



Original Research

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



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ARTICLE INFO

Article history:

Received 28 March 2016

Received in revised form 14 April 2016

Accepted 15 April 2016

Available online 30 April 2016

Keywords:

Cactus

Energy feed

Fecal inoculum

Gas production

Soybean hulls

Yeast

ABSTRACT

The aim of the study was to evaluate the fecal fermentation of partial replacing steam rolled corn with soybean hulls (SH) or prickly pear cactus (PC) as energy source in horse diets, in the presence of *Saccharomyces cerevisiae*. Steam rolled corn was replaced with SH at 0% (control), 7.5% (SH75), and 15% (SH150) in the first trial, whereas it was replaced with PC at 0% (control; the same of the first trial), 7.5% (PC75), and 15% (PC150) in the second trial. Yeast of *S. cerevisiae* was added at 0, 2, and 4 mg/g dry matter (DM) of incubated substrates. Fecal inoculum was obtained from four adult English Thoroughbred horses fed on an amount of commercial concentrate and oat hay *ad libitum*. Interactions observed between PC rations and yeast doses for the asymptotic gas production (GP), the rate of GP and carbon dioxide (CO₂) production during some incubation hours. Moreover, with no effect due to SH rations ($P > .05$), increased ($P < .05$) rate of GP was observed with the ration PC75 compared with other rations. Besides, PC75 and PC150 rations with 0 mg yeast/g DM linearly decreased ($P < .05$) CO₂ production at some incubation hours. However, SH75 and SH150 ration had increased ($P = .005$) DM degradability (DMD). Yeast addition at 2 mg/g DM increased the asymptotic GP ($P = .048$) with the SH75 and PC150 rations. The level of 4 mg yeast/g DM increased the asymptotic GP ($P = .048$) from the SH150 ration. Yeast addition at 2 and 4 mg/g DM increased ($P < .05$) the asymptotic GP from PC75 and PC150 rations, respectively, with increasing DMD with the both doses. Yeast addition increased ($P < .05$) CO₂ production from SH75, SH150, PC75, and PC150 rations. It could be concluded that SH and PC can replace steam rolled corn at levels of 7.5% to 15% without negative effect of fermentation kinetics and with better fermentation performance in the presence of yeast at 2 mg/g DM of substrates.

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1. Introduction

Grains represent important sources of energy to horses. Feeding diets with high-grain contents is associated with some feeding disorders and less feed utilization [1] due to microbial profile disturbance [2] and impaired fibrolytic activity in the hindgut [3]. Therefore, partial replacement of grains (energy

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sources) with other feeds, rich in fibers, would appear to be a good alternative solution to prevent such problems and increase feed utilization and reduce feeding costs.

Soybean hulls (SH) can provide animals with adequate energy without causing some of the common feeding disorders associated with high-grain diets feeding [4]. The SH has been used successfully to feed ruminant with economic advantages, as it contains high digestible fiber with very low starch content [4]. Moreover, SH is characterized by its rapid degradation in the horse's digestive system by cecal microflora [5] due to its high cellulose and hemicellulose contents with low lignin content. These characteristics give the SH an extensive ability for bacterial fermentation [6], mainly in the cecum [7]. Studies [5,6] showed favorable responses when SH substituted for hay in diets, including improved fermentation and increased digestibility of feed nutrients. To the best of our knowledge, no information is available about the annual production of SH in Mexico.

Because of special characteristics of prickly pear cactus (PC) including drought and salinity resistance, high biomass yield, and high palatability, it can be used as a livestock feed in arid and semiarid zones. Mexico is one of the highest producers of PC with about 140,045.36 tons annually [8]. However, little information is available about its nutritive value in animal feeding because not much effort has been used to improve its utilization as livestock feed [9]. It contains high levels of water-soluble and nonfiber carbohydrates with low concentrations of structural fibers, making it a rapid ruminal degradable feed [10]. In Mexico, only a few experiments have been carried out to use PC in animal feeding; however, PC is a common feed in times of drought. For efficient utilization, PC must be combined with other feeds to complete the diet because PC is low in proteins, although rich in carbohydrates and calcium [11].

Yeast feeding, to horses, positively influences feed utilization (i.e., feed intake and digestion) and hindgut microbial population dynamics [12]. Yeast feeding to horses improved digestion and fermentation kinetics of feeds in both in vitro [13,14] and in vivo [12] studies. Yeast supplementation has been shown to increase the total number of hindgut microorganisms [15], increase hindgut cellulolytic bacteria numbers, and raise fermentation pH. However, some in vitro [15] and in vivo [16] studies reported no effect with feeding yeast to horses. This inconsistency may be due to the natural and type yeast culture products as well as types of diets [12].

