Original Research

In Vitro Gas, Methane, and Carbon Dioxide Productions of High Fibrous Diet Incubated With Fecal Inocula From Horses in Response to the Supplementation With Different Live Yeast Additives

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ABSTRACT

In a randomized block design experiment, the effect of fecal inocula from horses supplemented with live yeast (*Saccharomyces cerevisiae*) in diets containing 50% oat straw on in vitro total gas (gas production [GP]), methane (CH_4), and carbon dioxide (CO_2) productions as indicators of hindgut activity was assessed. Three commercial products of *S. cerevisiae* were tested (1) Biocell F53 (YST53), (2) Procreatin 7 (YST07), and (3) Biosaf SC47 (YST047). For the incubations, each product was added at 0 (control without yeast addition), 2, or 4 mg/g dry matter (DM). Fecal inocula for incubations with each treatment was obtained from Quarter Horse mares fed the same yeast additives for 15 days, resulting in four different fecal inocula (FI53, FI07, FI47, and FI00). The fecal content mixed with the culture media were used to inoculate three identical runs of incubation in bottles containing 1-g DM of substrate (a mixture of concentrate and oat straw [1:1 DM]). The GP, CH_4, and CO_2 productions were measured at 2, 4, 6, 8, 10, 12, 24, and 48 hours post-incubation. Addition of additives YST53 and YST07 at 2 mg/g DM resulted in higher asymptotic GP (linear effect, $P = .021$) and GP during the first 12 hours of incubation (linear effect, $P < .05$) compared with control without yeast addition, with the highest value being for the dose 2 mg/g DM with the fecal inoculum FI53. The additive YST47 at all doses with fecal inoculum FI47 had lower GP (linear effect, $P < .05$) at different incubation hours compared with control. The additive YST53 increased GP, CH_4, and fermentation kinetics at the dose 2 mg/g DM with decreasing CH_4 production by 78% at 4 mg/g DM at 24 hours of incubation. Addition of YST53 at 2 and 4 mg/g DM with fecal inoculum FI53 enhanced fermentation kinetics ($P < .05$) compared with control and other additives at different doses. It can be concluded that the yeast additive Biocell F53 was the most effective at doses of 2 and 4 mg/g DM compared with other *Saccharomyces* strains to attain a more favorable hindgut fermentation to digest fibrous roughages by horses.

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1. Introduction

Feeding starchy grains to horses represents an important source of energy to meet their energy requirements. However, feeding high-grain diets is associated with some feeding disorders such as gastric ulceration, hindgut acidosis, laminitis, and colic [1]. In addition, feeding high-grain diets may decrease the starch digestibility in the small intestine and cause microbial disturbance and impairment of the fibrolytic activity in the hindgut [2], thus reducing energy utilization of the diet [3]. Feeding high-fiber diets would be an alternative solution to overcome these nutritional problems. Oat straw is commonly fed to horses in Mexico [4]. In 2013, Mexico produced about 11.2 million tonnes of oat straw with poor nutritive value due to its low protein content and nutrient digestibility. It would be of much interest to develop feeding strategies to ensure gut health and integrity of horses while meeting their energy requirements [5].

Yeast supplementation of horse diets can influence nutrient digestibility and microbiota dynamics in the horse hindgut. In some in vitro [6] and in vivo [4] studies, yeast addition to the diets improved digestion of low-quality forages. It has been shown that yeast supplementation can alter the microbial environment by increasing the total number of hindgut microorganisms [7]. As a result, feed digestion in the hindgut can be enhanced, especially that of the fiber fraction, most likely due to increased numbers of cellulolytic bacteria in the hindgut [8]. In contrast, other studies have reported no effect of yeast addition to equine diets on nutrient digestibility in vitro [7] or in vivo [9].

It is hypothesized that yeasts can enhance the digestion of poor-quality high-fiber feeds (such as oat straw) in the hindgut of horses. The aim of the present study was to assess how the supplementation of high-fiber diets with yeast could modify the microbial fermentation activity in the hindgut of horses and affect the digestion of a high-fiber substrate (oat straw). Feces from horses fed oat straw diets and supplemented with live yeast (*Saccharomyces cerevisiae*) were used to inoculate batch cultures, and total fermentation gas, methane (CH_4), and carbon dioxide (CO_2) produced after incubation in vitro were used as indicators of the fermentative activity in the hindgut.

