PAPER

Effects of *Saccharomyces cerevisiae* at direct addition or pre-incubation on *in vitro* gas production kinetics and degradability of four fibrous feeds

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Abstract

The objective of this study was to evaluate the effects of *Saccharomyces cerevisiae* on *in vitro* gas production (GP) kinetics and degradability of corn stover, oat straw, sugarcane bagasse and sorghum straw. Feedstuffs were incubated with different doses of yeast [0, 4, 8 and 12 mg/g dry matter (DM)] at direct addition or 72 h pre-incubation. Rumen GP was recorded at 2, 4, 6, 8, 10, 12, 14, 24, 30, 48, 54 and 72 h of incubation. After 72 h, rumen pH and methane were determined and contents were filtrated for DM, neutral (NDF) and acid detergent fibre (ADF) degradability. Fibrous species×method of application×yeast interactions occurred (P<0.001) for all measured ruminal GP parameters and degradability. The direct addition or 72 h pre-incubation of *S. cerevisiae* with corn stover improved (P<0.05) GP and methane and decreased (P<0.05) the lag time (*L*) and NDF degradability (NDFD). The direct addition of *S. cerevisiae* to oat straw increased (P<0.05) rate of GP (*c*) and decreased (P<0.05) asymptotic GP (*b*). However, 72 h pre-incubation increased (P<0.05) *c* with linearly decreased *b*, DM degradability (DMD) and NDFD. Applying *S. cerevisiae* for 72 h pre-incubation decreased (P<0.001) methane emission. The direct addition or 72 h pre-incubation of *S. cerevisiae* to sorghum straw increased (P<0.05) *b*, *c*, *L*, DMD and NDFD. Overall, the effect of dose varied among different feedstuffs and different application methods. Results suggested that the direct addition of *S. cerevisiae* could support and improve ruminal fermentation of low-quality forages at 4 to 12 g/kg DM.

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Introduction

Direct-fed microbial offer a great potential for manipulation of ruminal fermentation and *Saccharomyces cerevisiae* is an especially attractive organism. *S. cerevisiae* addition was reported to increase nutritional value of poor quality forages. *S. cerevisiae* have the ability to increase dry matter (DM) and neutral detergent fibre (NDF) digestion (Carro *et al*., 1992), increase initial rates of fibre digestion (Williams *et al*., 1991). Numerous studies (Kumar *et al*., 2013; Pinloche *et al*., 2013) documented positive effects of yeast, not only on the rumen environment, but also on the improvement of microbial activities. *S. cerevisiae* supplementation leads to increase in the number of total anaerobic and cellulolytic bacteria (Newbold *et al*., 1996; Jouany, 2001). *Saccharomyces cerevisiae* can provide the rumen with important nutrients and nutritional cofactors in addition to vitamins such as biotin and thiamine, which reported to be required for microbial growth and activity (Callaway and Martin, 1997; Mao *et al*., 2013).

Many reports illustrated that administration of yeast is paralleled with increased gas production (GP). Many studies suggested that *S. cerevisiae* might stimulate the acetogens to compete or co-metabolise hydrogen with methanogens, thereby reducing CH_4 emissions (Hristov *et al*., 2013). However, others reported increased CH_4 production (Martin *et al*., 1989; Martin and Nisbet, 1990), while Mathieu *et al*. (1996) reported no effects. These conflicting results on CH_4 production are likely due to strain difference of *S. cerevisiae* and type of diets (Patra, 2012). Supplementing diets with *S. cerevisiae* were shown to increase total volatile fatty acids (VFA) and propionic acid production (Mao *et al*., 2013). *S. cerevisiae* can enhance fungal colonisation of plant cell walls leading for increased DM and NDF digestion (Patra, 2012), increased initial rate of fibre digestion (Williams *et al*., 1991), improved *in situ* crude protein (CP) and NDF degradation.

