Original Research

Fecal Gas Production of Ten Common Horse Feeds Supplemented With *Saccharomyces cerevisiae*

Mona M.Y. Elghandour [a], Miguel Mellado [b], Ahmed E. Kholif [c], Abdelfattah Z.M. Salem [a,*], Alberto Barbabosa [a], Susana Ballinas [a], Alejandro Esquivel [a], Nicholas E. Odongo [d]

[a] Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, México
[b] Department of Animal Nutrition, Autonomous Agrarian University Antonio Narro, Saltillo, Mexico
[c] Dairy Science Department, National Research Centre, Giza, Egypt
[d] Department of Animal Sciences, School of Agriculture, Pwani University, Kilifi, Kenya

ARTICLE INFO

ABSTRACT

The study aimed to assess the nutritive value of 10 feeds (grains and forages) commonly used in horse nutrition in Mexico, on the basis of their chemical composition, in vitro organic matter digestibility (IVOMD) and in vitro gas production measurements with or without the supplementation of *Saccharomyces cerevisiae* (SC) at 4 mg/g DM. Fecal inoculum was obtained from 4 adult English Thoroughbred horses fed on restricted amount of concentrate and oat hay ad libitum. Substrates tested were: 6 concentrates (corn gluten meal, soybean meal, steam-rolled corn, steam-rolled barley, oat grain, and wheat bran) and 4 roughages (soybean hulls, corn stover, alfalfa hay, and oat hay). Gas production (GP) was recorded at 2, 4, 6, 8, 10, 12, 14, 24, 48, and 70 hours using the pressure transducer technique. Some ingredient × yeast interactions were observed ($P \le .020$) for the asymptotic GP and GP at 48 and 70 hours of incubation. Yeast addition increased ($P < .001$) the asymptotic GP of concentrates compared to roughages. Concentrate feeds had higher ($P < .05$) GP and lower ($P < .001$) rate of GP compared to roughages without yeast. From 24 to 70 hours of incubation, forages with or without yeast had lower ($P < .05$) GP compared to concentrates supplemented with SC. Forages had higher fermentation pH compared to concentrates but lower ($P < .05$) metabolizable energy, IVOMD, and microbial protein production compared to concentrates. Supplementation with SC increased ($P < .05$) the asymptotic GP of oat grain, soybean meal, soybean meal, steam-rolled barley, steam-rolled corn, wheat bran, corn stover, and oat hay, without affecting the rate of GP or lag time of oat grain, soybean meal, wheat bran, corn stover, and oat hay. Moreover, supplementation with SC increased ($P < .05$) metabolizable energy, IVOMD, and microbial protein production of steam-rolled barley, wheat bran, and corn stover, without affecting ($P > .05$) the fermentation of other feeds. Supplementation with SC improved fermentation of feeds with higher effects on concentrates compared to roughages. It was concluded that although SC mainly improves concentrate utilization by horses, it also improves fiber digestion when used on high-roughage diets fed to horses.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

In Mexico, the horse industry within the agriculture economy has become a strong sector. For top performance, horses must be fed adequately. A well-balanced ration in terms of energy, protein, minerals, and vitamins should be

* 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).

provided to fulfill their nutritional needs for good health and performance [1]. Horse rations can be made from locally available ingredients including roughages (e.g., hays and crops) and concentrates (e.g., grains and meals) [2]. The choice of feed ingredient for horse feeding depends on the horses' requirements, availability, and cost of commercially prepared feeds, and horse activity.

Concentrate feeds are required for growing and working horses which require extra energy and protein to support higher production levels. To prevent metabolic disorders associated with high-grain concentrate feeding, concentrates should be fed as a supplement to a forage-based diet and should not be more than 50%–60% of the total diet [1,2]. Oat, corn, and barley are the most widely used grains in horse diets in Mexico. Grains can be cracked, coarsely ground, rolled, or steam flaked. Wheat bran is one of the most valuable feed ingredient in the nutrition of horses due to its mild laxative effect and its bulky nature [1,2].

Concentrate feeds are needed when a horse cannot meet its energy and protein requirements from forage alone. Straws and hays are the most popular and less expensive sources of fiber for horses. Moreover, forage feeding to horses can provide many of the essential nutrients and prevent nutritional disorders because forage fibers maintain gastrointestinal health and well-being of horses [2]. Increasing dietary fiber to at least 1% of the horse's body weight with decreasing starch and sugar levels can reduce such disorders [2]. Therefore, feeding adequate amounts of fibrous feeds is required for normal digestive system function.

Addition of yeast (e.g. *Saccharomyces cerevisiae*) to the horse's diet has been shown to improve feed utilization and nutritive value [3–5] with positive effect on the hindgut microbial population [4]. Moreover, in vitro experiments [3,6,7] showed improved digestion and fermentation kinetics of feeds. The improved feed utilization is related to increased total number and activity of hindgut microorganisms, especially cellulolytic bacteria [8]. In addition, raising fermentation pH or at least maintaining fermentation pH with yeast feeding is another justification for using yeast [9]. On the other side, Lattimer et al [8] in an *in vitro* study and Glade and Biesik [10] in an *in vivo* study reported no effect of yeast-treated feed in horses, probably because the fermentation process used (Daisy II incubator) is a closed system and therefore does not allow for a continuous flow of microbes and nutrients. This may also be related to different yeast culture products and different diet types used [6,7].

