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Original Research

The Effect of Feeding Horses a High Fiber Diet With or Without Exogenous Fibrolytic Enzymes Supplementation on Nutrient Digestion, Blood Chemistry, Fecal Coliform Count, and In Vitro Fecal Fermentation

Abdelfattah Z.M. Salem [a,*], Mona M.Y. Elghandour [a], Ahmed E. Kholif [b], Nicholas E. Odongo [c], Francisco J.P. Jiménez [d], Roberto Montes-de-Oca [a], Ignacio A. Domínguez [a], Jorge A. Dibarrat [a]

[a] Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Toluca, México
[b] Dairy Science Department, National Research Centre, Giza, Egypt
[c] Department of Animal Sciences, School of Agriculture and Environmental Sciences, Pwani University, Kilifi, Kenya
[d] Instituto de Ciencias Agropecuarias, Universidad Autónoma de Hidalgo, Rancho Universitario, Tulancingo, Hidalgo, México

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ABSTRACT

Sixteen Quarter Horse mares (450 to 500-kg body weight) were used in a complete randomized design to determine the effects of feeding a high fiber diet with or without exogenous fibrolytic enzymes on nutrient digestion, blood chemistry, fecal coliform count, and in vitro fecal fermentation. The treatments comprised feeding the horses (1) a basal diet without enzyme addition (control); (2) control diet plus cellulase at 10 mL/mare/d (CELL); (3) control diet plus xylanase at 10 mL/mare/d (XYL); or (4) control diet plus a mixture of 5 mL cellulase and 5 mL xylanase/mare/d (CX). The basal concentrate diet consisted of a mixture of 50% commercial concentrate and 50% wheat bran fed at 4 kg/horse, offered twice daily at 04:00 and 16:00 hours, and oat straw offered ad libitum at 05:00 and 17:00 hours. The enzyme allocation for each day was mixed with 1 kg of concentrate diet at 04:00 hours, and the experiment lasted for 15 days comprising 10 days of adaptation and 5 days for sample collection. The in vitro cecal fermentation with addition of 2 μL/g dry matter (DM) of each enzyme (CELL, XYL, and CX) to a basal diet of oat straw and concentrates mixture (1:1 DM) as a substrate was carried out. The mares fed enzyme-supplemented diets had greater ($P < .01$) oat straw and total nutrients intakes compared with the control diet. Feeding enzyme-supplemented diets increased total nutrients digestibility ($P < .05$) and blood total protein ($P = .0277$) compared with the control. Feeding XYL-supplemented diet increased blood alanine transaminase and aspartate aminotransferase concentrations ($P < .05$) compared with control treatment. Lower fecal coliform count was obtained ($P = .0114$) with mares fed CX diet compared with control mares. The XYL and CX treatments had decreased asymptotic gas production (GP) ($P = .0173$) with lower rate of GP ($P = .0412$) compared with CELL treatment. CELL and XYL treatments had decreased ($P = .0394$) lag times compared with control and CX treatments. At 24 hours of incubation, CELL and XYL treatments decreased methane production ($P = .0131$), whereas CX treatment increased its production at 48 hours ($P = .0202$) compared with control treatment. No effect was observed ($P > .05$) with enzymes addition on carbon dioxide production at different hours of incubation compared with control treatment. Higher in vitro DM degradability values ($P = .0092$) were obtained with the enzyme

* Corresponding author at: Abdelfattah Z.M. Salem, Universidad Autónoma del Estado de México, 50000 Toluca, Mexico.
E-mail address: asalem70@yahoo.com (A.Z.M. Salem).

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treatments compared with control treatment. Fermentation pH was lower ($P = .0396$) with CX treatment and increased with CELL treatment compared with the control and XYL, showing a greater pH with CELL than the other treatments. It can be concluded that addition of fibrolytic enzymes at 10 mL/mare/d improved feed intake and nutrients digestibility without affecting mare's health.

1. Introduction

Horses are free-ranging herbivores adapted to eating large amounts of high fiber diets for the normal function of the equine digestive system. However, pathologies such as gastric ulceration, laminitis, hindgut acidosis, and colic are associated with feeding diets high in cereal grain [1]. Therefore, there is increasing interest in feeding fiber-based diets with low levels of starch and sugar to meet the energy demands of the horse and reduce incidences of such disorders. Feeding horses a minimum of 1% of their body weights (BWs) as fibrous feedstuffs can minimize occurrence of colic, gastric ulcers, hindgut acidosis, and stereotypical behaviors [2].

