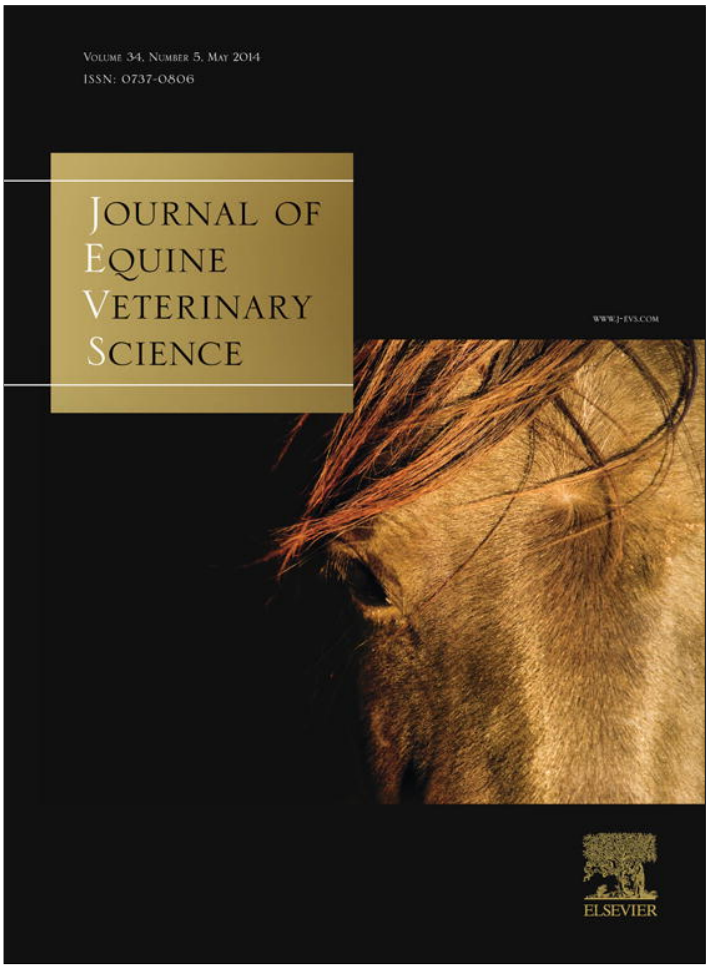

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Original Research

In Vitro Fermentative Capacity of Equine Fecal Inocula of 9 fibrous Forages in the Presence of Different Doses of *Saccharomyces cerevisiae*

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ABSTRACT

This experiment was conducted to evaluate *in vitro* effects of equine fecal inocula fermentative capacity on 9 fibrous forages in the presence of *Saccharomyces cerevisiae*. The fibrous feeds were corn stover (*Zea mays*), oat straw (*Avena sativa*), sugarcane bagasse and leaves (*Saccharum officinarum*), llanero grass leaves (*Andropogon gayanus*), Taiwan grass leaves (*Pennisetum purpureum*), sorghum straw (*Sorghum vulgare*), and steria grass leaves (*Cynodon plectostachyus*). Fibrous feed samples were incubated with several doses of *S. cerevisiae*; 0 (control), 1.25 (low), 2.5 (medium) and 5 (high) mg/g dry matter (DM) of a commercial yeast product containing 1×10^{10}/g. Fecal inoculum was collected from 4 adult horses were fed on an amount of commercial concentrate and oat hay ad libitum. Gas production (GP) was recorded at 2, 4, 6, 8, 10, 12, 24, and 48 hours post inoculation. An interaction occurred between feeds and yeast dose for fecal pH ($P < .01$), asymptotic GP (*b*, ml/g DM); rate of GP (*c*, /hr); initial delay before GP began (*L*, hours), GP at 4 hours and 48 hours ($P < .01$), and GP at 8 hours ($P < .01$) and at 24 hours ($P < .01$). Differences in fecal fermentation capacity between the tropical and template grass ($P < .05$) occurred for fecal pH, *c*, and GP during first 12 hours, whereas differences occurred ($P < .05$) between the agriculture byproducts and the grasses for fecal pH, *b*, and GP from 8 to 48 hours. Fermentation capacity between straws versus not straws ($P < .05$) differed for fecal pH, *b*, and GP after 12 hours between straws *versus* not straws. Addition of *S. cerevisiae* to *Z. mays* stover reduced ($P < .01$) fecal pH and the *c* fraction with a higher ($P < .01$) *b* fraction *versus* the other feeds. From 4 to 24 hours, *S. officinarum* bagasse improved GP to the highest values *versus* *S. officinarum* leaves. After 24 hours, *Z. mays* stover had the highest GP, whereas *C. plectostachyus* leaves had the lowest. There were no differences among the yeast doses for all measured parameters with the exception of *L* values (linear effect; $P < .01$). The *Z. mays* stover had the highest nutritive compared to the other fibrous feeds. However, addition of *S. cerevisiae* at 2.5 to 5.0 g/kg DM improved fecal fermentation capacity of low-quality forages.