2. Materials and Methods

2.1. Substrate and Yeast Additives

A basal diet consisting of a mixed ration containing 50% concentrate mixture and 50% oat straw (1:1 DM) was used as the substrate for the incubations. The concentrate mixture contained 50% commercial concentrate (Pell Rol Cuarto de Milla, Mexico) and 50% wheat bran. The chemical composition of the concentrate mixture was (per kg DM): 902 g of organic matter (OM), 112 g of crude protein (CP), 511 g of neutral detergent fiber (NDF), and 203 g of acid detergent fiber (ADF). The chemical composition of the oat straw was (per kg DM): 929 g of OM, 26.7 g of CP, 669 g of NDF, and 405 g of ADF.

Three commercial yeast additives of *S. cerevisiae* (Lesaffre Feed Additives, Toluca, Mexico) were tested: (1)

Biocell F53 (YST53) with a minimum guaranteed concentration of live yeast cells of 2×10^{10} colony forming unit (CFU) of *S. cerevisiae*/g, (2) Procreatin 7 (YST07) with a minimum guaranteed concentration of live yeast cells of 1.5×10^{10} CFU of *S. cerevisiae*/g, and (3) Biosaf SC47 (YST47) with a minimum guaranteed concentration of live yeast cells of 1.5×10^{10} CFU of *S. cerevisiae*/g. Each yeast additive was fed to horses from which inocula for the in vitro incubations were obtained and also supplemented to the batch cultures at three rates, namely 0 (control with no yeast), 2, or 4 mg additive/g diet DM.

2.2. In Vitro Fecal Incubations

Before the start of the experiment, fecal contents (i.e., the inoculum source) were collected from the 16 Quarter Horse mares used in the experiment of Salem et al [4] and offered for 15 days the same basal diet of a mixture of concentrate and oat straw that was used as substrate for the in vitro incubations [4]. The mares consumed the offered concentrates and oat hay at about 2:1 DM, respectively. In the experiment of Salem et al [4], horses were divided into four treatments (n = 4 mares/treatment) to receive the basal diet without yeast culture (control) or the basal diet supplemented with the three yeast cultures at the rate of 11 g Biocell/animal/d or 11 g Procreatin 7/animal/d or 15 g Biosaf SC47/animal/d for 15 days. Diets were balanced to cover animal's needs according to nutrient requirements proposed for horses by the National Research Council [10].

Fecal contents were collected from the rectum of each horse before the morning feeding. The fecal content of horses fed Biocell F53 culture was used as a source of fecal inoculum (FI) for the treatment of FI53, whereas those fed Procreatin 7 culture were used as inoculum for the treatment of FI07, and those fed Biosaf SC47 culture were used as a source of inoculum for the treatment of FI47. Feces from horses not receiving any yeast were considered control or treatment FI00. About 10% of individual fecal samples of each mare within each treatment were mixed and homogenized to obtain a homogenized sample of feces for each treatment. With the exception of the preparation of the microbial inocula, the method of Theodorou et al [11] was used to measure gas production (GP). A subsample of the composite fecal contents of each in vivo treatment was mixed with the Goering and Van Soest [12] buffer solution without trypticase in the ratio of 1:4 vol/vol. The four incubation media were strained through four layers of cheesecloth and poured into a flask with an O_2-free headspace. The fecal content mixed with the culture media were used to inoculate three identical runs of incubation in bottles containing 1-g DM of substrate (a mixture of concentrate and oat straw [1:1 DM]). Both oat straw and the concentrate mixture were grounded separately through a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA) using a 2-mm screen and then mixed together before the incubation. A total number of 252 bottles (3 fecal inocula [FI53, FI07, or FI47] $\times$ 3 yeast additives [YST53, YST07, or YST47] $\times$ 3 yeast doses [0, 2, or 4 mg/g DM incubated] $\times$ 3 replicates $\times$ 3 runs + 3 replicates of control [FI00 and no yeast additive] $\times$ 3 runs) plus three bottles without substrate and with no yeast used as blanks. Thereafter, all the