Forage feeds have low protein content and low nutrients digestibility [3,4]. There is a need for developing new feeding strategies to meet horse nutrient requirements while maintaining gut health and integrity. In ruminant diets, exogenous fibrolytic enzymes have been shown to improve the digestion of plant fiber fractions by improving ruminal fermentation working synergetically with endogenous rumen microbial enzymes [4]. The large intestine of the horse is a fermentation system similar to the rumen [5]. Microorganisms living in the rumen of ruminant animals and in the cecum of horses give them the ability to breakdown fibers by microbial fermentation to meet energy demands [6].

Numerous studies have been conducted with ruminants to investigate the potential benefits of exogenous fibrolytic enzyme supplementation in improving total tract nutrient digestibilities [7]. Supplementing the diet of horses with exogenous fibrolytic enzymes has gained substantial interest in recent years [6,8]. However, the potential of exogenous enzymes to enhance the digestion of plant structural carbohydrates in the hindgut of the equine working in synergism with endogenous microorganisms is inconclusive [8]. Hainze et al [8] fed horses a diet of grass hay with textured concentrate, pelleted concentrate, whole oats, or alfalfa-lucerne (*Medicago sativa*) supplemented with cellulase and reported that cellulase administration improved the digestion of neutral detergent fiber (NDF) and acid detergent fiber (ADF) in the oats and textured feeds, but decreased the digestion of NDF and ADF in the alfalfa-lucerne. In contrast, Murray et al [9] reported a significant reduction in in vivo digestibility of the fibrous fractions of enzyme-treated high-temperature dried lucerne and ensiled lucerne. Similarly, O'Connor-Robison et al [6] fed Arabian geldings a hay-based diet supplemented with cellulase and noted that cellulase addition decreased digestion of the fiber components.

Therefore, the aim of the present study was to determine the effect of feeding cellulase, xylanase, and their mixture (1:1 vol/vol) on nutrient digestion, blood chemistry, fecal coliform count, and in vitro fecal fermentation in horses fed a high fiber-based diet.

2. Materials and Methods

All procedures involved in handling animals during the experimental period were conducted according to the official Mexican standard of animals care number NOM-051-ZOO-1995.

2.1. Study Location

The experiment was conducted at the Sierra Morelos National Park, De la Barrera Teresona, Toluca, State of Mexico, Mexico. The park is at an altitude of 2,715 m above sea level. The climate in this area is temperate, semihumid with rains in summer. The average annual temperature is 15°C with a maximum of 37°C and a minimum of 3°C. The average annual rainfall is 970 mm with prevailing winds from the north.

2.2. Treatments and Experimental Design

Sixteen Quarter Horse mares (450 to 500-kg BW; 10–12 years of age) were used in this study. In a complete randomized design, four animals were randomly assigned to each of the treatments which were fed individually in 3.6 m × 3.6 m stalls. The basal concentrate diet consisted of a mixture of 50% commercial concentrate (2 kg of total diet/horse) (Pell Rol Cuarto de Milla, Mexico City, Mexico) and 50% wheat bran plus oat straw (2 kg of total diet/horse). The basal concentrate diet (4 kg of total diet/horse) was fed twice daily at 04:00 and 16:00 hours, whereas the forage component of oat straw was offered ad libitum two times daily at 05:00 and 17:00 hours. Diets were balanced to cover animal's requirements according to nutrient requirements of horses of National Research Council [2] as 0.63-kg crude protein (CP) + 16.39 Mcal digestible energy daily. The concentrates mixture contained (dry matter [DM] g/kg) organic matter (OM): 901.8, CP: 112.0, NDF: 511.0, and ADF: 202.8. The wheat bran contained (DM g/kg) OM: 931.0, CP: 169.8, NDF: 460.0, and ADF: 131.2. The oat straw contained (DM g/kg) OM: 929.4, CP: 26.7, NDF: 668.7, and ADF: 405.0. Horses were given 3 to 4 hours in the stalls to allow sufficient time for feeding. When not in the stalls, horses were maintained on a drylot for socialization and exercise.

The treatments comprised feeding the horses (1) both concentrates and oat straw without enzyme addition (control); (2) control diet plus cellulase at 10 mL/mare/d (CELL); (3) control diet plus xylanase at 10 mL/mare/d

(XYL); or (4) control diet plus a mixture of cellulase and xylanase at 5 mL each/mare/d (CX). The experiment lasted for 15 days comprising 10 days of adaptation and 5 days for sample collection. The enzyme allocation for each day was sprayed and mixed with 1 kg of concentrate component at 04:00 hours and left for animal for 1 hour to ensure total consumption before adding the remaining 3 kg of concentrate. Water and salt were available ad libitum.