In the current experiment, it was hypothesized that partial replacement of steam rolled corn grains with SH or PC as feed ingredients in the diet of horses in the presence of yeast would significantly improve feed digestibility. Therefore, the present study aimed to assess the effect of partial replacement of steam rolled corn at different levels with SH (trial 1) or PC (trial 2) in the presence of live *Saccharomyces cerevisiae* at different levels on in vitro total gas production (GP) and carbon dioxide (CO₂) productions.

2. Materials and Methods

2.1. Substrate and Yeast Cultures

In the first trial, three total mixed rations (TMRs) were formulated and used as incubation substrates (Table 1). Steam rolled corn was replaced by SH at 0% (control), 7.5% (SH75), and 15% (SH150). In the second trial, three TMRs were formulated as above and used as incubation substrates with replacing the steam rolled corn by PC at 0% (control), 7.5% (PC75), and 15% (PC150). Yeast of *S. cerevisiae* of Procreatin 7 (Safmex/Fermex S.A. de C.V., Toluca, Mexico) in

Table 1
Ingredients (g/kg DM) and chemical composition (g/kg DM) of incubated substrates.^{a,b}

Ingredients	Control	Trial 1		Trial 2	
		SH75	SH150	PC75	PC150
Oat hay	249	248	248	248	248
Steam rolled corn	250	175	100	175	100
Steam rolled barley	250	250	250	250	250
Wheat bran	120	110	120	110	120
Corn gluten feed	30	30	30	30	30
Soybean meal	30	30	20	30	20
Soybean hulls	0	75	150	75	150
Molasses	70	80	80	80	80
Vit/Min ^c	1	2	2	2	2
Chemical composition					
Dry matter	864.4	864.2	867.0	864.1	864.3
Organic matter	963.8	967.6	957.6	940.4	957.3
Crude protein	129.6	117.5	129.6	119.0	113.4
Neutral detergent fiber	355.7	385.0	394.5	428.3	340.2
Acid detergent fiber	120.9	114.7	193.3	130.2	122.3
Ether extract	24.0	24.1	17.9	22.3	23.2
Non-structural carbohydrates	454.5	441.0	415.6	370.8	480.5
Digestible energy (Mcal/kg)	35.0	34.0	33.0	33.0	32.0
Digestible crude protein (g/kg DM)	89.0	90.0	90.0	88.0	85.0

Abbreviation: DM, dry matter.

^a In trial 1: steam rolled corn was replaced with soybean hulls as energy source at 0% (control), 7.5% (SH75), and 15% (SH150).

^b In trial 2: steam rolled corn was replaced with prickly pear cactus as an energy source at 0% (control), 7.5% (PC75), and 15% (PC150).

^c Mineral/vitamin premix contains (mg/kg): Se (100), Co (55), I (125), Cu (25,000), Fe (25,000), Zn (40,000), Mn (20,000), Ca (103 g/kg), vit A (2,125,000 IU), vit D3 (212,500 IU), and vit E (5,000 IU).

powdered form containing 1×10^{10} cells/g of the product was added to each TMR at 0, 2, and 4 mg/g dry matter (DM).

2.2. In Vitro Incubations

Before starting incubation, fecal contents (the inoculum source) were collected from the rectum of four adult English Thoroughbred horses at the hospital de grandes especies de la facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, México (aged 7 to 9 years and weighing 490 ± 20.1 kg) before the morning feeding. Horses were daily fed 2 kg of commercial concentrate (Pel Rol Cuarto de Milla, Mexico) and oat hay *ad libitum*. Individual fecal samples were equally collected with rectal grab of each animal and then were mixed and homogenized to obtain a homogenized sample of feces which were mixed with the Goering and Van Soest [17] buffer solution without trypticase in the ratio of 1:4 weight/volume. The incubation media was then mixed and strained through four layers of cheesecloth into a flask with an O₂-free headspace and used to inoculate three identical runs of incubation in 120-mL serum bottles containing 1-g DM of substrate.

