

# Linking rumen disorders to immunity in cattle: old and new paradigms<sup>1</sup>

QENDRIM ZEBELI<sup>1</sup> AND BURIM N. AMETAJ<sup>2</sup>

<sup>1</sup>Institute of Animal Nutrition and Functional Plant Compounds, Department for Farm Animals and Veterinary Public Health, Vetmeduni Vienna, Veterinaerplatz 1, 1210 Vienna, Austria;

<sup>2</sup>Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada T6G 2P5

## Abstract

Maintaining a healthy rumen ecosystem is crucial to avoid systemic disorders in cattle, but also critical to ensure that milk and meat are produced from healthy animals in an efficient and cost-effective manner. In fact, today's intensive management systems of cattle typically encourage the inclusion of large amounts of easily degradable carbohydrates in the diet to support rapid weight gain or high milk yields, and enhance cost efficiency. Although these feeding practices are useful to maximize production in a cost-effective manner, they do not cope with the digestive physiology of cattle. Therefore, rumen acidosis, and in particular its subacute form, is one of the most prevalent animal health and welfare issues in intensive cattle production systems. New data indicate that rumen acidosis may lead to severe dysbiosis and the release of bacterial compounds, with immunogenic properties, which also adversely affect the host. One of these compounds is endotoxin or lipopolysaccharide, a component of the outer leaflet of the external membrane of all Gram-negative bacteria. This mini-review will primarily focus on rumen acidosis, trying to link this "old" metabolic disorder of cattle to more "new" paradigms that imply an association between rumen acidosis and endotoxin with other metabolic disorders of cattle. It is clear that the more we know about the metabolic adaptive capacity and health status of the rumen, the better we can shape our future feeding strategies for more efficient and healthy ruminant production.

**Keywords:** rumen acidosis, endotoxin, cattle health

## 1. Introduction

Rumen is harbored by a large diversity of microbiota which confers important metabolic capabilities to the host animal. These metabolic capabilities primarily include degradation of otherwise indigestible nutrients by the host, such as dietary fibres, and the generation of volatile fatty acids (VFA), mainly acetate, propionate and butyrate, as the major end-products of ruminal fermentation [1, 2]. For instance, depending also on the amount of dry matter ingested, rumen of dairy cows may generate roughly up to 120-130 Mol (6 - 7 kg) VFA daily. These large amounts of organic acids are absorbed across the stratified squamous epithelium of the reticulorumen. Their absorption is instrumental in supplying energy to the host and also in lowering the risk of rumen acidosis [1-4]. When the symbiotic relationship in the rumen is disturbed, such as during severe dysbiosis, rumen health becomes a systemic concern of the inner homeostatic mechanisms [3]. The next sections of this mini-review will: i) emphasize

the role of proper rumen metabolism for the host cattle, ii) revise the role of rumen acidosis on the release of endotoxins, and iii) discuss the latest findings about their major consequences for host health.

## 2. From normal ruminal metabolism to rumen acidosis – how does it happen?

Rumen plays a central role in the digestive physiology of ruminants including nutrient degradation, production of VFA, and the synthesis of microbial protein and important vitamins [2]. Rumen health and functioning are therefore important in supplying energy and utilizable protein as well as vitamins and several minerals to the host. In particular, the reticuloruminal absorption of VFA has a dual and thereby central role in digestive efficiency and health of ruminants [1]. The absorption of VFA ensures direct recovery of energy substrates from the rumen into the metabolic pool of the animal. On the other hand, absorption also regulates the intraruminal

<sup>1</sup> This work is dedicated to our former colleague and dear friend, Prof. Dr. Dashmir Xhaxhiu (1957-2012), for his long-standing contributions both as an adorable teacher, a high-profile scientist, and a visionary leader of Veterinary Sciences and Education in Albania.

milieu and pH by the extraction of protons together with VFA, hence lowering the risk of rumen fermentation disorders [1]. In fact, cattle develop these metabolic disorders associated with severe dysbiosis when rumen buffering capacity cannot keep pace with the accumulation and/or neutralization of the VFA [3].

Besides the luminal environment, the integrity and health status of the rumen epithelium is also a very important factor in cattle health. The integrity of the rumen epithelium is usually maintained by the interplay of certain intercellular structures called tight-junctions and gap junctions [3, 5]. When these cell-cell junctional structures are disrupted and the integrity of rumen epithelium is damaged, such as during rumen dysbiosis, several toxic compounds, including bacterial endotoxin released in the rumen or other toxins coming from diet, can be translocated into the bloodstream having fatal consequences for the host [3].