2.3. Enzyme Activity

The activities of the fibrolytic enzymes (Dyadic PLUS, Dyadic international, Inc, Jupiter, FL, USA) celluase plus and xylanase plus were determined as described by Robyt and Whelan [10]. Briefly, the enzyme products were assayed for endoglucanase and xylanase activity by catalytic hydrolysis of xylan from oat spelt and determining the liberated reducing groups using alkaline copper reagent. The cellulase product contained 30,000 to 36,000 units of cellulase/mL and 7,500 to 10,000 units of beta-glucanase/mL. The xylanase product contained 34,000 to 41,000 units of xylanase/mL, 12,000 to 15,000 units of beta-glucanase/mL, and 45,000 to 55,000 units of cellulase/mL.

2.4. Nutrient Digestibility

For feed intake determination, the individual amount of feed offered was recorded daily and orts were collected and weighed daily throughout the experimental period. During the adaptation period (i.e., first 10 days), horses were trained for feed and fecal collections. On collection days (i.e., last 5 days), fecal samples were collected daily with rectal grab at 4-hour intervals and pooled for each mare. Daily pooled individual feces samples were analyzed for DM, OM, CP, NDF, ADF, and acid insoluble ash (AIA) concentrations. Additionally, feeds were sampled daily and dried at 60°C to constant weight and stored for later chemical analysis for DM, OM, CP, NDF, ADF, and AIA concentrations. Apparent nutrient digestibilities were calculated using AIA concentrations as described by Agazzi et al [11] using the following equation:

$$\textit{Apparent nutrient digestibility} = 100 - \left(100 \times \frac{\%AIA\ in\ feed \times \%component\ in\ feces}{\%AIA\ in\ feces \times \%component\ in\ feed}\right)$$

For each nutrient digestibility within each treatment, 20 samples (4 mares × 5 days) were applied independently for the mentioned equation.

At day 0 (the day before experiment beginning—no enzyme was fed) and also on the last day of the experiment, one sample of rectal feces from each mare was collected and put into sealable plastic bags and stored at 4°C for analysis of total coliform count.

Dried feed, orts, and feces samples were ground through a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA) using a 2-mm screen and analyzed for DM (#930.15) and N (#954.01) according to Association of Official Analytical Chemists [12]. Neutral detergent fiber and ADF were analyzed according to Van Soest et al [13]. Acid insoluble ash concentrations in feed and fecal samples were determined using the method of Van Keulen and Young [14].

2.5. Blood Sampling and Analysis

On the last day of the experiment (i.e., day 15), all mares of each treatments (i.e., four mares/treatment) were sampled for blood. The blood was collected from the jugular vein before feeding into a 10-mL clean dry tube, with anticoagulants. Blood samples were centrifuged at 4,000×*g* at 4°C for 20 minutes. Serum was separated into 2-mL clean dried Eppendorf tubes and frozen at −20°C until analysis. The serum samples were analyzed spectrophotometrically using a BTS 350 Chemistry System Analyzer (Instrumentation Laboratory, Mexico City, Mexico) and IL test's using specific kits for concentrations of total protein (Cat.# 0018481300), urea (Cat.# 0018480400), alanine transaminase (ALT; Cat.# 0018480700), aspartate aminotransferase (AST; Cat.# 0018480800), glucose (Cat.# 0018480000), creatinine (Cat.# 0018480900), calcium (Cat.# 0018258840), phosphorus (Cat.# 0018481900), magnesium (Cat.# 0018481600), and gamma-glutamyl transpeptidase (GGT, Cat.# 0018480785).

2.6. Coliform Isolation and Quantification

Approximately 150 g of fecal samples were collected at days 0 and 15 from each mare as described by Weaver et al [15] and placed in sterile plastic bags and taken to the laboratory. The fresh fecal samples were thoroughly mixed in the plastic bags, and 1 g was taken for coliform count by membrane filtration. Briefly, the 1-g sample was transferred into a 120-mL diluent bottle containing 99 mL of 0.31 mmol KH_2PO_4/L buffer solution and shaken vigorously by hand for approximately 60 seconds before making serial dilutions for plating by membrane filtration. Membranes were placed on m-TEC agar (m-TEC HiCrome Agar, Sigma-Aldrich, Mexico City, Mexico) and incubated for 24 hours at 44.5°C in a water bath before counting yellow colonies typical of *Escherichia coli.*

2.7. In Vitro Fecal Incubations

The basal diet of oat straw and the 1:1 mixture of the concentrates that was fed to horses in the in vivo study were used as a substrate for the in vitro study. With the exception of the preparation of the microbial inocula, the method of Theodorou et al [16] was used to measure gas production (GP). On the last day of the in vivo experiment, fecal samples were collected directly from the rectum of each horse and a composite sample of each treatment obtained for the in vitro incubation. A representative sample of the composite fecal contents for each treatment was mixed with the Goering and Van Soest [17] buffer solution without trypticase in the ratio of 1:4 to form four incubation media. The formed 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 were used to inoculate three identical runs of incubation in bottles containing 1 g DM of substrate (a 1:1 mixture of concentrates plus oat straw). Enzyme (CELL, XYL, and CX) was added at 2 μL/g DM of incubated substrates.

