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"*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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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

This study was aimed 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 v/v; CX) were tested at three levels (µL/g DM): 0, 1 and 3, in addition to control without enzyme addition (EP0). Fecal inocula were collected from 16 Quarter Horse mares supplemented with enzyme at 0 (FCO-without enzyme), or fed 10 ml enzyme/mare/day of CE (FCE), XY (FXY) or CE+XY (1:1 v/v; 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 (CO_2) productions were measured at 2, 4, 6, 8, 10, 12, 24 and 48 h post-incubation. Interactions occurred ($P<0.05$) between fecal type × enzyme product for the asymptotic GP, the rate of GP, CH_4 production, and fermentation kinetic parameters. Moreover, interactions were observed ($P<0.05$) between fecal type × 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 ($P<0.05$) the asymptotic GP and calculated fermentation parameters. At 24 h and 48 h and without enzyme, FCX and FXY, respectively, had the highest ($P<0.05$) CH_4 production. It can be concluded that xylanase enzyme at 3 µL/g DM was the most effective compared to other treatments.

Keywords: Fibrolytic enzymes, fecal inoculum, *in vitro* gas production, methane production.

Running head: *Fibrolytic enzymes and in vitro fecal fermentation*

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 and low nutrient digestibility, and low crude protein (CP) content [3,4] feeding horses a minimum of 1% of their 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 walls degradation in ruminants [7,8] and in equines [10].

In ruminants, supplementing diets with fibrolytic enzymes has been shown to improve feed utilization and animal performance [9,11]. Supplementing the diet of horses with exogenous fibrolytic enzymes has gained substantial interest in recent years [10,12]. 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 breakdown 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. [10] 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 current study was to assess the effect of fecal inocula from horses supplemented with exogenous fibrolytic enzymes in diets on *in vitro* total gas, 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 DM) was used as the substrate for the incubations. The concentrate portion contained 50% commercial concentrate (Pell Rol® Cuarto de Milla®, Mexico) and 50% wheat bran which contained (g/kg DM): 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: OM: 929.4, CP: 26.7, NDF: 668.7 and ADF: 405.0.

Celluase® 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 xylanase activity as described by Robyt and Whelan [15]. The CE product contained 30,000 to 36,000 units of cellulase/g and 7,500 to 10,000 units of beta-glucanase/g. The XY product contained 34,000 to 41,000 units of xylanase/g, 12,000 to 15,000 units of beta-

glucanase/g and 45,000 to 55,000 units of cellulase/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 (450-500 kg BW; 10-12 years of age) used in the experiment of Salem et al [10] offered the same basal diet of a mixture of concentrate (restricted amount daily) and oat straw (*ad libitum*) (1:1 DM) that was used as substrate for the *in vitro* incubations as described above. 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 v/v; CX) at 10 ml/mare/day 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: fecal from mares fed control diet without enzyme addition (FCO), fecal from mares fed CE (FCE), fecal from mares fed XY (FXY) or fecal from mares fed CE + XY at 1:1 v/v (FCX). With the exception of the preparation of the microbial inocula, the method of Theodorou et al [16] was employed to measure gas production (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 v/v. 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 (3 fecal types × 3 enzyme doses (/g DM): 0 μL, 1 μL and 3 μL) × 3 enzyme products × 3 replicates × 3 runs + 3 replicates of control × 3 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 ºC. Gas, CH_4 and CO_2 productions were recorded at 2, 4, 6, 8, 10, 12, 24 and 48 h after inoculation. Gas production was recorded using the pressure reading technique (Extech instruments, Waltham, USA) of Theodorou et al [16], while 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 h, bottles were uncapped and the pH was measured using a digital pH meter (Conductronic pH15, Puebla, Mexico). The content of each bottle was then filtered under vacuum through glass crucibles with a sintered filter (coarse porosity no. 1, pore size 100 to 160 μm; Pyrex, Stone, UK) and fermentation residues dried at 65 °C for 72 h to estimate DM disappearance (DMD).

2.3. Calculations and Statistical Analyses

To estimate kinetic parameters of GP, gas volumes (ml/g DM) were fitted using the NLIN procedure of SAS [18] according to France et al [19] model as:

$$y = A \times [1 - e^{-c\,(t-L)}]$$

where y is the volume of GP at time t (h); A is the asymptotic GP (ml/g DM); c is the fractional rate of fermentation (/h), and L (h) is the discrete lag time prior to any gas is released.