bottles flushed with CO_2 and 50 mL of the buffered fecal fluid were dispensed. Then, the bottles were immediately closed with rubber stoppers, shaken and placed in an incubator set at 39°C. Gas, CH_4, and CO_2 productions were recorded at 2, 4, 6, 8, 10, 12, 24, and 48 hours post-inoculation. Gas production was recorded using the pressure transducer technique (Extech instruments, Waltham, USA) of Theodorou et al [11], whereas the CH_4 and CO_2 concentrations were measured using Gas-Pro detector (Gas Analyzer CROWCON Model Tetra3, Abingdon, UK).

At the end of incubation after 48 hours, bottles were open and the pH was measured using a digital pH meter (Conductronic pH15, Puebla, Mexico). The content of each bottle was then filtered under vacuum through glass crucibles with a sintered filter (coarse porosity no. 1, pore size 100–160 μm; Pyrex, Stone, UK), and fermentation residues dried at 65°C for 72 hours to estimate DM disappearance (DMD).

2.3. Calculations and Statistical Analyses

To estimate the fermentation kinetics parameters, the equation of France et al [13] was fitted using the NLIN procedure for non-linear regression of SAS [14]; as:

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

where A is the volume of GP (mL/g DM incubated) at time t (h); b is the asymptotic GP (mL/g DM incubated); c is the fractional fermentation rate (/h), and L (h) is the discrete lag time.

Metabolizable energy (ME, MJ/kg DM) and in vitro organic matter digestibility (OMD, %) were estimated according to Menke et al [15] as:

$$ME \ (MJ/Kg \ DM) = 2.20 + 0.136 \ GP + 0.057 \ CP$$

$$OMD \ (\%) = 14.88 + 0.889 \ GP + 0.45 \ CP + 0.0651 \ XA$$

where DM, dry matter; CP, crude protein in percent; XA, ash in percent; and GP, the net GP in milliliters from 200 mg dry sample after 24 hours of incubation. The partitioning factor at 24 hours of incubation (PF_{24}), as a measure of fermentation efficiency, was calculated as the ratio of in vitro DMD (mg/g DM) to the volume of gas (mL) produced at 24 hours (i.e., DMD/total GP [GP_{24}] according to Blümmel et al [16]).

Gas yields (GY_{24}) were calculated as the volume of gas produced after 24 hours (mL gas/g DM) of incubation divided by the amount of DMD (g) as:

$$Gas \ yields \ (GY_{24}) = mL \ gas \ per \ g \ DM/g \ DMD$$

Short-chain fatty acids (SCFA) were calculated according to Getachew et al [17] as:

$$SCFA \ (mmol/200 \ mg \ DM) = 0.0222 \ GP - 0.00425$$

where GP is 24 hours net GP (mL/200 mg DM).

Microbial crude protein (MCP) production was calculated according to Blümmel et al [16]:

$$MCP \ (mg/g \ DM) = mg \ DMD - (mL \ gas \times 2.2 \ mg/mL)$$

where 2.2 mg/mL is a stoichiometric factor that expresses mg of C, H, and O required for the SCFA gas associated with production of one mL of gas [16].

The data were analyzed with FI (FI00, FI53, FI07, or FI47), yeast additive (YST53, YST07, or YST47), and doses of yeast added (0, 2, or 4 mg/g DM) as the sources of variation using PROC MIXED procedure of SAS [14] in a factorial design. Data of each of the three runs for each treatment were averaged before the statistical analysis, and the mean of each individual sample was considered the experimental unit. The statistical model was:

$$Y_{ijkl} = \mu + F_i + S_j + D_k + (F \times S)_{ij} + (F \times S \times D)_{ijk} + E_{ijkl}$$

where Y_{ijkl} = is every observation when using one of the ith F_i on the addition of one of the jth yeast additives (S_j) at the kth yeast dose (D_k); μ is the general mean; F_i ($i = 1$–4) is the FI effect; S_j is the yeast additive effect ($j = 1$–3); D_k is the effect of yeast dose; $(F \times S)_{ij}$ is the interaction between FI and yeast additive; $(F \times S \times D)_{ijk}$ is the interaction between FI, yeast additive and yeast dose; E_{ijkl} is experimental error. Linear and quadratic polynomial contrasts were used to examine responses in GP to increasing addition levels of the yeast additives. Tukey's test was used for the multiple comparisons among means.