Thirty-six bottles (3 replicates × 4 treatments × 3 runs) plus three bottles without substrate and enzyme as blank were used for GP. Once all bottles were filled, bottles were flushed with carbon dioxide (CO_2) and immediately closed with rubber stoppers, shaken and placed in the incubator at 39°C. Gas production, methane (CH_4) production, and CO_2 production 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, CT, USA) of Theodorou et al [16], whereas the CH_4 and CO_2 productions were recorded using 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, Mexico). After pH measurement, the content of each bottle was filtered under vacuum through glass crucibles with a sintered filter (coarse porosity no. 1, pore size 100–160 μm; Pyrex, Stone, UK). Fermentation residues were dried at 65°C for 72 hours overnight to estimate DM degradability (DMD), with loss in weight after drying being the measure of degradable DM.

For estimating kinetic parameters of GP, results of GP (mL/g DM) were fitted using the NLIN option of SAS [18] according to France et al [19] as:

$$A = b \times \left(1 - e^{-c(t-L)}\right)$$

where A is the volume of GP at time t; b is the asymptotic GP (mL/g DM); c is the rate of GP (/h), and L (h) is the discrete lag time before GP. Metabolizable energy (ME, MJ/kg DM) and in vitro OM digestibility (OMD, mg/g DM) were estimated according to Menke et al [20]. The partitioning factor at 24 hours 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 hours according to Blümmel et al [21]. Gas yields (GY_{24}) were calculated as the volume of gas produced after 24 hours (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]. Microbial crude protein (MCP) production was calculated as by Blümmel et al [21].

2.8. Statistical Analyses

All data were analyzed as a completely randomized design using PROC MIXED of SAS [18]. The statistical model was $Y_{ij} = \mu + E_j + \varepsilon_{ij}$, where Y_{ij} represents every observation of the ith animal fed in the jth enzyme product, μ is the general mean, E_j is the enzyme product, and ε_{ij} is the experimental error.

However, total coliform counts were transformed to $\log_{10}$ per gram of feces before analysis, and data of fecal in vitro GP of each of the three runs within the same sample of the substrate were averaged before statistical analysis.

Tukey test was used for multiple comparisons of mean values for each parameter. Significance was declared at a level of $P < .05$, and the absence of effect should be stated as $P > .05$.

3. Results

3.1. Feed Intake and Nutrient Digestibility

The mares fed enzyme-supplemented diets had greater ($P < .01$) oat straw and total nutrient intakes compared with the control diet. Moreover, feeding enzyme-supplemented diets increased total nutrients digestibility ($P < .05$) compared with the control diet. Mares fed CELL diet had the highest CP digestibility ($P < .05$) compared with mares fed other diets (Table 1).

Table 1
Feed intake and nutrient digestibility of the diets supplemented with cellulase, xylanase, and cellulase + xylanase (1:1 vol/vol) enzymes in mares (n = 4).

Items	Diets				SEM	*P* Value
	Control	CELL	XYL	CX		
Oat straw intake (kg/d)						
Dry matter	5.67[b]	10.36[a]	10.40[a]	9.46[a]	0.630	.0001
Organic matter	5.27[b]	9.64[a]	9.66[a]	8.79[a]	0.585	.0012
Crude protein	0.150[b]	0.275[a]	0.278[a]	0.253[a]	0.0167	.0014
Neutral detergent fiber	3.79[b]	6.93[a]	6.95[a]	6.33[a]	0.421	.0009
Acid detergent fiber	2.30[b]	4.20[a]	4.21[a]	3.83[a]	0.255	.0013
Total intake (kg/d)						
Dry matter	9.13[b]	13.82[a]	13.86[a]	12.92[a]	0.630	.0011
Organic matter	8.39[b]	12.75[a]	12.78[a]	11.91[a]	0.586	.0013
Crude protein	0.538[b]	0.665[a]	0.663[a]	0.640[a]	0.0174	.0015
Neutral detergent fiber	5.56[b]	8.70[a]	8.72[a]	8.09[a]	0.421	.0001
Acid detergent fiber	3.00[b]	4.90[a]	4.92[a]	4.53[a]	0.255	.0001
Digestibility (g absorbed/kg ingested)						
Dry matter	557.4[b]	748.5[a]	732.7[a]	723.2[a]	13.42	.0057
Organic matter	577.0[b]	757.3[a]	741.7[a]	734.5[a]	13.21	.0064
Crude protein	597.4[c]	741.1[a]	702.6[b]	695.6[b]	14.17	.0153
Neutral detergent fiber	423.7[b]	687.1[a]	656.8[a]	645.3[a]	4.94	.0029
Acid detergent fiber	339.0[b]	661.4[a]	621.2[a]	603.0[a]	6.05	.0107

Abbreviations: CELL, fed the control diet plus cellulase at 10 mL/animal/d; control, fed oat straw and concentrates; CX, fed the control diet plus cellulase and xylanase at 5 mL of CELL plus 5 mL of XYL/animal/d; SEM, standard error of the mean; XYL, fed the control diet plus xylanase at 10 mL/animal/d.
[a,b,c]Means in the same row with different superscript letters differ ($P < .05$).