Metabolizable energy (ME, MJ/kg DM) and *in vitro* OM digestibility (OMD, %) were estimated according to Menke et al [20] as:

ME (MJ / kg DM) = 2.20 + 0.136 GP + 0.057 CP

OMD (%) = 14.88 + 0.889 GP + 0.45 CP + 0.0651 XA

where: DM dry matter, CP, crude protein in percent; XA, ash in percent; and GP, the net GP in mL from 200 mg dry sample after 24 h of incubation.

The partitioning factor at 24 h of incubation (PF_{24}), as a measure of fermentation efficiency, was calculated as the ratio of *in vitro* DMD (mg/g DM) to the volume of gas (mL) produced at 24 h (*i.e*., DMD/total GP (GP_{24}) according to Blümmel et al [21].

Gas yields (GY_{24}) were calculated as the volume of gas produced after 24 h (mL gas/g DM) of incubation divided by the amount of DMD (g) as:

Gas yields (GY_{24}) = mL gas per g DM/ g DMD

Short chain fatty acids (SCFA) were calculated according to Getachew et al [22] as:

SCFA (mmol/200 mg DM) = 0.0222 GP - 0.00425

where: GP is 24 h net GP (mL/200 mg DM).

Microbial crude protein (MCP) production was calculated according to Blümmel et al. [21]:

MCP (mg/g DM) = mg DMD-(mL gas $\times$ 2.2 mg/mL)

where 2.2 mg/mL is a stoichiometric factor that expresses mg of C, H and O required for the SCFA gas associated with production of one mL of gas [21].

The data was analyzed with fecal type as a random effect and yeast product and doses as fixed effects using PROC MIXED procedure of SAS [18] in a randomized block design. Data of

each of the three runs for each treatment were averaged before the statistical analysis and the mean of each individual sample was considered the experimental unit. The statistical model was:

$$Y_{ijkl}=\mu+ F_i+ Z_j + D_k+ (F*Z)_{ij} + (F*Z*D)_{ijk} +E_{ijkl}$$

where: Y_{ijkl}= is every observation of the *i*th fecal type (F_i) when incubated in the *j*th enzyme product (Z_j) and *k*th enzyme dose (D_k); μ is the general mean; F_i is the fecal type effect; Z_j is the enzyme product effect; D_k is the effect of enzyme dose; $(F*Z)_{ij}$ is the interaction between fecal type and enzyme product; $(F*Z*D)_{ijk}$ is the interaction between fecal type, enzyme product and enzyme dose; E_{ijkl} is experimental error. Linear and quadratic polynomial contrasts were used to examine responses in GP to increasing levels of the enzyme products. Tukey's test was used for the multiple comparisons of means.

3. Results

3.1. Fecal In Vitro Gas Production

There were interactions ($P<0.05$) between fecal type and enzyme product for the asymptotic GP, the rate of GP and GP at 2, 4, 6, 8 and 10 h after incubation. Moreover, three-way interactions were observed ($P<0.05$) between fecal type × enzyme product × enzyme dose for the rate of GP, and GP at 2 h and 4 h of incubation. Compared to the control treatment (FCO fecal and without enzyme addition), XY addition at 3 μL/g DM with FXY inoculum increased ($P<0.05$) the asymptotic GP and GP until 8 h of incubation. Enzymes addition had no effects ($P>0.05$) on the rate of GP and lag time (Table 1).

3.2. Methane and Carbon Dioxide Production

Interactions were observed ($P<0.05$) between fecal type × enzyme product, and between fecal type × enzyme product × enzyme dose at 10 h, 12 h, 24 h and 48 h of incubation. No CH_4 was produced during the first 8 h of incubation. Methane production started at 10 h of incubation without significant effect ($P>0.05$) for enzymes or fecal at 10 h and 12 h of incubation. At 24 h of incubation, FCX inoculum without enzyme had the highest CH_4 production ($P=0.020$), while FXY inoculum without enzyme addition had greater ($P=0.040$) CH_4 production at 48 h of incubation compared to other treatments (Table 2).

