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"Effect of *Saccharomyces cerevisiae* on *In Vitro* Fecal Digestion of Four Feed Ingredients Commonly Used to Feed Horses in Mexico"

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Effect of *Saccharomyces cerevisiae* on *In Vitro* Fecal Digestion of Four Feed Ingredients Commonly Used to Feed Horses in Mexico

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Running head: *Yeast and in vitro fecal fermentation*

ABSTRACT: The study aimed to assess the nutritive value in vitro of 4 feeds (grains and forages) commonly used in horses nutrition in Mexico, in the absence or presence of *Saccharomyces cerevisiae* 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. The incubated substrates included were corn gluten meal, soybean meal, oat grain and alfalfa hay. Gas production was recorded at 2, 4, 6, 8, 10, 12, 14, 24, 48 and 70 h using the Pressure Transducer Technique. Some ingredient × yeast interactions were observed ($P < .020$) for the asymptotic gas production (GP) and GP at 48 and 70 h of incubation. Yeast addition increased ($P < .001$) the asymptotic GP of concentrates compared to forages. Concentrate feeds had higher ($P < .05$) GP and lower ($P < .001$) rate of GP compared to forages without yeast. From 24 to 70 h of incubation, forages with or without yeast had lower ($P < .05$) GP compared to concentrates with yeast addition. Forages had higher fermentation pH compared to concentrates, but lower ($P < .05$) metabolizable energy (ME), in vitro organic matter digestibility (IVOMD) and microbial protein production (MBP) compared to concentrates. Yeast addition increased ($P < .05$) the asymptotic GP of oat grain and soybean meal, without affecting the rate of GP or lag time of both. Yeast treatment improved fermentation of feeds with higher effects on concentrates compared to forage. It was concluded that concentrate feeds had higher nutritive value than forages commonly fed to horses.

Keywords: Feeds, fecal inoculum, gas production, nutritive value, yeast.

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 provided to fulfill their needs for good health and good 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 condensed energy and protein feeds. 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 to 60 % of the total diet. Oat, corn, and barley are the most widely used grains in horse diets. Grains can be cracked, coarsely ground, rolled or steam-flaked.

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 fiber maintain gastrointestinal health of horses [2].

Addition of yeast to the horse's diet has been shown to improve feed utilization and nutritive value [3,4] with positive effect on the hindgut microbial population [4]. Moreover, in vitro experiments [3,5,6] 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 reason 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. This may be related to different yeast culture products and different diet types used [5,6].

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

Four feeds were used as incubation substrates corn gluten meal (*Zea mays*), soybean meal (*Glycine max*), oat grain (*Avena sativa*) and alfalfa hay (*Medicago sativa*) - (Table 1).

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 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 to 9 years of age and weighing 490 ± 20.1 kg at the hospital of Faculty of Veterinary Medicine, University of the State of Mexico, Mexico and these were used as the

inoculum sources. The donor horses were fed 2 kg of commercial concentrate (Pell Rol Cuarto de Milla, Mexico; 26.7 g protein/kg DM) and oat hay *ad libitum*. Fecal contents of all horses were 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 was then mixed and saturated with CO_2 for about 20 minutes and then strained through four layers of cheesecloth into a flask with an O_2-free headspace. After filtration, the filtrates were used to inoculate three 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 three bottles without substrate and yeast as blanks were used. After filling, bottles were flushed with CO_2 for 1 minutes and immediately closed with rubber stoppers, shaken and placed in an incubator set at 39 ºC for 70 h. Gas production was recorded at 2, 4, 6, 8, 10, 12, 14, 24, 48 and 70 h using the Pressure Transducer Technique (Extech instruments, Waltham, USA) of Theodorou et al [12]. At the end of incubation after 70 h, 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, USA) without use

of an alpha amylase but with sodium sulfite in the neutral detergent solution. Both NDF and ADF are expressed without residual 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:

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

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 prior to 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 three runs within the same sample of the four 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 *i*th feed (F_i) with *j*th 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 $P < .05$.