3.2. Blood Chemistry and Fecal Coliform Count

Feeding enzymes increased blood total protein (P = .0277) compared with the control. Feeding XYL-supplemented diet increased blood ALT (P = .0314) and AST (P = .0481) concentrations compared with control diet. No effects (P > .05) were observed for blood urea, creatinine, glucose, phosphorus, magnesium, and GGT due to enzyme supplementation. Lower fecal coliform count was obtained (P = .0114) with mares fed CX diet compared with mares fed control diet at the end of the experiment after 15 days (Table 2).

3.3. In Vitro Fecal Fermentation and Digestibility

The CELL treatment had greater asymptotic GP (P = .0173) with lower rate of GP (P = .0412) compared with XYL and CX treatments, with no difference (P > .05) between CELL, XYL, CX treatments, and control treatment. Both of CELL and XYL treatments had decreased (P = .0394) lag times compared with control and CX treatments. At 24 hours of incubation, CH_4 production was decreased (P = .0131) with CELL and XYL treatments compared with control and CX treatments, whereas at 48 hours of incubation, CX treatment increased CH_4 production (P = .0202) compared with control treatment. CELL treatment had the lowest CH_4 production (P = .0131) compared to other treatments (Fig. 1). No effect was observed (P > .05) with enzymes addition on CO_2 production during different incubation hours compared to control treatment (Fig. 2). Higher in vitro DMD values (P = .0092) were obtained with the enzyme treatments compared with control treatment with the highest value for CELL treatment. Fermentation pH was lower (P = .0396) with CX compared with control and XYL treatments, with CELL showing a greater pH than the other treatments. No treatment effects (P > .05) were observed on CO_2 production at 24 hours of incubation, ME, in vitro OMD, SCFA, PF_{24}, MCP, and GY_{24} (Table 3).

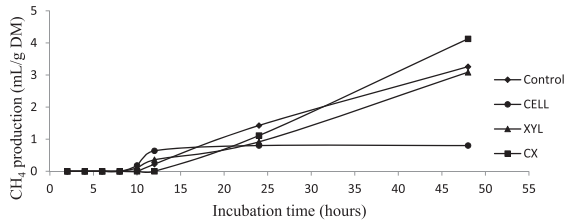

Fig. 1. Effect of addition of fibrolytic enzymes to different treatments on in vitro fecal CH_4 production (mL/g DM) after 48 hours of incubation. Abbreviations: CELL, control diet plus cellulase at 2 μL/g DM; CH_4, methane; control, oat straw and concentrate (1:1 DM) without enzyme; CX, control diet plus cellulase + xylanase (1:1 vol/vol) at 2 μL/g DM; DM, dry matter; XYL, control diet plus xylanase at 2 μL/g DM.

4. Discussion

4.1. Feed Intake and Nutrient Digestibility

The intake of oat straw was higher by 67% to 83% with addition of enzymes. This may be partly due to the greater nutrients digestibility with enzyme supplementation, which is consistent with previous results [9]. However, O'Connor-Robison et al [6] did not find a feed intake effect with cellulase in horse diets. To our knowledge, there are no in vivo studies available on cecal fermentation in horses. Because the large intestine of the horse is a fermentation system similar to the rumen [5], our explanations will borrow from studies with ruminant animals.

Hainze et al [8] reported reduced fiber digestibility of an enzyme-treated lucerne-based diet fed to horses and explained their results that the exogenous enzymes blocked enzyme-binding sites that would otherwise have been occupied by endogenous microbial enzymes.

In another study, Murray et al [9] fed Welsh-cross pony geldings on diet based on lucerne and enzyme addition at 8.9 L/tonne DM and reported that DM intake was significantly greater, whereas OMD was significantly reduced without affecting NDF digestibility compared with the

Table 2
Blood chemistry and total fecal coliform count of mares fed diets supplemented with cellulase, xylanase, and cellulase + xylanase (1:1 vol/vol) enzymes (n = 4).