The evaluation of the nutritive value of feed ingredients in each country is very important for nutritionists for establishing feed inventory and for formulating diets for horses. Therefore, the present experiment aimed to evaluate the fermentative capacity of 10 feed ingredients commonly used in equine feeding in Mexico in the presence or absence of *S. cerevisiae*.

2. Materials and Methods

2.1. Substrate and Yeast Cultures

Ten common horse feeds were used as incubation substrates (Table 1). The incubated concentrates included corn gluten meal (*Zea mays*), soybean meal (*Glycine max*), steam-rolled corn (*Zea mays*), steam-rolled barley (*Hordeum vulgare*), oat grain (*Avena sativa*), and wheat bran (*Triticum aestivum*). The incubated forages included soybean hulls (*Glycine max*), corn stover (*Zea mays*), alfalfa hay (*Medicago sativa*), and oat hay (*Avena sativa*). Procreatin 7 (Safmex/ Fermex S.A. de C.V., Toluca, Mexico) yeast product of *S. cerevisiae*, in powdered form, containing 1×10^{10} cells/g of the product, was used at 0 and 4 mg/g of feed dry matter (DM).

2.2. In Vitro Incubations

Before the morning feeding, fecal contents were collected from the rectum of 4 adult English Thoroughbred horses of 7–9 years of age and weighing 490 ± 20 kg at the animal hospital of Faculty of Veterinary Medicine, University of the State of Mexico, Mexico, and these were used to inoculate fermentation with different substrates. The donor horses were fed 2 kg of commercial concentrate (Pell Rol Cuarto de Milla, Mexico; 26.7 g crude protein (CP)/kg DM) and oat hay *ad libitum*. About 100 g of fecal contents was collected from each horse and equally mixed and homogenized and then mixed with the Goering and Van Soest [11] buffer solution without trypticase at 1-g feces to 4-mL buffer. The incubation media were then mixed and saturated with CO_2 for about 20 minutes and then strained through 4 layers of cheesecloth into a flask with an O_2-free headspace. After filtration, the filtrates were used to inoculate 3 identical runs of incubation at 50-mL solution in 120-mL serum bottles containing 0.5 g DM of substrate and yeast at either 0 or 4 mg/g DM.

A total of 180 bottles (2 yeast levels × 3 replicates × 3 runs × 10 substrates) plus 3 bottles without substrate and yeast were used as blanks. After filling, bottles were flushed with CO_2 for 1 minute and immediately closed with rubber

Table 1
Chemical composition (g/kg DM) of the feed ingredients used as substrates.

	Concentrate						Roughage			
	Corn Gluten Meal	Soybean Meal	Steam-Rolled Corn	Steam-Rolled Barley	Oat Grain	Wheat Bran	Soybean Hulls	Corn Stover	Alfalfa Hay	Oat Hay
Organic matter	918	927	989	979	968	877	952	941	883	940
Crude protein	211	398	76	132	117	168	121	65	220	83
Ether extract	11.9	16.2	6.5	14.3	41.8	53	8.3	11.2	26.8	18.3
Neutral detergent fiber	425	251	234	410	250	429	637	700	337	530
Acid detergent fiber	99	61	21	53	66	126	438	385	215	361
Nonstructural carbohydrates	271	263	672	423	559	227	185	164	299	309

stoppers, shaken and placed in an incubator equilibrated at 39°C for 70 hours. Gas production was recorded at 2, 4, 6, 8, 10, 12, 14, 24, 48, and 70 hours using the pressure transducer technique (Extech instruments, Waltham) of Theodorou et al [12]. At the end of incubation after 70 hours, bottles were uncapped, and the pH was immediately measured using a digital bench pH meter (Hanna instrument, Italy).

2.3. Chemical Analyses and Calculations

Samples of the feed ingredients were analyzed for DM (#934.01), ash (#942.05), N (#954.01), and EE (#920.39), according to AOAC [13]. The neutral detergent fiber (NDF) [14] and acid detergent fiber (ADF) content of both feeds and fermentation residues were determined using an ANKOM^{200} Fiber Analyzer Unit (ANKOM Technology Corp., Macedon, NY). Alpha amylase was used for NDF and ADF determination of the concentrate feeds and without alpha amylase for forage feeds but with sodium sulfite in the neutral detergent solution. Both NDF and ADF are expressed without residual ash. Organic matter (OM) and nonstructural carbohydrates (NSC) contents were calculated as: OM (%) = 100 − ash (%); and NSC (%) = 100 − moisture (%) − crude protein (%) − crude fat (%) − NDF − ash (%).

To estimate the kinetic parameters of GP, results of GP (mL/g DM) were fitted using the NLIN option of SAS [15] according to the equation of France et al [16] 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 organic matter digestibility (IVOMD, g/kg DM) were estimated according to Menke et al [17].

2.4. Statistical Analyses

Data of each of the 3 runs within the same sample of the 10 individual samples of ingredients were averaged before statistical analysis. Mean values of each individual sample were used as the experimental unit. Data of measured parameters were analyzed using the PROC GLM option of SAS [15] as:

$$Y_{ijk} = \mu + F_i + D_j + (F \times D)_{ij} + E_{ijk}$$

Where Y_{ijk} is every observation of the ith feed (F_i) with jth yeast level (D_j); μ is the general mean; $(F \times D)_{ij}$ is the interaction between feed ingredient and yeast level; E_{ijk} is the experimental error. Statistical significance was declared at $P < .05$.

