J. Dairy Sci. 98:1–14
http://dx.doi.org/10.3168/jds.2015-9913

Treatment of grain with organic acids at 2 different dietary phosphorus levels modulates ruminal microbial community structure and fermentation patterns in vitro

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ABSTRACT

Recent data indicate positive effects of treating grain with citric (CAc) or lactic acid (LAc) on the hydrolysis of phytate phosphorus (P) and fermentation products of the grain. This study used a semicontinuous rumen simulation technique to evaluate the effects of processing of barley with 50.25 g/L (wt/vol) CAc or 76.25 g/L LAc on microbial composition, metabolic fermentation profile, and nutrient degradation at low or high dietary P supply. The low P diet [3.1 g of P per kg of dry matter (DM) of dietary P sources only] was not supplemented with inorganic P, whereas the high P diet was supplemented with 0.5 g of inorganic P per kg of DM through mineral premix and 870 mg of inorganic P/d per incubation fermenter via artificial saliva. Target microbes were determined using quantitative PCR. Data showed depression of total bacteria but not of total protozoa or short-chain fatty acid (SCFA) concentration with the low P diet. In addition, the low P diet lowered the relative abundance of *Ruminococcus albus* and decreased neutral detergent fiber (NDF) degradation and acetate proportion, but increased the abundance of several predominantly noncellulolytic bacterial species and anaerobic fungi. Treatment of grain with LAc increased the abundance of total bacteria in the low P diet only, and this effect was associated with a greater concentration of SCFA in the ruminal fluid. Interestingly, in the low P diet, CAc treatment of barley increased the most prevalent bacterial group, the genus *Prevotella*, in ruminal fluid and increased NDF degradation to the same extent as did inorganic P supplementation in the high P diet. Treatment with either CAc or LAc lowered the abundance of *Megasphaera elsdenii* but only in the low P diet. On the other hand, CAc treatment increased the proportion of acetate in the low P diet, whereas LAc treatment decreased this variable at both dietary P levels. The propionate proportion was significantly increased by LAc at both P levels, whereas butyrate increased only with the low P diet. Treatments with CAc or LAc reduced the degradation of CP and ammonia concentration compared with the control diet at both P levels. In conclusion, the beneficial effects of CAc and LAc treatment on specific ruminal microbes, fermentation profile, and fiber degradation in the low P diet suggest the potential for the treatment to compensate for the lack of inorganic P supplementation in vitro. Further research is warranted to determine the extent to which the treatment can alleviate the shortage of inorganic P supplementation under in vivo conditions.
Key words: lactic acid, citric acid, phosphorus, rumen microbiota, Rusitec

INTRODUCTION

Cereal grains are important ingredients in the diet of high-producing ruminants. Besides supplying energy for rumen microbes and the host, grains are important sources of minerals, especially of phosphorus (P). Phosphorus nutrition has received renewed interest due to its potential environmental effects and the limitations of the global raw phosphate stores (Kincaid and Rodehutscord, 2005). From a nutritional point of view, a major challenge is to meet the requirements of the animal while minimizing P excretion to the environment (Humer and Zebeli, 2015). On the other hand, in vitro ruminant studies have shown that low P supply decreases microbial protein synthesis, cellulose degradation, and the formation of short-chain fatty acids (**SCFA**; Komisarczuk et al., 1987a,b), indicating decreased ruminal microbial activity and fermentation efficiency due to insufficient P supply.

Received June 6, 2015.
Accepted July 5, 2015.
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In cereal grains and legumes, 60 to 80% of P is stored as phytic acid (*myo*-inositol 6 hexakisphosphate, **$InsP_6$**; Reddy et al., 1982; Viveros et al., 2000), potentially reducing its ruminal solubility and consequently the availability for ruminants (Field, 1981). In contrast to monogastric animals, the $InsP_6$ is completely available for ruminants, due to a high phytase activity of ruminal microbes (Clark et al., 1986; Morse et al., 1992; Feng et al., 2013). However, other studies have shown that the rumen degradation of $InsP_6$ may be incomplete (Park et al., 2000; Kincaid et al., 2005; Jarrett et al., 2014), whereby various factors such as feed physical properties, short ruminal retention time (Kincaid et al., 2005; Jarrett et al., 2014), and processing of feedstuffs with formaldehyde or heat (Konishi et al., 1999; Park et al., 2000; Bravo et al., 2000) seem to play a role. Newer research data indicate that ruminal degradability of $InsP_6$ is increased by supplementing exogenous phytase to dairy cow diets, resulting in a higher P availability for ruminal microbiota and the host (Kincaid et al., 2005; Brask-Pedersen et al., 2013; Jarrett et al., 2014). These findings indicate that ruminants can still benefit from native plant $InsP_6$ hydrolysis triggered by exogenous phytase supplementation, thereby likely alleviating their dependency on inorganic P supplementation.

