Animal Feed Science and Technology 230 (2017) 1–12

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Modulation of ruminal fermentation profile and microbial abundance in cows fed diets treated with lactic acid, without or with inorganic phosphorus supplementation

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ARTICLE INFO

Keywords:
Grain processing
Phosphorus nutrition
Ruminal microbial abundance
Ruminal fermentation

ABSTRACT

This study evaluated the effects of lactic acid (LA) treatment of concentrates without or with inorganic P supplementation on ruminal fermentation profile and microbial abundances in non-lactating cows. Six rumen-fistulated Holstein cows were assigned to a double 3 × 3 Latin square design with 3 experimental periods. Each period lasted 14 d, whereby the measurements were performed during the last 2 days. Cows were fed 3 diets containing untreated control concentrate supplemented with inorganic P, and two LA-treated concentrates, either without (LA − P) or with (LA + P) the inorganic P supplementation. The concentrate mixtures of the LA diets were soaked in 5% LA for 24 h before feeding, whereas the concentrate of control diet was not. All diets were offered as a total mixed ration (forage to concentrate ratio of 53:47). Ruminal pH, ammonia and short-chain fatty acid (SCFA) concentrations were determined in free ruminal liquid (FRL) and particle associated ruminal liquid (PARL) that was collected at 0, 2, 4, 8, and 12 h post-morning feeding. Target ruminal microbes in FRL and rumen solid digesta collected at 2 h post-feeding were analyzed using quantitative PCR. Cows consumed on average 17 ± 1.0 kg DM/d (mean ± SEM), irrespective of the treatment. The concentration of total SCFA in FRL was increased by LA treatment ($P < 0.001$) without affecting the pH. Irrespective of P supplementation, feeding of LA-treated diets shifted SCFA profile towards more propionate in the FRL and PARL. The LA − P diet lowered ammonia concentration compared to the other diets ($P < 0.05$). The LA − P diet also reduced the fungal gene copies in solid digesta by 7.6% compared to the LA + P diet ($P < 0.05$). The total bacterial abundance in both ruminal fractions was not affected by diet; however, compared to the control, LA treatment enhanced ($P < 0.05$) the relative abundance of genus *Prevotella* in FRL (18%) and in solid digesta (27%). In contrast, the same treatment decreased the abundances of *Clostridium* cluster IV (23%) in FRL and *Selenomonas ruminantium* group (30%) in solid digesta ($P < 0.05$). Abundances of fibrolytic microbes

Abbreviations: A:P, acetate to propionate ratio; CON + P, control diet with the untreated concentrate mixture supplemented with inorganic phosphorus; CP, crude protein; DM, dry matter; FRL, free ruminal liquid; LA, lactic acid; LA + P, lactic acid-treated concentrates with supplemental inorganic phosphorus; LA − P, lactic acid-treated concentrates without inorganic phosphorus; SCFA, short-chain fatty acid; PARL, particle associated ruminal liquid; P, phosphorus; TMR, total mixed ration

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http://dx.doi.org/10.1016/j.anifeedsci.2017.05.017
Received 12 February 2017; Received in revised form 22 May 2017; Accepted 24 May 2017

correlated with acetate and butyrate only in the rumen solid digesta. In conclusion, the LA treatment of concentrates caused major bacterial shifts, an increase of ruminal fermentation output, and enhancement of propionate fermentation without affecting ruminal pH. The lack of inorganic P supplementation did not impair rumen variables measured, but additional P supply and LA treatment of concentrates beneficially affected ruminal fungi. Lowered ruminal ammonia and branched-chain SCFA concentrations by the LA − P diet suggest lowered protein breakdown in the rumen by this treatment.

1. Introduction

Phosphorus is a key mineral in animal nutrition but at the same time one of the main environmental contaminants from livestock production. Phosphorus nutrition research has renewed interest over the last decades due to increase environmental concerns and also the shortage of global raw phosphate stores (Kincaid and Rodehutscord, 2005). Decreasing supplemental P concentrations in the diet is one option to lower P excretion and environmental contamination (Ekelund et al., 2005; Kebreab et al., 2005). However, decreasing dietary P concentrations below requirements impairs health and productivity of dairy cows (Puggaard et al., 2014). Another viable option to reduce inorganic P supplementation and also minimize the P excretion is enhancing the utilization of P in dietary ingredients, especially in concentrate feeds such as grains and by-products of cereals and oilseeds which are P rich sources (Humer and Zebeli, 2015).