Regression coefficients between feed type (concentrates and forages) in the absence or presence of yeast with the asymptotic GP were estimated using the Microsoft Excel program.

3. Results

3.1. Chemical Composition

The chemical composition differed between concentrate feed ingredients and the forage feeds (Table 1). A high CP content was observed with soybean meal (concentrate), alfalfa hay (forage), and the corn gluten meal (concentrate). On the other hand, higher NDF contents were observed in forages than concentrates. Corn stover, soybean hulls, and oat hay had the highest NDF contents. Concentrates had more NSC contents compared with roughages. The highest NSC contents were observed with steam-rolled corn, oat grain, and steam-rolled barley. For the forage ingredients, oat hay followed by alfalfa hay had the highest NSC contents. However, the chemical composition of all feed ingredients was comparable with those reported in the National Research Council [2] for horse nutrition.

3.2. In Vitro Gas Production

Interactions between ingredients × yeast level were found ($P \le .020$) for the asymptotic GP and GP at 48 and 70 hours of incubation (Table 2). Moreover, the asymptotic GP, the rate of GP, GP at 24, 48, and 70 hours of incubation, fermentation pH, ME, IVOMD, and microbial protein production (MCP) were different ($P < .05$) between roughages and concentrates. Yeast supplementation increased ($P < .001$) the asymptotic GP of concentrates compared to forages with or without yeast addition. However, yeast supplementation decreased ($P < .001$) the rate of GP from concentrates and forage compared to forage without yeast, with no effect ($P > .05$) on lag time. During fermentation (2 hours of incubation), concentrates with yeast addition had higher ($P < .05$) GP compared to concentrates without yeast, with no difference ($P > .05$) compared to forages either with or without yeast; however, during the incubation from 24 to 70 hours forages with or without yeast has lower ($P < .05$) GP compared to concentrates with yeast. With no yeast effect ($P = .574$), forage increased fermentation pH compared to concentrates. Concentrates with yeast had higher ($P < .05$) ME, IVOMD, and MCP compared to concentrates without yeast and compared to forages with or without yeast supplementation (Table 2).

3.3. Regression Analysis of Data

Data on Table 3 show the occurrence of ingredient × yeast interactions ($P < .01$) for the asymptotic GP, GP, ME, IVOMD, and MCP. All measured parameters differed ($P < .002$) among the incubated substrates. Moreover, yeast supplementation affected ($P < .01$) all measured variables, except the lag time and fermentation pH. Yeast had no effect ($P > .05$) on GP or fermentation kinetics of corn gluten meal. On the contrary, yeast supplementation increased ($P < .05$) the asymptotic GP of oat grain, soybean meal, steam-rolled barley, steam-rolled corn, wheat bran, corn stover, and oat hay. Besides, yeast addition had no effect ($P > .05$) on the rate of GP or lag time of oat grain, soybean meal, wheat bran, corn stover, and oat hay. Yeast supplementation increased ($P < .05$) GP during fermentation with increased effect ($P < .05$) during the incubation at 24–70 hours of incubation. However, yeast did not affect GP ($P > .05$) of soybean hulls and alfalfa hay. Yeast addition increased ($P < .05$) ME, IVOMD, and MCP of steam-rolled barley, wheat bran, and corn stover, with no effect ($P > .05$) on the fermentation kinetic of other feed ingredients (Table 3).

Table 2

In vitro fecal gas kinetics and cumulative gas production of some concentrates and forages during 70 hours of incubation as affected by the addition of 4 mg/g DM (+) or no addition (−) of yeast cultures.

	Concentrate		Roughage		SEM	Ingredient	Yeast	Ingredient × Yeast
	−	+	−	+				
Gas production parameters[a]								
b	181.4b	301.8a	137.2b	182.9b	13.44	<.001	<.001	.007
c	0.043bc	0.033c	0.075a	0.054b	0.0037	<.001	<.001	.166
L	1.33	1.13	1.29	1.27	0.156	.760	.479	.568
In vitro gas production (ml/g DM) at:								
2 h	14.7b	17.7ab	18.3a	18.2ab	0.93	.032	.132	.100
4 h	28.1	34.19	34.1	34.4	1.71	.079	.066	.100
6 h	40.4b	49.6a	47.6ab	48.9ab	2.37	.172	.031	.104
8 h	51.6b	63.9a	59.3ab	62.0ab	2.93	.334	.014	.104
10 h	61.9b	77.4a	69.4ab	73.6ab	3.39	.584	.005	.103
12 h	71.3b	89.9a	78.1ab	84.1ab	3.79	.899	.002	.102
14 h	80.0b	101.6a	85.7b	93.5ab	4.12	.773	.007	.098
24 h	113.5b	150.0a	110.8b	128.1b	5.24	.022	<.001	.070
48 h	154.2bc	219.6a	131.5c	164.6b	6.69	<.001	<.001	.020
70 h	169.1b	252.3a	135.7c	175.7b	7.96	<.001	<.001	.009
Fermentation kinetic[b]								
pH	6.41b	6.52ab	6.80a	6.59ab	0.086	.012	.574	.069
ME	6.35b	7.35a	5.78b	6.25b	0.247	.001	.005	.293
IVOMD	437.7b	502.7a	394.9b	425.5b	18.23	.002	.011	.350
MCP	488.2b	556.5a	483.3b	515.5b	9.79	.023	<.001	.070

Abbreviation: SEM, standard error of the mean.

