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Increasing dietary doses of an *Aspergillus oryzae* extract with alpha-amylase activity on nutrient digestibility and ruminal fermentation of lactating dairy cows

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ABSTRACT

This study was designed to evaluate increasing dietary doses of an *Aspergillus oryzae* extract with alpha-amylase activity on nutrient intake and total tract digestion, sorting index, ruminal fermentation, milk yield and composition, blood metabolites and nitrogen utilization of mid- to late lactating dairy cows. Twenty-four multiparous Holstein cows (162.3 ± 107.9 days in milk, 636 ± 62 kg of live weight, and 31.6 ± 6.5 kg/d of milk yield) were used in a replicated 4 × 4 Latin square design experiment. Eight cows used in the experiment had rumen cannulas to assess ruminal fermentation variables. Periods had 21 days, in which 14 days were allowed for treatment adaptation and 7 days for sampling. Cows were randomly assigned to the following treatments: control (A0), and *Aspergillus oryzae* extract to supply 150,300 or 450 FAU/kg DM. One FAU (fungal amylase unit) is the amount of enzyme which will dextrinize soluble starch at the rate of 1-mg per minute at 30 °C and pH 4.8. Treatments did not affect DM and nutrient intake, as well as the sorting index of cows. Alpha-amylase supplementation linearly increased ($P = 0.031$) crude protein digestibility and tended to linearly increase ($P = 0.060$) DM digestibility. Treatments did not affect ruminal pH, acetate, butyrate, propionate and total branched-chain fatty acids. Alpha-amylase linearly increased ($P = 0.023$) isovalerate production and tended to quadratically affect ($P = 0.065$) ammonia nitrogen concentration in rumen. Milk yield and composition, and efficiency of milk production were not affected ($P \geq 0.275$) by alpha-amylase supplementation. Treatments tended to linearly decrease ($P = 0.061$) N excreted in feces. Treatments linearly increased ($P \leq 0.039$) live weight and body condition score of cows. Finally, alpha-amylase supplementation did not affect ($P \geq 0.234$) serum glucose, urea, and hepatic enzymes concentration. Increasing doses of an *Aspergillus oryzae* extract up to 450 FAU/kg DM did not alter starch digestibility, ruminal propionate production, microbial protein synthesis, and milk yield and composition of mid-lactating cows.

Abbreviations: ADF, acid detergent fiber; aNDF, neutral detergent fiber; BFCA, branched-chain fatty acids; CP, crude protein; DM, dry matter; EC, enzyme commission number; ECM, energy-corrected milk; FAU, fungal amylase unit; FCM, fat-corrected milk; iADF, indigestible acid detergent fiber; LW, live weight; NFC, non-fiber carbohydrate; NH_3-N, ammonia nitrogen; PD, purine derivatives; PD_{abs}, absorbed; SCFA, short-chain fatty acids

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1. Introduction

Starch in a lactating cow diet ranges from 210 up to 300 g/kg DM, and a large proportion of dietary starch is fermented by microorganisms in the rumen; however, substantial amounts of starch can be digested in the small intestine or fermented in the large intestine. Earlier studies found improvements on cattle performance when processed starch sources were supplied to increase ruminal digestion (Theurer et al., 1999). Huntington (1997) described that the ruminal starch digestion is beneficial because it increases the microbial protein duodenal flow. For example, the microbial protein flow increased from 2.2 to 2.8 kg/d when cows were fed starch sources with different ruminal starch degradation rates (Poore et al., 1993).

The addition of amylolytic enzymes to cattle diets may support maximal ruminal digestibility of starch (Tricarico et al., 2008) and there are evidences from *in vitro* and *in vivo* studies that amylolytic enzymes can promote animal productive performance. Amylase from *Bacillus licheniformis* increased the ruminal digestibility of starch from sorghum and corn in lambs (Rojo-Rubio et al., 2005). Tricarico et al. (2005) found a quadratic response in milk production when supplied amylolytic enzymes to dairy cows, with an increase of milk yield up to 1.5 kg/d. However, other studies did not show improvements on nutrient digestibility and animal performance when supplementing alpha-amylase (Weiss et al., 2011; Nozière et al., 2014).

