



Supplementing phytogenic compounds or autolyzed yeast modulates ruminal biogenic amines and plasma metabolome in dry cows experiencing subacute ruminal acidosis

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ABSTRACT

Subacute ruminal acidosis (SARA) causes ruminal dysbiosis, thereby increasing the risk of systemic metabolic disorders in cattle. We recently showed that supplementation with phytogenic compounds (PHY) or autolyzed yeast (AY) counteracted negative effects of SARA by improving ruminal pH and microbiome. This study investigated the effects of an intermittent SARA challenge on the ruminal concentration of biogenic amines (BA) and lipopolysaccharides (LPS), as well as on the blood metabolome. We also evaluated effects of PHY and AY on the latter variables. Eight rumen-cannulated nonlactating Holstein cows were arranged in an incomplete 4×3 Latin square design with 4 experimental runs and 3 treatment groups. During each run, cows were switched from an all-forage diet (baseline) to an intermittent concentrate-challenge diet with a forage:concentrate ratio of 35:65 (dry matter basis) to induce SARA for 1 (SARA1) or 2 (SARA2) wk, separated by 1 wk of forage-only feeding. The 3 treatment groups were no additive as control, PHY, or AY. During baseline, SARA1 and SARA2 rumen fluid samples were collected for analysis of BA and LPS. Blood samples were taken during baseline and SARA1 for a targeted metabolomics approach. High-concentrate feeding caused a 9-fold increase in ruminal LPS during SARA1 and an 11-fold increase in SARA2 compared with the baseline. Elevated concentrations of ruminal BA were found during both SARA periods, with histamine showing the strongest increase during SARA1. Moreover, a decrease in phosphatidylcholines, lysophosphatidylcholines, sphingomyelines, and several

AA in the blood during SARA1 were detected. Supplementation of PHY decreased concentrations of LPS (−43%), histamine (−66%), pyrrolidine (−38%), and spermine (−54%) in SARA1 and cadaverine in SARA2 (−50%). Moreover, cows that received PHY had higher concentrations of cholesterol (+26%), several AA, and phosphatidylcholines in SARA1 compared with control cows. For AY, decreases in ruminal ethanolamine (−21%), methylamine (−52%), histamine (−54%), spermidine (−44%), and spermine (−80%) in SARA1 were observed, whereas in the blood an increase in tryptophan was noticed. In conclusion, the SARA was associated with markedly increased concentrations of LPS and BA in the rumen fluid and undesirable shifts in the plasma metabolome. Supplementation of PHY and AY counteracted some of these changes and therefore may help in attenuating negative effects of high-concentrate feeding in dairy cattle.

Key words: feed additive, subacute rumen acidosis, biogenic amine, blood metabolome, lipopolysaccharide

INTRODUCTION

Concentrate-rich diets have become a common practice in commercial cattle production increasing the risk of SARA. This digestive disorder is characterized by a prolonged and intermittent decline in ruminal pH, causing major microbial imbalances in the rumen (Tajima et al., 2000; Khafipour et al., 2009), commonly known as dysbiosis. Growing evidence indicates that gastrointestinal dysbiosis during SARA promotes the production of microbe-derived toxic compounds, including bacterial LPS and biogenic amines (BA; Ametaj et al., 2010; Wang et al., 2013; Mao et al., 2016), which have been suggested as biomarkers of dysbiosis during SARA in cattle (Plaizier et al., 2012).

The release of large amounts of bacterial LPS and BA in the rumen has been reported during a single

Received March 14, 2018.

Accepted May 31, 2018.

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SARA bout (Ametaj et al., 2010; Saleem et al., 2012); however, it is not clear if the release of such toxic compounds differs with duration of SARA or when SARA occurs repeatedly. Recent research of our team has shown that intermittent SARA affects pH and causes important bacterial shifts in the rumen. These effects were stronger during the first versus the second SARA bout, despite feeding the same diet (Neubauer et al., 2018). Most interestingly, this research showed that supplementation of phytogenic compounds (**PHY**) or autolyzed yeast (**AY**) alleviated the dysbiosis mainly by reducing the drop of ruminal pH, promoting chewing activity, and beneficially affecting microbial community structure. These effects were observed particularly when severest pH drops occurred (Kröger et al., 2017; Neubauer et al., 2018). However, the extent to which repeated SARA bouts affect the indicators of ruminal dysbiosis such as the release and accumulation of BA and LPS and whether **PHY** or **AY** supplementation is also able to modulate these indicators have not yet been evaluated.