Different superscripts following means in the same row indicate differences at $P < .05$.

[a] b is the asymptotic gas production (mL/g DM), c is the rate of gas production (/h), L is the initial delay before gas production begins (h).

[b] IVOMD, in vitro organic matter digestibility (mg/g DM); MCP, microbial protein production (mg/g DM); ME, metabolizable energy (MJ/kg DM).

Regression analysis showed that a strong relationship between the asymptotic GP and CP for forage without yeast addition ($R^2 = 0.87$), between the asymptotic GP and NSC for forages without yeast supplementation ($R^2 = 0.64$) and between the asymptotic GP and NDF of forage with yeast addition ($R^2 = 0.62$). Yeast supplementation increased the correlation between the asymptotic GP and CP of concentrates, asymptotic GP and NDF of forage, and asymptotic GP and NSC of concentrates but decreased the association between the asymptotic GP and CP and asymptotic GP and NSC of forage. There was a strong relationship ($R^2 = 0.79$) between GP at 24 hours of incubation and CP content of forages, a good relationship ($R^2 = 0.75$) between GP at 24 hours of incubation and NSC content of forage, and a moderate relationship ($R^2 = 0.45$) between GP at 24 hours of incubation and NDF content of forages in the absent of yeast (Table 4).

4. Discussion

The in vitro technique of Theodorou et al [12] has been used successfully to study the nutritive value of ruminant feeds in vitro. Moreover, in equine nutrition, the technique of Theodorou has been used successfully to evaluate feed nutritive value [4,18]. Besides, using in vitro fermentation technique to evaluate feed nutritive value and utilization is the common method in ruminant and equine nutrition [4,6,7,18,19]. The only difference between ruminant and equine studies is the use of feces as the source of inoculum in equine studies instead of rumen fluid [4,18]. Using rumen fluid or feces as a source of inoculum showed the same amounts of gases from feeds [20].

4.1. Chemical Composition

Within feeds (concentrates vs. forages) and also among different feed ingredients, the chemical composition widely varied due to the nature of the feed, the growing conditions, production environments, and the interaction between environment and feed [21]. Variations in climate, soil, harvesting conditions, and postharvesting treatments cannot be ignored [21]. This was reflected as different individual fermentation characteristics with different incubated substrates.

4.2. In Vitro Fermentation

The interactions between type of feed and yeast supplementation revealed that the asymptotic GP and the accumulated GP from 48 to 70 hours of incubation differed among feeds and the supplementation of yeast. Besides, the asymptotic GP, the rate of GP, and fermentation kinetics including pH, ME, IVOMD, and MCP were different between forages and concentrates. Therefore, the main effect of feed and yeast will be discussed instead of individual feeds. The chemical composition varied amply between concentrates and forages and also between individual feeds, and this explains the different fermentation kinetics. The chemical composition and in vitro fermentation kinetics showed that concentrates had higher nutritive value (i.e., availability of nutrients for ruminal microflora activity) than forages [4,7,19]. Availability of essential nutrients required for rumen microorganisms activity will stimulate the degradability of different nutrients [22]. The production of gases from roughages depends on the protein and fiber contents of feeds [22]. Increased CP content of feeds was inversely related to fiber content, as it has been observed

Table 3

In vitro fecal gas kinetics and cumulative gas production of 10 horse feeds during 70 hours of incubation as affected by the addition of 4 mg/g DM (+) or no addition (-) of yeast cultures.

