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Feeding barley grain-rich diets altered electrophysiological properties and permeability of the ruminal wall in a goat model

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ABSTRACT

High-producing ruminants are commonly fed large amounts of concentrate to meet their high energy demands for rapid growth or high milk production. However, this feeding strategy can severely impair rumen functioning, leading to subacute ruminal acidosis. Subacute ruminal acidosis might have consequences for electrophysiological properties by changing the net ion transfer and permeability of ruminal epithelia, which may increase the uptake of toxic compounds generated in the rumen into the systemic circulation. The objective of the present study was to investigate the effects of excessive barley feeding on the electrophysiological and barrier functions of the ruminal epithelium and serum inflammation and ketogenesis markers after a long-term feeding challenge, using growing goats as a ruminant model. A feeding trial was carried out with growing goats allocated to 1 of the 3 groups (n = 5–6 animals/group), with diets consisting exclusively of hay (control diet) or hay with 30 or 60% barley grain. Samples of the ventral ruminal epithelium were taken after euthanasia and instantly subjected to Ussing chamber experiments, where electrophysiological properties of the epithelium were measured in parallel with the permeability of marker molecules of different sizes [fluorescein 5(6)-isothiocyanate and horseradish peroxidase] from luminal to apical side. Additionally, ruminal fluid and blood samples were taken at the beginning of the experiment as well as shortly before euthanasia. Ruminal fluid samples were analyzed for volatile fatty acids and pH, whereas blood samples were analyzed for lipopolysaccharide, serum amyloid A, and β-hydroxybutyrate. Electrophysiological data indicated that barley feeding increased the epithelial short-circuit current compared with the control. Tissue conductance also increased with dietary barley inclusion. As shown with both marker molecules, permeability of ruminal epithelia increased with barley inclusion in the diet. Despite a lowered ruminal pH associated with increased volatile fatty acids (such as propionate and butyrate) concentrations as well as altered epithelial properties in response to high-grain feeding, no signs of inflammation became apparent, as blood serum amyloid A concentrations remained unaffected by diet. However, greater amounts of grain in the diet were associated with a quadratic increase in lipopolysaccharide concentration in the serum. Also, increasing the amounts of barley grain in the diet resulted in a tendency to quadratically augment serum concentrations of β-hydroxybutyrate and, hence, the alimentary ketogenesis. Further studies are needed to clarify the role of barley inclusion in the development of subacute ruminal acidosis in relation to ruminal epithelial damage and the translocation of toxic compounds in vivo.

Key words: Ussing chamber, short-circuit current, subacute ruminal acidosis, ruminal barrier

INTRODUCTION

The intensive production systems of ruminants encourage the inclusion of large amounts of cereal grains or easily degradable byproducts in the diet to support high milk yields or rapid weight gain. Although these feeding practices might be helpful to enhance cost efficiency in the short term, they do not comply optimally with the ruminant's digestive physiology. The most important consequence thereof is a disturbed rumen ecosystem, which is often perceived in the form of a prolonged decline in ruminal pH to acidotic values <5.8, a rumen metabolic disorder commonly referred to as SARA (Zebeli and Metzler-Zebeli, 2012).

Subacute ruminal acidosis is associated with important microbial and metabolic alterations that also affect the epithelial lining of the rumen (Penner et al., 2011). One of the crucial functions of the reticuloruminal epi-

