Journal of Equine Veterinary Science 70 (2018) 13–17

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

Effect of Natuzyme Enzyme on Fecal Digestion and Fermentation of Wheat Straw and Alfalfa Hay in Arabian Horses

Tahereh Mohammadabadi [a,*], Maryam Harsini Shakarami [a], Mona M.M.Y. Elghandour [b], Abdelfattah Z.M. Salem [b,*], José Cedillo Monroy [c]

[a] Department of Animal Science, Faculty of Animal Science and Food Technology, Khuzestan Agricultural Sciences and Natural Resources University, Iran
[b] Depto. de Nutrición Animal, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Toluca, Edo de México, México
[c] Centro Universitario UAEM-Temascaltepec, Universidad Autónoma del Estado de México, Edo de México, México

ARTICLE INFO

Article history:
Received 28 May 2018
Received in revised form
1 July 2018
Accepted 2 July 2018
Available online 2 August 2018

Keywords:
Alfalfa hay
Horse
Gas production
Digestibility
Natuzyme enzyme

ABSTRACT

The aim of this study was to evaluate the effect of Natuzyme enzyme mixture (0, 3, and 6 g/kg dry matter [DM]) on microbial digestion as well as fermentation of wheat straw and alfalfa hay in Arabian horse. Four female Arabian horses (3–4 years, average body weight 400 kg) were fed with diet containing 35% commercial concentrate +65% forage (wheat straw and alfalfa hay) for 2 weeks. The fermentation and gas production of wheat straw and alfalfa hay with different levels of Natuzyme enzyme were determined using standard in vitro gas production protocol (6 replicates). The DM and neutral detergent fiber (NDF) digestibility were determined using specific media (four replicates per time). Addition of enzyme had no effect on gas production rate of alfalfa hay ($P > .05$), but the potential of gas production was increased with alfalfa hay at 3 and 6 g/kg DM of enzyme compared with control. The addition of enzyme to alfalfa did not affect partitioning factor, microbial biomass, microbial biomass efficiency, and cell wall degradability ($P > .05$). DM digestibility of alfalfa hay after 24, 48, and 72 hours of incubation with mixed cecum contents was not affected by enzyme ($P > .05$), but NDF digestibility at 24, 48, and 72 hours was increased ($P < .05$). The enzyme supplementation decreased microbial biomass efficiency and increased potential of gas production, DM, and NDF digestibility at 24 hours for straw. The highest value was obtained for straw treated with 3 and 6 g/kg DM of enzyme. Gas production parameters, DM, and NDF digestibility of straw at 48 and 72 hours of incubation were not influenced by enzyme addition. In conclusion, addition of Natuzyme enzyme mixture caused the proper fermentation, gas production, and digestibility of alfalfa hay and straw without affecting the DM digestibility by cecal microorganisms, thereby suggesting the additive role of this enzyme in Arabian horse nutrition.

1. Introduction

Fermentation system of the large intestine of the horse is similar to the rumen. The size and diversity of the microbial population in the horse digestive tract caused to use fiber feeds [1]. These microorganisms provide them the ability to breakdown fibers to meet energy demands [2]. Digesta remains for 6 to 12 hours in the cecum, 24 to 50 hours in the large colon and 8 to 12 hours in the small colon [3]. These retention times allow the microbes to extract energy from the remaining carbohydrates and helping to save the entire energy in the horses. Under normal nutritional conditions, fermentation of carbohydrates is a very slow process [4].

The fibrolytic bacteria form the dominant population in horse cecum and colon when diet contains huge amounts of fiber [5]. Most horses can supply their nutrients with 100% forage in the diet.

Animal welfare/ethical statement: The research was performed in accordance with the ethical standard laid down in the 1996 declaration of Helsinki and its later amendments.

Conflict of interest statement: The authors declare that they have no conflict of interest.

* Corresponding authors at: Dr. Tahereh Mohammadabadi, Department of Animal Science, Faculty of Animal Science and Food Technology, Khuzestan Agricultural Sciences and Natural Resources University, Iran; and Prof. Dr. Abdelfattah Z.M. Salem, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Toluca, Edo de México, Mexico.

