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Original Research

Horse Fecal Methane and Carbon Dioxide Production and Fermentation Kinetics Influenced by *Lactobacillus farciminis*–Supplemented Diet

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ABSTRACT

The effect of equine fecal inocula on the in vitro gas, methane (CH_4), and carbon dioxide (CO_2) production was elucidated in the present study. Fecal inocula were obtained from four Azteca horses (aged 5–8 years, 480 ± 20.1 kg). In vitro fermentation (up to 48 hours) was performed with substrate consisting of 50% (w/w) oat straw and 50% (w/w) of a commercial concentrate in the presence of a commercial *Lactobacillus farciminis* product (0–6 mg/g DM of substrate). Incorporation of *L. farciminis* resulted in increased levels of asymptotic gas (GP), CH_4, and CO_2 production ($P < .05$). The lag time and the rate of GP were shown to be independent from *L. farciminis* addition (linear, $P > .05$; quadric, $P > .05$). Furthermore, a slight reduction in fermentation pH (linear, $P = .029$) and higher metabolizable energy values ($P = .001$) were obtained with *L. farciminis* supplementation in a dose-dependent manner. No significant impact of *L. farciminis* on dry matter degradability values was estimated ($P > .05$). In vitro gas, CH_4, and CO_2 production were increased (linear, $P \leq .001$) in the presence of *L. farciminis* from 6 hours of incubation onward. In conclusion, addition of *L. farciminis* at a dose-dependent manner (2–6 mg/g DM of diet) was observed to be persuasive in terms of attaining amicable hindgut fermentation by improving fecal gas kinetics viz. gas, CH_4, and CO_2 production without any side effect.

1. Introduction

Horses belong to non-ruminant herbivores and therefore, the hindgut represents a fermentative chamber for dynamic and diversified microbiota. These microbes ferment the fibers gradually and endow horses to prosper on a high-fiber forage-rich feed to retain their normal digestive system. Volatile fatty acids, obtained by fermentation of fibers, are the dominant energy sources (>50%) for horses [1]. Similarly, starch-rich forage such as cereal grains are relevant sources of energy in concentrate feeds for horses. However, feeding disorders including hindgut acidosis, gastric ulceration, laminitis, endotoxemia, and colic have been linked to feeding high-starch grain diets [2]. Furthermore, an alteration of small intestinal starch digestibility was reported, thereby resulting in an alteration of the microbial population and fibrolytic activity in the hindgut [3] and therefore, a significant reduction of energy utilization from the diet. There is an urgent need for fiber-based diets containing lower amounts of sugars and starch to acquire the energy demands of horses and to maintain their health and integrity by reducing incidence of feeding disorders. In addition, there is an increased demand to enhance the athletic high-level performances of modern horses. This aim could be achieved by establishing new

Animal welfare/ethical statement: All procedures and animals used in this study were in compliance with the guidelines of the Mexican Official Mexican Standard 062-ZOO-1999.

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feeding strategies to meet the necessary nutrient requirements of horses.

The health benefits of consuming probiotics, especially lactic acid bacteria (LAB), have created immense interest among researchers globally. Lactic acid bacteria are generally regarded as safe [4], and some LAB have been reported to tolerate the conditions of the gastrointestinal tract [5]. Furthermore, LAB are known to affect gas production (GP) in monogastric animals. Tsukahara et al [6] reported a significant reduction in intestinal GP, particularly carbon dioxide (CO_2) in pigs in the presence of LAB. However, hydrogen sulphide production was increased, and a negative correlation between the hydrogen sulphide and methane (CH_4) production was reported. Takahashi et al [7] demonstrated the impact of LAB on rumen methanogenesis and reported an increase in total gas, CH_4, and CO_2 production. The ability of LAB to affect fermentation in an animal is strongly dependent on the chemical composition of the diets.

Currently, *Lactobacilli* have not been used effectively playing promising role in horse feeding. However, *Lactobacilli* are of interest not only because of their potential contribution to fermentation but also because of their ability to combat several pathogens [8]. To the best of our knowledge, no research activities have been performed in the field of equine probiotic strategy, revealing the impact of *Lactobacilli*, particularly *Lactobacillus farciminis* on the GP in horses. However, the beneficial impact of exogenous *Lactobacilli* and other probiotics in horses toward digestibility and fermentation end-products as well as acute enterocolitis treatment has been reported [9,10].

