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Investigation of ammonium lactate supplementation on fermentation end products and bacterial assimilation of nitrogen in dual-flow continuous culture

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ABSTRACT

Supplements investigated throughout the present study are produced by fermenting lactose that is present in whey to lactate, yielding products differing in ammonium relative to lactate concentrations and in physical form (liquid or dry). Trials 1 and 2 investigated Lacto-Whey (LW; Fermented Nutrition Corp., Luxemburg, WI) and GlucoBoost (GB; Fermented Nutrition Corp.), respectively, using dual-flow continuous culture systems (n = 4), each with a 4 × 4 Latin square design. A greater proportion of nonprotein nitrogen was present in GB than in LW. In trial 1, the treatment with LW was isonitrogenously dosed against soybean meal (SBM) as a control (no LW) and factorialized with either a wheat- or corn-based concentrate (55% inclusion rate, dry matter basis). We hypothesized that LW would increase propionate production and that the combination of +LW with wheat would increase bacterial assimilation of NH_3-N into cellular N. No differences were observed for total volatile fatty acid (VFA) production per day. However, treatment × time interactions revealed that +LW increased lactate concentration at 0, 0.5, and 1 h and tended to increase molar percentage of propionate at 1 and 1.5 h postfeeding, documenting the immediate availability of lactate converted to propionate in the +LW treatments. The main effect of corn increased the proportion of bacterial N derived from NH_3-N. Trial 2 was designed to investigate GB; isonitrogenous treatments included an SBM control, crystal GB, liquid GB (LGB), and LGB with yeast culture, which were dosed twice daily. We hypothesized that GB would increase propionate production and bacterial assimilation of NH_3-N; the combination of LGB and yeast culture was expected to have a positive additive effect, yielding the greatest VFA production and bacterial NH_3-N assimilation. No differences were observed for total VFA production; however, LGB decreased molar percentage of acetate and increased propionate and butyrate molar percentages. There were no differences in non-NH_3-N flow or microbial N flow. Under the conditions of our studies, lactate in LW and GB was fermented extensively to propionate, and microbial protein synthesis in these treatments was comparable with that in SBM controls.
Key words: ammonium lactate, rumen fermentation, microbial protein synthesis, continuous culture

INTRODUCTION

With increases expected in the global demand for traditional grains, use of by-products will become increasingly important as a way to decrease feed costs within US animal production systems (Johansson, 2015). Some producers include whey in their dairy rations for added energy and protein; however, supplementing sugars, such as lactose in whey, has yielded inconsistent results, specifically with respect to microbial protein synthesis (Oba, 2011) and animal efficiency of N utilization (Broderick et al., 2008; Penner and Oba, 2009). Compared with feeding raw whey products, fermenting lactose to lactate might help circumvent potential complications by improving stability during storage and lowering transportation costs of a drier product. Moreover, the nutritional quality might be improved because of decreased variation in nutrient concentrations and a more consistently increased yield of gluconeogenic precursors absorbed by the dairy cow.

Lactate is a key intermediate in ruminal fermentation and is metabolized via different pathways, resulting in the production of different VFA. Some strains of *Megasphaera elsdenii* will preferentially utilize lactate over glucose (Weimer and Moen, 2013), producing either propionate via the acrylate pathway or acetate and butyrate via pyruvate. In addition, *Selenomonas*

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ruminantium ssp. *lactilytica* can also utilize lactate, producing acetate, propionate, and succinate (Nagaraja and Titgemeyer, 2007). Protozoa, most notably *Entodinium* spp., also metabolize lactate primarily to butyrate (Brossard et al., 2004). Greater ruminal lactate concentrations in defaunated (protozoa removed) animals suggest that protozoa play a key role in lactate metabolism (Newbold et al., 2015). Increased lactate availability and subsequent utilization by microbes should facilitate cellular carbon conversion into bacterial products and increase the assimilation of NH_3-N into bacterial AA and nucleic acids. For this reason, supplementing lactate and ammonium together has the potential for a positive additive effect.