Items	Diets				SEM	*P* Value
	Control	CELL	XYL	CX		
Blood chemistry						
Alanine transaminase (U/L)	12.5[b]	11.8[b]	20.0[a]	14.8[ab]	3.23	.0314
Aspartate aminotransferase (U/L)	341.0[b]	389.8[b]	416.8[a]	384.3[b]	43.93	.0481
Urea (mmol/L)	16.2	15.1	15.8	14.3	1.26	.7330
Creatinine (mmol/L)	182.0	184.9	186.3	170.5	16.17	.3030
Total protein (mmol/L)	4.49[b]	5.14[a]	5.09[a]	5.19[a]	0.274	.0277
Glucose (mmol/L)	7.50	7.50	7.25	7.50	0.346	.9402
Calcium (mmol/L)	3.45	3.63	4.05	3.60	0.153	.0837
Phosphorus (mmol/L)	1.12	1.08	1.16	1.03	0.071	.6386
Magnesium (mmol/L)	0.65	0.67	0.71	0.88	0.056	.4481
Gamma-glutamyl transpeptidase (U/L)	16.0	15.8	15.0	15.5	0.976	.9002
Fecal coliform count (log_{10}/g feces)						
Day 0	7.36	7.48	7.47	7.45	0.073	.6810
Day 15	4.26[a]	4.07[ab]	3.70[ab]	3.53[b]	0.139	.0114

Abbreviations: CELL, fed the control diet plus cellulase at 10 mL/animal/d; control, fed oat straw and concentrate; CX, fed the control diet plus cellulase and xylanase at 5 mL of CELL plus 5 mL of XYL/animal/d; SEM, standard error of the mean; XYL, fed the control diet plus xylanase at 10 mL/animal/d.
[a,b]Means in the same row with different superscript letters differ (P < .05).

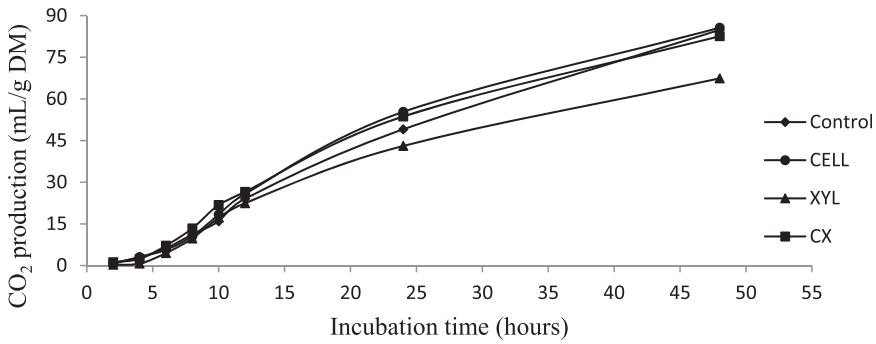

Fig. 2. Effect of addition of fibrolytic enzymes to different treatments on in vitro fecal CO2 production (mL/g DM) after 48 hours of incubation. Abbreviations: CELL, control diet plus cellulase at 2 μL/g DM; CO_2, carbon dioxide; control, oat straw and concentrate (1:1 DM) without enzyme; CX, control diet plus cellulase + xylanase (1:1 vol/vol) at 2 μL/g DM; DM, dry matter; XYL, control diet plus xylanase at 2 μL/g DM.

control. However, they reported that addition of enzyme at 2.3 and 4.7 L/ton DM resulted in a significant reduction in the apparent digestibility of OM, NDF, and total nonstarch polysaccharide digestibilities compared with control. In the present study, enzyme treatment resulted in a significant greater apparent digestibility of different nutrients relative to the control diet. Increased digestibility of enzyme-treated diets has also been observed in small ruminants by others [4,7].

Dietary nutrients digestion especially the fiber fraction can be affected by many factors including the chemical composition of the diet [23], the size of the indigestible fiber fraction, the digestion rate of potentially digestible fiber fractions, and rumen or cecal outflow rate, as well as the use of feed additives [7]. The suggested modes of action for the improved digestibility as a result of fibrolytic enzyme administration include increased digestion rate of the potentially digestible fiber fractions, altered fermentation kinetics, and enhanced ruminal microorganism attachment to feed particles and colonization of the plant cell wall [7,24]. Moreover, improved synergism between exogenous and endogenous enzymes [24] and increased numbers of fibrolytic and nonfibrolytic bacteria in the rumen and maybe in the cecum are other possible mechanisms.

4.2. Blood Parameters and Fecal Coliform

Blood parameters AST, ALT, phosphorus, and GGT were within normal physiological ranges of 212 to 449 (U/L), 3 to 23 (U/L), 0.77 to 1.67 (mmol/L), and 4 to 22 (U/L),

Table 3
In vitro fecal gas kinetics and cumulative gas production (mL/g DM) after 48 hours of incubation with fibrolytic enzyme addition at 2 μL/g DM.