Based on previous investigations, *Lactobacillus* sp. is supposed to enhance digestion of high-fiber feeds of poor quality in the hindgut of horses. Thus, this study was aimed to elucidate the impact of exogenous *Lactobacilli* (*L. farciminis*) on fecal gas kinetics (in vitro GP, CH_4, and CO_2 emission) through oat straw digestion as indicators of the fermentative activity in the hindgut of horses.

2. Materials and Methods

All procedures implied in handling animals of this study were in compliance with the guidelines of the Mexican Official Mexican Standard 062-ZOO-1999.

2.1. Preparation of Bacterial Inoculum

Rumen medium dispersed in a buffer as described by Goering and Van Soest [11] was inoculated with 3×10^{11} CFU/g (colony forming unit/gram) of *L. farciminis* (a commercial product of SAFISIS, Toluca, Mexico) and then incubated for 24 hours at 30°C under static conditions after saturation with CO_2 for 10 minutes.

2.2. Substrate and Treatments

The mixture of oat straw and a commercial concentrate (PURINA, Toluca, Mexico) at 1:1 (w/w DM) was used as a substrate for the in vitro fecal evaluation (Table 1). Before usage, the substrate was dried at 60°C for 48 hours. Four doses (0, 2, 4, and 6 mg/g DM of substrate) of *L. farciminis* were applied in the in vitro fecal fermentation study.

Table 1
Chemical composition of ingredients and total mixed ration used (g/kg DM).

Substrate	Organic Matter	Crude Protein	Neutral Detergent Fiber	Acid Detergent Fiber
Concentrate	901.8	112	511	202.8
Oat straw	929.4	26.7	668.7	405.1
Total mixed ration	915.6	69.4	589.8	303.9

2.3. In Vitro Incubation

Four Azteca horses (aged 5–8 years, 480 ± 20.1 kg) were used for fecal content (inoculum source) collection. The horses were fed ad libitum with the substrate mentioned above. Fresh water was made available to the animals 1 week before the collection phase. Fecal samples were obtained from the rectum of the horses. Culture broth was added to the fecal contents in a ratio of 4:1. The mixture was kept under CO_2 environment throughout the entire in vitro incubation process (39°C; 48 hours). All incubations were performed in triplicate and either rumen fluid or fecal fluid was used as a blank. Gas, CO_2, and CH_4 production were estimated after 2, 4, 6, 8, 10, 12, 14, 24, and 48 hours of incubation using the pressure reading technique [12] or a Gas-Pro detector (Gas Analyzer CROWCON Model Tetra3, Abingdon, UK). Furthermore, pH was measured and after filtration, dry matter degradability (DMD) was estimated [13].

2.4. Chemical Analyses and Calculations

Dry matter, ash, nitrogen, acid detergent fiber, and lignin content of the substrate were determined according to the Association of Official Analytical Chemists [14]. For neutral detergent fiber quantification, the methodology of Van Soest et al [15] was employed. Kinetic parameters of GP (mL/g DM) were obtained by fitting the data according to France et al [16] using the NLIN option of SAS [17]. Metabolizable energy (ME) and DMD were calculated according to Menke et al [18].

2.5. Statistical Analyses

Fecal fermentation data were analyzed as a completely randomized design using the PROC GLM option of SAS:

$$Y_{ij} = \mu + B_i + \varepsilon_{ij}$$

where, Y_{ij} = observation obtained with i^{th} level of LAB; B_i = level of LAB (I = 1–4); μ = general mean; ε_{ij} = experimental error.

Linear and quadratic polynomial contrasts were used to evaluate responses for increasing levels of *L. farciminis*. Turkey's test was employed to measure multiple comparisons among means. Significance level was declared at $P < .05$.

3. Results

Asymptotic GP (linear effect, $P = .001$), asymptotic CH_4 production (linear, $P = .021$; quadric, $P = .034$), and asymptotic CO_2 production (linear, $P = .042$; quadric, $P = .031$) were observed to be higher in the presence of *L. farciminis* in a dose-dependent manner. However, no effect ($P > .05$) of *L. farciminis* supplementation on the rate of gas, CH_4, and CO_2 production as well as on the lag times was observed (Table 2).

Furthermore, an increase in ME (linear, $P = .001$) was observed in the presence of *L. farciminis*. *L. farciminis* addition did not result in a significant effect on DMD values ($P > .05$). Fermentation pH was shown to be slightly lower ($P = .029$) in the presence of *L. farciminis* (Table 2).