Haraldsson et al. (2004) showed that treating cereals with low concentrations of lactic acid (**LAc**), a mild organic acid widely used in food technology, promoted the hydrolysis of $InsP_6$. More recently, Metzler-Zebeli et al. (2014) reported a linear increase in the hydrolysis of $InsP_6$ by treating grain with graded levels of LAc from 7.63 to 76.25 g/L LAc. In addition, we have observed that processing grain with 76.25 g/L LAc results in an increase of slowly digestible starch (Deckardt et al., 2014, 2015). Our more recent studies have shown that treatment with citric acid (**CAc**), another organic acid frequently used in the feed and food industries, increased mineral solubility and concentration of soluble fiber fractions in barley (Harder et al., 2015a,b), with potential benefits for gastrointestinal microbial activity.

Because of these changes in $InsP_6$ hydrolysis and fermentation products in grains, we hypothesized that treatment with CAc or LAc may modulate the abundance of key ruminal microbes, their fermentation patterns, and nutrient degradation, thus compensating potential impairment of rumen microbial activity due to the lack of inorganic P supplementation. Therefore, this study used a targeted quantitative PCR (**qPCR**) approach coupled with the fermentation profile to investigate the effects of treatment of grain with CAc and LAc at different dietary P supplies on changes in the abundance of fibrolytic and amylolytic microbiota and their patterns of fermentation in a standardized semicontinuous rumen simulation technique (**Rusitec**), which has been used in similar studies (Deckardt et al., 2015).

MATERIALS AND METHODS

Grain Processing

Samples of a 2-row summer barley cultivar 'Espinosa' (Saatzucht Edelhof, Austria) were ground with a knife mill (Grindomix, GM200, Retsch, Haan, Germany) for 17 s at 5,000 rpm. Subsequently, these barley grain samples were soaked in distilled water as control (**CON**) in a ratio of 1:1.2 (wt/vol) or in 76.25 g/L LAc (DL-lactate, 80% wt/wt, Brenntag CEE GmbH, Vienna, Austria) or 50.25 g/L CAc (99.5%, Solan Kraftfutterwerk Schmalwieser GmbH, Bachmanning, Austria) for 24 h at 22°C. The soaking procedure was the same as used in our previous work (Harder et al., 2015a,b). We used concentrations of 50.25 g/L CAc and 76.25 g/L LAc because these concentrations showed the best results both in terms of $InsP_6$ hydrolysis (Metzler-Zebeli et al., 2014) and modification of other chemical components of barley (Harder et al., 2015a,b). On average, each gram of barley grain absorbed 0.75 mL of fluid. Subsequently, the treated barley samples were spread onto petri dishes (94 × 16 mm) and air-dried at 22°C for 72 h.

Experimental Diets

The basal diet consisted of meadow hay (second cut), solvent-extracted soybean meal, barley grains (i.e., CON, CAc, or LAc), and 1 of 2 vitamin-mineral premixes (Biomin M16; Biomin GmbH, Herzogenburg, Austria) for dairy cows, one containing no inorganic P source and the other containing inorganic P sources (Table 1). Hay was ground to pass a 2-mm sieve (Pulverisette Type 14702, Fritsch GmbH, Idar-Oberstein, Germany) and subsequently mixed with other ingredients, resulting in 3 different diets (i.e., CON, CAc, and LAc) for the Rusitec experiment (Table 1).

Rumen Simulation Technique

The Rusitec system (Czerkawski and Breckenridge, 1977), consisting of 12 incubation units, was used as described in previous studies (Deckardt et al., 2015). A total amount of 349.92 mL of buffer solution per day (flow rate: 0.243 mL/min) were infused continuously in each fermenter using a 12-channel peristaltic pump (IPC-N 12/ISM 937, IDEX Health & Science GmbH, Wertheim, Germany). Two buffer solutions were prepared to investigate our hypotheses (Table 2). The artificial saliva was prepared according to McDougall