There was no interaction observed ($P>0.05$) between fecal type × enzyme product or between fecal type × enzyme product × enzyme dose for CO_2 production throughout incubation hours. Enzyme addition had no effect ($P>0.05$) on CO_2 production throughout incubation hours (Table 3).

3.3. Fermentation Profile

There were interaction ($P<0.05$) between fecal type × enzyme product for pH, ME, DMD, SCFA, PF_{24}, MCP and GY_{24}. Three-way interaction occurred ($P=0.014$) between fecal type × enzyme product × enzyme dose for DMD. Addition of XY enzyme at 1 μL/g DM linearly increased DMD ($P=0.026$) with FXY inoculum. Addition of XY enzyme at 3 μL/g DM quadratically increased SCFA production ($P=0.043$) and MCP production ($P=0.039$) with FXY inoculum. The XY treatment had the lowest PF_{24} ($P=0.033$) compared to other treatments. Enzyme treatments had no effect ($P>0.05$) on pH, ME, OMD and GY_{24} (Table 4).

4. Discussion

The *in vitro* fermentation technique is a simple, powerful and sensitive screening tool for evaluating substrate fermentation and for testing the efficacy of feed additives. Like in ruminants, the technique can be used for studying the nutritive value of equine diet using either cecal contents or feces as a source of inoculum [13,23]. The use of feces as the source of microbial inoculum for *in vitro* fermentation has proved to be a successful alternative source of microbial inoculum in equine studies [13,22].

Agazzi et al [24] have showed that the average mean retention time for feed passing through the gut of the horse ranges between 36-38 h; however, in the current *in vitro* study, incubations were extended to 48 h. Addition of CE or XY resulted in inconsistent fermentation kinetics and GP results probably due to the enzyme activities and the diets of inoculum donor animals [25,26].

4.1. In Vitro Fecal Gas Production

The occurrence of interactions between fecal type and enzyme product suggests that the asymptotic GP, the rate of GP and gas volumes are fecal type and enzyme product dependent. The fermentation of the diet depends on many factors including the diet and nutrient availability for inocula microorganisms during fermentation [10,23]. Availability of nutrients for inocula activity and growth will stimulate the degradability of different nutrients [23].

Xylanase addition at 3 μL/g DM increased GP without affecting the lag time or the rate of GP, which suggests a stimulated fecal fermentation. As GP is closely correlated with the amount of feed fermented, these findings suggest that XY enzyme could degrade some cell wall constituents and facilitate the access of fecal microorganisms [27]. Fibrolytic enzyme (e.g. XY

enzyme) can stimulate fibrolytic and non-fibrolytic bacteria due to release of carbohydrates from feeds that are readily utilized by the bacteria [28]. Addition of fibrolytic enzymes facilitates the access of microorganisms to feed components enabling a faster microbial growth [27]. In their study, Mao et al [25] observed that addition of XY enzyme increased the numbers of total bacteria and *Fibrobacter succinogens* in the incubation medium and improved *in vitro* fermentation. Different GP with different enzyme doses, support the hypothesis that a suitable enzyme level could improve the fermentation of feeds during the initial stages of fiber digestion [10].

4.2. Methane and Carbon Dioxide Production

Enzyme addition had no effect on CO_2 production throughout the incubation. However, some interactions between fecal type × enzyme product × enzyme dose were observed. Interaction occurrence shows that CH_4 production is fecal type, enzyme product and enzyme dose dependent. To our knowledge, very few number of experiments studied the effect of the effect of fibrolytic enzymes on CH_4 production from equines compared to ruminants [10]. Fermentation of dietary carbohydrates produces mainly acetate, propionate, butyrate and gases of H, CO_2 and CH_4, with different proportions at different incubation times. In the current study, CH_4 started to be produced at 10 h of incubation with rapid increase to reach its peak concentration at the end of incubation, while gases started early at the beginning of the incubation which reflects the nature of produced gases during fermentation. Methane production for horses are between those for pigs and ruminants by the methanogenic *Archaea*, which represent the main hydrogenotrophic microbial community [29], with about 3-4% of the digestible energy or 2-3% of the gross energy intake. Salem et al [10] showed that CE and XY enzymes at 2 mL/g DM of the same substrate

used in the current study decreased CH_4 production, whereas CE + XY mixture (1:1 v/v) increased its production at 48 h.