A total of 135 bottles (3 yeast doses \times 3 replicates \times 3 runs \times 5 substrates) plus three bottles without substrate and yeast as blanks were used. After filling all bottles, they were flushed with CO₂ and immediately closed with rubber stoppers, shaken, and placed in an incubator set at 39°C. Gas and CO₂ productions were recorded at 2, 4, 6, 8, 10, 12, 14, 16, 18, 24, 36, 48, and 72 hours using the Pressure Transducer Technique (Extech instruments, Waltham) of Theodorou et al [18]. The production of CO₂ was recorded using Gas-Pro detector (Gas Analyzer CROWCON Model Tetra3, Abingdon, UK).

As described in Rodriguez et al [19], at the end of incubation after 72 hours, bottles were uncapped and the pH was measured using a digital pH meter (Conductronic pH15, Puebla, Mexico), and the residual of each bottle was filtered under vacuum through glass crucibles with a sintered filter, then fermentation residues dried at 65°C for 72 hours to estimate DM degradability (DMD) [20].

2.3. Chemical Analyses and Calculations

Samples of the TMRs were analyzed for DM (#934.01), ash (#942.05), N (#954.01), and ether extract (EE) (#920.39) according to AOAC [21]. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) analyses were determined using an ANKOM²⁰⁰ Fiber Analyzer unit (ANKOM Technology Corporation, Macedon, NY, USA). The NDF was assayed with alpha amylase in the NDF. Both NDF and ADF are expressed without residual ash.

Digestible energy (DE; Mcal/kg) was calculated as: $DE = (3.6 + 0.211 \text{ crude protein [CP]} + 0.421 \text{ EE} + 0.015 \text{ crude fiber [CF]})/4.184$ [22]. Data of CF are not shown.

Digestible CP (DCP; g/kg DM) was calculated as: $DCP = 4.49 + 0.8533 \text{ CP}$ [22].

To estimate the kinetic parameters of GP, results of GP (mL/g DM) were fitted using the NLIN option of SAS [23] according to the equation of France et al [24] 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 before GP.

2.4. Statistical Analyses

Data of each of the three runs within the same sample of each of the three individual samples of TMRs were averaged before statistical analysis. Mean values of each individual sample were used as the experimental unit. Results of in vitro GP and rumen fermentation parameters were analyzed as a factorial experiment using the PROC GLM option of SAS [23] as:

$$Y_{ijk} = \mu + R_i + A_j + (R \times A)_{ij} + E_{ijk}$$

where Y_{ijk} is every observation of the i th TMR (R_i) with j th yeast dose (A_j); μ is the general mean; $(R \times A)_{ij}$ is the interaction between ration type and yeast dose; E_{ijk} is the experimental error. Linear and quadratic polynomial contrasts were used to examine responses to increasing addition levels of test (steam rolled corn replacement and yeast doses). Statistical significance was declared at $P < .05$.

3. Results

The chemical composition of ingredients and incubation substrates is shown in Table 1. The NDF content of the diets increased with increasing levels of SH, while decreased with increasing PC levels. The DCP contents were similar with different levels of SH but decreased in value with increasing levels of PC in the diet.

3.1. Trial 1

3.1.1. Interaction Effects

There were no interaction between TMR type and yeast dose for GP parameters and DMD (Table 2). Interaction effects were observed ($P < .05$) between ration type and yeast dose for CO₂ production at different times of incubation (Table 3).

3.1.2. Diet Effects

Replacing steam rolled corn with SH had no effect ($P > .05$) on GP parameters and GP during different incubation hours. Moreover, fermentation pH did not differ ($P > .05$) between different TMR. However, replacing steam rolled corn with SH linearly ($P = .005$) increased DMD (Table 2).

The main effects of TMR type without yeast addition showed decreased ($P < .05$) CO₂ production at most incubation hours when steam rolled corn was replaced with SH (Table 3).

3.1.3. Yeast Effects

Yeast addition affected the asymptotic GP (quadratic effect, $P = .048$) and lag time (linear effect, $P = .018$). The effect of yeast differed across the three rations. For the control and SH150 ration, yeast addition quadratically decreased the asymptotic GP ($P = .048$), whereas for the

Table 2
In vitro fecal gas kinetics and fermentation profile of replacing steam rolled corn with three levels of soybean hulls^a (SH) at three levels of *Saccharomyces cerevisiae* in horse.