Research has also established that during SARA and the resulting dysbiosis, the permeability of the rumen mucosa increases (Aschenbach and Gäbel, 2000), offering the opportunity for the microbe-derived toxic compounds to translocate into the systemic circulation. This event could lead to systemic inflammation and metabolic derailments such as displaced abomasum, laminitis, fatty liver, and plasma mineral disturbances in cattle exposed to such a SARA challenge (Zebeli and Metzler-Zebeli, 2012). Most conventional studies investigating the effect of SARA on the metabolism of cows focused on single metabolites (Zebeli et al., 2011; Marchesini et al., 2013). In recent years, however, metabolomics techniques have been developed, allowing the detection of multiple classes of metabolites that reflect changes of key metabolic pathways and help to gain a deeper understanding of the mechanisms behind metabolic disorders (Hailemariam et al., 2014a,b; Humer et al., 2016). Metabolomics-based technologies, investigating the effect of high-concentrate feeding in dairy cows carried out on rumen fluid samples, revealed substantial effects of SARA on the rumen metabolome, such as an increased concentration of several potentially toxic metabolites as well as perturbations in AA (Ametaj et al., 2010; Saleem et al., 2012). Given the potential of metabolomic technologies to extend our understanding of the mechanisms behind metabolic disorders, we hypothesized that the characterization of the blood metabolomic profile of dairy cows fed either an all-forage diet or a high-concentrate diet would provide comprehensive information on how SARA as a metabolic disease affects the cow's systemic metabolism and how these changes interact with the accumulation

of toxic compounds in the rumen. Therefore, besides analyzing BA and LPS in the rumen fluid, we aimed at conducting additional blood analysis during the period of severest pH drops (i.e., the first SARA challenge; Kröger et al., 2017) to verify the proposed hypothesis. We used a SARA feeding challenge model with non-lactating cows to be able to investigate the effects of diet-induced ruminal disturbances on the blood metabolome, while ruling out potential confounding influences of lactation (e.g., hormonal changes, nutritional demands, and mobilization) on systemic metabolism and blood metabolome.

The aims of this study were to investigate changes in the concentration of BA and LPS in the rumen fluid as well as in the blood metabolome of cows experiencing SARA and to investigate possible modulatory effects of the supplementation of feed additives. Our respective hypothesis was that **PHY** and **AY** would help in reducing the concentration of LPS and BA in cows experiencing SARA, especially when pH is lowest, thereby resulting in less detrimental changes in the blood metabolome.

MATERIALS AND METHODS

Animals, Diets, and Experimental Design

The experiment was part of a larger study and detailed information about cows, feeding, and experimental setup as well as results according to reticularruminal pH, chewing activity, and ruminal microbiome have been reported in our companion papers (Kröger et al., 2017; Neubauer et al., 2018). In brief, 8 rumen-cannulated (100 mm i.d.; Bar Diamond, Parma, ID), nonlactating Holstein cows (average BW: 863 ± 65 kg, mean \pm SD) were kept in a loose-housing stable at the research dairy farm of Vetmeduni Vienna (Pottenstein, Austria). Cows were arranged to an incomplete double 4×3 Latin square design with 4 runs and 3 treatments ($n = 8$ per treatment). Cows were randomly allocated to 1 of the 3 treatment groups: control (**CON**, no additive), **PHY** (Digestarom Dairy, BIOMIN Holding GmbH, Getzersdorf, Austria; 3 g per animal and day) or **AY** (Levabon Rumen E, BIOMIN Holding GmbH; 15 g per animal and day). The **PHY** consisted of a blend of spices, herbs, and essential oils with major active compounds deriving from mint, thyme, rosemary, and clove, whereas **AY** contained autolyzed spray-dried yeast (*Saccharomyces cerevisiae*). Each of the 4 runs lasted for 43 d and was followed by a 3-wk washout period to allow cows to recover from high-concentrate feeding and to avoid carry-over effects.

During each experimental run, cows were exposed to an intermittent high-concentrate feeding and thus

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