1. Introduction

The presence of microorganisms in the hindgut allows the horse to efficiently digest fiber and roughage and often

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leads to their selection as the main component of mature horse diets [1]. Although the horse is able to nutritionally use diets high in fibrous feeds, their digestive system is not as efficient as that of ruminants [1].

There is increasing interest in feeding fiber-based feeds as an alternative to high-starch cereal grains to horses as a means of meeting the energy demands and reduce various pathologies, such as gastric ulceration, hind-gut acidosis, laminitis, and colic [2,3], associated with feeding high levels of cereal grains. Depending on the forage type and time of harvest, forages of moderate to high nutritive value may meet the energy and crude protein (CP) requirements of horses [4]. Horses naturally use forage as a primary component of their diet, and forage is a basic necessity for normal function of the equine digestive system. High forage rations are desirable because they contain low levels of starch and sugar. Feeding a minimum 1% of body weight as fiber is very important to minimize the incidence of hindgut acidosis [5], colic [6], gastric ulcers [7], and stereotypical behaviors [8]. In tropical areas such as Mexico, forages used as feeds are generally low in digestibility and low in true protein [9]. Therefore, there is a need to develop feeding strategies which meet the requirements of performance horses while maintaining gut health and integrity.

Yeast cultures can increase the number of lactate-using bacteria and result in increased cecal pH [10]. In some studies, supplementation of equine diets with a yeast culture has improved digestion of low-quality forages, which would be advantageous to the horse's health [11]. Yeast cultures can provide enhanced microbial environmental conditions and/or increase the total number of hindgut microorganisms, resulting in improved digestibility of forages in the hindgut [1].

However, previous research results with *Saccharomyces cerevisiae* supplementation of horse diets has been variable and inconsistent. Addition of *S. cerevisiae* to equine diets may stimulate hindgut digestion by altering microbial population, and some research has indicated that adding *S. cerevisiae* to the diet of equines can improve nutrient digestibility, [12] increase microbial populations [13,14], and maintain cecal pH [13,15]. However, other studies have reported no improvement in nutrient digestibility when *S. cerevisiae* supplements were given to horses *in vivo* [16,17] and *in vitro* [14].

The purpose of this experiment was to evaluate the fermentative capacity of 9 fibrous forages in the presence of various doses of *S. cerevisiae*, using the *in vitro* gas production (GP) technique of Theodorou et al [18] as an indicator of hindgut activity using equine fecal inocula.