Fecal of FCX or FXY and without enzyme addition increased CH_4 production compared to other treatments, even with enzyme addition. This means that within each treatment, the enzyme addition reduced CH_4 production. Methane production depends on the quality of the diet fed. Feeding highly fibrous diets produces greater CH_4 than when fed better quality forages [26]. This reflects expected better feed utilization with addition of enzyme to the mare's diet. Agazzi et al [24] showed that the mechanisms involved in the digestion and fermentation of plant cell wall component are very similar in both ruminants and equines; therefore, the probable mode of action in the ruminant may be applied to horses. Decreased CH_4 production may be due to affected acetogens with enzyme addition, to compete or to co-metabolize H_2 for other process than its utilization with methanogens thereby, reducing CH_4 formation and emissions [30]. Decreased CH_4 can refer to decreased acetate and increased propionate production resulting in reduced loss of energy to the host [30]. Reddish and Kung [31] have shown that supplementing fiber degrading enzymes in animal diets may improve feed utilization by enhancing fiber degradation and reducing CH_4 production per unit of animal by-products [28].

4.3. Fermentation Kinetic parameters

Fermentation parameters of pH, ME, DMD, SCFA, PF_{24}, MCP and GY_{24} were fecal type and enzyme product dependent as interactions were observed. Xylanase addition increased DMD. The increased DM digestion may be related to enhanced attachment and colonization to the plant cell wall material by rumen microorganisms [28]. A synergism interaction between the endogenous and the exogenous enzymes applied has been considered as the most likely mode of

action [32]. Salem et al [10] stated that the addition of CE, XY and CE + XY (the same preparations used in the current study) improved DMD of diets containing 50% oat straw *in vitro*.

Increased SCFA and MCP productions were obtained with XY addition. The increased SCFA concentrations could be associated to an improved digestion of structural carbohydrates [23]. Tang et al [26] observed increased concentrations of SCFA due to enzymatic treatments for maize stover, rice straw and wheat straw.

Improved fermentation kinetic can be explained based on increased *in vitro* cecal MCP production as a result of enzyme supplementation, which affected positively and modified microbial population of the digestive system and increased DM digestibility that help stimulate and increase the growth of cecal and colon bacteria. Partitioning factor is an index of the distribution of truly degraded substrate between microbial biomass and fermentation end products. The decreased PF with enzymes addition reflects less substrate converting into microbial biomass [23].

Enzyme had no effect on pH, which could be due to the very high buffering capacity of the *in vitro* fermentation processes because 4 parts of buffer solution were added to 1 part diluted fecal fluid [6].

5. Conclusions

Addition of xylanase at 3 μL/g DM resulted in greater gas production and improved fermentation kinetics. However, more studies are warranted to delineate the interactions between

fecal type and different enzyme products at different doses on nutritive value and fermentation kinetics of mare's diet.

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Table 1

In vitro fecal gas kinetics and cumulative gas production (GP) after 48 h of incubation as affected by fibrolytic enzymes addition

Fecal type (FT)	Enzyme product (EP)	Dose (D) µL/g	GP parameters			*In vitro* GP (mL/g DM) at:							
			A (mL/g DM)	*c* (/h)	*L* (h)	2 h	4 h	6 h	8 h	10 h	12 h	24 h	48 h
FCO	EP0	0	292.1	0.062	0.99	34.0	64.0	90.5	113.9	134.5	152.8	225.4	276.6
FCE	CE	0	340.7	0.048	1.21	30.9	59.0	84.5	107.7	128.8	147.9	231.2	305.0
		1	341.3	0.041	1.80	26.5	50.9	73.3	93.9	112.9	130.4	209.9	288.9
		3	346.6	0.043	1.42	29.4	56.3	80.9	103.5	124.2	143.1	228.2	311.0
FCX	CX	0	249.9	0.069	1.24	31.8	59.5	83.6	104.7	123.0	139.1	200.4	239.9
		1	276.3	0.057	1.53	29.6	56.0	79.6	100.7	119.4	136.2	205.0	257.7
		3	276.5	0.063	1.26	32.4	60.9	86.1	108.4	128.0	145.3	214.2	262.3
FXY	XY	0	358.0	0.041	1.45	27.7	53.3	76.8	98.4	118.4	136.8	220.5	303.9
		1	384.6	0.061	1.12	32.8	61.8	87.4	110.0	130.0	147.7	218.3	268.7
		3	396.0	0.074	1.58	42.0	78.3	109.6	136.5	159.8	179.9	254.0	297.2