Ration	Yeasts Dose (mg/g DM)	Gas Production Parameters ^b			In Vitro Gas Production (ml/g DM) at:												Fermentation Kinetics		
		b	c	L	2 hr	4 hr	6 hr	8 hr	10 hr	12 hr	14 hr	16 hr	24 hr	36 hr	48 hr	60 hr	72 hr	pH	DMD
Control	0	225.6	0.033	1.45	14.4	27.9	40.5	52.2	63.3	73.6	83.3	92.3	123.1	146.7	178.7	193.9	204.1	6.92	253.9
	2	165.8	0.050	1.19	14.1	27.0	38.6	49.2	58.9	67.7	75.7	83.1	106.6	123.1	143.2	151.6	156.8	6.86	248.4
	4	191.7	0.030	0.58	11.1	21.5	31.3	40.5	49.3	57.5	65.2	72.5	97.7	117.6	145.6	159.4	169.1	6.76	240.1
			179.5	0.036	1.61	12.7	24.4	35.4	45.5	54.9	63.7	71.8	79.4	104.6	123.4	147.9	158.9	166.0	6.84
SH75	0	202.0	0.029	0.77	11.2	21.7	31.6	41.0	49.9	58.3	66.2	73.7	99.7	120.5	150.1	165.0	175.6	6.96	293.9
	2	192.1	0.035	1.12	12.4	23.9	34.0	44.8	54.2	62.0	71.2	78.9	105.0	125.0	152.1	164.9	173.6	6.93	306.2
	4	194.7	0.035	0.94	12.9	25.0	36.2	46.6	56.2	65.2	73.6	81.4	107.6	127.4	153.9	166.3	174.8	6.94	296.7
			174.1	0.043	1.04	14.4	27.7	39.8	50.9	61.0	70.4	78.9	86.7	112.1	130.0	151.7	160.6	165.9	6.88
SH150	0	206.9	0.037	1.22	13.7	26.4	38.2	49.2	59.4	68.9	77.7	86.0	113.7	134.7	163.0	176.4	185.5	6.62	268.5
	2	35.63	0.0051	0.032	2.21	4.24	6.13	7.88	9.51	11.03	12.45	13.78	18.31	21.85	26.88	29.40	31.20	0.062	20.81
	4	0.915	0.336	0.731	0.538	0.554	0.570	0.586	0.601	0.616	0.630	0.644	0.693	0.734	0.795	0.827	0.850	0.206	0.005
		0.9728	0.447	0.827	0.505	0.524	0.542	0.560	0.578	0.597	0.615	0.633	0.703	0.766	0.865	0.918	0.957	0.135	0.289
Pooled LSD Ration	Linear	0.917	0.802	0.018	0.597	0.603	0.610	0.617	0.624	0.631	0.639	0.647	0.679	0.710	0.766	0.801	0.830	0.020	0.403
	Quadratic	0.048	0.106	0.503	0.805	0.844	0.041	0.917	0.952	0.985	0.984	0.954	0.850	0.769	0.658	0.606	0.571	0.150	0.443
	Linear × Yeast	0.816	0.119	0.304	0.814	0.833	0.850	0.865	0.878	0.888	0.897	0.905	0.923	0.929	0.952	0.905	0.889	0.055	0.807

Abbreviations: DM, dry matter; LSD, least significant difference.

^a Steam rolled corn was replaced with soybean hulls as an energy source at 0% (control), 7.5% (SH75), and 15% (SH150).

^b b is the asymptotic gas production (ml/g DM); c is the rate of gas production (/h); DMD is the DM degraded substrate (mg/g DM); L is the initial delay before gas production begins (h).

SH75 ration, yeast addition quadratically increased the asymptotic GP. For the control and SH150 rations, yeast addition linearly decreased fermentation pH, whereas for the SH75 ration, yeast addition linearly increased it ($P = .020$) (Table 2).

For all rations, yeast addition quadratically increased ($P < .05$) CO₂ production at 2 hours of incubation and from 14 hours to 72 hours of incubation. The most effective dose differed across rations, but the highest CO₂ was produced with 2 mg/g DM yeast for the SH150 ration (Table 3).

3.2. Trial 2

3.2.1. Interaction Effects

Interactions between ration type and yeast dose were observed for the asymptotic GP ($P = .015$), the rate of GP ($P = .018$), and at 2 hours of incubation ($P = .034$; Table 4). In addition, interactions were observed between ration type and yeast dose for DMD ($P = .046$; Table 4) and CO₂ production during incubation hours of 4 hours to 36 hours (Table 5).