Feed Type	Feed Ingredient	Yeast	Gas Production Parameters[a]			In Vitro Gas Production (ml/g DM) at										Fermentation Kinetic[b]			
			b	c	L	2 h	4 h	6 h	8 h	10 h	12 h	14 h	24 h	48 h	70 h	pH	ME	IVOMD	MCP
Concentrate	Corn gluten meal	−	211.2	0.049	1.47	19.3	36.9	52.8	67.2	80.8	92.2	103.0	143.8	189.3	203.3	6.77	7.31	504.1	545.0
		+	264.9	0.037	1.37	18.6	35.9	52.0	66.9	80.3	93.7	105.7	154.0	218.1	243.5	6.68	7.59	522.3	564.0
		P value	.109	.071	.632	.595	.711	.827	.949	.931	.827	.734	.427	.202	.149	.041	.429	.428	.428
		SEM	18.47	0.0037	0.137	0.86	1.66	2.42	3.13	3.84	4.54	5.18	8.17	13.34	15.94	0.022	0.223	14.55	15.28
	Oat grain	−	177.8	0.028	0.92	9.6	18.7	27.3	35.5	43.2	50.5	57.3	86.5	130.7	152.1	6.65	5.2	354.7	437.8
		+	313.0	0.028	1.06	17.1	33.3	48.6	63.0	76.7	89.5	101.7	153.3	231.2	268.5	6.67	7.0	473.5	562.7
		P value	.004	.807	.816	.003	.003	.003	.003	.002	.002	.002	.001	.006	.004	.467	.001	.001	.001
		SEM	8.79	0.0018	0.379	0.84	1.60	2.28	2.90	3.43	3.92	4.34	5.86	7.13	7.40	0.021	0.159	10.44	10.98
	Soybean meal	−	167.7	0.053	1.55	17.0	32.2	45.9	58.2	69.3	79.2	88.1	120.8	154.4	163.5	6.65	7.99	565.5	501.9
		+	234.2	0.046	1.02	20.4	39.1	56.1	71.6	85.7	98.6	110.4	155.5	207.4	224.1	6.65	8.94	627.1	566.7
		SEM	3.63	0.0037	0.475	1.34	2.44	3.31	4.02	4.57	4.98	5.30	5.77	4.46	3.71	0.014	0.157	10.24	10.76
		P value	.002	.216	.477	.141	.118	.097	.078	.063	.051	.041	.013	.001	.003	.752	.013	.013	.013
	Steam-rolled barley	−	195.6	0.037	1.82	13.8	26.5	38.4	49.4	59.7	69.2	78.0	113.6	160.7	179.5	6.13	5.87	397.7	488.5
		+	420.7	0.019	1.05	14.9	29.3	43.1	56.3	69.1	81.3	93.1	145.6	237.4	292.4	6.35	6.74	454.6	548.3
		SEM	38.18	0.0035	0.109	1.35	2.55	3.58	4.49	5.25	5.92	6.47	7.94	6.19	3.12	0.088	0.217	14.12	14.85
		P value	.014	.025	.008	.574	.490	.412	.341	.274	.221	.176	.046	.009	<.001	.152	.047	.047	.046
	Steam-rolled corn	−	185.6	0.036	1.09	12.7	24.4	35.4	45.6	55.1	63.9	72.1	105.4	150.2	168.5	5.83	5.53	373.4	473.1
		+	339.7	0.020	0.33	13.5	26.5	38.9	50.8	62.3	73.3	83.8	130.6	210.5	256.2	6.19	6.22	418.2	520.2
		SEM	17.01	0.0033	0.185	0.87	1.65	2.36	3.02	3.61	4.16	4.66	6.70	9.90	11.87	0.275	0.181	11.92	12.53
		P value	.003	.026	.044	.550	.425	.354	.287	.228	.186	.149	.057	.013	.006	.411	.055	.057	.057
	Wheat bran	−	150.5	0.056	1.66	15.8	29.9	42.6	53.9	64.0	73.1	81.2	110.7	139.9	147.4	6.47	6.23	431.0	483.0
		+	238.4	0.047	1.43	21.5	41.1	58.8	75.0	89.6	103.0	115.1	161.0	212.8	228.9	6.62	7.61	520.6	577.2
		SEM	13.93	0.0030	0.225	1.85	3.43	4.80	5.99	6.97	7.83	8.57	11.00	12.97	13.55	0.182	0.298	19.55	20.56
		P value	.011	.123	.515	.095	.082	.075	.068	.060	.054	.049	.032	.017	.013	.592	.031	.032	.032
Forage	Alfalfa hay	−	189.6	0.059	0.91	20.9	39.5	56.0	70.7	83.6	95.2	105.5	142.0	177.1	185.8	6.68	7.03	484.1	541.6
		+	228.0	0.038	1.16	16.6	31.9	46.1	59.3	71.5	82.8	93.3	135.1	189.5	210.6	6.65	6.84	471.7	528.6
		SEM	21.95	0.0052	0.345	2.13	3.94	5.48	6.83	7.97	8.96	9.84	13.00	17.12	19.25	0.015	0.352	23.12	24.31
		P value	.284	.047	.635	.224	.246	.272	.304	.342	.385	.430	.726	.637	.415	.336	.722	.724	.725
	Corn stover	−	96.1	0.076	1.30	13.3	24.7	34.5	42.9	50.0	56.2	61.5	78.8	92.4	95.1	6.51	4.68	320.2	423.4
		+	152.4	0.065	1.28	18.4	34.5	48.6	61.0	71.9	81.5	89.9	119.0	144.7	150.3	6.40	5.77	391.7	498.6
		SEM	6.02	0.0090	0.326	1.13	1.89	2.37	2.58	2.64	2.57	2.38	1.16	3.66	5.17	0.121	0.030	2.07	2.18
		P value	.003	.437	.968	.034	.022	.014	.008	.004	.002	.001	<.001	.005	.002	.545	<.001	<.001	<.001
	Oat hay	−	109.5	0.088	1.13	17.4	31.9	44.0	54.1	62.6	69.8	75.8	94.3	106.9	109.0	7.33	5.02	340.9	452.4
		+	186.2	0.049	0.52	17.5	33.3	47.5	60.4	72.0	82.5	92.1	127.7	166.8	178.8	6.77	5.93	400.2	514.8
		SEM	4.21	0.0110	0.255	1.92	3.28	4.26	4.94	5.39	5.64	5.76	5.29	3.27	3.10	0.080	0.144	9.42	9.89
		P value	.002	.067	.167	.963	.778	.589	.419	.285	.185	.117	.011	.002	<.001	.007	.011	.011	.011
	Soybean hulls	−	153.7	0.077	1.82	21.6	40.1	56.0	69.7	81.4	91.4	100.1	128.2	149.3	152.8	6.68	6.38	434.5	515.7
		+	165.0	0.065	2.11	20.2	37.9	53.5	67.1	79.1	89.6	98.8	130.4	157.6	163.2	6.55	6.44	438.4	519.8
		SEM	7.55	0.0053	0.640	0.52	0.87	1.14	1.38	1.63	1.91	2.21	3.90	6.54	7.27	0.141	0.107	6.96	7.33
		P value	.350	.203	.331	.139	.144	.190	.254	.374	.527	.692	.715	.419	.369	.551	.711	.712	.713
	SEM pooled		17.22	0.0056	0.279	1.38	2.51	3.44	4.23	4.89	5.45	5.93	7.60	9.56	10.57	0.126	0.206	13.51	14.20
	Ingredient		<.001	<.001	.002	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
	Yeast		<.001	<.001	.308	.008	.002	.003	<.001	<.001	<.001	<.001	<.001	<.001	<.001	.769	<.001	<.001	<.001
	Ingredient × Yeast		<.001	.121	.244	.003	.002	.002	.001	.001	.009	.008	.006	.003	<.001	.075	.006	.006	.006

[a] b is the asymptotic gas production (mL/g DM), c is the rate of gas production (/h), L is the initial delay before gas production begins (h).