E-mail addresses: mohammadabadi@ramin.ac.ir (T. Mohammadabadi), salem@uaemex.mx, asalem70@yahoo.com (A.Z.M. Salem).

https://doi.org/10.1016/j.jevs.2018.07.008
0737-0806/

Forage, in addition to providing nutrients for this animal, also plays an important role in the health of the digestive tract and helps to maintain its normal functioning. The feeding of horses using fibrous feedstuffs can minimize occurrence of colic, gastric ulcers, hindgut acidosis, and stereotypical behaviors [6]. Fiber is the main component of the feed of these animals; therefore, it should be tried to use higher digestibility forage in the feeding of this animal [3].

The pivotal part of agricultural waste is lignocellulosic material, whose nutritional value for animals is low. Despite the limitations, these materials have potentiality for their utilization as an energy source in the livestock nutrition. The nutritional value of these materials increases with the use of physical, chemical, and biological processing [7]. Over the past 20 years, significant attempts have been made to use exogenous fibrolytic enzymes to improve the quality of forage and animal performance [7]. However, exogenous fibrolytic enzymes improved digestion of insoluble fiber in neutral detergent in horses [8] and rabbits [9,10]. Researchers also reported that the exogenous enzymes differed in their effects on fermentation of wheat straw, corn stalks, and sugarcane bagasse ensiled with multienzymes [11]. The fibrolytic enzymes increase the nutrient content of forage due to breaking of special chemical bonds of forage that are not degraded by animal enzymes [12].

Although, Hainze et al [13] reported that the use of exogenous xylanase and cellulase enhances cell wall digestibility in horse forage rations and the use of these enzymes can reduce the need for concentrate feeds for providing of digestible energy in the horse. However, limited research activity has been carried out on the use of exogenous enzymes to improve microbial digestion and fermentation of agricultural byproducts using horse digestive tract microorganisms. Since, 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 [13]. Therefore, this investigation was conducted to study of effect of Natuzyme enzyme mixture on microbial digestibility as well as fermentation of wheat straw and alfalfa hay in Arabian horse.

2. Materials and Methods

2.1. Animals and Diets

Four female Arabian horses (3–4 years, average body weight 400 kg) were fed with diet containing 65% forage (wheat straw and alfalfa hay) and 35% commercial concentrate for 2 weeks. Total mixed diets were fed in two equal meals and had free access to fresh water. After 2 weeks of feeding to horses, samples of fresh feces (approximately 100 g) were collected from each horse before the morning feeding. Further, it was mixed with McDuggal buffer (1 feces and 3 buffer), strained through four layers of cheesecloth, and transferred into prewarmed thermos flasks that were flushed with CO_2 during transport to the laboratory.

Experimental samples include wheat straw and alfalfa hay with different levels of Natuzyme enzyme mixture (0, 3, and 6 g/kg dry matter [DM]). The gas production and fermentation of samples were determined using diluted feces content of Arabian horses (six replicates per treatment). The enzyme mixture composition was cellulase, xylanase, beta-glucanase, alpha-amylase, pectinase, phytase, lipase, and protease as 6, 10, 0.7, 0.7, 0.07, 1.5, 0.03, and 3 MU/kg, respectively (Bioproton Pty Ltd Co Australia).

2.2. In Vitro Gas Production

In vitro gas production was determined as described by Blümmel et al [14]. About 300 mg of substrate samples (wheat straw and alfalfa hay) were weighed accurately into 100 mL glass vials. Further, buffered feces content and reducing solution were added to each vial. Vials were fitted with plungers and placed in a prewarmed incubator at 39°C. Gas production was manually measured at 2, 4, 6, 8, 12, 24, 36, 48, 72, and 96 hours using a digital pressure gauge. Cumulative gas production data were fitted to the exponential equation as given in the following [15].

$$Y = b\left(1 - e^{-ct}\right)$$

where Y = gas volume at time t (mL), b = potential of gas production (mL), t = time (h), and c = fractional rate of gas production (mL/h).

