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### Effects of dietary fibrolytic enzymes on chewing time, ruminal fermentation, and performance of mid-lactating dairy cows



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#### ABSTRACT

Exogenous fibrolytic enzymes (EFE) can increase ruminal digestion of neutral detergent fiber (NDF) and improve its fermentation for cattle. Twenty-four multiparous Holstein cows  $(33.72 \pm 7.63 \text{ kg milk/d} \text{ and } 176 \pm 82.27 \text{ days in milk} - \text{DIM}$ , at the start of the experiment) were used in a replicated 4 × 4 Latin square experimental design. Increasing doses of a commercial product was used to evaluate the effect of xylanase activity (100 units per gram of product) on intake and total-tract digestion of nutrients, sorting index, chewing time, milk yield and composition, N use, and ruminal fermentation. Treatments consisted of 0, 8, 16 or 24 g/d per cow of EFE product mixed into the concentrate. Corn silage was used as forage source. The basal diet had a forage-to- concentrate ratio of 50:50 (dry matter basis). Even though EFE supplementation had a positive linear effect on dry matter and NDF intake, it had no impact on total-tract digestion of nutrients. Moreover, this enzyme promoted a linear increase of the intake of feed with larger particle size (>19 mm) and quadratically affected rumination and chewing activity (hours), reaching the highest values at intermediate doses (8 g/d and 16 g/d). Treatments had no effect on milk yield and composition; however, the N use efficiency was linearly decreased by EFE, reducing thus the ability to synthesize milk and quadratically decreasing N retention. No treatment effect was found on ruminal pH, whereas a negative guadratic effect on ruminal NH<sub>3</sub>-N concentration was significant. Regarding ruminal parameters, this enzyme supplementation provided linear increased in acetate, propionate, butyrate, and total short-chain fatty acids synthesis. As result, EFE supplementation improved DM and NDF intake, increasing the time spent chewing and ruminating, leading to a greater total short-chain fatty acids production in rumen. Nonetheless, EFE did not improve milk yield and composition of mid-lactating dairy cows. © 2016 Elsevier B.V. All rights reserved.

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*Abbreviations:* aADF, acid detergent fiber; aNDF, neutral detergent fiber; BFCA, branched-chain fatty acids; CP, crude protein; DM, dry matter; EE, ether extract; FCM, fat corrected milk; iADF, indigestible acid detergent fiber; N, nitrogen; NH<sub>3</sub>-N, ammonia nitrogen; SD, standard error of the mean; SCFA, short-chain fatty acids.

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### 1. Introduction

Ruminants are able to use nutrients from the digestion of fibrous material, and convert them into products for human nutrition (Burns, 2008). However, forage digestion is lower and slower than the digestion of other carbohydrates, limiting nutrient intake and performance of dairy cows (Yang et al., 2000; Mertens and Huhtanen, 2007). In addition, forages produced in Brazil, notably corn silages, have higher neutral detergent fiber (NDF) content and lower digestibility whether compared to those from temperate climate countries (545 g/kg vs. 450 g/kg, respectively; Valadares Filho et al., 2016; NRC, 2001). The relatively low digestion of forage coupled with the NDF contents of Brazilian corn silages may impair milk production efficiency, thus being necessary to explore ways to enhance fiber digestion (Varga and Kolver, 1997; Vicini et al., 2003; Allen and Piantoni, 2014).

The addition of exogenous fibrolytic enzymes (EFE) to dairy cow diets aims to increase ruminal enzyme activity and fiber digestion, to improve animal performance (Morgavi et al., 2000; Meale et al., 2014). Dehority and Tirabasso (1998) suggested that the enzyme binding sites in fiber substrates might not be fully occupied by ruminal microorganisms and, thus, the EFE supplementation could led to rise in NDF degradability. Beauchemin et al. (2003a,b) reviewed a dietary supplementation with EFE to dairy cows and observed increases in dry matter intake  $(1.0 \pm 1.3 \text{ kg/d})$  and in milk yield  $(1.1 \pm 1.5 \text{ kg/d})$ . However, there have been reported inconsistent findings regarding the effect of such enzyme supplementation on nutrient digestion, metabolism and performance of dairy cows (Yang et al., 2000; Beauchemin et al., 2000; Reddish and Kung, 2007; Arriola et al., 2011).