[b] IVOMD, in vitro organic matter digestibility (mg/g DM); MCP, microbial protein production (mg/g DM); ME, metabolizable energy (MJ/kg DM); PF, partitioning factor at 24 hours of incubation.

Table 4
Regression coefficients (R^2) between some nutrients from 10 horse feeds and the asymptotic gas production and gas production (GP) at 24 hours of incubation.

Ingredient Group	Yeast	Variable 1	Variable 2	Equation	Regression Coefficient
Concentrate	−	CP	b	$y = -0.3915x + 188.6$	$R^2 = 0.02$
	+	CP	b	$y = -3.8787x + 373.01$	$R^2 = 0.30$
Forage	−	CP	b	$y = 5.9365x + 64.592$	$R^2 = 0.87$
	+	CP	b	$y = 4.1984x + 131.53$	$R^2 = 0.48$
Concentrate	−	CP	GP at 24 hours	$y = 0.7778x + 99.188$	$R^2 = 0.15$
	+	CP	GP at 24 hours	$y = 0.4877x + 141.05$	$R^2 = 0.14$
Forage	−	CP	GP at 24 hours	$y = 3.825x + 64.054$	$R^2 = 0.79$
	+	CP	GP at 24 hours	$y = 0.8428x + 117.74$	$R^2 = 0.12$
Concentrate	−	NDF	b	$y = 0.3985x + 168.13$	$R^2 = 0.01$
	+	NDF	b	$y = 0.1121x + 298.06$	$R^2 = 0.02$
Forage	−	NDF	b	$y = -2.0236x + 248.74$	$R^2 = 0.53$
	+	NDF	b	$y = -2.0844x + 297.77$	$R^2 = 0.62$
Concentrate	−	NDF	GP at 24 hours	$y = 1.0578x + 78.216$	$R^2 = 0.20$
	+	NDF	GP at 24 hours	$y = 0.4875x + 133.76$	$R^2 = 0.10$
Forage	−	NDF	GP at 24 hours	$y = -1.2665x + 180.64$	$R^2 = 0.45$
	+	NDF	GP at 24 hours	$y = -0.3485x + 147.26$	$R^2 = 0.10$
Concentrate	−	NSC	b	$y = 0.5637x + 144.45$	$R^2 = 0.07$
	+	NSC	b	$y = 4.0262x + 37.806$	$R^2 = 0.57$
Forage	−	NSC	b	$y = -6.2904x + 412.57$	$R^2 = 0.64$
	+	NSC	b	$y = -4.216x + 367.44$	$R^2 = 0.32$
Concentrate	−	NSC	GP at 24 hours	$y = -0.5397x + 148.86$	$R^2 = 0.13$
	+	NSC	GP at 24 hours	$y = -0.5937x + 188.93$	$R^2 = 0.35$
Forage	−	NSC	GP at 24 hours	$y = -4.6211x + 313.13$	$R^2 = 0.75$
	+	NSC	GP at 24 hours	$y = -1.1753x + 179.5$	$R^2 = 0.15$

b is the asymptotic gas production (mL/g DM), CP is the crude protein, NDF is the neuter detergent fiber, NSC is the nonstructural carbohydrates.

previously [19,23]. This phenomenon had a great effect on the asymptotic GP and in vitro GP at different hours of incubation.

Higher GP from concentrates compared to forages reveals that the higher content of highly fermentable constituents in concentrates compared to the slowly fermented constituents of forages. In addition, the effect of yeast supplementation on the asymptotic GP was more evident with concentrates than forages. Regression analysis showed a strong relationship between CP and NSC contents of concentrates and a weak relationship between GP and NDF content in forages. The response of feedstuffs to the addition of yeast depends on many factors including yeast source, feed type and composition, method of application, and yeast level [19,24,25]. Besides, yeast supplementation increased the asymptotic GP of oat grain, soybean meal, soybean meal, steam-rolled barley, steam-rolled corn, wheat bran, corn stover, and oat hay, without affecting other feeds tested. This is related to the chemical composition of each feed [4,7,19]. Saccharomyces cerevisiae has the ability to stimulate the growth and activity of cellulolytic bacteria in the hindgut resulting in an improved fiber digestion [5,26]. The main end products of dietary carbohydrates fermentation are acetate, propionate, and butyrate as well as gases such as hydrogen, carbon dioxide, and methane [27]. Yeast not only has the ability to increase GP but also can induce qualitative changes in the produced gases; decrease methane and ammonia production [28].