After 96 hours of incubation, the contents of the vials were filtered and the residues were placed in an oven and dried at 105°C, and cell wall degradability was measured by calculating difference between weights of primary substrate and weight after incubation. At the end of each incubation period, the content of some vials was transferred into an Erlenmeyer flask, mixed with 20 mL neutral detergent fiber (NDF) solution, boiled for 1 hour, filtered, dried, and ashed. The fermentation parameters such as partitioning factor and microbial biomass were estimated according to the method of Makkar and Becker [16] as follows:

$$\text{Partitioning factor } (mg/mL) = \frac{\text{Digested organic matter}(mg)}{\text{Gas production}(mL)}$$

$$\text{Microbial biomass}(mg) = \text{Digested organic matter}(mg) - (\text{Gas production} \times 2.2)$$

$$\text{Microbial biomass efficiency}(\%) = \frac{\text{Microbial biomass}(mg)}{\text{Digested organic matter}(mg)} \times 100$$

2.3. In Vitro Digestibility by Horse Cecum Bacteria

About three to four samples of fresh feces of each animal fed with diet containing 65% forage (wheat straw and alfalfa hay) and 35% concentrate for 2 weeks were taken and mixed with McDugal buffer. It was then strained by two layers of cheesecloth into prewarmed thermo flasks for transportation to the laboratory. The isolation of anaerobic bacteria was carried out using specific media [17]. The media contained 15 mL of Mineral Solution I (KH_2PO_4 3.0 g; $(NH_4)_2SO_4$ 6.0 g; NaCl 6.0 g; $MgSO_4$ 0.6 g; $CaCl_2 \cdot 2H_2O$ 0.795 g/L), 15 mL of Mineral Solution II (K_2HPO_4 3 g/L), 0.5 g yeast extract, 0.5 g Trypticase, 0.1 mL Resazurin (0.1%), 0.2 g microcrystalline cellulose, 0.1 g cellubiose, 0.8 g sodium carbonate, 30 mL clarified feces fluid, 0.5 g L-cysteine HCl-H_2O, 0.05 g benomyl and metalaxyl (200 U/mL), and distilled water 100 mL. One gram of sample (wheat straw and alfalfa hay with 0, 3 and 6 g/kg DM of Natuzyme enzyme

mixture), specific media of bacteria, and reducing solution was added to each vial, and were autoclaved at 120°C for 15 minutes [18]. Vials were placed in an incubator for 24, 48, and 72 hours of incubation (four replicates per each time). During sampling, vials were removed from the incubator after 24, 48, and 96 hours of incubation. Then the content of each vial was filtered and oven-dried at 105°C for DM determination.

2.4. Statistical Analyses

A completely randomized design was used to determine the effect of Natuzyme enzyme mixture on the various parameters. All data were analyzed using the GLM procedure in SAS [19] based on the statistical model as given in the following:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where Y_{ij} is the observation, μ is the general mean, T_i is the effect of treatment on the observed parameters, and e_{ij} the standard error of term. Means were compared by the Duncan multiple comparison test at $P < .05$.

3. Results

The use of different levels of enzyme did not affect the rate of gas production. However, increasing enzyme doses improved ($P < .05$) the potential of gas production. The use of different levels of enzyme on partitioning factor and microbial biomass efficiency of wheat straw was significant ($P < .05$) but it showed nonsignificant ($P > .05$) effect on microbial biomass and cell wall degradability. Microbial biomass efficiency of wheat straw was increased with increase in the enzyme level, but the value of partitioning factor was observed to be decreased. However, the addition of enzyme did not affect partitioning factor, microbial biomass, cell wall degradability, and the microbial biomass efficiency of alfalfa hay (Tables 1 and 2).

Increasing the level of enzyme increased the DM and NDF digestibility of wheat straw at 24 hours. On the other hand, NDF digestibility of alfalfa hay was increased at 24, 48, and 72 hours of incubation ($P < .05$). But DM and NDF digestibility of straw at 48 and 72 hours of incubation and DM digestibility of alfalfa hay at 24, 48, and 72 hours of incubation with mixed cecum contents was not affected due to enzyme addition (Tables 3 and 4).