3.2.2. Diet Effects

Increasing levels of PC in the ration linearly increased asymptotic GP ($P = .008$) and the rate of GP ($P = .005$; Table 4). Lag time, GP at incubation hours of 2 hours to 72 hours, fermentation pH, and DMD were not affected ($P > .05$; Table 4).

Increasing levels of PC in the ration linearly decreased ($P < .05$) CO₂ production at different incubation hours (Table 5).

3.2.3. Yeast Effects

Increasing the dose of yeast linearly ($P = .037$) decreased asymptotic GP but had no effect ($P > .05$) on lag time (Table 4). Increasing yeast levels quadratically increased ($P = .044$) the rate of GP and quadratically ($P = .025$) increased DMD. Fermentation pH was not affected across rations (Table 4).

Yeast addition decreased ($P < .05$) CO₂ from the control ration but increased CO₂ production from PC75 and PC150 rations at various incubation hours (Table 5).

4. Discussion

Using in vitro fermentation technique to evaluate feed nutritive value and utilization is common method in ruminant and equine nutrition. Using feces as a source of inoculum is a popular method in equine feeds in vitro evaluation [13,25]. However, using rumen fluid or feces as a source of inoculum showed the same amounts of gases from feeds [26]. Moreover, the technique of Theodorou et al [18] has been used successfully for studying the in vitro fecal fermentation with some modifications including the use of feces as inoculum source [13,25,27] with longer lag phase with faces compared with rumen liquor inoculum, which may be due to the different number of microorganisms per gram of rumen liquor or feces.

Because no interaction was observed between SH rations and yeast doses for GP parameters, the main effect

Table 3

In vitro fecal carbon dioxide production of replacing steam rolled corn with three levels of soybean hulls^a (SH) at three levels of *Saccharomyces cerevisiae* in horse.

Ration	Yeast Dose (mg/g DM)	In Vitro Carbon Dioxide Production (mL/g DM) at:												
		2 hr	4 hr	6 hr	8 hr	10 hr	12 hr	14 hr	16 hr	24 hr	36 hr	48 hr	60 hr	72 hr
Control	0	1.8	13.4	13.4	17.1	21.7	21.7	24.8	37.3	56.3	70.6	70.6	70.6	70.6
	2	3.0	3.9	3.9	3.9	7.8	10.8	10.8	16.2	44.1	69.7	69.7	73.3	73.3
	4	1.6	11.2	11.2	12.8	17.8	17.8	17.8	25.9	54.1	82.5	82.5	90.7	90.7
SH75	0	2.2	7.1	7.1	13.1	19.9	19.9	19.3	24.7	26.7	30.3	43.2	47.0	47.0
	2	1.7	6.1	6.1	13.0	17.4	18.7	18.7	30.4	44.1	56.1	59.1	76.8	76.8
	4	4.1	13.2	13.2	19.9	19.9	21.6	27.5	27.5	56.1	78.7	78.7	78.7	78.7
SH150	0	4.1	10.5	10.5	13.0	13.0	13.9	21.0	27.5	36.2	54.2	54.2	55.6	55.6
	2	9.0	15.5	15.5	15.5	28.0	28.0	42.0	50.3	76.5	115.4	117.1	134.4	134.4
	4	6.2	14.0	14.0	19.8	19.8	27.3	19.9	37.2	59.6	85.1	85.1	86.5	86.5
Pooled LSD		1.33	2.92	2.92	3.95	4.14	4.49	5.06	6.81	4.14	5.00	4.97	6.12	6.12
Ration														
	Linear	0.639	0.762	0.762	0.228	0.343	0.378	0.319	0.848	0.014	0.002	0.003	0.046	0.046
	Quadratic	0.004	0.059	0.059	0.336	0.349	0.161	0.006	0.030	0.002	<0.001	<0.001	0.003	0.001
Yeast														
	Linear	0.247	0.306	0.306	0.348	0.771	0.320	0.437	0.950	<0.001	<0.001	<0.001	<0.001	<0.001
	Quadratic	0.206	0.157	0.157	0.082	0.754	0.708	0.933	0.633	0.035	0.001	0.002	<0.001	<0.001
	Ration × Yeast	0.213	0.158	0.158	0.326	0.040	0.128	0.031	0.068	<0.001	<0.001	<0.001	<0.001	<0.001

Abbreviations: DM, dry matter; LSD, least significant difference.