### 3. Endotoxin – just a bacterial toxin?

Bacterial endotoxin or lipopolysaccharide (LPS) is a component of the outer leaflet of the external membrane of all Gram-negative bacteria (GNB) – with high pro-inflammatory properties - that function as a permeability barrier of GNB, protecting them from host defense mechanisms, and it may also contribute to bacterial attachment to surfaces and in biofilm formation [3, 6]. Endotoxin is a complex molecule of lipid and carbohydrate nature containing three units: (1) variable O antigen, (2) core polysaccharide, and (3) lipid A. Despite the fact that endotoxin is bound to the outer membrane, it is shed during the growth and stationary phases of bacterial growth, and released following cell disintegration and lysis [7]. Rumen endotoxin is variable in its composition and toxicity; the chemical structure of endotoxin is from GNB species to species different [6], whereas the toxicity is believed to be influenced not only by species of GNB but also the environment [3]. The rate of endotoxin release varies among different species and strains of GNB [6] and the acidic environment, which largely depend on the diet composition [4]. For example, endotoxin from *Escherichia coli* is more toxic than that of other GNB typically found in the rumen fluid of cattle, and a severe acidosis is believed to further increase the virulence of *E. coli* [8], and probably the toxicity of rumen endotoxin [3].

### 4. pH, acidosis and endotoxin in the rumen – hows and whys?

To support high milk yields or daily gains, cattle typically are fed large amounts of cereal grains or easily degradable by-products. This feeding strategy is often associated with rumen disorders. The most prevalent form of rumen disorders is the sub-acute rumen acidosis (SARA), which is often perceived as intermittent drops of ruminal pH below 5.8 for at least 5-6 h/d [9], and is characterized by dysbiosis with major changes in the profile of microbiota in the rumen, often in the favor of GNB [8]. Indeed, a grain-induced SARA is dominated by *E. coli* in the rumen fluid [6, 8]. Even a mild grain-induced SARA can be dominated by another GNB, *Megasphaera elsdenii* [6, 8]. Interestingly, the rumen of cows with forage pellet-induced SARA had greater counts of *Prevotella albensis*, another GNB in the rumen [8]. The increase of counts from different strains of GNB during SARA explains why this disorder is typically accompanied with dramatic increase in the concentration of endotoxin in the rumen fluid of dairy cows [3, 5, 8, 10].

Rumen pH plays a modulatory role in the release and accumulation of endotoxin due to its effects on the metabolic processes and changes in the cell membrane of rumen bacteria and maintenance of bacterial ecological balances [11]. In an acidic rumen environment, some of GNB cannot survive the low pH and end-up dying releasing large amounts of free endotoxin in the rumen fluid; some others adjust quickly to the new environment and grow rapidly, releasing additional endotoxin during their growth phase [3]. In fact, among the factors responsible for disruption of barrier function of the rumen are the luminal load of endotoxin [12], overgrowth of rumen GNB with certain virulence factors [8], and adaptive changes occurring in the rumen epithelium in dependence of the duration and severity of the acidotic challenge [3].

### 5. Rumen endotoxin and risks for host health – a new paradigm?

Translocation of endotoxin from rumen or hindgut into the bloodstream results in the activation of a non-specific acute phase response (APR) in cattle [3, 6, 8, 10]. The reason for the activation of a systemic APR is that endotoxin stimulates the release of pro-inflammatory cytokines such as tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1, and IL-6 by liver

macrophages, resulting in enhanced secretion of acute phase proteins (APP) from hepatocytes [3, 8, 10]. Acute phase response is viewed as part of multifaceted innate immune and metabolic responses of the host, aiming to eliminate the agent(s) that caused the interference and to bring the homeostasis back to normality [13]. However, the presence of ruminal endotoxin and subsequent APR for a longer time period, such as during early lactation when cows are fed diets rich in concentrates, might be associated with negative consequences for the host. It has been discussed that the host may become refractory to the prolonged presence of endotoxin, and these states generally are associated by suppression of the immune system and the subsequent increased susceptibility to various diseases [3]. On the other hand, the activation of an APR has greater requirements in energy and nutrients, which results in lowered efficiency of energy and feed use by the animal [14]. Furthermore, potential involvement of rumen endotoxin in the ethiopathogenesis of different metabolic diseases is also discussed recently [3, 6]. For example, endotoxin has been shown to be associated with perturbations of plasma minerals [15] and endocrine mediators responsible for regulation of energy and lipid metabolism in dairy cattle [15, 16].