Callaway and Martin [29] suggested that S. cerevisiae has the ability to provide ruminal microflora with some important nutrients and nutritional cofactors required for their activities. Some authors have validated the ability of S. cerevisiae to scavenge excess oxygen from the rumen creating an optimal environment for rumen anaerobic

bacteria [30,31]. In addition, S. cerevisiae has the ability to provide a focal point for the development of a stable microbial consortium and an environment that promotes the growth of beneficial microorganisms around substrates [31]. Live yeasts positively altered the microbial balance in the hindgut of horses [6]. Besides, yeast feeding stimulates the population of cellulolytic bacteria and their activity [32]. In their experiment, Lattimer et al [8] suggested that S. cerevisiae caused an improved energetics of the microflora resulting in improved microbial balance in the hindgut, stimulated cellulolytic bacteria activity, increased nutrients digestibility, and increased GP. In the present study, IVOMD and MCP were higher for steam-rolled barley, wheat bran, and corn stover with S. cerevisiae and with concentrates than with forages.

Forages increased fermentation pH compared with concentrates, with no effect of yeast supplementation on this variable. Moreover, for individual feeds, yeast did not affect fermentation pH and lag time. Concentrates compared to forage showed increased fermentation pH with no effect of yeast before incubation revealing that fecal pH depend on the fermented substrate [19]. Fermentation of concentrates produced higher concentration of lactate which is known to lower the pH compared to forages which produce less lactate and maintain a more desirable pH in the cecum [26,33].

Yeast supplementation was more effective from 24 to 70 hours of incubation. This may be due to the time required for the release of slowly fermented materials from forages compared to concentrates. For forages, more time was necessary for hydrolysis of their nutrients, and therefore, less gas was produced in the first hours of incubation. This is in line with previous observations showing lower gas volume as the roughage level increases

in the diet [34,35]. Increased cell-wall components with forages compared with concentrates were considered to suppress microbial activity through a reduction in the availability of rapidly fermented carbohydrates [36]. Yeast increased ME of steam-rolled barley, wheat bran, and corn stover. Increased ME concentrations are associated with high activities of microbes in the rumen when yeast was added [37].

5. Conclusions

Nutrient contents, IVOMD, and GP of different feedstuffs used in the equine diet with or without *S. cerevisiae* supplementation varied amply. The effect of *S. cerevisiae* supplementation was greater with concentrates than with forages. However, the addition of *S. cerevisiae* improved fermentation kinetics and GP of the 4 forages tested. These results suggest that the strain of *S. cerevisiae* used in the present study can improve forages fermentation in the large intestine of horses at 4 g/kg DM.

Acknowledgments

References

[1] Brooks CC, Campbell CM. Horse nutrition. Honolulu: University of Hawaii; 1981.

[2] Nutrient requirements of horses (NRC). 6th ed. Washington, DC: National Academy Press; 2007.

[3] Clarke LL, Roberts MC, Argenzio RA. Feeding and digestive problems in horses: physiologic responses to a concentrate meal. Vet Clin North Am Equine Pract 1990;6:433–51.

[4] Elghandour MM, Kholif AE, Lopez S, Mendoza GD, Odongo NE, Salem AZM. *In vitro* gas, methane and carbon dioxide productions of high fibrous diet incubated with fecal inocula from horses fed live yeasts in response to the supplementation with different yeast additives. J Equine Vet Sci 2016;38:64–71.

[5] Jouany JP, Medina B, Bertin G, Juiliand V. Effect of live yeast culture supplementation on hindgut microbial communities and their polysaccharides and glycoside hydrolase activities in horses fed high-fiber or high-starch diet. J Anim Sci 2009;87:2844–52.

[6] Salem AZM, Elghandour MMY, Kholif AE, Barbabosa A, Camacho LM, Odongo NE. The Effect of feeding horses a high fiber diet with or without live yeast cultures supplementation on feed intake, nutrient digestion, blood chemistry, fecal coliform count and *in vitro* fecal fermentation. J Equine Vet Sci 2016;39:12–9.

[7] Elghandour MMY, Chagoyán JCV, Salem AZM, Kholif AE, Castañeda JSM, Camacho LM, Cerrillo-Soto MA. Effects of Saccharomyces cerevisiae at direct addition or pre-incubation on in vitro gas production kinetics and degradability of four fibrous feeds. Ital J Anim Sci 2014;13:295–301.

[8] Lattimer JM, Cooper SR, Freeman DW, Lalman DA. In: Effects of Saccharomyces cerevisiae on *in vitro* fermentation of a high concentrate or high fiber diet in horses. Proceedings of the 19th Symposium of the Equine Science Society, Tucson, AZ; 2005. p. 168–73.

[9] Velázquez AE, Kholif AE, Elghandour MM, Salem AZM, de Oca Jiménez RM, Pliego AB, Odongo N, Bórquez JL, Cipriano M, Olivares J. Effect of partial replacement of steam-rolled corn with soybean hulls or prickly pear cactus in the horse's diet in the presence of live *Saccharomyces cerevisiae* on in vitro fecal gas production. J Equine Vet Sci 2016;42:94–101.

[10] Glade MJ, Biesik LM. Enhanced nitrogen retention in yearling horses supplemented with yeast culture. J Anim Sci 1986;62:1635–40.

[11] Goering MK, Van Soest PJ. Forage fibre analysis (apparatus, reagents, procedures and some applications). Washington, DC, USA: Agricultural Research Service, USDA; 1970.

[12] Theodorou MK, Williams BA, Dhanoa MS, McAllan AB, France J. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Anim Feed Sci Technol 1994;48:185–97.

[13] Association of Official Analytical Chemists (AOAC). Official methods of analysis. 16th ed. Arlington, VA, USA: AOAC; 1997.