2. Materials and Methods

2.1. Fibrous Feed Species and Yeast Culture Levels

Three individual samples of each of the fibrous feeds of corn stover and cobs (*Zea mays*), oat straw (*Avena sativa*), sugarcane bagasse and leaves (*Saccharum officinarum*), llanero grass leaves (*Andropogon gayanus*), Taiwan grass leaves (*Pennisetum purpureum*), sorghum straw (*Sorghum vulgare*), and steria grass leaves (*Cynodon plectostachyus*) were randomly and manually harvested in triplicate from different sites in the state of Mexico in Mexico. Samples of stover, straws, and leaves were collected at the last stages of maturity and dried at 60°C for 48 hours in a forced air oven to constant weight, ground in a Wiley mill to pass through a 1-mm sieve, and stored in plastic bags for subsequent determination of chemical components and *in vitro* GP. Four levels of Saccharomyces *cerevisiae* commercially available as a feed additive (Procreatin 7; Safmex/Fermex S.A. de C.V., Toluca, Mexico) in powdered form containing 1×10^{10} colony-forming units (CFU)/g of yeast product were used at levels (mg/g dry matter [DM] of substrate) of control (0 mg), low (1.25 mg), medium (2.50 mg), and high (5.00 mg). Feed samples were incubated with yeast doses which were added to the bottles immediately before incubation. A stock solution of yeast culture doses was prepared before treatments in distilled water in order to obtain suitable doses in each 1 ml of stock solution.

2.2. In Vitro Incubation

Fecal inoculum was collected from 4 adult horses ranging from 5 to 8 years of age and weighing 480 ± 20.1 kg. Horses were fed daily on an amount of commercial concentrate (Purina, Toluca, Mexico) and oat hay as ad libitum. Fresh water was available to the horses at all times.

The methods used for the GP technique were as described by Theodorou et al [18]. Fecal contents were collected directly from the rectum of each horse and immediately transferred to the laboratory for *in vitro* incubation. Fecal contents were combined with culture medium in a ratio of 1:4 and kept dispensed under CO_2 immediately after extraction and during the incubation process. Fecal inoculum mixed with culture medium was used to inoculate 3 identical series (runs) of bottles containing 1 g of DM of each 1 of the fibrous feed species as substrates. For each inoculum, 3 substrate-negative controls (blank) were also included. This resulted in a total of 324 bottles for GP (9 fibrous feeds × 3 individual samples × 3 runs × 4 yeast doses). Once all bottles were filled, they were immediately closed with rubber stoppers, shaken, and placed in the incubator at 39°C. Gas production readings were made at 2, 4, 6, 8, 10, 12, 24, and 48 hours post-inoculation, using the pressure reading technique (Extech instruments; Waltham, CT, USA). At the end of incubation (ie, 72 hours), bottles were uncapped, and pH was measured using a pH meter (Conductronic pH15, Puebla, Mexico).

2.3. Calculations and Statistical Analyses

To estimate kinetic parameters of GP, results (ml/g DM) were fitted using the NLIN option of SAS [19] according to France et al [20] 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 (/hr), and L (hours) is the discrete lag time prior to GP.

The experimental design was a completely randomized design considering, as fixed factors, feed species (S) and

Table 1
Sample types and chemical compositions of the 9 fibrous feeds (g/kg DM)

Species	Sample	Organic Matter	Crude Protein	Acid Detergent Fiber	Neutral Detergent Fiber
Zea mays	Straw-tropical	959.7	62.9	274.4	476.7
Avena sativa	Straw- tropical	923.6	37.2	380.0	537.8
Zea mays	Cobs leaves- tropical	976.1	21.4	428.9	698.9
Saccharum officinarum	Bagasse- tropical	982.0	25.7	324.4	458.9
Saccharum officinarum	Leaves- tropical	929.7	42.9	385.6	614.4
Andropogon gayanus	Leaves- temperate grass	948.5	22.9	485.2	697.8
Pennisetum purpureum	Leaves- temperate grass	948.5	22.9	482.2	697.8
Sorghum vulgare	Straw- tropical	944.3	40.0	377.8	556.7
Cynodon plectostachyus	Leaves- temperate grass	912.2	42.0	362.2	584.4

yeast culture doses (D) in the linear model [21]. Data from each of the 3 runs within the same sample were averaged prior to statistical analysis. Mean values of each individual sample within each species (ie, 3 samples of each) were used as the experimental unit. The statistical model was:

$$Y_{ijk} = \mu + S_j + D_k + (S \times D)_{jk} + E_{ijk}$$

where: Y_{ijk} = is every observation of the ith fibrous specie (S_j) when incubated in the jth yeast (D_k; yeast culture doses); μ is the general mean; S_j (j = 1 to 9) is the feed effect; D is the yeast doses effect (k = 1 to 4); $(S \times D)_{jk}$ is the interaction between feed and yeast dose; and E_{ijk} is experimental error. Linear and quadratic polynomial contrasts were used to examine responses of feeds to increasing addition levels of the yeast culture.

3. Results

3.1. Chemical Composition

Chemical analysis showed that the organic matter (OM) did not differ and ranged between 912 and 982 g/kg DM for *C. plectostachyus* leaves and *S. officinarum* bagasse, respectively. Corn stover (*Z. mays*) had the highest ($P < .05$) CP and the lowest ($P < .05$) acid detergent fiber (ADF) and neutral detergent fiber (NDF). However, both the leaves of *Z. mays* and *P. purpureum* had the highest ($P < .05$) ADF and NDF and the lowest ($P < .05$) CP (Table 1).

3.2. In Vitro Gas Production

Both the fibrous species and yeast dose effects had an interaction for fecal pH ($P < .01$), asymptotic GP (b, ml/g DM); rate of GP (c, /hr); initial delay before GP began (L, hours) and GP at 4 and 48 hours ($P < .01$). Interactions occurred also for GP at 8 ($P < .01$), and 24 hours ($P < .01$).

Contrasting effects of fecal fermentation capacity occurred between tropical and temperate grasses for fecal pH ($P < .00$), c ($P < .05$), GP during the first 12 hours ($P < .05$) and also occurred for fecal pH ($P < .01$), b ($P < .01$), and GP from 8 to 48 hours ($P < .05$) between agriculture byproducts and grasses. Straw and no-straw fibrous feeds had differences in their fermentation for fecal pH ($P < .01$), b ($P < .01$), and GP after 12 hours ($P < .05$).

Fermentation of *Z. mays* stover with *S. cerevisiae* reduced ($P < .01$) fecal pH and the rate of GP (/hr), but asymptotic GP (b, ml/g DM) was higher ($P < .01$) than in the other fibrous species. In contrast, *C. plectostachyus* leaves had the lowest ($P < .01$) asymptotic GP with the highest ($P < .01$) rate of GP (c, /hr). The *S. officinarum* bagasse reduced ($P < .01$) the fermentation lag to its lowest level compared to *P. purpureum* leaves, which had ($P < .01$) the highest values. During the period from 4 to 24 hours, *S. officinarum* bagasse improved *in vitro* GP to maximum recorded values compared to *S. officinarum* leaves, which had the lowest values. After 24 hours (ie, GP between 24 and 48 hours), *Z. mays* stover had the highest, whereas *C. plectostachyus* leaves had the lowest *in vitro* GP. The other species varied in terms of *in vitro* GP, with intermediate values at different times.

No effects were observed among yeast doses for fecal pH, the asymptotic GP (b, ml/g DM), the rate of GP (c, /hr), and *in vitro* GP at any measured time. Addition of *S. cerevisiae* caused varied responses for the initial delay before GP began between fibrous species. However, addition of *S. cerevisiae* linearly reduced ($P < .01$) the initial delay before GP began for *Z. mays* stover (linear effect, $P < .01$; quadratic effect, $P = .07$), *S. officinarum* bagasse (linear effect, $P < .01$), and leaves (linear effect, $P < .05$), *P. purpureum* leaves (linear effect, $P < .01$; quadratic effect, $P < .05$), and *C. plectostachyus* leaves (linear effect, $P < .01$; quadratic effect, $P < .05$) compared to the other fibrous species, which increased with a dose of 1.25 mg/g DM for *A. sativa* straw (linear effect, $P < .05$; quadratic effect, $P < .01$) and *A. gayanus* (quadratic effect, $P < .01$) leaves (Table 2). Effect of different *S. cerevisiae* doses with different fibrous species was negligible for *in vitro* GP (mL/g DM) (Table 2).