## 6. Conclusions

Rumen acidosis is one of the most important health and welfare issues in intensive rearing systems of cattle worldwide; it lowers both ruminal feed degradation and feed efficiency. The most important consequence of rumen acidosis, which has taken enormous research interests recently, is that this disorder increases the risk of systemic metabolic and immune alterations due to the release of large amounts of bacterial endotoxins and disruption of rumen barriers. Despite the advances gained in the recent years, which have increased our understanding on the role of the rumen in health and disease in cattle, more research is warranted, in particular, in the development of more efficient feeding strategies that help alleviating the conflict existing between high biochemical output of the rumen and the release of large amounts of endotoxin and the disruption of the barrier functions of the rumen epithelia, especially in high-producing cattle. The more we know about the metabolic adaptive capacity of the gastrointestinal tract, at a molecular level, the better we can shape the future feeding strategies for more efficient and healthy ruminant production with lowered ecological

footprints of meat and milk production to feed the increasing world human population.

## 7. References

1. Aschenbach JR, Penner GB, Stumpff F, Gäbel G: **Ruminant nutrition symposium: Role of fermentation acid absorption in the regulation of ruminal pH.** *Journal of Animal Science* 2011, **89**:1092-1107.
2. Khiaosa-Ard R, Zebeli Q: **Dietary modulation of rumen metabolism: a key factor to enhancing ruminant production.** *Albanian Journal of Agricultural Sciences* 2012, **3**:131-140.
3. Ametaj BN, Zebeli Q, Iqbal S: **Nutrition, microbiota, and endotoxin-related diseases in dairy cows.** *Revista Brasileira de Zootecnia* 2010, **39** (supl. especial):433-444.
4. Ametaj BN, Zebeli Q, Saleem F, Psychogios N, Lewis MJ, Dunn SM, Xia J, Wishart DS: **Metabolomics reveals unhealthy alterations in rumen metabolism with increased proportion of cereal grain in the diet of dairy cows.** *Metabolomics* 2010, **6**:583-594.
5. Zebeli Q, Metzler-Zebeli B, Ametaj BN: **Meta-analysis reveals threshold level of dietary concentrate that triggers systemic inflammation in cattle.** *Journal of Dairy Science* 2012, **95**:2662-2672.
6. Plaizier JC, Khafipour E, Li S, Gozho GN, Krause DO: **Subacute ruminal acidosis (SARA), endotoxins and health consequences.** *Animal Feed Science and Technology* 2012, **172**:9-21.
7. Hurley JC: **Endotoxemia: Methods of detection and clinical correlates.** *Clinical Microbiology Reviews* 1995, **8**:268-292.
8. Khafipour E, Plaizier JC, Aikman PC, Krause DO: **Population structure of rumen *Escherichia coli* associated with subacute ruminal acidosis (SARA) in dairy cattle.** *Journal of Dairy Science* 2011, **94**:351-360.
9. Zebeli Q, Dijkstra J, Tafaj M, Steingass H, Ametaj BN, Drochner W: **Modeling the adequacy of dietary fiber in dairy cows based on the responses of ruminal pH and milk fat production to composition of the diet.** *Journal of Dairy Science* 2008, **91**:2046-2066.
10. Emmanuel DGV, Dunn SM, Ametaj BN: **Feeding high proportions of barley grain stimulates an inflammatory response in dairy cows.** *Journal of Dairy Science* 2008, **91**:606-614.

11. Russell JB, Rychlik JL: 2001: **Factors that alter rumen microbial ecology**. *Science* **292**:1119–1122.
12. Emmanuel DGV, Madsen KL, Churchill TA, Dunn SM, Ametaj BN: **Acidosis and lipopolysaccharide from Escherichia coli B:055 cause hyperpermeability of rumen and colon tissues**. *Journal of Dairy Science* 2007, **90**:5552-5557.
13. Gabay C, Kushner I: **Acute-phase proteins and other systemic response to inflammation**. *New England Journal of Medicine* 1999, **340**:448-454.
14. Elsasser TH, Caperna TJ, Li C-J, Kahl S, Sartin JL: **Critical control points in the impact of the proinflammatory immune response on growth and metabolism**. *Journal of Animal Science* 2008, **86**:E105-125E.
15. Waldron MR, Nonnecke BJ, Nishida T, Horst RL, Overton TR: **Effect of lipopolysaccharide infusion on serum macromineral and vitamin D concentrations in dairy cows**. *Journal of Dairy Science* 2003, **86**:3440-3446.
16. Zebeli Q, Sivaraman S, Dunn SM, Ametaj BN: **Intermittent parenteral administration of endotoxin triggers metabolic and immunological alterations typically associated with displaced abomasum and retained placenta in periparturient dairy cows**. *Journal of Dairy Science* 2011, **94**:4968–4983.