4. Discussion

The findings of current experiment indicated that with increase in enzyme dose, the gas production potential of wheat straw and alfalfa hay by horse feces improved without affecting the rate of gas production and cell wall degradability. However, Salem et al. [20] concluded that mares fed diets containing enzyme had greater total nutrients digestibility with respect to the control diet. At 24 hours of incubation, CH_4 production was decreased due to cellulase and xylanase addition, whereas it was increased at 48 hours of incubation due to xylanase supplementation. Authors further reported that cellulase supplementation had the highest gas production and the lowest gas production rate. Cellulase improved cecal fermentation and enhanced attachment and colonization of cecal microorganisms to the plant cell wall and acts as synergism with cecal endogenous microbial enzymes. The researcher reported that cellulase administration to grass hay in horses improved the digestion of NDF and acid detergent fiber in the oats and textured feeds, but decreased the digestion of NDF and acid detergent fiber in the alfalfa hay [13,20]. Murray et al [21] reported a significant reduction in in vivo digestibility of the fibrous fractions of dry alfalfa hay treated with enzyme. Moreover, O'Connor-Robison et al [2] noted that feeding of hay supplemented with cellulase to Arabian geldings decreased fiber digestion.

The fibrolytic enzymes increase the content of DM, crude protein, and the soluble portion of the feed that improve the energy availability and also lead to rapid microbial growth [22]. Increase in the bacteria counts and the microbial colonization on the particles may be resulted in an increase in total gas production [23]. This could be one of the reasons for improving the produced gas potential with increasing the enzyme level in the present study. According to the reports of Mao et al [24], the addition of cellulase and xylanase increased the production of methane gas. Wallace et al [25] indicated that cellulase and other commercially fibrolytic enzymes could increase gas produced from forage fermentation under in vitro condition. However, some studies have shown that the fibrolytic enzyme does not affect the gas production potential [26]. In general, limited studies have been performed on the effects of exogenous enzymes on methane emissions from animal. The

Table 1
Effect of Natuzyme enzyme levels on gas production parameters of wheat straw.

Enzyme (g/kg DM)	B	C	Cell Wall Degradability (%)	Pf	Microbial Biomass	Microbial Biomass Efficiency (%)
0	15.76[b]	0.020	16.55	20.11[a]	126.17	88.97[a]
3	20.62[a]	0.013	17.27	16.70[b]	123.69	86.79[b]
6	21.69[a]	0.018	17.32	14.47[c]	122.16	84.77[c]
SEM	0.841	0.001	0.465	0.660	3.090	0.442
P-value	.001	.117	.247	.0007	.664	.0003

Abbreviations: Pf, partitioning factor; SEM, standard error of the mean.
B—Potential of gas production.
C—Gas production rate.
[a, b, c] Means with common letter (s) within each column do not differ significantly ($P > .05$).

Table 2
Effect of Natuzyme enzyme levels on gas production parameters of alfalfa.

Enzyme (g/kg DM)	B	C	Cell Wall Degradability (%)	Pf	Microbial Biomass	Microbial Biomass Efficiency (%)
0	14.41[c]	0.038	17.37	19.86	145.02	88.88
3	16.44[b]	0.042	18.24	18.95	145.08	88.37
6	17.95[a]	0.045	19.05	18.47	147.10	88.009
SEM	0.416	0.0039	0.710	0.460	0.954	0.266
P-value	.0007	.4556	.292	.1528	.2648	.1585

Abbreviations: Pf, partitioning factor; SEM, standard error of the mean.
B—Potential of gas production.
C—Gas production rate.
[a, b, c] Means with common letter (s) within each column do not differ significantly ($P > .05$).

Table 3
Effects of Natuzyme enzyme levels on dry matter (DM) and acid neutral detergent fiber (NDF) digestibility (%) of wheat straw.

Enzyme (g/kg DM)	Time (h)					
	24		48		72	
	DM	NDF	DM	NDF	DM	NDF
0	24.76[b]	7.90[b]	27.54	12.70	38.04	16.19
3	25.55[b]	9.44[b]	28.40	13.76	38.35	16.66
6	29.055[a]	12.66[a]	29.04	13.78	39.66	17.52
SEM	0.719	0.871	0.724	0.658	0.988	0.680
P-value	.0027	.0069	.0995	.4354	.1968	.40403

Abbreviation: SEM, standard error of the mean.
[a, b] Means with common letter (s) within each column do not differ significantly ($P > .05$).

Table 4
Effects of Natuzyme enzyme levels on dry matter (DM) and acid neutral detergent fiber (NDF) digestibility (%) of alfalfa.