3. Results

3.1. Fecal In Vitro GP

There were no interactions ($P > .05$) between FI, yeast additive, and yeast dose. However, interactions occurred ($P < .05$) between FI and yeast additive for the asymptotic GP, the rate of GP, and cumulative GP at different incubation hours (Table 1).

Addition of YST53 and YST07 cultures at 2 mg/g DM resulted in higher asymptotic GP (linear effect, $P = .021$) and GP during the first 12 hours of incubation (linear effect, $P < .05$) compared to FI00 (control without yeast addition), with the highest value being for the dose 2 mg YST53/g DM with the FI FI53. However, the additive YST47 with FI FI47 had lower GP (linear effect, $P < .05$) at different incubation hours compared with FI00 at all doses (Table 1).

3.2. CH_4 and CO_2 Productions

There were interactions ($P < .05$) between FI and yeast additive and interactions among FI, yeast additive, and doses at 12 and 24 hours of incubation for CH_4 production. No CH_4 was produced until 10 hours of incubation. The doses 2 and 4 mg YST53/g DM at 10 and 12 hours of incubation had the highest (linear effect, $P = .028$) CH_4 production compared with the other doses and other yeast additives (Table 2).

For CO_2 production, no interactions ($P > .05$) occurred among FI, yeast additive and yeast doses at different incubation hours; however, interactions occurred ($P = .022$) between FI and yeast additive after 2 hours of incubation, with no interactions at other incubation hours. Addition of yeast products at all levels had no effect ($P > .05$) on CO_2 production (Table 3).

Table 1
In vitro fecal gas kinetics and total cumulative gas production[a] (GP) during 48 hours of incubation as affected by different yeast cultures addition at different levels.[b]

Fecal From Mares Fed on:	Yeast Additive	Dose mg/g DM	GP Parameters			In Vitro GP, mL/g DM at:							
			b, mL/g DM	c, /h	L, h	2 hours	4 hours	6 hours	8 hours	10 hours	12 hours	24 hours	48 hours
No yeast	Without yeast	0	292.1	0.062	0.99	34.0	64.0	90.5	113.9	134.5	152.8	225.4	276.6
Biocell F53	YST53	0	280.8	0.054	1.04	28.7	54.4	77.5	98.3	116.9	133.6	203.5	259.4
		2	346.5	0.053	1.18	33.9	64.4	91.9	116.6	139.0	159.1	244.5	315.8
		4	321.7	0.061	1.73	36.6	68.9	97.5	122.8	145.2	165.0	244.5	302.3
Procreatin 7	YST07	0	300.8	0.052	1.02	29.7	56.4	80.5	102.2	121.8	139.4	213.9	275.4
		2	316.9	0.048	1.35	28.7	54.9	78.6	100.2	119.9	137.7	215.5	284.4
		4	320.0	0.047	1.35	28.4	54.3	77.9	99.4	119.0	136.8	215.1	285.5
Biosaf SC47	YST47	0	245.2	0.062	1.38	28.7	54.0	76.4	96.0	113.4	128.7	189.4	232.1
		2	257.8	0.064	1.31	30.7	57.7	81.5	102.5	120.9	137.1	201.2	245.2
		4	286.5	0.059	1.24	32.0	60.4	85.6	108.0	127.9	145.6	217.2	269.7
SEM			16.37	0.0037	0.202	1.60	2.91	3.98	4.88	5.63	6.28	9.09	12.97
P value													
Doses:													
Linear			.021	.856	.096	.021	.017	.014	.011	.009	.008	.006	.011
Quadratic			.224	.660	.909	.712	.665	.618	.572	.530	.490	.328	.244
Fecal inoculum × yeast additive			.007	.031	.412	.021	.019	.016	.014	.012	.011	.006	.006
Fecal inoculum × yeast additive × yeast dose			.388	.374	.292	.120	.117	.116	.116	.117	.120	.164	.289