^a Steam rolled corn was replaced with soybean hulls as an energy source at 0% (control), 7.5% (SH75), and 15% (SH150).

of SH rations and yeast doses will be discussed. In the contrary, interactions occurrence between PC rations and yeast doses for the asymptotic GP, the rate of GP, and CO₂ production revealing that the effective dose will be ration dependent. Ration containing either steam rolled corn or SH produced insignificantly different amount of gases, revealing that SH has almost the same nutritive value of steam rolled corn. Soybean hulls contain large concentration of nonamylose polysaccharides, which are considered as rich energy sources in the diets of horses [28]. Rich content of nonamylose polysaccharides with poor starch content make SH an ideal feed component in the diet of horses with less potential to cause digestive disturbances, thus less bypass of starch to the large intestine. Almost no study substituted the main energy source of equine's diets with SH or PC; however, no almost no study used PC in horses, where many used SH as a substitute for the forage portion of diet [4,28] with promising results.

There is limited data both in vitro and in vivo on the use of PC in the diet of horses. Some parallels with experimentation with ruminants will therefore be highlighted. Ration of PC75 increased rate of GP without affecting the asymptotic GP or the lag time. This may be due to the high contents of readily available carbohydrates and nonfiber carbohydrates in the PC, which are rapidly degraded in the rumen [10] resulting in stimulated microbial growth, as it serves as a source of energy for ruminal microflora. However, increasing PC level (i.e., at PC150) may result in increased soluble carbohydrates content in the rumen thus depressing ruminal cellulolytic activity and decreasing rate of GP [29]. In addition, in their review, Ben Salem et al [29] reported that increasing PC level in diets might impair the microbial growth in the rumen due to high levels of minerals. In agreement with the current results, Tegegne et al [30] observed that in vitro rate of ration degradation increased with increasing PC level.

The effect of yeast differed between SH rations and was dose dependent. The asymptotic GP was increased with yeast addition to SH containing rations (i.e., SH75 and SH150). Moreover, yeast addition to PC containing rations increased the asymptotic GP and the rate of GP. Several studies [13,31] have shown that the responses to *S. cerevisiae* addition were substrate and dose dependent. In many reports, yeast showed improve microbial profile and balance the hindgut of horses, in addition to stimulating the cellulolytic bacterial number and activity [32] resulting in increased digestibility and GP [13,14]; however, the DMD of SH containing rations was not affected in the present study with yeast addition, but the positive effect was clear with PC containing rations (i.e., PC75 and PC150). Moreover, yeast addition decreased the lag time with SH rations. In addition, Newbold et al [33] stated that *S. cerevisiae* might scavenge O₂, which is toxic to anaerobic bacteria in the cecum, and activate cellulolytic bacteria attachment to plant cell wall components. Besides, the small peptides and other active nutrients required by cellulolytic bacteria to induce growth is present in *S. cerevisiae* [33] resulting in improved fermentation activity inside the cecum.

The dose of 2 mg yeast/g DM of PC150 ration increased the asymptotic GP and the rate of GP compared to the dose 4 mg yeast/g DM with PC75 ration. In contrary, the doses 2 mg and 4 mg/g DM were the effective doses with rations SH75 and SH150, respectively. The different responses to yeast dose with incubated substrates concur with the assumption of Elghandour et al [13] that responses to yeast are highly variable and substrates composition dependent. *S. cerevisiae* addition did not affect the DMD of SH rations, which is in agreement with the results of Elghandour et al [14] who obtained no change in in vitro DMD with *S. cerevisiae* addition to a fiber-based equine diet. However, *S. cerevisiae* increased DMD of PC rations with different effective doses as the interactions between PC ration and yeast doses were significant for DMD.

Table 4 In vitro fecal gas kinetics and fermentation profile of replacing steam rolled corn with three levels of prickly pear cactus^a (PC) at three levels of *Saccharomyces cerevisiae* in horse.