4. Discussion

Based on chemical composition and *in vitro* fermentation kinetics of our fibrous feeds, the tropical species had higher nutritive value than the temperate grasses. The same occurred where agriculture byproducts had a higher nutritive value than grasses. Moreover, straw had higher nutritive value than the not straws. However, many studies have shown that temperate grasses have advantages over subtropical grasses, including a higher nutritive value. Temperate grasses generally have a higher DM digestibility, due primarily to lower lignin content [22]. Compared with temperate forages, tropical forages typically have increased annual DM yield, although this increased yield is usually associated with decreased forage quality [23]. These differences may be based on geographic regions or unknown factors.

Although the technique of Theodorou et al [18] for studying *in vitro* fermentation initially relied upon rumen

Table 2
In vitro fecal gas kinetics of nine low quality roughages as affected by different levels of *Saccharomyces cerevisiae* (mg/g DM)

Species	Fibrous Species	Fecal pH	Gas Production Parameters[a]			*In Vitro* Gas Production (ml/g DM)					
			b	*c*	*L*	Gas4	Gas8	Gas10	Gas12	Gas24	Gas48
Zea mays (stover)	0	6.5	202.4	0.024	2.57	26.0	48.3	70.2	83.7	129.4	171.0
	1.25	6.5	182.3	0.030	1.77	30.4	55.7	79.9	94.4	139.8	172.4
	2.5	6.6	191.4	0.025	1.64	26.6	49.5	72.2	86.2	133.6	173.9
	5.0	6.6	190.2	0.025	1.78	26.4	49.1	71.6	85.4	132.3	172.3
	P value										
	Linear	0.002	0.161	0.769	0.001	0.895	0.870	0.841	0.824	0.755	0.745
	Quadratic	0.4723	0.045	0.153	0.070	0.286	0.306	0.335	0.353	0.478	0.993
Avena sativa (straw)	0	6.8	115.0	0.051	2.19	30.2	52.4	71.1	80.9	104.8	114.1
	1.25	6.8	115.2	0.051	2.73	30.3	52.6	71.3	81.1	105.1	114.3
	2.5	6.8	115.3	0.050	1.57	29.8	51.9	70.6	80.4	104.7	114.3
	5.0	6.8	113.5	0.048	1.68	28.4	49.7	67.7	77.4	101.8	112.2
	P value										
	Linear	.860	.948	.821	.015	.857	.883	.899	.908	.979	.963
	Quadratic	.760	.987	.965	.001	.904	.887	.895	.894	.922	.978
Zea mays (cobs leaves)	0	6.8	94.50	0.053	1.32	24.1	41.6	56.3	64.0	83.4	92.6
	1.25	6.8	139.2	0.025	1.43	19.5	36.3	53.0	63.2	97.7	126.8
	2.5	6.8	89.49	0.048	1.83	22.0	38.5	52.7	60.3	79.8	88.4
	5.0	6.8	154.2	0.030	1.45	25.1	45.4	64.4	75.5	110.7	139.0
	P value										
	Linear	.291	.686	.656	.079	.701	.730	.752	.763	.780	.727
	Quadratic	.052	.002	.028	.538	.468	.631	.876	.922	.174	.008
Saccharum officinarum (bagasse)	0	6.7	119.6	0.090	2.50	50.0	79.1	98.0	105.8	118.0	119.6