Abbreviation: SEM, standard error of the mean.
[a] b, asymptotic gas production; c, rate of gas production; L, initial delay before gas production begins.
[b] YST53, basal diet incubated with Biocell F53; YST07, basal diet incubated with Procreatin 7; YST47, basal diet incubated with Biosaf SC47.

3.3. Fermentation Profile

There were no interactions among FI, yeast additiveand yeast doses for all measured fermentation parameters. However, interactions between yeast additive and FI were observed for ME ($P = .006$), OMD ($P = .006$), SCFA ($P = .006$), MCP ($P = .009$), and GY_{24} ($P = .006$). Addition of YST53 at 2 and 4 mg/g DM with inoculum FI53 had the highest ME (linear effect, $P = .006$), OMD (linear effect, $P = .006$), SCFA ($P = .006$), MCP (linear effect, $P = .006$), and GY_{24} (linear effect, $P = .005$) compared with FI00 and other cultures at different doses. However, the additive YST53 with inoculum FI53 had the lowest PF_{24} (linear effect, $P = .005$) (Table 4).

4. Discussion

The use of in vitro fermentation procedures for studying the nutritive value of equine diet using feces as a source of inoculum is a popular method in equine feeds

Table 2
In vitro fecal methane production after 48 hours of incubation as affected by different yeast cultures addition at different levels.[a]

Fecal From Mares Fed on:	Yeast Additive	Dose mg/g DM	In Vitro Methane Production (mL/g DM) at:		
			12 hours	24 hours	48 hours
No yeast	Without yeast	0	0.00	1.04	1.52
Biocell F53	YST53	0	0.00	1.51	5.32
		2	0.23	2.68	2.68
		4	0.23	0.23	0.23
Procreatin 7	YST07	0	0.00	0.34	0.81
		2	0.22	2.38	2.38
		4	0.00	1.07	3.43
Biosaf SC47	YST47	0	0.00	0.31	1.52
		2	0.00	1.34	1.34
		4	0.00	1.46	2.40
SEM			0.006	0.015	0.028
P value					
Doses:					
Linear			1.000	.838	.247
Quadratic			.025	.033	.013
Fecal inoculum × yeast additive			.002	.026	.304
Fecal inoculum × yeast additive × yeast dose			.040	.048	.341

Abbreviation: SEM, standard error of the mean.
[a] YST53, basal diet incubated with Biocell F53; YST07, basal diet incubated with Procreatin 7; YST47, basal diet incubated with Biosaf SC47.

Table 3
In vitro fecal carbon dioxide production during 48 hours of incubation as affected by different yeast cultures addition at different levels.[a]

Fecal From Mares Fed on:	Yeast Culture	Dose mg/g DM	In Vitro Carbon Dioxide Production (mL/g DM) at:							
			2 hours	4 hours	6 hours	8 hours	10 hours	12 hours	24 hours	48 hours
No yeast	Without yeast	0	0.0	11.2	44.4	77.0	110.2	130.6	181.4	233.7
Biocell F53	YST53	0	17.0	25.0	58.3	81.6	104.8	120.4	182.6	238.1
		2	20.2	50.6	63.3	66.3	99.6	105.4	128.2	177.1
		4	0.0	0.0	1.1	19.1	52.4	78.0	126.8	174.0
Procreatin 7	YST7	0	13.3	38.2	61.5	92.7	108.0	101.3	205.5	252.0
		2	0.0	0.0	2.1	3.3	36.6	69.8	135.0	150.6
		4	0.0	14.4	31.4	64.6	64.6	82.8	145.7	212.3
Biosaf SC47	YST47	0	0.0	0.0	0.7	25.0	27.6	53.7	109.8	176.3
		2	23.3	33.3	33.3	66.5	93.9	108.9	152.7	197.7
		4	0.0	0.0	8.7	41.9	75.2	80.8	122.8	189.3
SEM			10.55	8.59	15.12	13.20	14.82	18.66	12.48	16.79
P value										
Doses:										
Linear			.057	.968	.118	.871	.949	.687	.931	.118
Quadratic			.196	.590	.149	.144	.468	.956	.211	.100
Fecal inoculum × yeast additive			.022	.124	.069	.152	.655	.686	.524	.058
Fecal inoculum × yeast additive × yeast dose			.051	.120	.063	.281	.630	.425	.412	.053