[14] Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fibre, neutral detergent fibre, and non-starch carbohydrates in relation to animal nutrition. J Dairy Sci 1991;74:3583–97.

[15] SAS. User's guide: statistics, version 9.0. Cary, NC: SAS Institute; 2002.

[16] France J, Dijkstra J, Dhanoa MS, López S, Bannink A. Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed *in vitro*: derivation of models and other mathematical considerations. Br J Nutr 2000; 83:143–50.

[17] Menke KH, Raab L, Salewski A, Steingass H, Fritz D, Schneider W. The estimation of the digestibility and metabolizable energy content of ruminant feedstuffs from the gas production when they are incubated with rumen liquor in vitro. J Agr Sci 1979;92: 217–22.

[18] Kholif AE, Baza-García LA, Elghandour MM, 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.

[19] Elghandour MMY, Vázquez Chagoyán JC, Salem AZM, Kholif AE, Martínez Castañeda JS, Camacho LM, Buendía G. In Vitro Fermentative capacity of equine fecal Inocula of 9 fibrous forages in the presence of different levels of *Saccharomyces cerevisiae*. J Equine Vet Sci 2014;34:619–25.

[20] Lowman RS, Theodorou MK, Hyslop JJ, Dhanoa MS, Cuddeford D. Evaluation of an in vitro batch culture technique for estimating the *in vivo* digestibility and digestible energy content of equine feeds using equine faeces as the source of microbial inoculum. Anim Feed Sci Technol 1999;80:11–27.

[21] Welch RW. The chemical composition of oats. In: Welch R, editor. The oat crop: production and utilization. London: Chapman & Hall; 1995.

[22] Paya H, Taghizadeh A, Janmohammadi H, Moghadam GA. Nutrient digestibility and gas production of some tropical feeds used in ruminant diets estimated by the in vivo and in vitro gas production techniques. Am J Anim Vet Sci 2007;2:108–13.

[23] Radhakrishnan L, Murugan M, Sivakumar T. Biomass yield, chemical composition and nutritive value of Desmanthus virgatus (hedge lucerne) for sheep. Anim Feed Sci Technol 2007;7:119–23.

[24] Yalçın S, Yalçın S, Can P, Gürdal AO, Bagci C, Eltan Ö. The nutritive value of live yeast culture (Saccharomyces cerevisiae) and its effect on milk yield, milk composition and some blood parameters of dairy cows. Asian-Australas J Anim Sci 2011;24:1377–85.

[25] Patra AK. The use of live yeast products as microbial feed additives in ruminant nutrition. Asian J Anim Vet Adv 2012;7:366–75.

[26] Glade MJ. Dietary yeast culture supplementation of mares during late gestation and early lactation: effects on dietary nutrient digestibilities and fecal nitrogen partitioning. J Equine Vet Sci 1991; 11:10–6.

[27] Makkar HPS. Applications of the in vitro gas method in the evaluation of feed resources, and enhancement of nutritional value of tannin-rich tree/browse leaves and agro-industrial by-products. In: Development Field Evaluation Animal Feed Supplementation Packages, IAEA-TECDOC-1294, IAEA, Vienna; 2002. p. 23–40. Available at: http://www-naweb.iaea.org/nafa/aph/public/review-3.pdf.

[28] Hristov AN, Oh J, Firkins JL, Dijkstra J, Kebreab E, Waghorn G, Makkar HPS, Adesogan A, Yang W, Lee C, Gerber PJ, Henderson B, Tricarico J. Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. J Anim Sci 2013;91:5045–69.

[29] Callaway ES, Martin SA. Effects of a Saccharomyces cerevisiae culture on ruminal bacteria that utilize lactate and digest cellulose. J Dairy Sci 1997;80:2035–44.

[30] Newbold CJ, Wallace RJ, McIntosh FM. Mode of action of the yeast *Saccharomyces cerevisiae* as a feed additive for ruminants. Br J Nutr 1996;76:249–61.

[31] Jouany JP. A new look to at yeast culture as probiotics for ruminants. Feed Mix 2001;9:17–9.

[32] Medina M, Girard ID, Jacotot E, Julliand V. Effect of a preparation of Saccharomyces *cerevisiae* on microbial profiles and fermentation patterns in the large intestine of horses fed a high fiber or a high starch diet. J Anim Sci 2002;80:2600–9.

[33] Hall MM, Miller-Auwerda PA. In: Effect of a Saccharomyces cerevisiae pelleted product on cecal pH in the equine hindgut. Proceedings of the 19th Equine Science Society Symposium. AZ: Arizona State University; 2005. p. 45–6.

[34] Reddy B. Utilization of red gram (Cajanus cajan) by-products for intensive goat production. Hyderabad, India: Ranga Agricultural University; 2003. PhD.

[35] Elghandour MMY, Kholif AE, Marquez-Molina O, Vazquez-Armijo JF, Puniya AK, Salem AZM. Influence of individual or mixed cellulase and xylanase mixture on in vitro rumen gas production kinetics of total mixed rations with different maize silage and concentrate ratios. Turk J Vet Anim Sci 2015;39:435–42.

[36] Wilson JR, Hatfield RD. Structural and chemical changes of cell wall types during stem development: consequences for fibre degradation by rumen microflora. Aust J Agric Res 1997;48:165–80.

[37] Chiquette J. Evaluation of the protective effect of probiotics fed to dairy cows during a subacute ruminal acidosis challenge. Anim Feed Sci Technol 2009;153:278–91.