SEM	17.88	0.0045	0.312	2.33	4.29	5.93	7.30	8.44	9.39	12.50	14.21
P value											
Doses:											
Linear	0.012	0.053	0.606	0.032	0.037	0.043	0.050	0.059	0.069	0.165	0.541
Quadratic	0.235	0.355	0.572	0.116	0.119	0.122	0.124	0.127	0.129	0.139	0.159
FT×EP	0.001	0.001	0.057	0.010	0.015	0.023	0.034	0.049	0.067	0.172	0.621
FT×EP×D	0.074	0.002	0.396	0.030	0.042	0.060	0.085	0.118	0.161	0.573	0.485

A, asymptotic gas production; *c*, rate of gas production; CE, cellulase; CX, cellulase + xylanase (1:1); D, Dose; DM, dry matter; EP, enzyme product; FCO, fecal from horses fed control diet; FCE, fecal from horses fed cellulase; FCX, fecal from horses fed cellulase + xylanase (1:1); FT, fecal type; FXY, fecal from horses fed xylanase; GP, gas production; *L*, initial delay before gas production begins; XY, xylanase.

Table 2

In vitro fecal methane production after 48 h of incubation as affected by fibrolytic enzymes addition

Fecal type (FT)	Enzyme product (EP)	Dose (D) µL/g	*In vitro* methane production (mL/g DM) at:							
			2 h	4 h	6 h	8 h	10 h	12 h	24 h	48 h
FCO (control)	EP0 (without enzyme)	0	0.00	0.00	0.00	0.00	0.00	0.00	1.04	1.52
FCE	CE	0	0.00	0.00	0.00	0.00	0.00	0.14	0.83	1.09
		1	0.00	0.00	0.00	0.00	0.18	0.64	0.80	0.80
		3	0.00	0.00	0.00	0.00	0.32	0.84	1.19	2.92
FCX	CX	0	0.00	0.00	0.00	0.00	0.12	0.12	2.95	3.71
		1	0.00	0.00	0.00	0.00	0.00	0.00	1.11	4.13
		3	0.00	0.00	0.00	0.00	0.20	0.20	1.00	2.50
FXY	XY	0	0.00	0.00	0.00	0.00	0.33	0.98	2.27	7.47
		1	0.00	0.00	0.00	0.00	0.11	0.36	0.92	3.09
		3	0.00	0.00	0.00	0.00	0.25	0.25	2.75	4.26
			0.00	0.00	0.00	0.00	0.00	0.14	0.83	1.09

SEM	0.0000	0.0000	0.0000	0.0000	0.062	0.051	0.039	0.049
Doses:								
Linear	1.000	1.000	1.000	1.000	0.427	0.658	0.579	0.774
Quadratic	1.000	1.000	1.000	1.000	0.363	0.914	0.020	0.040
FT×EP	1.000	1.000	1.000	1.000	0.007	0.030	<0.001	<0.001
FT×EP×D	1.000	1.000	1.000	1.000	0.010	0.038	0.009	0.005

CE, cellulase; CX, cellulase + xylanase (1:1); D, dose; DM, dry matter; EP, enzyme product; FCO, fecal from horses fed control diet; FCE, fecal from horses fed cellulase; FCX, fecal from horses fed cellulase + xylanase (1:1); FT, Fecal type; FXY, fecal from horses fed xylanase; XY, xylanase.