Enzyme (g/kg DM)	Time (h)					
	24		48		72	
	DM	NDF	DM	NDF	DM	NDF
0	37.40	16.70[b]	42.41	19.23[c]	53.28	24.40[b]
3	38.48	19.63[b]	42.58	23.02[b]	54.24	24.52[b]
6	38.92	26.88[a]	45.03	28.30[a]	56.68	28.20[a]
SEM	0.969	1.562	1.59	0.399	1.011	0.911
P-value	.4684	.0007	.3786	.0001	.6179	.0191

Abbreviation: SEM, standard error of the mean.
[a, b, c] Means with common letter (s) within each column do not differ significantly ($P > .05$).

difference in mode of action of this enzyme on alfalfa hay and maize straw may be due to differences in cell wall structure.

The fibrolytic enzymes have led to the release of plant polysaccharides through the breakdown of specific chemical bonds of forages that are not degraded by animal enzymes [12]. They increase the number of fibrolytic bacteria that will improve the forage digestibility. Finding showed that xylanase plays a limited role in the fermentation of alfalfa hay and corn forage [27]. Most of the exogenous enzymes contain cellulase and xylanase that usually have synergistic effects on cell wall hydrolysis [28,29]. In general, the effect of the addition of the fibrolytic enzymes is affected by the combination of the diet, the type of enzyme, the enzyme level, the enzyme stability, its method of application [30], level of animal productivity, and exogenous enzyme interaction with feed, host, and rumen microorganisms [31]. Probably, microbial enzymes from herbivores species that use different types of pasture forage in the hydrolysis of the plant cell wall, are different [32].

The present study showed that the supplementation of Natuzyme enzyme levels on wheat straw digestibility had a significant effect at 24 hours of incubation and increasing the content of enzyme had increased the DM digestibility. DM digestibility of alfalfa hay had not been affected by treatments. According to the reports, higher in vitro DM digestibility values were obtained with the cellulase enzyme treatment in comparison with control treatment [20]. Gado et al [11] showed that the increased DM digestibility 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.

It is stated that anaerobic bacteria with a relatively high growth rate and high digestibility of insoluble cellulose could be used as a higher source of cellulase enzymes and used industrially for enzymatic hydrolysis. Rowe et al [33] showed that the addition of exogenous enzyme into diet containing wheat and high nonstarch polysaccharides improved starch prececal digestion, and increased the profitability of wide range of seeds in the horse diet. However, increased degradation of NDF using exogenous enzymes of high concentrate diet was reported by López et al. [34].

Most of the enzyme products caused the subtle changes in the cell wall structure by enzymatic action, which allows ruminal microbes to obtain earlier access to fermentable substrate [34]. Gado et al [11] concluded a positive correlation between xylanase activity and the NDF degradation of alfalfa hay in vitro, which was attributed to a modification of the fiber structure by enzyme action. Gado et al. [35] reported that enzyme supplementation increased in vitro digestibility of DM and cell wall fractions, whereas Bowman et al [36] reported a 25% increase in total-tract NDF digestibility with a fibrolytic enzyme product. Enzymes applied to feed increased solubility of DM and NDF and possibly released more nutrients that were available to support the production of glycocalyx, which is produced by bacteria and permits adhesion between bacteria and substrate [37]. The researchers reported that 5 g/kg DM Natuzyme exogenous enzyme increased the disappearance of sesame straw DM and NDF by in vitro rumen bacteria [18]. Wang et al [38] also applied a fibrolytic multienzyme (1.5 g/kg) to wheat straw and reported the rapidly degradable fraction of DM tended to increase, compared with the control.

5. Conclusion

Addition of Natuzyme enzyme mixture showed the proper fermentation and gas production of alfalfa hay and wheat straw without affecting the DM digestibility by cecum bacteria. Therefore, the findings of the study suggested that this enzyme can be used as a feed additive in Arabian horse nutrition.

Acknowledgments

The authors gratefully acknowledge the Khuzestan Agricultural Sciences and Natural Resources University for their laboratory and financial support.