	1.25	6.7	116.6	0.131	1.81	57.3	88.3	104.2	109.5	116.0	116.6
	2.5	6.7	143.5	0.087	1.40	57.2	91.6	114.7	124.5	140.9	143.5
	5.0	6.8	115.7	0.075	0.72	40.3	67.6	87.3	96.1	112.5	115.6
	P value										
	Linear	.003	.048	.861	.008	.044	.023	.016	.021	.041	.047
	Quadratic	.044	.129	.019	.628	.193	.023	.658	.344	.139	.129
Saccharum officinarum (leaves)	0	6.9	90.47	0.039	2.50	18.6	33.1	46.3	53.6	74.7	87.3
	1.25	7.0	103.0	0.030	1.63	16.2	29.7	42.7	50.4	75.4	94.8
	2.5	7.0	104.7	0.029	1.50	15.9	29.3	42.2	49.9	74.9	94.9
	5.0	7.0	87.37	0.047	1.58	21.1	37.1	50.9	58.3	77.5	86.2
	P value										
	Linear	.291	.164	.204	.035	.302	.334	.371	.422	.963	.110
	Quadratic	.587	.520	.535	.313	.625	.652	.690	.737	.809	.346
Andropogon gayanus (leaves)	0	6.9	127.3	0.030	1.90	20.7	38.1	54.8	64.7	96.3	119.6
	1.25	6.8	131.6	0.027	2.83	19.6	36.1	52.3	62.1	94.4	120.6
	2.5	6.8	116.3	0.04	1.70	23.7	42.3	58.9	68.3	95.0	111.5
	5.0	6.8	118.3	0.033	1.49	21.5	39.1	55.6	65.3	94.5	113.5
	P value										
	Linear	.135	.240	.196	.550	.241	.272	.322	.380	.652	.200
	Quadratic	.236	.225	.241	.006	.230	.218	.218	.221	.608	.341
Pennisetum purpureum (leaves)	0	6.9	88.97	0.052	2.73	22.1	38.2	51.7	58.8	77.1	86.6
	1.25	7.0	104.7	0.045	2.42	24.3	42.7	58.7	67.4	90.6	102.3
	2.5	6.9	95.60	0.051	1.43	22.3	38.6	52.4	59.8	79.6	91.4
	5.0	7.0	120.7	0.034	1.04	19.2	34.6	49.1	57.6	84.6	106.9
	P value	.691	.803	.918	<.0001	.960	.922	.891	.863	.821	.811
	Linear	.191	.592	.662	.031	.461	.314	.194	.1560	.230	.452
	Quadratic										
Sorghum vulgare (straw)	0	6.8	124.2	0.038	2.50	24.2	43.4	61.0	71.1	100.5	119.0
	1.25	6.7	120.9	0.032	1.92	20.3	37.0	52.7	62.0	91.0	112.4
	2.5	6.7	100.4	0.044	1.63	21.5	37.9	52.2	60.2	82.8	96.6
	5.0	6.7	53.73	0.377	0.80	45.4	52.1	53.4	53.6	53.7	53.7
	P value										
	Linear	.361	.234	.921	.001	.453	.283	.201	.165	.143	.202
	Quadratic	.641	.607	.869	.381	.418	.405	.493	.568	.946	.747
Cynodon plectostachyus (leaves)	0	6.8	51.90	0.202	2.58	35.6	46.4	50.2	51.1	51.9	51.9
	1.25	6.8	61.93	0.117	1.86	29.0	43.2	51.6	54.8	60.5	61.9
	2.5	6.8	60.27	0.167	1.52	38.2	52.1	57.7	59.2	60.3	60.3
	5.0	6.8	59.23	0.121	1.08	30.1	44.7	52.7	55.4	58.9	59.2
	P value										
	Linear	.863	.153	.329	<.0001	.489	.089	.014	.016	.099	.150
	Quadratic	.335	.238	.048	.016	.031	.046	.282	.893	.285	.239
LSD pooled		0.052	8.654	0.0404	0.397	5.45	8.22	10.23	11.24	12.06	10.24
P value:											
Fibrous species		<.0001	<.0001	<.0001	.0004	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

(*continued on next page*)

Table 2 (*continued*)