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thelia is to maintain barrier integrity while regulating the selective absorption of VFA (Aschenbach et al., 2011). The barrier function of the rumen is particularly important in animals during long episodes of SARA to prevent translocation of the accumulated LPS or other potentially toxic compounds (e.g., enterotoxins and biogenic amines) into the systemic circulation (Ametaj et al., 2010b; Plaizier et al., 2012). Changes in the morphological and histological properties of ruminal papillae (Steele et al., 2009) as well as an increased permeability of the ruminal epithelium through deterioration of cellular junctions and thickness of this epithelium (Steele et al., 2011) have been reported during SARA insults. Under normal physiological conditions, the ruminal epithelium serves as a barrier and prevents the paracellular transport of toxic compounds into the blood circulation (Plaizier et al., 2012). However, at high acidity, as in case of SARA, high LPS concentrations and osmolality of the digesta can decrease the barrier function and increase the epithelial permeability, resulting in an increased uptake of LPS (Emmanuel et al., 2007; Khafipour et al., 2009a), which initiates an acute-phase response (**APR**). Serum amyloid A (**SAA**) is an acute-phase protein (**APP**) that participates in the neutralization of LPS during the process of APR (Emmanuel et al., 2008; Plaizier et al., 2012). Serum amyloid A was demonstrated to significantly increase during SARA in cattle (Gozho et al., 2006; Emmanuel et al., 2008; Khafipour et al., 2009a). Although the APR is regarded as a protective reaction of the body aiming to reestablish the disturbed homeostasis, in the long term, the inflammatory state has important implications for animal health and production efficiency (reviewed by Zebeli and Metzler-Zebeli, 2012).

Despite the importance of barrier function for systemic health, little data exist evaluating the effects of high-grain feeding and SARA on the electrophysiological characteristics and barrier function of the ruminal epithelium. Most of the studies conducted so far have evaluated the effects of in vitro acidic medium on the functionality of ruminal epithelia (Aschenbach and Gäbel 2000; Emmanuel et al., 2007; Penner et al., 2010). Although these in vitro data serve as indications for implication of SARA in the changes of ruminal epithelial properties, these experiments cannot replace in vivo conditions. Consequently, a need exists to examine whether individual changes in the diet (i.e., higher concentrate level/low fiber content), are involved in the electrophysiological changes and barrier function of the ruminal epithelia.

The objective of the present study was to evaluate changes in the electrophysiological properties and barrier function of the ruminal epithelium, using growing goats after a long-term feeding challenge of excessive amounts of barley grain as an in vivo ruminant model. Long feeding periods are crucial to explain potential dietary effects on the functionality of the ruminal epithelia under in vivo conditions. Ruminal pH and the concentration of VFA were measured in the ruminal digesta to evaluate the fermentation characteristics in vivo and relate it to findings obtained in the Ussing chamber experiments. Additionally, clinical signs were evaluated and serum SAA and BHBA were used as indicators of APR and ketogenesis, respectively.

MATERIALS AND METHODS

Animals, Housing, and Feeding Trial

The animal experimentation protocol of this research was approved by the institutional ethics committee of the University of Veterinary Medicine Vienna in accordance with Good Scientific Practice guidelines and national legislation (Protocol no. 15/03/97/2011). For the feeding trial, 1 female and 17 noncastrated male growing goats (6 Boer breed, 6 White German Noble breed, and 6 Toggenburg breed) were used at the experimental station of the Institute for Organic Farming and Biodiversity in Wels, Austria. Upon arrival, all goats were treated for internal parasites with Hapadex 5% (1.5 mL of netobimin/10 kg of BW; Intervet GmbH, Vienna, Austria). Upon the commencement of the experiment, the goats were between 3 and 4 mo old and ranged between 13 and 22 kg in BW. Prior to the experiment, goats consumed a high-forage diet of 1.5 kg of meadow hay (second cut) and 200 g of hammermilled barley grain per animal and day (as-fed basis) for a period of 2 wk.

After this adaptation period, the animals were gradually switched to the experimental diets, which they received for another 6 wk. Due to health problems, 1 animal had to be removed from the experiment. Animals were allocated in a blocked, randomized design, balanced for weight and breed, to 1 of 3 treatments consisting of either pure chopped hay, considered as the control (**CTR**) diet (n = 5), or a low-barley (**LB**) diet containing 30% barley grain and 70% hay (n = 6), or a high-barley (**HB**) diet containing 60% barley grain and 40% hay (n = 6; Table 1). It has been shown in dairy cows that diets including the rather moderate amount of 30% barley can already increase ruminal LPS and plasma SAA concentrations (Emmanuel et al., 2008). Diets containing 60% grain were shown to induce SARA in dairy cows (Steele et al., 2011) and were considered to cause changes in the epithelial barrier function. Accordingly, we chose the 2 levels 30 and 60% to compare them with a control treatment containing no barley to investigate the effects of dietary barley in-