Abbreviation: SEM, standard error of the mean.
[a] YST53, basal diet incubated with Biocell F53; YST07, basal diet incubated with Procreatin 7; YST47, basal diet incubated with Biosaf SC47.

evaluation [6]. In the present study, the incubation was extended to 48 hours although Agazzi et al [18] have shown that the average mean retention time for feed passing through the gut of the horse ranges between 36–38 hours. The use of either rumen fluid or feces as a source of inoculum has been shown to produce similar volumes of fermentation gas when either grains or forages were incubated [19]. Many studies stated that the technique of Theodorou et al [11] could be used successfully for studying the in vitro fecal fermentation with the use of feces as the source of microbial inoculum [6,20]. However, the lag phase appears to be longer when feces are used as the inoculum source compared with

rumen liquor. This may be attributed to the different concentration of microorganisms per millimeter of rumen liquor or feces because the microbial population (bacteria, protozoa, and fungi) is essentially similar in the hindgut and in the rumen [21].

4.1. In Vitro Fecal Gas Production

Interactions occurred between FI and yeast additive for most of the measured parameters, which suggests that the responses to *S. cerevisiae* addition are affected by both sources of variation. Moreover, numerous studies have indicated that the responses to *S. cerevisiae* addition were

Table 4
In vitro fecal fermentation profile[a] after 48 hours of incubation as affected by different yeast cultures addition at different levels.[b]

Fecal From Mares Fed on:	Yeast Additive	Dose mg/g DM	pH	ME	OMD	DMD	SCFA	PF$_{24}$	MCP	GY$_{24}$
No yeast	Without yeast	0	6.64	8.73	586.2	643.7	4.98	5.30	697.5	188.8
Biocell F53	YST53	0	6.74	8.13	547.4	546.0	4.49	5.44	656.6	183.9
		2	6.67	9.25	620.2	562.3	5.41	5.21	733.2	192.1
		4	6.75	9.24	620.2	552.0	5.41	5.20	733.2	192.3
Procreatin 7	YST07	0	6.73	8.41	565.8	571.7	4.73	5.36	676.0	186.5
		2	6.79	8.46	568.7	578.7	4.76	5.35	679.0	186.8
		4	6.74	8.44	567.9	583.7	4.75	5.35	678.2	186.8
Biosaf SC47	YST47	0	6.73	7.75	522.3	588.7	4.18	5.53	630.2	180.8
		2	6.82	8.07	543.1	628.3	4.44	5.44	652.2	183.7
		4	6.79	8.50	571.6	622.3	4.80	5.35	682.1	187.1
SEM			0.048	0.250	16.17	20.99	0.202	0.055	17.01	1.91
P value										
Dose:										
Linear			.506	.006	.006	.328	.006	.005	.006	.005
Quadratic			.631	.322	.329	.415	.324	.354	.328	.345
Fecal inoculum × yeast additive			.597	.006	.006	.133	.006	.360	.009	.006
Fecal inoculum × yeast additive × yeast dose			.508	.164	.164	.929	.161	.852	.212	.164

Abbreviation: SEM, standard error of the mean.
[a] ME, metabolizable energy (MJ/kg DM); OMD, in vitro organic matter digestibility (mg/g DM); DMD, in vitro dry matter digestibility (mg/g DM); SCFA, short-chain fatty acids (mmol/g DM); GY$_{24}$, gas yield at 24 hours of incubation (gas/g DMD); MCP, microbial crude protein production (mg/g DM); PF$_{24}$, partitioning factor at 24 hours of incubation (mg DMD/mL gas).
[b] YST53, basal diet incubated with Biocell F53; YST07, basal diet incubated with Procreatin 7; YST47, basal diet incubated with Biosaf SC47.

related to diet type, diet composition, application method, and dose, in addition to interactions among yeast and diet [6,22].