The costs of forage and cereals have been increased during the last years, and farmers are seeking for technologies to enhance the feed conversion efficiency in livestock. In this context, the objective of this study was to determine the influence of increasing dietary levels of an extract derived from *Aspergillus oryzae* culture with amylolytic activity (Amaize®, Alltech Inc., Springfield, KY) on nutrient intake and total tract digestion, sorting index, ruminal fermentation, milk yield and composition, metabolic profile and nitrogen utilization of mid- to late lactating dairy cows. Our hypothesis was that the extract with alpha-amylase activity would enhance ruminal fermentation, microbial protein synthesis and productive performance of cows in a dose dependent manner.

2. Material and methods

The experiment was authorized by the Bioethics Committee of School of Veterinary Medicine and Animal Science, University of Sao Paulo (protocol number: 1746040814).

2.1. Cows and design

Twenty-four multiparous Holstein cows (162.3 ± 107.9 days in milk, 636 ± 62 kg of live weight (LW), and 31.6 ± 6.5 kg/d of milk yield, at the start of experiment) were blocked according to the milk production, days in milk and LW into a replicated 4 × 4 Latin square design experiment. Eight cows used in the experiment had rumen cannulas to assess ruminal fermentation variables. Periods lasted 21 days, in which 14 days were allowed for treatment adaptation and 7 days for sampling. Cows were randomly assigned to the following treatments: control (A0), and supply of 150, 300 or 450 FAU/kg DM of Amaize® (Alltech Inc.; batch: 432715-1). The intermediate dose of Amaize® was recommended by manufacturer and the other doses were based on positive outcomes on milk production of the enzyme product (Tricarico et al., 2005).

The Amaize® consists of an *Aspergillus oryzae* culture extract with known alpha-amylase activity (EC 3.2.1.1; 600 FAU/g of product) commercially available as a brown dry powder. One FAU (fungal amylase unit) is the amount of enzyme which will dextrinize soluble starch at the rate of 1-mg per minute at 30 °C and pH 4.8 (Food Chemicals Codex, 1996). Diets (Table 1) were formulated according to the NRC (2001), the supplement was weighed daily and hand mixed into the concentrate before the morning feeding. Ground corn was obtained after corn grains were milled to passed through 2 mm screen in hammer mill. Diets were supplied as a total mixed ration (TMR, 50:50 at 0700 h and 1300 h) and orts were maintained at 5–10% of feed offered. Cows were housed in individual pens (17.5 m^2 of area), containing individual feed bunks, sand bedding, and forced ventilation.

2.2. Nutrient total tract digestion

Maize silage and ort samples were obtained on a daily basis during each 7-d sampling period and pooled into one sample (2.1 kg) for each period. Concentrate ingredients were collected along with the preparation of the mixture (n = 4). Samples of dietary ingredients, orts and feces (collection is described below) were partially dried in a forced-air oven at 55 °C for 72 h, and ground to pass through a 1 mm screen (Wiley Mill, Arthur H. Thomas, Philadelphia, PA). All samples were assessed for DM (method 930.15), total nitrogen [N × 6.25 = crude protein (CP); method 984.13], and ether extract (method 920.39) according to AOAC (2000). Neutral detergent fiber (aNDF), acid detergent fiber (ADF), and lignin (sulfuric acid method) content of samples were determined as described by Van Soest et al. (1991). Neutral detergent fiber analysis was carried out using α-amylase but no sodium sulfide using a fiber analyzer (TE-149 fiber analyzer, Tecnal Equipment for Laboratory Inc., Piracicaba, Brazil). Starch content of samples was determined by enzymatic degradation (Termamyl® 300L and Amyloglucosidase AMG 300L, Novozymes, Basal, Sweden) and readings performed in a spectrophotometer as described by Bach Knudsen (1997). Net energy for lactation was estimated according to NRC (2001) equations. Non-fiber carbohydrate (NFC) was calculated as follows: NFC = 100 − [(CP − CP from urea + urea) + NDF + ether extract + ash].

Feed intake was determined based on the daily feed offered and refusals. Samples of TMR and refusals of each cow were sampled throughout days 16–19 of each period and assessed for particle size distribution using a particle separator sieves system (Penn State Particle Separator, Nasco, Fort Atkinson, WI, USA). The particle separator used can sort 4 particle fractions (P1 > 19.0, P2 > 8.0, P3 > 4.0 and P4 < 4.0 mm).