Species	Fibrous Species	Fecal pH	Gas Production Parameters[a]			*In Vitro* Gas Production (ml/g DM)					
			b	*c*	*L*	Gas4	Gas8	Gas10	Gas12	Gas24	Gas48
Yeast doses:											
Linear		.865	.946	.612	<.0001	.585	.470	.460	.474	.561	.688
Quadratic		.595	.079	.281	.168	.399	.735	.987	.840	.247	.059
Fibrous species × yeast doses		.001	<.0001	<.0001	<.0001	<.0001	.005	.103	.090	.001	<.0001
Contrasts effects:											
Tropical vs. temperate grasses		<.0001	.831	.032	.201	.001	.002	.009	.022	.274	.710
Agriculture byproducts vs. grasses		<.0001	.0001	.307	.360	.079	.006	.002	<.0001	<.0001	<.0001
Straws vs. no straws		<.0001	<.0001	.968	.259	.920	.547	.138	.036	.002	<.0001

DM, dry matter; LSD, least significant difference.

[a] *b* is the asymptotic gas production (ml/g DM); *c* is the rate of gas production (/hr); *L* is the initial delay before gas production begins (hours).

fluid as the source of microbial inoculum, use of feces as the source of microbial inoculum has proved to be a successful alternative source of microbial inoculum in ruminants [14,24-26] and equine [27-30] studies. Use of inocula for *in vitro* GP from either rumen fluid or feces resulted in few differences in accumulation of gas, although the lag phase appeared to be higher when feces were used. This may be due to the different number of microorganisms per gram of rumen digesta or feces. A similar situation is evident in equines, wherein studies have shown a similar pattern of GP using feces fluid or equine feces as the inoculum but with a lower difference in the lag phase noted between cecal fluid and feces compared than that seen with rumen fluid and feces [30,31]. Microorganisms such as bacteria, protozoa, and fungi are found in the hindgut [32] and are similar to the microbes in the rumen [33]. However, bacteria and fungi seem to play a much bigger role in fiber digestion than protozoa do [32].

Use of *in vitro* fermentation procedures to study diet digestion and fermentative end products has become increasingly popular in equine nutrition, based on validation of the use of equine feces as the source of inoculum. Macheboeuf and Jestin [34] and Lowman et al [35] have shown that grains and forages incubated with equine feces produced GP profiles similar to known gas concentrations. Furthermore, Ringler et al [36,37] found that combined use of equine fecal inoculum yielded valid *in vitro* estimates of DM, NDF, and ADF digestibility.

Fibrous feeds can be the main sources of nutrients for equines for long periods of time, especially during high-latitude winters and in the dry season when resources are in short supply [38]. The chemical composition of different fibrous species and varieties varies widely, but there are many factors which cause this variation. The most important factor is the growing conditions, such as the genotype of the crops, differences among production environments, and the interaction between environment and genotypes [35]. Environmental differences include variations in climate, soil, and agronomic practices. In addition, variations arise from differences in harvesting conditions and postharvesting treatments [39,40]. In contrast, pasture management techniques cannot be ignored [41], as there is usually an inverse relationship between the CP and fiber content in a forage species [42], and this occurred in our study where the CP content was highest with *Z. mays* stover, *S. officinarum* leaves, and *A. gayanus* leaves where low contents of both NDF and ADF occurred, but vice versa, in the case of *Z. mays* cobs leaves and Taiwan grass. This phenomenon will affect the asymptotic GP and *in vitro* GP with advancing times of incubation.

Responses to the addition of dietary *S. cerevisiae* are dependent upon yeast source, fibrous species type, forage composition, application method, and dose-dependent interactions between yeast and diet [43,44]. In our study, the same yeast with the same doses and application methods resulted in different responses to the different fibrous feeds. Production of gases from the tested roughages depends on the CP and fiber contents of the feeds [45]. The different substrates used in our study caused different individual fermentation characteristics as to process dynamics and products while incubated with equine feces. As the volume of GP reflects the fermentation potential of the fiber fraction [26], the linearly higher GP during the first period of fermentation (ie, during the first 12 hours) of both *S. officinarum* bagasse and leaves refers to its high content of highly fermentable constituents versus the other fibrous feeds. In contrast, the fermentation process of *Z. mays* stover is dependent upon their content of slowly fermented constituents; GP depends on nutrient availability for inocula microorganisms [46].

