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Original Research

In Vitro Assessment of Fecal Inocula From Horses Fed on High-Fiber Diets With Fibrolytic Enzymes Addition on Gas, Methane, and Carbon Dioxide Productions as Indicators of Hindgut Activity

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ABSTRACT

The aim of this study was to assess the effect of fecal inocula from horses fed on concentrate (restricted amount daily) and oat straw (*ad libitum*) supplemented with fibrolytic enzymes on in vitro hindgut activity. Cellulase (CE), xylanase (XY), and CE + XY (1:1 vol/vol; CX) were tested at three levels (μL/g dry matter [DM]): 0, 1, and 3, in addition to control without enzyme addition. Fecal inocula were collected from 16 Quarter Horse mares supplemented with enzyme at 0 (without enzyme), or fed 5-mL enzyme/mare/d of CE (FCE), XY (FXY), or CE + XY (1:1 vol/vol; FCX) for 15 days. The fecal content mixed with the culture media were used for incubation in bottles containing 1-g DM of substrate (a mixture of concentrate and oat straw [1:1 DM]). Gas (GP), methane (CH_4), and carbon dioxide productions were measured at 2, 4, 6, 8, 10, 12, 24, and 48 hours after incubation. Interactions occurred ($P < .05$) between fecal source × enzyme product for the asymptotic GP, the rate of GP, CH_4 production, and fermentation kinetic parameters. Moreover, interactions were observed ($P < .05$) between fecal source × enzyme product × enzyme dose for the rate of GP, CH_4 production, and DM digestibility. Xylanase at 3-μL/g DM with FXY fecal increased ($P < .05$) the asymptotic GP, short-chain fatty acids, and microbial protein productions with lowering ($P < .05$) partitioning factor. At 24 and 48 hours and without enzyme, FCX and FXY, had the highest ($P < .05$) CH_4 production. It can be concluded that XY enzyme at 3-μL/g DM was the most effective compared with other treatments.

1. Introduction

Feeding horses on fibrous diets is important to overcome some feeding disorders such as gastric ulceration, hindgut acidosis, laminitis, and colic associated with high-starch diets [1]. Such disorders could impair the fibrolytic activity in the horse's hindgut and cause microbial profile disturbance with the proliferation of *Streptococcus bovis* as the dominant microbe causing a reduced energy yield of the fed diet [2] and reducing whole-diet digestibility. However, fibrous feeds are characterized by poor palatability, high lignocellulose content, low nutrient digestibility, and low crude protein (CP) content [3,4].

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Feeding horses a minimum of 1% of their body weight (BW) as fibers can minimize occurrence of such disorders [5]. Oat straw is one of the most common agriculture residues in Mexico with low nutritive value as low protein content and low nutrients digestibility and with about 11.2 million of tones produced during 2013. Therefore, there is a need to develop feeding strategies that meet the energy requirements of the horse fed high-fiber diets and maintain gut health and integrity [6]. For an effective utilization of fibrous feeds, exogenous fibrolytic enzymes have been used to improve carbohydrate and cell wall degradation in ruminants [7,8] and in equines [9].

In ruminants, supplementing diets with fibrolytic enzymes has been shown to improve feed utilization and animal performance [10,11]. Supplementing the diet of horses with exogenous fibrolytic enzymes has gained substantial interest in recent years [9,12]. Because the large intestine in the horses is a fermentation system similar to the rumen [13], improvements in feed utilization and animal performance should be expected with horses with fibrolytic enzymes supplementation. In the rumen of ruminants and in the cecum of equines, living microorganisms give them the ability to break down fibers to meet their energy demands. Consequently, the application of exogenous enzymes to fibrous feeds may help release starches, sugars, proteins, vitamins, and minerals for digestion and absorption in the small intestine [14]. However, the potential of exogenous enzymes to enhance the digestion of fibers in the hindgut of the equine is inconclusive. Salem et al [9] observed in vivo improved neutral detergent fiber (NDF) and acid detergent fiber (ADF) digestion of oat straw when mares were fed fibrous diet supplemented with fibrolytic enzymes. In contrast, Murray et al [12] reported a significant reduction in in vivo digestibility of the fibrous fractions of enzyme-treated diets.