In vitro gas and CH_4 production were increased (linear, $P = .001$) in the presence of *L. farciminis* from 6 hours of incubation onward (Table 3). Carbon dioxide production was also increased significantly (linear, $P < .001$) after 6, 24, and 48 hours of incubation in the presence of *L. farciminis* (Table 3).

Table 2
In vitro horse fecal gas kinetics, CH_4, and CO_2 production and fermentation kinetics of a total mixed ration of oat straw and concentrate (1:1) as affected by different levels of *Lactobacillus farciminis* (mg/g DM of substrate).

L. farciminis Doses	GP[a]			CH_4 Production[b]			CO_2 Production[c]			Fecal Fermentation Kinetics		
	b	*c*	*L*	*b*	*c*	*L*	*b*	*c*	*L*	pH	ME	DMD
0	150.6	0.136	1.56	32.1	0.016	3.48	93.8	0.0178	5.11	6.82	7.01	0.500
2	166.2	0.132	1.79	35.5	0.015	3.99	103.5	0.0173	5.86	6.80	7.42	0.469
4	192.0	0.141	1.76	41.0	0.016	3.92	119.6	0.0184	5.77	6.73	8.14	0.477
6	208.8	0.148	1.91	44.6	0.017	4.26	130.0	0.0194	6.26	6.73	8.61	0.503
SEM	5.90	0.0079	0.082	2.34	0.0011	0.126	3.65	0.0012	0.362	0.026	0.142	0.0125
Linear	0.001	0.709	0.128	0.021	0.122	0.235	0.042	0.142	0.32	0.029	0.001	0.228
Quadratic	0.498	0.501	0.251	0.034	0.241	0.521	0.031	0.354	0.421	0.451	0.405	0.237

Abbreviations: CH_4, methane; CO_2, carbon dioxide; DMD, dry matter degradability; GP, gas production; ME, metabolizable energy; SEM, standard error of the mean.
[a] *b* is the asymptotic GP (mL/g DM); *c* is the rate of GP (/h); and *L* is the initial delay before GP begins (h).
[b] *b* is the asymptotic CH_4 production (mL/g DM); *c* is the rate of CH_4 production (/h); and *L*, is the initial delay before CH_4 production begins (h).
[c] *b* is the asymptotic CO_2 production (mL/g DM); *c* is the rate of CO_2 production (/h); and *L*, is the initial delay before CO_2 production begins (h).

4. Discussion

In the last few years, equine nutritionists have concentrated to mitigate the risks associated with feeding high-starch concentrates to horses. One focus in this respect has been the supplementation of probiotics into the diets. High concentrate fed diets supplemented with direct-fed microorganisms or probiotics was shown to reduce the risk of several disorders. Probiotics are used as feed additives that enhance intestinal microbial balance and digestive health in the host animal. Among probiotics, the genus *Lactobacillus* is the most frequently exploited LAB [19]. In spite of the broad applications of LAB in the feed preparations intended for equine, no peer-reviewed research has evidenced the efficacy of either a single strain or a multiple strain, particularly in terms of nutrient digestion, in vitro gas, CH_4 and CO_2 production, and fermentation kinetics in mature horses.

Previously, a tremendous effort had been undertaken to assess the impact of LAB supplementation on fermentation end-products and digestibility in horses fed high- and low-starch concentrates [10]. Addition of *Enterococcus faecium* was shown to result in higher ether extracts ($P < .05$) and a decreased sodium ($P < .1$) digestibility. The supplementation of LAB was reported to increase copper ($P < .05$), iron, and zinc digestibility in horses. Furthermore, a limited effect on the nutrient digestibility and the lack of acidosis associated with feeding high-starch concentrates was observed.

Probiotic supplements intended for horses may aid in supporting digestive health, promote efficient digestion, inhibit the growth of pathogenic bacteria, reduce side effects associated with antibiotic administration, increase lactation in mares, increase growth in foals, and reduce the incidence of various disorders. In the present study, a successful attempt was undertaken to fulfill the gap in equine research and to demonstrate an increase in in vitro cumulative gas, CH_4, and CO_2 production from a high-fiber substrate in the presence of *L. farciminis*.