Probiotics offer potential to modulate lactate accumulation, perhaps explaining why supplemental yeast culture (**YC**) sometimes increases total VFA concentration (Desnoyers et al., 2009). Yeast culture might increase abundance of lactate-utilizing bacteria, such as *M. elsdenii* (Newbold et al., 1996; Callaway and Martin, 1997) and *S. ruminantium* (Martin, 1998), which should increase conversion of lactate to propionate (the key gluconeogenic precursor) or to butyrate (a key fuel and metabolite for the rumen epithelium; Oba, 2011).

We aimed to evaluate the effects of ammonium lactate–based supplements on VFA production and bacterial assimilation of NH_3-N. Trial 1 investigated Lacto-Whey (**LW**; Fermented Nutrition Corp., Luxemburg, WI) from the standpoint of conversion of lactate to VFA with contrasting grain sources (corn or wheat). Lactate is neutralized with NH_3 to maintain a pH of about 5.5 to maximize lactate yield (37% lactate on an as-is basis), with the CP containing about 75 to 77% NH_3-N, 17% whey protein, and 6 to 8% spent *Lactobacillus bulgaricus* cells (Reddy et al., 1976). Although LW was researched decades ago as a substitute for soybean meal in dairy rations, presumably through assimilation into rumen microbial protein (Huber et al., 1976), no direct comparison has been made for microbial N measurements. Renewed interest in fermented whey products has justified the need to study assimilation of LS NH_3-N into microbial N. We hypothesized that LW in combination with the wheat-based diet would increase propionate production while also increasing bacterial assimilation of NH_3-N. Next-generation sequencing was performed to evaluate a potential shift in the bacterial and archaeal communities.

Trial 2 evaluated the effects of feeding GlucoBoost (**GB**; Fermented Nutrition Corp.) as a crystal (**CGB**) or liquid (**LGB**) without and with YC compared with soybean meal (**SBM**). The LGB is similar to LW but with slightly less NH_3-N, whereas the CGB uses Ca in whey to crystallize the ammonium lactate (Marriott, 1985). Because *Lactobacillus bulgaricus* produces L- and potentially considerable (up to 90%) D-lactate (Bernard et al., 1991), we surmised that addition of YC might increase conversion of total lactate, particularly D-lactate, primarily to propionate (via succinate), as did an extract of *Aspergillus oryzae* (Nisbet and Martin, 1993). We hypothesized that GB inclusion would increase propionate production; the combination of liquid GB and YC was expected to have a positive additive effect and yield the greatest VFA production and bacterial NH_3-N assimilation. The combination of both studies provides an opportunity to evaluate lactate metabolism in continuous cultures, which allows direct quantification of net VFA production while maintaining both bacterial and protozoal lactate-metabolizing communities.

MATERIALS AND METHODS

Experimental Design

Trial 1. Rumen fluid was collected and pooled from 2 ruminally cannulated Jersey cows housed at The Ohio State University's Waterman Dairy Center to be used as inocula. Donor cows were fed a typical lactating diet that contained corn silage (58.1%), grain mix (23.2%), alfalfa (14.2%), and whole cottonseed (4.3%); the remaining amount represented mineral supplements to meet or slightly exceed nutrient requirements. All procedures were approved by The Ohio State University Institutional Animal Care and Use Committee. Collected material was immediately squeezed through 2 layers of cheesecloth into prewarmed beverage coolers and maintained at 39°C during transportation back to the laboratory. In less than 40 min from sampling, the inoculum was delivered with an equal part of buffer (totaling half of the 1.71 ± 0.25 L of working volume) into each of the 4 individual fermentation systems that featured a continuous influx of buffer solution, a heating element to maintain the culture at 39°C, and an impeller (50 rpm) to maintain adequate mixing. The buffer solution was made according to Weller and Pilgrim (1974) except that it excluded urea to maintain (and not exceed) desired NH_3-N concentrations (5–10 mg/dL, approximately 4 h postfeeding). Total buffer input and liquid passage rate were maintained at 7%/h; passage rate of solids was 5%/h. Modified filters (50-μm pore size) were used on filtrate pumps to retain protozoa (Karnati et al., 2009).

Fermentors were administered a pelleted diet (60 g of DM/d; 55:45 concentrate:forage diet; Table 1) and their respective treatments (10 mL of slurry) twice daily at 0800 and 2000 h. Diets contained either a