Addition of yeast culture had a positive overall effect on GP from most of the substrates. Yeast supplementation is likely to stimulate the microbial cellulolytic activity in the hindgut causing an improved fiber digestion [47]. Previous studies indicated that live yeasts can improve the microbial balance in the hindgut of horses, stimulating the population of cellulolytic bacteria and their activity [13], thereby increasing the digestibility of dietary nutrients [48,49]. Lattimer et al [14] suggested that yeast culture supplementation resulted in improved energetics of the microflora and, as a result of the improved the microbial balance in the hindgut with stimulated cellulolytic bacteria activity and increased digestibility, the amount of gas produced increased.

However, diet composition-related ability of live yeasts to modify microbial digestion and fiber degradation in horses has not been extensively studied [47]. The DM, NDF, and ADF digestibilities were enhanced in mature horses fed

a forage diet supplemented with yeast culture [48], and McDaniel et al [50], using cecal fluid from mature horses consuming a high-fiber diet, reported an increase in the acetate-to-propionate ratio as well as in the total volatile fatty acids (VFA) concentrations *in vitro* [10].

A shorted fermentation lag time with yeast addition is due to 2 basic mechanisms. The first was reported by Newbold et al [51] as the respiratory activity which scavenges O_2, which is toxic to anaerobic bacteria and causes inhibition of adhesion of cellulolytic bacteria to cellulose, and this peak in O_2 concentration occurs at approximately the time of feeding. The second mode of action is based on the fact that yeast contain small peptides and other nutrients which are required to stimulate ruminant cellulolytic bacteria to initiate growth [52,53]. In our study, addition of *S. cerevisiae* shortened the lag time to first GP compared to control (ie, 0 mg of *S. cerevisiae*/g DM). However, increasing doses of *S. cerevisiae* in most fibrous feeds decreased the lag time.

Effects of feeding yeast culture on fecal pH depend upon the fermented substrate. Because increased lactate concentrations are known to lower the pH and maintain a more desirable pH in the cecum [15] and thus increase fiber digestion, removal of excess lactate is beneficial. Jouany et al [47] showed an increase in lactate utilizing bacteria in the caecum of animals [54]. In some studies, there was a trend toward a higher cecal pH in horses fed the yeast culture, and *S. cerevisiae* supplementation appeared to minimize the level of adverse changes to pH in the cecum of the horse [15]. The response to increasing *S. cerevisiae* doses varied among our fibrous feeds, but, in general, desirable effects occurred with doses of 2.5 to 5.0 g yeast/kg DM in most of the fibrous feeds.

5. Conclusions

Addition of live yeast (*S. cerevisiae*) to 9 different fibrous feeds resulted in different *in vitro* GP from these substrates, which, if a similar scenario exists *in vivo*, has important implications for the overall energy balance of the equines. Based on the highest asymptotic GP and CP content occurring with the lowest contents of both NDF and ADF, *Z. mays* stover had the highest nutritive value compared to the other fibrous feeds. Low or nonexistent effects of *S. cerevisiae* addition on *in vitro* fecal gas kinetics of some feeds improved fecal fermentation kinetics with the other forages at 2.5 to 5.0 g/kg DM. Based on chemical composition and *in vitro* fermentation kinetics, higher nutritive values occurred with tropical species than with temperate grasses, with agricultural byproducts than grasses, and with straws than not straws.

Nevertheless, further research is required to elucidate the mode of action of *S. cerevisiae* and to investigate factors such as yeast supplementation level, substrate, application methods used, and yeast/substrate interactions on cecal fermentation and *in vivo* nutrient digestibility.

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