4.1. In Vitro Fecal Gas Production

Supplementation of the oat straw–containing diet with *L. farciminis* resulted in higher asymptotic gas, CH_4, and CO_2 production. This may be attributed to the ability of *L. farciminis* to metabolize components of the used substrate. In general, incorporation of *L. farciminis* in horse feed improved fecal fermentation, and therefore asymptotic GP. In line with the finding of the present investigation, a more balanced microbial population in the hindgut of horses and an increase in feed digestibility, and therefore more efficient energy utilization from the diets were reported when the feed was supplemented with live microorganisms [20]. The observed increase in GP is linked to a higher fermentation activity in the in vitro system [21]. This is due to a better availability of nutrients for microbial fermentation and results in an improved nutrient degradability [22]. Gases such as H_2, CH_4, and CO_2 are mainly produced due to the microbial activity on dietary carbohydrates.

In the present study, no effect of *L. farciminis* on the fermentation rate and the lag time of GP were observed. This is in contrast to previous results, where a reduction in the rate of GP was estimated in the presence of live microorganisms [21,23]. The different types and chemical composition of the substrates used in those studies might explain the observed differences.

4.2. Fecal Fermentation Kinetics

Supplementation of the diets with *L. farciminis* was found to affect fermentation pH, ME, and DMD. Fermentation pH was shown to be lowered in the presence of *L. farciminis*. This effect could be explained by the production of lactic acid by this group of microorganisms [24]. The observed increased in ME might be due to a stimulation of the microbial activity in the hindgut by *L. farciminis*,

Table 3
In vitro horse fecal gas, CH_4, and CO_2 production (mL/g DM) of a total mixed ration of oat straw and concentrate (1:1) at 6, 24, and 48 hours of incubation, as affected by different levels of *L. farciminis* (mg/g DM of substrate).

L. farciminis Doses	In vitro GP			CH_4 Production			CO_2 Production		
	6 h	24 h	48 h	6 h	24 h	48 h	6 h	24 h	48 h
0	83.2	144.1	150.3	17.8	30.8	32.1	51.8	89.7	93.6
2	90.7	159.1	165.9	19.4	34.0	35.4	56.5	99.1	103.3
4	109.3	185.3	191.8	23.3	39.6	40.9	68.1	115.4	119.5
6	123.0	202.8	208.6	26.3	43.3	44.5	76.6	126.3	129.9
SEM	3.02	5.23	5.85	0.64	1.12	1.25	1.9	3.3	3.6
Linear	0.001	0.001	0.001	<0.001	<0.001	<0.001	0.0	0.0	0.0
Quadratic	0.177	0.406	0.492	0.038	0.087	0.105	0.1	0.3	0.3

Abbreviations: CH_4, methane; CO_2, carbon dioxide; GP, gas production; SEM, standard error of the mean.

which should result in an improved nutrient digestion. However, *L. farciminis* supplementation did not affect DMD significantly.

4.3. Methane and Carbon Dioxide Production

A dose-dependent increase in the rate of in vitro gas, CH_4, and CO_2 production was observed after 48 hours of incubation in the presence of *L. farciminis*. The average mean retention time of feed in the gastrointestinal tract of a horse was reported to be between 36 and 38 hours [25,26]. Incubation of either grains or forages with feces as inoculum is known to result in the production of significant amounts of fermentation gas and an increase in the lag phase [27]. This might be due to the presence of different microorganisms in the feces [23]. An additional effect of probiotics was shown to be dependent on several factors such as source, type, and dose of the probiotic as well as the composition of the animal diet [27]. In contrast to the present study, Mwenya et al [28] reported that live microorganisms such as yeasts have the capability to shift H_2 utilization from methanogenesis to the production of acetate from CO_2 and H_2 using homoacetogenic bacteria. In addition, a 20% reduction in CH_4 production after 48 hours of alfalfa incubation in the presence of live microorganisms was observed [29]. Furthermore, Newbold and Rode [30] reported a significant reduction in CH_4 production due to the supplementation of live yeasts.

5. Conclusion

L. farciminis at doses from 2 to 6 mg/g DM of diet was shown to improve GP and fermentation kinetics. Thus, *L. farciminis* fed to horses at the above mentioned doses could improve hindgut digestion of high-fiber roughages such as oat straw. In vitro CH_4 production was increased in the presence of *L. farciminis* from 6 hours of incubation. Carbon dioxide emission was also increased after 6, 24, and 48 hours of incubation at diversified doses of *L. farciminis*. However, further in vivo studies certainly need to be performed to fully elucidate the potential of supplementing horse diets with diversified doses of *L. farciminis*, particularly in terms of fermentation kinetics in the hindgut of the animals.

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