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). In the other hand, higher NDF contents were observed with forage ingredients than concentrate ingredients. The highest NSC contents were observed with oat grain. However, the chemical composition of all feed ingredients was comparable with those reported in the NRC [2] of horse nutrition.

3.2. In Vitro Gas Production

Interactions between ingredients × yeast level occurred ($P \leq .020$) for the asymptotic GP and GP at 48 and 70 h of incubation (Table 2). Moreover, the asymptotic GP, the rate of GP, GP at 24, 48 and 70 h of incubation, fermentation pH, ME, IVOMD and MBP were different ($P < .05$) between forages and concentrates. Yeast addition increased ($P < .001$) the asymptotic GP of concentrates compared to forage with or without yeast addition. However, yeast 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 h 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 hours from 24 to 70 h forages with or without yeast has lower ($P < .05$) GP compared to concentrates with yeast addition. With no yeast effect ($P = .574$), forage increased fermentation pH compared

to concentrates. Concentrates with yeast had higher ($P < .05$) ME, IVOMD and MBP compared to concentrates without yeast and compared to forages with or without yeast addition (Table 2).

3.3. Regression Analysis of Data

Data on Table (3) shows the occurrence of ingredient × yeast interactions ($P < .01$) for the asymptotic GP, GP, ME, IVOMD and MBP. All measured parameters differed ($P \leq .002$) between the incubated substrates. Moreover, yeast addition affected ($P \leq .008$) all measured parameters 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 addition increased ($P < .05$) the asymptotic GP of oat grain and soybean meal. Besides, yeast addition had no effect ($P > .05$) on the rate of GP or lag time of oat grain and soybean meal. Yeast addition increased ($P < .05$) GP during fermentation with increased effect ($P < .05$) during the incubation at 24 to 70 h of incubation (Table 3).

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]. The only difference between ruminant and equine studies is the use of feces as the source of inocula 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 [19].

4.1. Chemical Composition

Within each ingredient type (concentrates vs. forages) and also between different feed ingredients, the chemical composition widely varied due to the genotype of the feed, the growing conditions, production environments, and the interaction between environment and genotypes [21]. Other factor including variations in climate, soil, harvesting conditions and post-harvesting 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 feed ingredient and yeast addition reveal that the asymptotic GP and the accumulated GP from 48 to 70 h of incubation differed between feed ingredients and yeast addition. Besides, the asymptotic GP, the rate of GP, and fermentation kinetics including pH, ME, IVOMD and MBP were different between forages and concentrates. Therefore, the main effect of feed ingredients and yeast will be discussed instead of individual feed ingredients. The chemical composition was varied between concentrates and forages, and also between individual feeds, and is the main reason for differed fermentation kinetics. The chemical composition and in vitro fermentation kinetics showed that concentrate ingredients had higher nutritive value (i.e. availability of nutrients for ruminal microflora activity) than the forage ingredients [4,6,7]. Availability of essential nutrients required for rumen microorganisms activity will stimulate the degradability of different nutrients [20]. The production of gases from roughages depends on the protein and fiber contents of feeds [20]. As shown in Table 1, increased CP content of feeds was

187 inversely related to fiber content [7,22]. This phenomenon had a great effect on the asymptotic
188 GP and in vitro GP at different hours of incubation.