The additive Biocell F53 (i.e., YST53) incubated with FI from horses fed Biocell F53 (i.e., FI53) resulted in higher GP than the other yeast additives with other fecal inocula. The different responses between yeast additives may be related to the number of active cells, strain of *S. cerevisiae,* other nutrients, and carrier materials presented in each additives. Feeding the donor horses with YST53 improved the cecal and fecal fermentation and affected positively, in balance, the activity and concentrations of fecal microbes thus resulting in higher GP during fermentation. Many reports have shown that live yeasts can improve the microbial balance in the hindgut of horses and stimulate the population of cellulolytic bacteria and their activity [23], thus increasing the digestibility of dietary nutrients [24] with increasing efficiency of energy utilization by the microbiota [7]. Newbold et al [25] stated that the high respiratory activity of *S. cerevisiae* might allow it to scavenge O_2, which is toxic to anaerobic bacteria, and causes inhibition of cellulolytic bacteria attachment to plant cell wall components. Moreover, *S. cerevisiae* contains small peptides and other nutrients that are required by cellulolytic bacteria to induce growth [25].

In this particular study, the volume of gas produced reflects the fermentation activity of the inoculum used in each case, and the potential of each additive to further stimulate such activity [6]. Moreover, GP depends on nutrient availability for inocula microorganisms during fermentation [26]. Fermentation of dietary carbohydrates to acetate, propionate, and butyrate produces gases, mainly hydrogen (H_2), CO_2, and CH_4. Availability of nutrients for inocula will stimulate the degradability of different nutrients [6].

Yeast additives and doses had no effects on both fermentation rate and lag time. This is in contrast to Murray et al [20] and Elghandour et al [6] who obtained decreased rate of GP in response to yeast supplementation. These differences may be related to incubated substrates. Elghandour et al [6] suggested that responses to yeast are highly variable and appear to be influenced by the composition of the substrate incubated.

4.2. CH4 and CO2 Productions

No effects were observed on CO_2 as a result of yeast addition at different doses. For CH_4 production, interaction effects were obtained between FI and yeast additive only at 12 and 24 when CH_4 started to be produced. Before the first 10 h of incubation, CH_4 production was negligible and then started to be increased quickly to reach its peak concentration at the end of incubation; however, GP started early with incubation. This reflects the nature of the produced gases during incubation hours. During fermentation process, many gases are produced within the cecum, which mainly constitutes of H_2, CO_2, and CH_4. There was no CH_4 production with Biocell F53 (FI53 and YST53) during the last 24 hours of incubation. However, CH_4 production increased from 0.70 to 3.53 mL/g DM with YST07. Compared with control (i.e., FI00), the additive YST53 at

doses 0 mg/g DM and using inoculum FI53 increased CH_4 production by 250% at 48 hours of incubation; however, the dose 4 mg/g DM decreased it by 85%. The response for the other additives at different doses varied between increasing and decreasing CH_4 production compared with control (i.e., FI00). These conflicting results on CH_4 production are likely due to strain difference of yeast additive [22].

In general, CH_4 yields for horses are between those for pigs and ruminants and amount to 3%–4% of the digestible energy or 2%–3% of the gross energy intake [27]. In both ruminants and horses, CH_4 is mainly produced by the methanogenic *Archaea*, which represent the main hydrogenotrophic microbial community [28].