Therefore, the aim of the present study was to assess the effect of fecal inocula from horses supplemented with exogenous fibrolytic enzymes in diets on in vitro total gas (GP), methane (CH_4), and carbon dioxide (CO_2) productions as indicators of hindgut activity of a diet containing 50% oat straw.

2. Materials and Methods

2.1. Substrate and Enzyme Products

A basal diet consisting of a mixture of concentrate and oat straw (1:1 dry matter [DM]) was used as the substrate for the incubations. The concentrate portion contained 50% commercial concentrate (Pell Roll Cuarto de Milla, Mexico) and 50% wheat bran which contained (g/kg DM) the following: organic matter (OM): 901.8, CP: 112.0, NDF: 511.0, and ADF: 202.8. The chemical composition (g/kg DM) of the oat straw was as follows: OM: 929.4, CP: 26.7, NDF: 668.7, and ADF: 405.0.

Cellulase plus (CE) and xylanase plus (XY) (Dyadic PLUS; Dyadic international, Inc, Jupiter, FL, USA) were used. The enzyme activities of the enzyme products were assayed for endoglucanase and XY activity as described by Robyt and Whelan [15]. The CE product contained 30,000 to 36,000 U of CE/g and 7,500 to 10,000 U of beta-glucanase/g. The XY product contained 34,000 to 41,000 U of XY/g, 12,000 to 15,000 units of beta-glucanase/g, and 45,000 to 55,000 U of CE/g.

2.2. In Vitro Fecal Incubations

Before the start of the experiment, fecal contents (i.e., the inoculum source) were collected from 16 Quarter Horse mares (450 to 500 kg BW; aged 10 to 12 years) used in the in vivo experiment of Salem et al [9] offered the same basal diet of a mixture of concentrate (restricted amount daily) and oat straw (*ad libitum*) at 1:1 DM that was used as a substrate for the in vitro incubations as described previously. However, the mares consumed the offered concentrates and oat hay at about 2:1 DM, respectively. The mare's daily diets were supplemented with CE, XY, or CE + XY (1:1 vol/vol; CX) at 5 mL/mare/d for 15 days.

Four composited fecal contents samples, collected from the rectum of each mare before the morning feeding on the last day (i.e., day 15), were used for the in vitro incubation. About 10% of individual fecal samples of each mare within each treatment were mixed and homogenized to obtain a homogenized sample of feces of each treatment. The four fecal treatments were compared in the presence of three levels of each enzyme product: fecal from mares fed control diet without enzyme addition (FCO) and without enzyme addition (EP0) before incubation, fecal from mares fed CE (FCE) and with CE addition before incubation, fecal from mares fed XY (FXY) and with XY addition before incubation, or fecal from mares fed CE + XY at 1:1 vol/vol (FCX) and with CE + XY (1:1 vol/vol) addition before incubation. With the exception of the preparation of the microbial inocula, the method of Theodorou et al [16] was used to measure GP. Briefly, a subsample of the composite fecal contents of each treatment was mixed with the Goering and Van Soest [17] buffer solution without trypticase in the ratio of 1:4 vol/vol. The incubation media were mixed and strained through four layers of cheesecloth into a flask with an O_2-free headspace. The fecal content mixed with the culture media was used to inoculate three identical runs of incubation in bottles containing 1-g DM of substrate (a mixture of concentrate and oat straw [1:1 DM]). Oat straw and concentrates were separately grounded through a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA) using a 2-mm screen and then mixed together before the incubation.

A total number of 252 bottles (three fecal sources × three enzyme doses [per gram DM: 0, 1, and 3 μL] × three enzyme products × three replicates × three runs + [three replicates of control × three runs]) plus three bottles without substrate and enzyme as blanks. After bottles filling, they were flushed with CO_2 and immediately closed with rubber stoppers, shaken, and placed in an incubator set at 39°C. Gas, CH_4, and CO_2 productions were recorded at 2, 4, 6, 8, 10, 12, 24, and 48 hours after inoculation. Gas production was recorded using the pressure reading technique (Extech instruments, Waltham, MA, USA) of Theodorou et al [16], whereas the CH_4 and CO_2 productions were recorded using a Gas-Pro detector (Gas Analyzer Crowcon, Model Tetra3, Abingdon, UK). At the end of incubation after 48 hours, bottles were uncapped and the pH was measured using a digital pH meter (Conductronic pH15, Puebla,