189 Higher GP from concentrates compared to forages reveals the concentrates higher content of
190 highly fermentable constituents compared to slowly fermented constituents with forage feeds. In
191 addition, the effect of yeast addition on the asymptotic GP was clearer with concentrates than
192 with the forage with or without yeast addition. Regression analysis showed a strong relationship
193 between CP and NSC contents of concentrate feeds and a weak relationship between GP and
194 NDF content of forage feeds. The response to the addition of dietary yeast depends on many
195 factors including yeast source, feed type and composition, method of application method, and
196 yeast level [7,23,24]. Besides, yeast addition increased the asymptotic GP of oat grain and
197 soybean meal. This is related to the chemical composition of each feed ingredient [4,6,7].
198 *Saccharomyces cerevisiae* has the ability to stimulate the microbial cellulolytic growth and
199 activity in the hindgut resulting in an improved fiber digestion [25,26]. The main end-products of
200 dietary carbohydrates fermentation are acetate, propionate and butyrate as well as the gases,
201 hydrogen, carbon dioxide and methane [27]. Yeast not only has the ability to increase GP, but
202 also, can induce qualitative changes in the produced gases; decrease methane and ammonia
203 production [28].

204 Callaway and Martin [29] suggested that *S. cerevisiae* has the ability to provide ruminal
205 microflora with some important nutrients and nutritional cofactors required for their activities. In
206 another experiment, Newbold et al [30] and Jouany [31] validated the ability of *S. cerevisiae* to
207 scavenge excess oxygen from the rumen creating an optimal environment for rumen anaerobic
208 bacteria. In addition, *S. cerevisiae* has the ability to provide a focal point for the development of
209 a stable microbial consortium and an environment that promotes the growth of beneficial

210 microorganisms around substrates [31]. Salem et al [5] indicated that live yeasts positively
211 altered the microbial balance in the hindgut of horses. Besides, Medina et al [32] observed that
212 yeast feeding stimulated the population of cellulolytic bacteria and their activity. In their
213 experiment, Lattimer et al [8] suggested that *S. cerevisiae* addition caused an improved
214 energetics of the microflora resulting in improved microbial balance in the hindgut, stimulated
215 cellulolytic bacteria activity, increased nutrients digestibility, and increased GP.

216 Forages increased fermentation pH compared to concentrates, with no effect of yeast addition.
217 Moreover, for the individual feed ingredients, yeast did not affect fermentation pH and lag time.
218 Concentrates compared to forage showed increased fermentation pH with no effect of yeast
219 addition before incubation revealing that fecal pH depend on the fermented substrate [7].
220 Fermentation of concentrates produced higher concentration of lactate which is known to lower
221 the pH compared to the forage which produce less lactate and maintain a more desirable pH in
222 the cecum [25, 33].

223 Yeast addition was effective from 24 to 70 h of incubation. This may be due to the time required
224 for the release of slowly fermented materials from forage feeds compared to the concentrate
225 feeds. For forages, time was necessary for degradation of forage feeds, and therefore less gas was
226 produced in the first few hours of incubation. Reddy [34] and Elghandour et al [35] observed
227 lower gas volume as the roughage level increased in the diet. Increased cell-wall components
228 with forages compared to the concentrates was considered to suppress microbial activity through
229 a reduction in the availability of rapidly fermented carbohydrates [36].

230

231

5. Conclusions

The responses to *S. cerevisiae* addition varied among the tested feed ingredients. The effect was more effective with concentrates than with forages. However, the addition of *S. cerevisiae* improved fermentation kinetics and gas production of forage ingredients. The results of the present study suggest that the *S. cerevisiae* can support ruminal fermentation of forages at the level of 4 g/kg DM.

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Table 1.

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

	Corn gluten meal	Soybean meal	Oat grain	Alfalfa hay
Organic matter	918.1	927.3	967.8	883.3
Crude protein	210.6	397.6	117.2	220.3
Ether extract	11.88	16.15	41.80	26.82
Neutral detergent fiber	425.1	251.0	249.9	337.0
Acid detergent fiber	98.6	61.2	65.9	214.8
Non-structural carbohydrates	270.5	262.5	558.9	299.2

Table 2.

In vitro fecal gas kinetics and cumulative gas production of some concentrate versus forage feed ingredients during 70 hours of incubation as affected by addition of 4 mg/g DM (+) or absent (-) of yeast cultures.