Given the variability of literature findings allied to the relatively low digestibility of corn silage produced in Brazilian dairy farms, this study aimed to evaluate increasing dietary levels of EFE on intake and total-tract digestion of nutrients, sorting index, chewing time, milk yield and composition, N use, and ruminal fermentation in mid-lactating cows.

#### 2. Material and methods

The experimental procedures were approved by the Bioethics Committee of the School of Veterinary Medicine and Animal Science, University of São Paulo (approval number: 7066120214).

#### 2.1. Animals, experimental design and treatments

Twenty-four multiparous Holstein cows  $(33.72 \pm 7.63 \text{ kg milk/d}$  and  $176 \pm 82.27 \text{ days}$  in milk, mean  $\pm$  SD) were distributed into a replicated  $4 \times 4$  Latin square experimental design in which two squares consisted of rumen-cannulated cows used for ruminal fermentation assessment, beyond the other parameters evaluated. The animals were housed in individual pens  $(17.5 \text{ m}^2)$ , with sand bedding, feed bunks and forced ventilation. Experimental periods consisted of 21 d, within which the first 14 d were for treatment adaptation and the last 7 d for data collection. Animals were randomized to receive 0, 8, 16 or 24 g/d per cow of Fibrozyme (Alltech, Nicholasville, KY, USA; batch: 417990-2). This product is an extract from *Trichoderma longibarachiatum* fermentation with a minimum of 100 IU of xylanase activity per gram of product. According to the manufacture's directions, Fibrozyme consists of a dry mixture of inactivated yeast, dry brewery yeast, yucca extract and soluble extract of *Trichoderma longibarachiatum* fermentation. The EFE was daily supplied in the form of a brown dry powder hand-mixed into the concentrate before morning feeding. Total Mixed Ration (TMR) was prepared twice daily and supplied to the animals in equal amounts at 0700 h and 1300 h. Table 1 shows the diet composition that was formulated according to the NRC (2001) recommendations.

#### 2.2. Nutrient intake

Feed offered and refusals were weighed daily to estimate feed intake and restrict orts to 50-100 g/kg of total offered food (on as-fed basis). Throughout the sampling period, ingredients were collected during the concentrate preparation (4 samples, one per period); silage and ort samples of each cow were taken daily to provide a composite sample. Immediately after collections, samples were stored at  $-20 \,^{\circ}$ C and later dried in a forced air oven at  $55 \,^{\circ}$ C for 72 h and ground in 1-mm or 2-mm screen Willey mill (MA340, Marconi, Piracicaba, Brazil).

Dry matter (DM, AOAC 950.15), ether extract (EE, AOAC 920.39) and total N (AOAC, 984.13) contents were analyzed in all samples according to the methods described by AOAC (2000). The contents of NDF and ADF were estimated according to the methods described by Van Soest and Mason (1991). The aNDF analysis was performed using  $\alpha$ -amylase without sodium sulphide (TE-149 fiber analyzer, Tecnal Equipment for Laboratory Inc., Piracicaba, Brazil). Total digestible nutrient was calculated according to NRC (2001): TDN<sub>1X</sub> = tdNFC + tdCP + (tdEE × 2.25) + tdNDF – 7, wherein tdNFC is the total digestible non-fiber carbohydrate, tdCP is the total digestible crude protein, tdEE is the total digestible EE, and tdNDF is the total digestible NDF. Non-fiber carbohydrate was calculated according to Hall (2000) in which: NFC = 100 – (CP + NDF + EE + ash) and net energy of lactation (NEL) was calculated according to Weiss et al. (1992): NEL (Mcal/kg) = 0.0245 × TDN (%) – 0.12, using 1 as processing factor.

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