Approximately 40–50% of the diet DM in commercial dairy farms consists of concentrates. With such inclusion levels of concentrates, P requirements of the lactating cow can be met from dietary organic P sources. However, the vast majority of P in grains and particularly in by-products of grains and oilseeds used in ruminant feeding is stored as phytate (myo-inositol hexakisphosphate) (Humer and Zebeli, 2015). In ruminants, microbial phytase activity in the rumen plays an important role to degrade phytate and increase phytate-P availability for microbes and the host (Clark et al., 1986; Morse et al., 1992; Feng et al., 2015). Nevertheless, degradation of phytate-P may not be complete in the rumen, especially under feeding conditions that decrease rumen fermentation as well as reduce ruminal retention time due to short particle size of forages, and high feed intake levels (Kincaid et al., 2005; Jarrett et al., 2014). According to in situ trials conducted in dairy cows only 40–50% of total P of barley is degraded in the rumen by 8 h of incubation, although with prolonged incubation time in the rumen to 24 h almost 90% of P disappeared (Khol-Parisini et al., 2015). Exogenous phytase supplementation (Brask-Pedersen et al., 2013; Jarrett et al., 2014) and grain treatment with organic acids (Haraldsson et al., 2004; Metzler-Zebeli et al., 2014; Khol-Parisini et al., 2015) increase phytate-P availability.

Ruminal microbes have specific requirements for P for fermentation and growth (Durand and Komisarczuk, 1988). According to Kincaid and Rodehutscord (2005), a minimal dietary P level of 2.8–4.0 g/kg of digestible OM has been suggested to meet the requirements of ruminal microbes for N assimilation and cellulose fermentation. Cellulolytic bacteria were consistently reported to have higher P requirements than amylolytic bacteria (Bryant et al., 1959; Caldwell et al., 1973). Therefore, ruminal fiber degradation may be impaired with low dietary P supply (Durand and Komisarczuk, 1988; Harder et al., 2015a). Harder et al. (2015a) reported an increased total bacterial abundance and improved fermentation profile with treatment of barley grain with 5% lactic acid (LA) in a P-deficient diet (3.1 g P/kg DM), using rumen simulation technique. These results suggest that such LA treatment of grain increased P availability, thereby replacing readily available inorganic P supplementation to meet microbial P needs. However, this potential has not yet been evaluated in the complexity of in vivo rumen metabolism with cows fed diets rich in concentrates and without inorganic P supplementation. Accordingly, we hypothesized that diets including LA-treated concentrate may beneficially modulate the metabolic activity and abundance of ruminal microbiota of the liquid and solid phase in the rumen of cows fed diets without inorganic P supplementation. We explored rumen modulatory effects of treating a concentrate mixture with a 5% LA used in conjunction with or without inorganic P supplementation in non-lactating dairy cows. The main aim was to determine whether this LA treatment compensates the lack of readily available inorganic P supplementation for rumen microbes and fermentation. The chosen LA concentration of 5% was previously proved to be effective in enhancing the hydrolysis of phytate in barley (Metzler-Zebeli et al., 2014) and ruminal fermentation of diets including LA-treated barley in vitro (Harder et al., 2015a). Effects of the same LA treatment of concentrates with or without P supplementation on feed intake, metabolic health, and milk production were also tested in a separate longitudinal trial with early lactation dairy cows, and those results were reported elsewhere (Khol-Parisini et al., 2016).

2. Materials and methods

2.1. Animals, diets, and lactic acid treatment

All procedures involving animal handling and treatment were approved by the institutional ethics committee of Vetmeduni Vienna and the national authority according to § 26 of the Law for Animal Experiments, Tierversuchsgesetz 2012, BGBl. I Nr. 114/2012 (permission of animal experiment: BMWFW-68.205/0064-WF/II/3b/2014).

This trial was conducted with 6 ruminally fistulated non-lactating Holstein cows weighing 795 ± 109 kg (mean ± SD). They were kept in a loose-housing stable with straw bedding at the dairy research farm “Kremesberg” of the University of Veterinary Medicine Vienna, Vienna, Austria. The cows were blocked by body weight into two squares, 3 animals in each square were then arranged in a 3 × 3 Latin square design, with 3 dietary treatments (n = 6 per diet) and 3 experimental periods. Diets included a