References

[1] Tisserand JL. Microbial digestion in the large intestine in relation to monogastric and polygastric herbivores. Acta Vet Scand Suppl 1989;86:83.
[2] O'Connor-Robison CI, Nielsen BD, Morris R. Cellulase supplementation does not improve the digestibility of a high-forage diet in horses. J Equine Vet Sci 2007;27:535–8.
[3] Kohnke JR, Kelleher F, Trevor-Jones P. Feeding horses in Australia: a guide for horse owners and managers. Barton, Australia: Rural Industries Res. and Development Corp.; 1999.
[4] Argenzio RA, Southworth M, Stevens CE. Sites of organic acid production and absorption in the equine gastrointestinal tract. Am J Physiol 1974;226:1043–50.
[5] Al Jassim RAM, Rowe JB. Better understanding of acidosis and its control. Recent advances in animal nutrition in Australia, 12; 1999. p. 91–7.
[6] National Research Council. Nutrient requirements of horses. 4th ed. Washington, DC: National Academy Press; 1978.
[7] Salem AZM, Gado HM, Colombatto D, Eghandour MMY. Effect of exogenous enzymes on nutrient digestibility, ruminal fermentation and growth performance in beef steers. Livest Sci 2013;154:69–73.
[8] Kholif AE, Baza-García LA, Eghandour MMY, Salem AZM, Barbabosa A, Dominguez-Vara IA, Sanchez-Torres JE. 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. J Equine Vet Sci 2016;39:44–50.
[9] Gado HM, Kholif AE, Salem AZM, Elghandour MMM, Olafadehan OA, Martinez MA, Al-Momani AQ. Fertility, mortality, milk output and body-thermoregulation of growing Hy-Plus rabbits fed on diets supplemented with multi-enzymes preparation. Trop Anim Health Prod 2016;48:1375–80.
[10] Gado HM, Mellado M, Salem AZM, Zaragoza A, Seleem TST. Semen characteristics and sexual hormones of Hy-Plus rabbit bucks influenced by a dietary multi-enzymes additive. World Rabbit Sci 2015;23:111–20.
[11] Gado HM, Eghandour MMY, Cipriano M, Odongo NE, Salem AZM. Rumen degradation and nutritive utilization of wheat straw, corn stalks and sugarcane bagasse ensiled with multi-enzymes. J Appl Anim Res 2017;45:485–9.
[12] Beauchemin KA, Morgavi DP, McAllister TA, Yang WZ, Rode LM. The use of enzymes in ruminant diets. In: Garnsworthy PC, Wiseman J, editors. Recent advances in animal nutrition. England, Loughborough: Nottingham University Press; 2001. p. 297–322.
[13] Hainze MTM, Muntifering RB, McCall CA. Fiber digestion in horses fed typical diets with and without exogenous fibrolytic enzymes. J Equine Vet Sci 2003;23:111–5.
[14] Blümmel M, Makkar HPS, Becker K. In vitro gas production: a technique revisited. J Anim Physiol Anim Nutr 1997;77:24–34.
[15] Ørskov ER, McDonald I. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J Agric Sci 1979;92:499–503.
[16] Makkar HPS, Becker K. Do tannins in leaves of trees and shrubs from African and Himalayan regions differ in level and activity. Agroforest Syst 1998;40:59–68.
[17] Makkar HP, McSweeney CS, editors. Methods in gut microbial ecology for ruminants, vol. 10. Dordrecht, Netherlands: Springer; 2005. p. 25.
[18] Mohammadabadi T, Chaji M. Effect of exogenous enzyme on in vitro fermentation of sesame straw by rumen bacteria culture. J Appl Anim Res 2010;39:161–3.

