transit rates of a high-molecular-weight fluorophore. However, when the ruminal tissue was maintained at pH 5.1 with a physiological concentration of volatile fatty acids (VFA; 100 mM), both tissue conductance and fluorophore transit increased dramatically, indicating enhanced epithelial permeability. This finding was further supported by substantially decreased abundance of multiple tight junction proteins (Meissner et al., 2017). Despite the solid construction of the ruminal epithelium, it appears that chemical and/or physiological impacts of high VFA concentrations can lead to a decline in the “mortar” that holds cells tightly together. This mechanism may establish microlesions that can eventually develop into full-blown ulcerations. Either way, the loss of barrier integrity provides a path for intra-ruminal LPS or other toxins to impact the cow.

Confounded results

Despite a growing mechanistic underpinning connecting ruminal dysbiosis to systemic inflammation and illness behavior, there are also reasons to question some long-held assumptions regarding links between ruminal fermentation and whole-animal responses. First of all, it is clear today that nearly any significant disruption of normal ruminal fermentation also has substantial effects on the distal small intestine. For example, let's consider a case where some problem with diet formulation or presentation results in a dairy cow consuming much more starch than intended. We would expect ruminal pH to decline rapidly, which in turn would disrupt fermentation of potentially-digestible NDF (pdNDF). What is less commonly considered is that this increased outflow of pdNDF to the abomasum and eventually the hind gut will provide much more substrate to gut microbes in that ecosystem. As a result, we would expect at least some increase in acid production and a decrease in pH in the hind gut. In a more extreme scenario where even ruminal starch fermentation is impacted (or bypassed, for example by feeding intact corn), the impact on hind-gut pH may be even more dramatic than in the rumen. The point is that we have hundreds of published studies with various dietary challenges documenting declines in rumen pH and associated systemic responses including host release of inflammatory molecules, decreased feed intake, and other illness behaviors. Unfortunately, very few of those studies simultaneously measured fecal pH (much less ileal or colonic) at the same time. It's important to acknowledge that a correlation between ruminal acidosis and other responses does not

imply a causal relationship, especially when disruptions to other regions of the gastrointestinal tract occur essentially simultaneously.

As demonstrated above, the intestinal epithelium is the chain-link fence to the rumen's Great Wall. Although more or less ignored for decades in the study of ruminants, the distal intestine is a focus of intense research in species used as models for human health. As a result, we now know about many factors that can contribute to disruptions of the intestinal epithelium, including heat stress, dysbiosis, and even short-term feed restriction. Following up on this monogastric work, lactating Holstein cows undergoing feed intake restriction (50-60% of *ad libitum* intake) showed clear signs of LPS translocation out of the gut by day 5 and altered intestinal tissue morphology on day 7 of the challenge (Kvidera et al., 2017).

With the increasing interest in the hind-gut as the possible site connecting SAGA with systemic effects, several intensive studies have been conducted to challenge the hind gut microbiome with excess starch supply and see if classic acidosis responses (or even hemorrhagic bowel syndrome) could be induced (Gressley et al., 2011, 2016). In general, although fermentation and pH can certainly be affected by postruminal carbohydrate supply, these studies failed to consistently observe health effects of these rather extreme treatments. It seems unlikely that excessive postruminal carbohydrate supply *alone* is sufficient to induce illness behavior in most cattle.

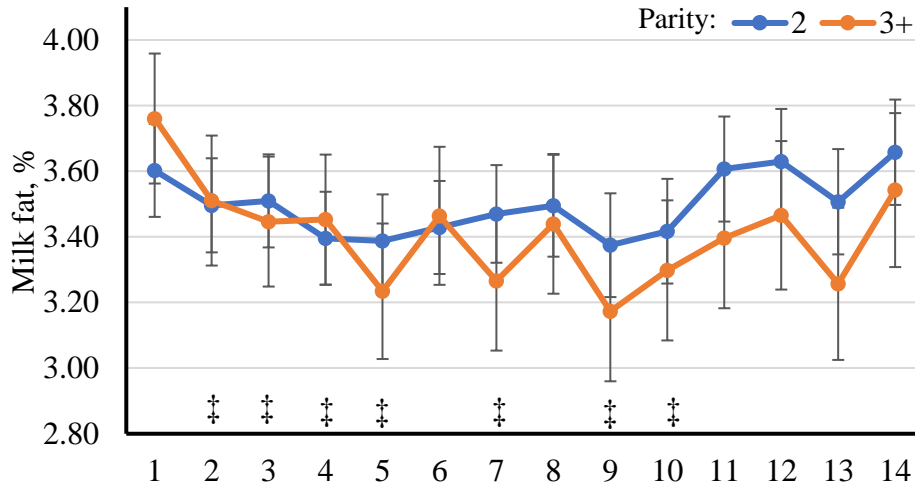
SAGA: a case study

Our research group recently dealt with an outbreak and resolution of digestive disorders among 15 control cows enrolled in a larger production study. Over 14 weeks, cows were individually fed, with milk yield and composition, blood variables, and health observations recorded. The diet included drought-stressed corn silage that introduced difficulties including low energy density, high dry matter content (making it unstable at feedout), and mycotoxin contamination. The diet included 31% NDF, but only 16% forage NDF, and particle size was marginal. Retrospective mycotoxin analysis showed TMR concentrations of ~1000 ppb trichothecenes and ~70 ppb zearalenone.