Items	Diets				SEM	*P* Value
	Control	CELL	XYL	CX		
b (mL/g DM)	298.1[ab]	341.3[a]	276.3[b]	284.6[b]	10.14	.0173
c (h)	0.052[ab]	0.041[b]	0.057[a]	0.061[a]	0.0025	.0412
L (h)	2.36[a]	1.80[b]	1.53[b]	2.12[a]	0.252	.0394
Gas at 2 hours	29.3	26.5	29.6	32.8	1.38	.0919
Gas at 4 hours	55.7[a]	50.9[b]	56.0[a]	61.8[a]	2.54	.0358
Gas at 6 hours	79.4[a]	73.3[b]	79.6[a]	87.4[a]	3.51	.0498
Gas at 8 hours	100.8[b]	93.9[b]	100.7[b]	110.0[a]	4.32	.0489
Gas at 10 hours	120.1[a]	112.9[b]	119.4[a]	130.0[a]	5.00	.0487
Gas at 12 hours	137.4[b]	130.4[b]	136.2[b]	147.7[a]	5.57	.0474
Gas at 24 hours	210.7	209.9	205.1	218.3	7.50	.9200
Gas at 48 hours	271.6[a]	288.9[a]	257.7[b]	268.7[a]	8.78	.0342
Methane at 24 hours	1.43[a]	0.80[b]	0.92[b]	1.11[a]	0.0521	.0131
Methane at 48 hours	3.26[b]	0.80[c]	3.09[b]	4.13[a]	0.677	.0202
Carbon dioxide at 24 hours	49.04	55.36	43.08	53.69	10.981	.8411
Carbon dioxide at 48 hours	84.85	85.65	67.40	82.58	14.117	.2212
pH	6.84[b]	6.97[a]	6.81[b]	6.69[c]	0.022	.0396
ME (MJ/kg DM)	8.33	8.31	8.17	8.53	0.204	.9194
In vitro OMD (mg/g DM)	560.2	558.7	550.1	573.7	13.33	.9201
In vitro DMD (mg/g DM)	533.3[c]	580.0[a]	543.3[b]	546.7[b]	4.37	.0092
SCFA (mmol/g DM)	4.66	4.64	4.53	4.83	0.166	.9204
PF_{24} (mg DMD/mL gas)	5.39	5.41	5.42	5.35	0.049	.9573
MCP (mg/g DM)	670.1	668.5	659.4	684.2	14.02	.9200
GY_{24} (gas/g DMD)	185.7	185.0	184.5	187.0	1.67	.9498

Abbreviations: *b*, asymptotic gas production; *c*, rate of gas production; CELL, control diet plus cellulase at 2 μL/g DM; control, oat straw and concentrate (1:1 DM) without enzyme; CX, control diet plus cellulase + xylanase (1:1 vol/vol) at 2 μL/g DM; DM, dry matter; GP, gas production; GY_{24}, gas yield at 24 hours of incubation; in vitro DMD, in vitro dry matter degradability; in vitro OMD, in vitro organic matter digestibility; *L*, initial delay before gas production begins; MCP, microbial crude protein production; ME, metabolizable energy; PF_{24}, partitioning factor at 24 hours of incubation; SCFA, short-chain fatty acid; SEM, standard error of the mean; XYL, control diet plus xylanase at 2 μL/g DM.
[a,b,c]Means in the same row with different superscript letters differ ($P < .05$).

respectively, as per the manufacturer kit. Blood content of total protein and magnesium was slightly lower than the provided normal physiological range of 5.3 to 7.1 (mmol/L) and 0.90 to 1.14 (mmol/L), whereas blood content of urea, creatinine, glucose, and calcium were greater than normal physiological range of 4.1 to 7.6, 88 to 156, 3.4 to 6.2, and 2.8 to 3.2 (mmol/L), respectively, but without significant differences between treatments. Values greater than normal physiological range may be due to normal and individual differences between horses as the control horses were not different from the treated horses. The lack of difference in urea and creatinine concentrations between treated and control horses suggests no catabolism of muscles protein and that the kidney function was not adversely affected by any the diets [25]. Generally, serum creatinine is an indicator of glomerular filtration in the kidney [25]. The higher glucose concentration observed compared to the normal physiological range may be due to increased OM, NDF, and ADF digestibilities resulting in enhanced energy utilization and increased propionate absorption through cecum wall, leading to a high rate of glucose synthesis.