There is little information about the potential effects of yeast on methanogenesis in the ruminants and almost no information in horses. However, the probable mode of action in the ruminant may be applied to horses as the cecum of horse shows similar conditions as those prevailing in the rumen. Yeast has the ability to shift H_2 utilization from methanogenesis to reductive acetogenesis through the homoacetogenic bacteria that can produce acetate from CO_2 and H_2 [29]. In vitro studies have shown beneficial effects of feeding live yeast strain on growth and H_2 utilization and acetate production by acetogenic bacteria isolated from a rumen of lambs, even in the presence of methanogens [30]. Lynch and Martin [31] reported a 20% reduction in CH_4 production after a 48 hours incubation of alfalfa supplemented with a live yeast product. In another study, yeast addition decreased CH_4 by about 58% [32]. Polyorach et al [33] noted that CH_4 production was decreased when animals fed *S. cerevisiae* fermented cassava chip protein instead of soybean meal due to the ability of *S. cerevisiae* to affect H_2 metabolism in the rumen with altering the fermentation process in a manner that reduces the formation of CH_4.

4.3. Fermentation Kinetics

Interactions between yeast additive and FI were observed for ME, OMD, SCFA, MCP, and GY_{24}. As previously mentioned, feeding donor horses with YST53 may make the hindgut environmental more appropriate to benefit and balance microbial community, resulting in increased ME, OMD, SCFA, MCP, and GY_{24} without affecting DMD. Moreover, fermentation kinetics was improved with YP53 at 2 mg/g DM with F53 fecal type. Addition of YST53 yeast is likely to stimulate the microbial activity in the hindgut causing an improved nutrient digestion [20]. However, the additives YST07 and YST47 at different doses affected hindgut fermentation to a lesser extent.

Improved fermentation kinetics can be explained based on increased in vitro cecal microbial protein production as a result of yeast supplementation. Yeast supplements can modify the microbial population of the digestive system and provide various growth factors and provitamins that stimulate the growth of cecal bacteria. Lattimer et al [5] demonstrated that addition of *S. cerevisiae* increased the proportion of microbial N, which might be explained by an increased ability of the microbiota to capture ammonia and convert it to microbial cell protein. In contrast, Medina et al

[23] reported no increase in microbial counts in the cecum or colon of horses supplemented with *S. cerevisiae*.

Increased SCFA with yeast addition contrasts with results of Lattimer et al [5] who reported no effect of yeast supplementation on SCFA concentration. Moreover, Warren and Hale [8] found that yeast addition during in vitro fecal fermentation had no effect in SCFA production. The increased SCFA was expected as a result of increased acetate, not propionate production as a result of increased oat straw portion in the diet. Medina et al [23] and Lattimer et al [5] reported that addition of *S. cerevisiae* increased the in vitro concentration of acetate without any effects on propionate production due to the addition of *S. cerevisiae*. With rumen inoculum, Elghandour et al [6] showed that addition of *S. cerevisiae* increased SCFA production and ME. They attributed their results to the high activities of microbes in the rumen as a result of produced growth factors for microbial growth and activity in the rumen and to the ability of *S. cerevisiae* to provide conducive anaerobic conditions to microbial growth [34].

As the PF is an index of the distribution of truly degraded substrate between microbial biomass and fermentation waste products, the lower PF in the present study reflects less substrate converting into microbial biomass [35]. Elghandour et al [36] showed that addition of *S. cerevisiae* decreased PF from different poor-quality roughages.

There was no effect of *S. cerevisiae* on pH in the bottles. The lack of difference observed in the present study could be due to the very high buffering capacity of the in vitro fermentation processes because four parts of buffer solution were added to one part diluted fecal fluid [5].

The lack of effect of *S. cerevisiae* supplementation on DMD coincides with Lattimer et al [5] who obtained unaffected in vitro DMD with *S. cerevisiae* supplementation of a high-concentrate or high-fiber diets.

5. Conclusions

Addition of yeast at 2 mg yeast/g DM resulted in increased GP and improved fermentation kinetics. The additive Biocell F53 was the most effective at doses of 2 and 4 mg yeast/g DM compared with other additives. Based on these results, Biocell F53 can be fed to horses in vivo at doses of 2–4 mg/g DM of diet, enhancing the hindgut digestion of high-fiber roughages such as oat straw. However, more studies are required to characterize the in vivo effects and modes of action of different yeast additives at different doses on fermentation kinetics in the hindgut of horses.

Acknowledgments

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