	Concentrate		Forage		SEM			
	-	+	-	+		Ingredient	Yeast	Ingredient × Yeast
Gas production parameters[1]								
B	181.4b	301.8a	137.2b	182.9b	13.44	<0.001	<0.001	0.007
C	0.043bc	0.033c	0.075a	0.054b	0.0037	<0.001	<0.001	0.166
L	1.33	1.13	1.29	1.27	0.156	0.760	0.479	0.568
In vitro gas production (ml/g DM)								
2h	14.7b	17.7ab	18.3a	18.2ab	0.93	0.032	0.132	0.100
4h	28.1	34.19	34.1	34.4	1.71	0.079	0.066	0.100
6h	40.4b	49.6a	47.6ab	48.9ab	2.37	0.172	0.031	0.104
8h	51.6b	63.9a	59.3ab	62.0ab	2.93	0.334	0.014	0.104
10h	61.9b	77.4a	69.4ab	73.6ab	3.39	0.584	0.005	0.103
12h	71.3b	89.9a	78.1ab	84.1ab	3.79	0.899	0.002	0.102
14 h	80.0b	101.6a	85.7b	93.5ab	4.12	0.773	0.007	0.098
24h	113.5b	150.0a	110.8b	128.1b	5.24	0.022	<0.001	0.070
48h	154.2bc	219.6a	131.5c	164.6b	6.69	<0.001	<0.001	0.020
70 h	169.1b	252.3a	135.7c	175.7b	7.96	<0.001	<0.001	0.009
Fermentation kinetic[2]								
pH	6.41b	6.52ab	6.80a	6.59ab	0.086	0.012	0.574	0.069
ME	6.35b	7.35a	5.78b	6.25b	0.247	0.001	0.005	0.293
IVOMD	437.7b	502.7a	394.9b	425.5b	18.23	0.002	0.011	0.350
MBP	488.2b	556.5a	483.3b	515.5b	9.79	0.023	<0.001	0.070

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

SEM is the standard error of the mean.

[1] *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).

[2] IVOMD is the in vitro organic matter digestibility (mg/g DM), MBP is microbial protein production (mg/g DM), ME is the metabolizable energy (MJ/kg DM.

Table 3.

In vitro fecal gas kinetics and cumulative gas production of 4 feed ingredients during 70 hours of incubation as affected by addition of 4 mg/g DM (+) or absent (-) of yeast cultures.

		Gas production parameters[1]			*In vitro* gas production (ml/g DM)										Fermentation kinetic[2]			
Feed ingredient	Yeast	*b*	*c*	*L*	2h	4h	6h	8h	10h	12h	14 h	24h	48h	70 h	pH	ME	IVOMD	MBP
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	0.109	0.071	0.632	0.595	0.711	0.827	0.949	0.931	0.827	0.734	0.427	0.202	0.149	0.041	0.429	0.428	0.428
	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	0.004	0.807	0.816	0.003	0.003	0.003	0.003	0.002	0.002	0.002	0.001	0.006	0.004	0.467	0.001	0.001	0.001
	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
	P-value	0.002	0.216	0.477	0.141	0.118	0.097	0.078	0.063	0.051	0.041	0.013	0.001	0.003	0.752	0.013	0.013	0.013
	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
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
	P-value	0.284	0.047	0.635	0.224	0.246	0.272	0.304	0.342	0.385	0.430	0.726	0.637	0.415	0.336	0.722	0.724	0.725
	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
	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	<0.001	<0.001	0.002	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Yeast	<0.001	<0.001	0.308	0.008	0.002	0.003	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.769	<0.001	<0.001	<0.001
	Ingredient × Yeast	<0.001	0.121	0.244	0.003	0.002	0.002	0.001	0.001	0.009	0.008	0.006	0.003	<0.001	0.075	0.006	0.006	0.006

[1] *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).

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