By weeks 4–5 on the study, sporadic diarrhea began to appear and milk fat content had dropped from 3.7% to 3.4%, on average (**Fig. 2**). Coincident with the onset of summer heat stress (mean daily THI > 65), three cows developed severe digestive disorders, resulting in a displaced abomasum in one cow. Fecal samples were collected to enumerate viable clostridia bacteria (Arm & Hammer, Waukesha, WI), revealing a mean of 10^3 CFU/g *Clostridium perfringens*, with individuals as high as 10^5 CFU/g. Furthermore, blood analyses showed significant increases in the inflammatory biomarker haptoglobin and the dysbiosis marker D-lactate (**Fig. 2**).

At that point, the diet was changed to replace some corn silage with wheat straw (3.5% of DM), a direct-fed microbial was added to the diet (Biofix Plus Pro; Biomin America, Overland Park, KS), and organic acid treatment (Ultra-Curb, Kemin, Des Moines, IA) of

the silage face was initiated. Within a month after these changes were implemented, essentially all signs of digestive problems resolved, including milk fat content, fecal consistency, and blood plasma concentrations of haptoglobin and D-lactate. This case study points to multiple factors that likely combined to lead to microbial and gastrointestinal disruptions resulting in clinical disease in a subset of cows.



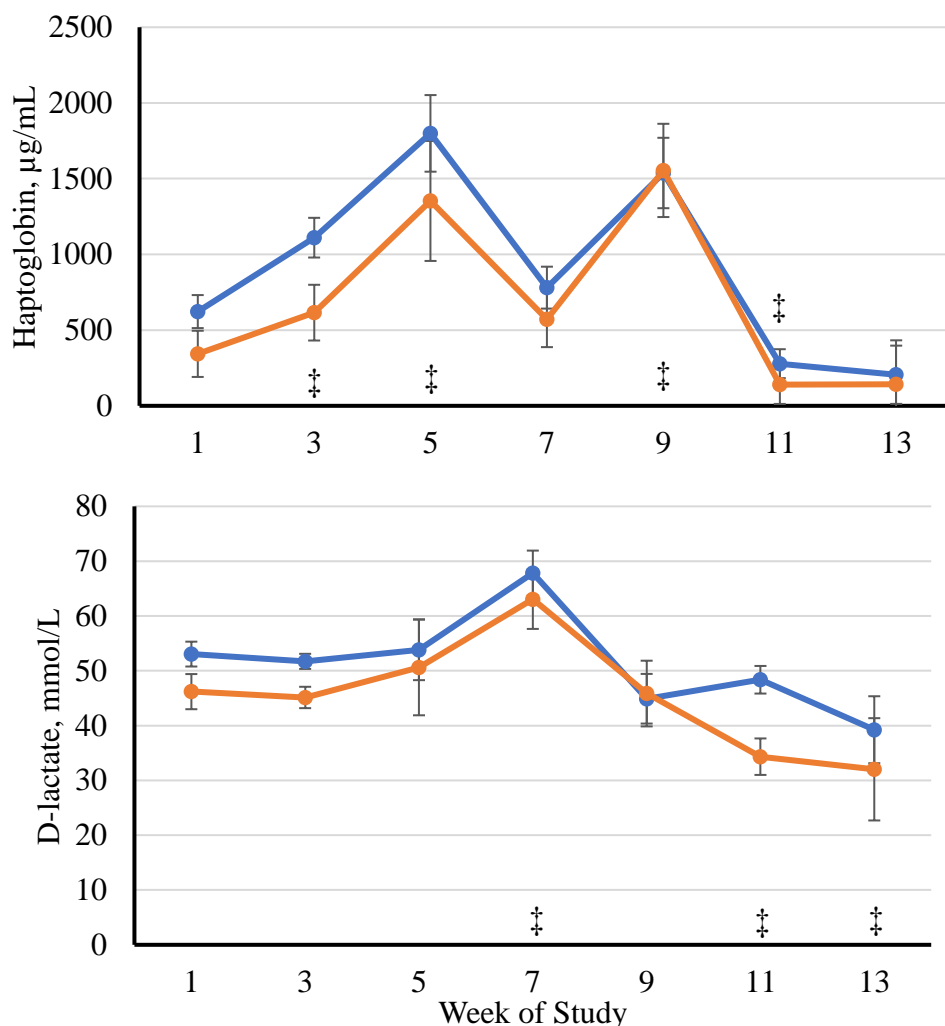


Figure 2. Milk fat, plasma haptoglobin, and plasma D-lactate concentrations of cows fed a ration low in peNDF and naturally contaminated with mycotoxins during the onset of summer heat stress. In week 8, dietary peNDF was increased with 3.5% wheat straw and a direct-fed microbial was added to combat mycotoxicosis. Values are means \pm standard errors, $n = 15$. ‡ $P < 0.05$ vs. week 1.

Where do we stand?

Ruminal acidosis certainly can and does occur, and we have good evidence now that many dairy cattle exhibit physical signs of damage to the ruminal wall during their lifetime. As a result, at least some systemic negative effects of SAGA are likely due to direct translocation of microbes or microbial products through a disrupted ruminal epithelium. However, it's also the case that the intestinal epithelium is susceptible to disruption, and dysbiosis in the hind gut is likely just as common as it is in the rumen. Furthermore, the intestine is likely more susceptible to compounded stressors, given the reported impacts of factors like mycotoxins and heat stress on intestinal health across species. Tracking down the root cause of a specific digestive health issue may need to

expand beyond a careful review of diet formulation and presentation, to include other factors that can adversely affect microbial or intestinal stability.

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