[19] Statistical Analysis System (SAS). SAS user's guide: statistics. Ver 9.0. Cary, NC, USA: SAS Institute; 2002. p. 956.
[20] Salem AZM, Elghandour MM, Kholif AE, Odongo NE, Jiménez FJ, Montes-de-Oca R, Dibarrat JA. 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. J Equine Vet Sci 2015;35:735–43.
[21] Murray JAM, Longland AC, Davies DR, Hastie PM, Moore-Colyer M, Dunnett C. The effect of enzyme treatment on the nutritive value of lucerne for equids. Livest Sci 2007;112:52–62.
[22] Salem AZM, Hassan AA, Gado HM, Khalil MS, Simbaya J. Effect of sun-drying and exogenous enzymes on nutrients intake and digestibility as well as nitrogen utilization in sheep fed *Atriplex halimus* foliages. Anim Feed Sci Technol 2012;171:128–35.
[23] Sipai SH, Dutta KS, Savsani HH, Murthy KS, Vataliya PH, Chavda JA, Gajera AP. Inclusion of different exogenous fibrolytic enzymes to dry jowar fodder and their effect on in vitro total gas production. Vet World 2013;6:839.
[24] Mao HL, Wu CH, Wang JK, Liu JX. Synergistic effect of cellulase and xylanase on in vitro rumen fermentation and microbial population with rice straw as substrate. Anim Feed Sci Technol 2013;13:477–87.
[25] Wallace RJ, Wallace SJ, McKain N, Nsereko VL, Hartnell GF. Influence of supplementary fibrolytic enzymes on the fermentation of corn and grass silages by mixed ruminal microorganisms in vitro. J Anim Sci 2001;79:1905–16.
[26] Kung L, Cohen MA, Rode LM, Treacher RJ. The effect of fibrolytic enzymes sprayed onto forages and fed in a total mixed ratio to lactating dairy cows1. J Dairy Sci 2002;85:2396–402.
[27] Eun JS, Beauchemin KA, Schulze H. Use of exogenous fibrolytic enzymes to enhance in vitro fermentation of alfalfa hay and corn silage. J Dairy Sci 2007;90:1440–51.
[28] Bhat MK, Hazlewood GP. Enzymology and other characteristics of cellulases and xylanases. In: Bedford MR, Partridge GG, editors. Enzymes in farm animal nutrition. Wallingford, Oxon, UK: CABI Publishing; 2001. p. 11–60.
[29] Hernandez A, Kholif AE, Lugo-Coyote R, Elghandour MMY, Cipriano M, Rodríguez GB, Odongo NE, Salem AZM. The effect of garlic oil, xylanase enzyme and yeast on biomethane and carbon dioxide production from 60-d old Holstein dairy calves fed a high concentrate diet. J Clean Prod 2017;142:2384–92.
[30] Yang WZ, Beauchemin KA, Rode LM. A comparison of methods of adding fibrolytic enzymes to lactating cow diets. J Dairy Sci 2000;83:2512–20.
[31] Beauchemin KA, Colombatto D, Morgavi DP, Yang WZ. Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. J Anim Sci 2003;81(14_suppl_2):E37–47.
[32] Elghandour MMY, Salem AZM, Ronquillo M, Bórquez JL, Gado HM, Odongo NE, Peñuelas CG. Effect of exogenous enzymes on in vitro gas production kinetics and ruminal fermentation of four fibrous feeds. Anim Feed Sci Technol 2013;179:46–53.
[33] Rowe J, Brown W, Bird S. Safe and effective grain feeding for horses. A report for the rural industries research and development corporation. Australia: Barton Act; 2001.
[34] López D, Elghandour MMY, Salem AZM, Vázquez-Armijo JF, Salazar MC, Gado HM. Influence of exogenous enzymes on in vitro gas production kinetics and dry matter degradability of a high concentrate diet. Anim Nutr Feed Technol 2013;13:527–36.
[35] Gado HM, Salem AZM, Camacho LM, Elghandour MMY, Salazar MC. Influence of exogenous enzyme on in vitro ruminal degradation of ensiled rice straw with DDGS. Anim Nutr Feed Technol 2013;13:569–74.
[36] Bowman GR, Beauchemin KA, Shelford JA. The Proportion of the diet to which fibrolytic enzymes are added affects nutrient digestion by lactating dairy cows. J Dairy Sci 2002;85:3420–9.
[37] Yang WZ, Beauchemin KA, Rode LM. Effects of an enzyme feed additive on extent of digestion and milk production of lactating dairy cows. J Dairy Sci 1999;82:391–403.
[38] Wang Y, McAllister T, Rode L, Beauchemin K, Morgavi D, Nsereko V, Yang W. Effects of exogenous fibrolytic enzymes on epiphytic microbial populations and in vitro digestion of silage. J Sci Food Agric 2002;82:760–8.