Table 3

In vitro fecal carbon dioxide production after 48 h of incubation as affected by fibrolytic enzymes addition

Fecal type (FT)	Enzyme product (EP)	Dose (D) μL/g	*In vitro* carbon dioxide production (mL/g DM) at:							
			2 h	4 h	6 h	8 h	10 h	12 h	24 h	48 h
FCO (control)	EP0 (without enzyme)	0	0.00	11.2	44.4	77.0	110.2	130.6	181.4	233.7
FCE	CE	0	15.0	31.4	72.0	89.2	139.1	159.7	209.3	276.0
		1	30.8	59.8	77.3	112.6	162.5	197.8	263.8	296.4
		3	48.3	68.8	72.8	122.7	172.6	222.5	294.2	330.5
FCX	CX	0	26.6	40.5	73.8	93.2	143.1	163.5	259.6	318.1
		1	40.8	42.7	89.7	132.7	182.6	194.9	261.9	320.5
		3	36.5	36.5	84.9	122.4	172.2	205.7	256.7	317.8
FXY	XY	0	25.1	28.1	43.2	89.7	139.6	189.5	244.5	309.9
		1	8.9	11.4	51.3	88.5	135.4	154.8	200.3	253.8
		3	9.4	31.1	47.7	58.8	105.7	126.2	207.7	262.4

SEM	3.08	4.00	14.00	12.12	28.31	12.26	22.46	20.65
Doses:								
Linear	0.584	0.797	0.740	0.792	0.966	0.815	0.950	0.455
Quadratic	0.999	0.885	0.624	0.621	0.941	0.528	0.622	0.550
FT×EP	0.053	0.187	0.056	0.231	0.316	0.086	0.223	0.447
FT×EP×D	0.465	0.053	0.108	0.262	0.060	0.086	0.420	0.142

CE, cellulase; CX, cellulase + xylanase (1:1); D, dose; DM, dry matter; EP, enzyme product; FCO, fecal from horses fed control diet; FCE, fecal from horses fed cellulase; FCX, fecal from horses fed cellulase + xylanase (1:1); FT, Fecal type; FXY, fecal from horses fed xylanase; XY, xylanase.

Table 4

In vitro fecal fermentation profile after 48 h of incubation as affected by fibrolytic enzymes addition

Fecal type (FT)	Enzyme product (EP)	Dose (D) µL/g	pH	ME (MJ/kg DM)	OMD (mg/g DM)	DMD (mg/g DM)	SCFA (mmol/g DM)	PF_{24} (mg DMD/mL gas)	MCP (mg/g DM)	GY_{24} (gas/g DMD)
FCO	EP0	0	6.64	8.73	586.2	643.7	4.98	5.30	697.5	188.8
FCE	CE	0	6.89	8.88	596.6	616.3	5.11	5.27	708.4	189.8
		1	6.97	8.31	558.7	521.0	4.64	5.41	668.5	185.0
		3	6.91	8.80	591.3	536.7	5.05	5.29	702.8	189.1
FCX	CX	0	6.72	8.04	541.8	604.3	4.43	5.45	650.8	183.5
		1	6.81	8.17	550.1	572.3	4.53	5.42	659.4	184.5
		3	6.83	8.42	566.3	546.0	4.73	5.37	676.5	186.4
FXY	XY	0	6.88	8.59	577.5	524.7	4.87	5.32	688.3	187.9
		1	6.79	8.53	573.7	654.3	4.83	5.35	684.2	187.0
		3	6.80	9.50	637.2	581.0	5.62	5.16	751.0	193.9

SEM	0.043	0.340	22.22	28.79	0.277	0.076	23.37	2.65
P value								
Doses:								
Linear	0.687	0.164	0.165	0.026	0.162	0.248	0.165	0.223
Quadratic	0.580	0.141	0.139	0.487	0.043	0.033	0.039	0.138
FT×EP	0.020	0.017	0.171	0.050	0.017	0.025	0.017	0.024
FT×EP×D	0.136	0.578	0.573	0.014	0.575	0.644	0.573	0.645

CE, cellulase; CX, cellulase + xylanase (1:1); D, Dose; DMD, *in vitro* dry matter disappearance; EP, enzyme product; FT, fecal type; FCE, fecal from horses fed cellulase; FCO, fecal from horses fed control diet; FCX, fecal from horses fed cellulase + xylanase (1:1); FXY, fecal from horses fed xylanase; GY_{24}, gas yield at 24 h of incubation; MCP, microbial crude protein production; ME, metabolizable energy; OMD, *in vitro* organic matter digestibility; PF_{24}, partitioning factor at 24 h of incubation; SCFA, short chain fatty acids; XY, xylanase.