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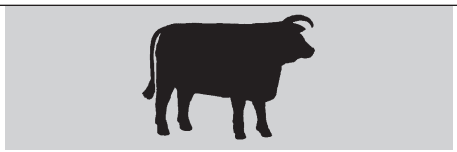

Aspergillus oryzae α-amylase supplementation on rumen volatile fatty acid profile and relative abundance of mRNA associated with nutrient absorption in ruminal and duodenal tissue from beef steers

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ABSTRACT

The objective of this experiment was to investigate the effect of Aspergillus oryzae α-amylase (AAM) supplementation on rumen VFA profile and relative abundance of mRNA associated with nutrient absorption in ruminal and duodenal tissue from beef steers. Nine crossbred beef steers (BW 622 ± 50 kg), fitted with rumen and duodenal fistulas, were used in this experiment. Steers were housed in individual stanchions and fed a high-concentrate finishing diet (6% forage NDF) twice daily for 8 d. Treatments included (1) control (corn meal; n = 5) and (2) AAM (fungal α-amylase 750 units/g; n = 4). Dietary treatment supplements were manufactured before each feeding by mixing 3 g of α-amylase or corn meal into 150 g of dried distillers grains for the morning feeding and 2 g of α-amylase or corn meal into 100 g of dried distillers grains for the afternoon feeding. Supplements were applied as a top dress for every feeding and thoroughly mixed by hand. On d 5, rumen fluid samples were obtained every 4 h for 24 h and analyzed for VFA. On d 9, rumen papillae and duodenal mucosal tissue samples were collected. Total tissue RNA was extracted for real-time PCR analysis. Sodium/potassium ATPase pump α1; glucose transporter 2 and 5; putative anion transporter isoform 1; sodium/hydrogen antiporter isoforms 1, 2, and 3; 3-hydroxy 3-methylglutaryl coenzyme A synthase isoform 2; downregulated in adenoma; monocarboxylate cotransporter isoform 1; and glyceraldehyde-3-phosphate dehydrogenase mRNA were tested. No treatment differences (P > 0.21) were detected for any of the genes analyzed in ruminal or duodenal tissue. Concentrations of VFA and the acetate-to-propionate ratio did not differ (P > 0.22) among treatments. However, the acetate-to-propionate ratio and molar percentages of butyrate were numerically greater (P = 0.17) in AAM steers compared with controls. Under the conditions of this experiment, AAM supplementation had no effect on relative expres-

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sion of mRNA associated with nutrient absorption and minimal effects on molar proportions of VFA.

Key words: duodenum, fungal α-amylase, gene, rumen, steer, volatile fatty acid

INTRODUCTION

Starch from cereal grains is the primary dietary energy source for finishing cattle, representing 50 to 70% of the finishing diet. Amylose and amylopectin comprise the majority of the starch in finishing beef cattle diets (Huntington, 1997). The rate and extent of starch digestion in the rumen have an effect on total-tract starch digestibility and animal performance (Lykos et al., 1997). Rapid and extensive ruminal starch digestion can have negative effects on the rumen microbial ecosystem. Opportunistic microbes may flourish in the rumen of cattle fed high-concentrate diets and may lead to the release of endotoxins (Owens et al., 1998). In addition, rapid starch fermentation could result in acidosis. Understanding the correct balance for optimizing ruminal starch fermentation while avoiding conditions leading to ruminal disturbance is desirable for efficient production in beef cattle.

Direct-fed microbials or probiotics are defined as "a live microbial feed supplement which beneficially affects the host by improving gastrointestinal tract microbial balance" (Heyman and Menard, 2002). Direct-fed microbials for ruminants can be supplied in the form of fungi. *Aspergillus oryzae*, a fungus, contains α-amylase that aids in the digestion of starch (Tricarico et al., 2005). The supplemental fungal α-amylase did not increase ruminal starch digestion but consistently increased the molar proportions of butyrate at the expense of propionate, indicating a shift in starch degrading microbial populations (Tricarico et al., 2005). We hypothesized that *A. oryzae* α-amylase supplementation to high-concentrate diets fed to steers consuming a high concentrate diet would increase microbial production of butyrate and decrease propionate production, resulting in increased expression of nutrient transporter genes in the epithelial wall of the rumen. Therefore, the objective of this experiment was to investigate the effect of *A. oryzae* α-amylase supplementation on VFA profiles in the rumen and relative abundance of genes related to nutrient absorption in ruminal and duodenal tissue from beef steers fed a high concentrate diet based on steam-flaked corn.

MATERIALS AND METHODS

Animal Care

Nine beef steers fitted with rumen and duodenum fistulas were used in this experiment. All animal care and handling described herein were conducted according to the guidelines approved by the Colorado State University Institutional Animal Care and Use Committee. Steers were housed in a climate controlled metabolism building. Individual stanchions were equipped with automatic waterers and feed troughs. Steers were fed a high concentrate finishing diet (6% forage NDF) twice daily for 8 d (Table 1). Treatments included (1) control (**CON**; corn meal, n = 5) and (2) **AAM** (α-amylase: Amaize; 750 fungal amylase units/g, n = 4; Alltech Inc. Nicholasville, KY). Each gram of Amaize contained 750 fungal α-amylase units. Dietary treatment supplements were manufactured before each feeding by mixing 3 g of α-amylase (AAM) or corn meal (CON) into 150 g of dried distillers grains for the morning feeding and 2 g of α-amylase (per animal) or corn meal into 100 g of dried distillers grains for the afternoon feeding. Supplements were applied as a top dress to each feed bunk for every feeding and thoroughly mixed by hand. On d 5, rumen fluid samples were obtained every 4 h for 24 h and analyzed for VFA. On d 9, rumen and duodenal epithelial biopsy samples were collected as described below.

Ruminal Papillae and Duodenal Mucosal Collection

On d 6 and 7, meloxicam (1.0 and 0.5 mg/kg of BW, respectively) was administered to each steer. On d 9, steers were fed 2 h before ruminal and duodenal tissue biopsies. Steers were transported to the veterinary teaching hospital at Colorado State University for the biopsy procedure. Each steer was weighed and given a single s.c. dose of oxytetracycline (200 mg/mL, 20 mg/kg of BW). An 8-mm diameter skin biopsy instrument (Integra Miltex, York, PA) was used to collect approximately 10 rumen papillae pinch biopsies from an area (approximately 100 cm^2) from the rumen caudal wall. Biopsy tissue was immediately rinsed with sterilized phosphate buffered solution (pH 7.4), treated with RNA later solution (Qiagen, Valencia, CA), and snap frozen in liquid N_2. Samples were stored at −80°C. Duodenal mucosal samples were obtained using a sterile medical spatula scraper approximately 100 cm aboral to the pyloric sphincter. Duodenal contents were aspirated, and a medical spatula was inserted into the duodenal fistula parallel to the intestinal lining approximately 15 cm into the duodenum aborally. Gentle pressure was applied with spatula tip to the mucosal lining of the intestine as the spatula was withdrawn. Tissue from the spatula was collected and treated as described for the rumen papillae pinch biopsies.

RNA Extraction and cDNA Synthesis

Total RNA was extracted from ruminal and duodenal tissue samples by homogenizing in Tri Reagent (Sigma, St. Louis, MO). Homogenates were then incubated at room temperature, mixed with chloroform, and incubated at room temperature for an additional 15 min followed by centrifugal separation at 13,000 × *g* force at 4°C for 15 min. The RNA was purified using RNEasy Mini kit (Qiagen) with RNase-free DNase (Qiagen) treatment to remove possible DNA contamina-