Ration	Yeast Dose (mg/g DM)	In Vitro Gas Production (mL/g DM) at:										Fermentation Kinetics							
		c	L	2 hr	4 hr	6 hr	8 hr	10 hr	12 hr	14 hr	16 hr	24 hr	36 hr	48 hr	60 hr	72 hr	pH	DMD	
Control	0	225.6	0.033	1.45	14.4	27.9	40.5	52.2	63.3	73.6	83.3	92.3	123.1	146.7	178.7	193.9	204.1	6.92	253.9
	2	165.8	0.050	1.19	14.1	27.0	38.6	49.2	58.9	67.7	75.7	83.1	106.6	123.1	143.2	151.6	156.8	6.86	248.4
PC75	0	191.7	0.030	0.58	11.1	21.5	31.3	40.5	49.3	57.5	65.2	72.5	97.7	117.6	145.6	159.4	169.1	6.76	240.1
	2	147.2	0.071	1.58	13.8	25.8	36.3	45.6	53.9	61.3	67.9	73.9	92.9	106.3	123.4	131.0	136.1	6.96	239.2
PC150	0	204.1	0.035	1.24	13.8	26.7	38.7	49.8	60.2	69.9	78.9	87.3	115.5	136.8	165.1	178.1	186.7	6.64	244.4
	2	86.1	0.094	1.56	14.3	26.2	36.1	44.3	51.2	56.9	61.7	65.6	76.0	81.1	84.8	85.6	85.9	6.71	273.4
Pooled LSD	0	170.2	0.034	1.28	10.5	20.3	29.5	38.1	46.2	53.7	60.8	67.4	90.0	107.4	131.4	143.0	151.0	6.66	233.8
	2	149.7	0.042	1.14	12.1	23.3	33.5	42.9	51.5	59.4	66.6	73.3	95.0	110.5	129.4	137.3	142.1	6.83	256.0
Ration	4	185.8	0.028	1.08	10.1	19.7	28.8	37.3	45.4	53.0	60.2	67.1	90.8	109.8	137.0	150.8	160.7	6.77	247.7
	LSD	12.91	0.0113	0.400	1.67	3.25	4.74	6.18	7.55	8.87	10.14	11.35	15.65	19.14	24.18	26.72	28.52	0.093	8.81
Linear	0.008	0.005	0.253	0.577	0.768	0.952	0.883	0.7438	0.629	0.535	0.459	0.278	0.195	0.129	0.110	0.110	0.100	0.317	0.511
	0.949	0.045	0.724	0.038	0.054	0.076	0.102	0.1314	0.164	0.199	0.235	0.376	0.497	0.665	0.745	0.799	0.426	0.518	
Quadratic	0.037	0.631	0.280	0.452	0.419	0.394	0.373	0.356	0.341	0.329	0.320	0.294	0.282	0.280	0.286	0.294	0.203	0.012	
	0.818	0.044	0.808	0.048	0.036	0.034	0.039	0.331	0.326	0.032	0.037	0.349	0.038	0.048	0.054	0.063	0.764	0.025	
Ration × Yeast	0.015	0.018	0.770	0.034	0.826	0.8779	0.899	0.899	0.884	0.857	0.821	0.645	0.490	0.312	0.248	0.212	0.144	0.046	

Abbreviations: DM, dry matter; LSD, least significant difference.

^a Steam rolled corn was replaced with prickly pear cactus as an energy source at 0% (control), 7.5% (PC75), and 15% (PC150).^b *b* is the asymptotic gas production (mL/g DM); *c* is the rate of gas production (/h); DMD is the DM degraded substrate (mg/g DM); *L* is the initial delay before gas production begins (h).

Interactions were observed between ration type and yeast dose for CO₂ production. This indicates that the effective dose will vary between rations. This was clear as both doses of yeast had almost the same effect with the ration SH75, whereas the dose 2 mg yeast was more effective than the dose 4 mg yeast when incubated with SH150 ration. Soybean hulls rations and PC rations decreased CO₂ production compared with the control ration. The volume of gas and gas types (i.e., hydrogen, CO₂, and CH₄) reflect the fermentation potential of the diet [13,14] as it depends on nutrient availability for inocula microorganisms during fermentation [34]. Soybean hulls contain higher fiber content than steam rolled corn, with higher protein contents. In the contrary, PC contains high concentration from readily available carbohydrates and nonfiber carbohydrates [30]. Fermentation of dietary carbohydrates produces mainly acetate, propionate, and butyrate, in addition to gases. Increasing fiber portions may direct the fermentation toward the production of acetate instead of gases, which resulted in unaffected GP between different results. Unaffected GP, with increasing CO₂ production with SH and PC, indicates increasing acetate production; however, it was not determined in the present study. In addition, the decreased CO₂ production of SH rations may be related to the higher CP contents of SH than in steam rolled corn (12.0 vs. 8.1%, respectively; data are not shown). Protein degradation in the rumen leads to the accumulation of ammonia-N in the incubation medium, and preventing the release of CO₂ in the incubation bottles [35].