Addition of XYL elevated liver enzymes of ALT and AST concentrations, but these were within normal physiological ranges of both ALT and AST enzymes, which are important indicators of liver activity and function suggesting there were no pathologic lesions in the liver [26]. Blood total protein was higher with enzyme addition to the diet than with the control. Increased total protein in the serum reflects a good nutritional status in the horses because there is a positive correlation between blood total protein and dietary protein intake [27]. Moreover, it would be possibly due to the greater CP intake and digestibility observed with fibrolytic enzymes addition [7].

At day 15 of the experiment, feeding horses with a mixture of cellulase and xylanase (i.e., CX treatment) lowered the fecal shedding of *E. coli* by about 17% compared with control. Pathogenic bacteria such as *E. coli* are known to cause intestinal diseases in horses [28]. However, there is limited information available on the microbial burden of equine feces. Nutrition is among the major factors influencing the conditions in the rumen and cecum. A relation between diet and the proliferation and resistance of *E. coli* has been suggested [29]. The energy content of the diet and the manner in which it is fermented in the rumen or cecum play a critical role in maintaining bacterial populations [30]. Feeding enzymes may improve the feed utilization and stimulate the immune response in the host animal as shown with the enzyme treatment as the fecal shedding of *E. coli* was decreased by about 17% compared to control with the CX treatment; however, there were no significant differences for CELL and XYL. Another probable reason is a lowered cecal pH as shown in the in vitro fecal fermentation where CX treatment had the lowest pH [31].

4.3. In Vitro Fecal Fermentation and Digestibility

The cellulase treatment (i.e., CELL) had the highest asymptotic GP and the lowest rate of GP. Administrations of exogenous fibrolytic enzymes have been shown to increase in vitro GP and the nutritive value of feeds with ruminal inoculum from cattle [32]. However, in the present study, only cellulase had any effect. Xylanase and cellulose and/or xylanase mixture had no effect. This may be related to the nature of the diet [32] or the fecal inocula used. The higher GP with CELL may allow greater voluntary feed intake by decreasing cecum physical fill and increasing the net energy intake of the diets [33]. The volume of GP reflects the fermentation potential of the diet and depends on nutrient availability for inocula microorganisms during fermentation [23]. Exogenous enzymes can enhance attachment of microorganisms to feed particles, creation of a stable enzyme–feed complexes [34], and/or the possibility of the fiber structure alteration within the cecum causing microbial colonization to feeds. In general, the enzyme effects are dependent on many factors such as source, type and dose of enzyme, type of diets fed to the animals, and enzyme application method [35]. In the present study, two different enzyme products were used with different responses; however, enzymes were used at the same dose and the same diet. Different enzyme products and different enzyme sources can explain the different response to the different tested enzyme products in the present study. The ability of CELL, XYL, or their mixture to increase fiber digestion may be limited by the lack of enzymes that degrade the core structure of lignin–cellulose complexes in low-quality forages [35].

Enzyme addition (CELL in the present study) may cause improved cecal fermentation and enhance attachment and colonization of cecal microorganisms to the plant cell wall and work in synergism with cecal endogenous microbial enzymes as previously mentioned.

Higher in vitro DMD values were obtained with enzyme treatments compared with control. Nsereko et al [36] showed that the increased DMD of diets with the addition of enzymes may have been due to increased fiber digestion and altered fermentation, enhanced attachment and colonization to the plant cell wall material by microorganisms. Results of in vitro DMD support those obtained in vivo (Tables 1 and 3).

At 24 hours of incubation, CELL and XYL decreased CH_4 production, while at 48 hours of incubation higher CH_4 production was observed with the CX treatment, and lower CH_4 production was observed with CELL treatment. Fermentation of dietary carbohydrates to acetate, propionate, and butyrate produce gases which mainly constitutes H_2, CO_2, and CH_4. During the first 12 hours of incubation, CH_4 production was negligible and then it started to increase rapidly to reach its peak concentration at the end of incubation. However, GP started with the onset of incubation. In general, CH_4 yields for horses are between those for pigs and for ruminants and they equal 3% to 4% of the digestible energy or 2% to 3% of the gross energy intake [37]. In both ruminants and horses, CH_4 is mainly produced by the methanogenic archaea, which represent the main hydrogenotrophic microbial community [38].

5. Conclusions

Addition of fibrolytic enzyme of cellulase, xylanase, and their mixture (1:1 vol/vol) at 10 mL/animal/d resulted in greater intake of oat straw. Improved feed intake was coupled with increased nutrient digestibilities. Blood

parameters were altered by the treatments with lower fecal shedding of *E. coli* of about 17% with the treatment of cellulase and/or xylanase mixture. In vitro fecal fermentation showed higher GP and DMD with enzyme treatment with the better results with cellulase addition at 2 μL/g DM of the substrate. These results show that addition of fibrolytic enzymes at 10 mL/mare/d improved feed intake and nutrients digestibility without affecting mare's health.

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