Yeast addition increased CO₂ production with higher production with yeast addition at 2 mg/g DM of SH and PC ration. Besides, yeast increased CO₂ production from PC75 and PC150 rations at both yeast levels. Little information is available on the effect of yeast on CO₂ production in ruminant and equines. However, increased CO₂ production can be considered as an indicator of improved fibers digestion by yeast addition [12]. The hydrolysis of feed fibers (i.e., cellulose and hemicelluloses) in the digestive tract of horses produces large amounts of hydrogen and CO₂ [36]. In an in vitro experiment [14] and in an in vivo experiment [12] observed unaffected CO₂ production with yeast addition to equine fiber-based diets.

5. Conclusions

Soybean hulls (SH) and prickly pear cactus (PC) can replace steam rolled corn in diets of horses at levels of 7.5% to 15% with no negative effect on in vitro fermentation. The ration PC75 increased rate of GP and decreased CO₂ production. Rations of SH75 and SH150 increased DM degradability. Yeast addition had positive impact on fermentation of diets containing SH and PC than that containing steam rolled corn. Yeast at 2 mg/g DM increased GP with the SH75 and PC150 TMRs, where as at 4 mg/kg DM yeast increased GP from the SH150 ration only. Yeast increased CO₂ production from SH75, SH150, PC75, and PC150 TMRs. More studies, including in vivo experiments, are recommended to investigate replacing corn and other grains with SH and PC at different levels.

Table 5

In vitro fecal carbon dioxide production of replacing steam rolled corn with three levels of prickly pear cactus^a (PC) at three levels of *Saccharomyces cerevisiae* in horse.

Ration	Yeast Dose (mg/g DM)	In Vitro Carbon Dioxide Production (mL/g DM) at:													
		2 hr	4 hr	6 hr	8 hr	10 hr	12 hr	14 hr	16 hr	24 hr	36 hr	48 hr	60 hr	72 hr	
Control	0	1.8	13.4	13.4	17.1	21.7	21.7	24.8	37.3	56.3	70.6	70.6	70.6	70.6	
	2	3.0	3.9	3.9	3.9	7.8	10.8	10.8	16.2	44.1	69.7	69.7	73.3	73.3	
	4	1.6	11.2	11.2	12.8	17.8	17.8	17.8	25.9	54.1	82.5	82.5	90.7	90.7	
PC75	0	4.5	4.5	4.5	12.4	12.4	12.4	20.0	32.3	49.2	52.3	52.3	52.3	52.3	
	2	1.9	8.3	8.3	15.5	21.4	26.8	39.8	47.6	58.8	68.9	77.1	77.1	77.1	
	4	7.6	15.7	15.7	27.7	27.7	34.5	37.1	37.1	44.1	61.0	81.6	81.6	82.9	
PC150	0	0.0	0.0	0.0	0.0	1.8	2.4	8.3	19.3	36.2	67.1	67.1	68.1	68.1	
	2	5.5	8.2	8.2	8.8	8.2	18.0	32.4	39.3	61.7	87.5	87.5	87.5	87.5	
	4	5.4	15.6	15.6	25.1	25.1	30.0	35.0	38.8	45.0	56.9	94.8	100.9	100.9	
Pooled LSD		1.67	2.72	2.72	2.75	2.90	4.34	4.12	4.98	4.17	5.22	8.99	8.27	8.43	
Ration															
Linear		0.082	0.999	0.999	0.005	0.061	0.040	0.002	0.053	0.075	0.003	0.596	0.257	0.293	
Quadratic		0.831	0.418	0.418	0.064	0.006	0.224	0.569	0.615	0.837	0.358	0.106	0.071	0.081	
Yeast															
Linear		0.058	0.002	0.002	<0.001	0.001	0.004	0.003	0.053	0.090	0.309	0.006	0.007	0.001	
Quadratic		0.989	0.109	0.109	0.003	0.019	0.679	0.108	0.203	0.003	0.009	0.613	0.751	0.783	
Ration × Yeast		0.095	0.020	0.020	0.007	0.001	0.010	0.004	0.001	0.001	0.009	0.656	0.711	0.734	

Abbreviations: DM, dry matter; LSD, least significant difference.

^a Steam rolled corn was replaced with prickly pear cactus as an energy source at 0% (control), 7.5% (PC75), and 15% (PC150).

Acknowledgment

The authors would like to thank the financial support from Universidad Autónoma del Estado de México of the project #3706/2014 CID.

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