Many methods can be used to *S. cerevisiae* administration. Most products contain a mixture of varying proportions of live and dead *S. cerevisiae* cells. Those with a predominance of live cells are sold as live yeasts, while others containing more dead cells and the growth medium are sold as *S. cerevisiae*. Hence, the method of *S. cerevisiae* administration may affect the potential response. The response depends on number of live or metabolically active yeast cells have shown to have a greater effect in stimulating rumen fermentation (Dawson *et al*., 1990). Direct-fed microbial are often recommended in various European countries than other administration methods (Doreau and Jouany, 1998).

The aim of this study was to determine effects of increasing doses of the yeast (*Saccharomyces cerevisiae*) in two methods of applications (direct or 72 h of pre-incubation) on *in vitro* GP, degradability and some ruminal fermentation parameters of the fibrous feedstuffs of corn stover, oat straw, sugarcane bagasse, and sorghum straw.

Materials and methods

Fibrous feed species and yeast product levels

Three individual samples of each of the fibrous feeds corn stover, oat straw, sugarcane bagasse, and sorghum straw were randomly and manually harvested in triplicate from different sites in the State of Mexico. Samples were dried at 60°C for 48 h in a forced air oven

to constant weight, ground in a Wiley mill to pass a 1 mm sieve and stored in plastic bags for subsequent determination of chemical components and *in vitro* GP. Four levels of commercial yeast product (*Saccharomyces cerevisiae* I-1077, LEVUCELL® SC20; Lalleland, Montréal, QC, Canada) contain 1×10^{10} per gram yeast product. Doses of yeast were (/g DM): control (0 mg), low (4 mg), medium (8 mg) and high (12 mg). Feed samples were incubated with yeast doses that were added into the bottles immediately before incubation (direct method) or 72 h pre-incubated at room temperature. Stock solution of each yeast product doses was prepared before treatments in distilled water in order to get the suitable doses in each 1 mL of the stock solution.

In vitro incubations

Rumen inoculum was collected from two Brown Swiss cows (400 to 450 kg body weight) fitted with permanent rumen cannula. Cows were fed *ad libitum* a total mixed ration made up of 50:50 commercial concentrate (PURINA®, St. Louis, MO, USA) containing (g/kg) 147.3 CP, 160.4 NDF, 27.7 acid detergent fibre (ADF). Alfalfa hay was formulated to meet all of their nutrient requirements (National Research Council, 2001). Fresh water was available to cows at all times during the rumen inoculum collection phase.

Ruminal contents from each cow was obtained before the morning feeding, flushed with CO_2, then mixed and strained through four layers of cheesecloth into a flask with O_2 free headspace. Samples (1 g) of each feed were weighed into 120 mL serum bottles with appropriate addition of *S. cerevisiae* doses/g DM. Consequently, 10 mL of particle free ruminal fluid was added to each bottle followed by 40 mL of the buffer solution according to Goering and Van Soest (1970), with no trypticase added, in a 1:4 (v/v) proportion.

During incubations, it was used 4 feedstuffs of 3 individual samples of each with the 4 doses of *S. cerevisiae* in 2 application methods (direct addition or 72 h pre-treatments) of *S. cerevisiae* and 4 bottles (replicates) were used for each incubated sample during 3 runs of incubation. Once all bottles were filled, they were immediately closed with rubber stoppers, shaken and placed in the incubator at 39°C. The volume of gas produced was recorded at times of 2, 4, 6, 8, 10, 12, 14, 24, 30, 48, 54 and 72 h of incubation using the pressure reading technique (Extech instruments, Waltham, MA, USA) of Theodorou *et al*. (1994). At the end of incubation (*i.e*. 72 h), bottles were uncapped, pH was measured using a pH meter (Conductronic pH15; Conductronic, Puebla, Mexico) and the contents of each bottle were filtered to obtain the non-fermented residue for determination of degraded substrate. After recording the final gas volume (*i.e*., 72 h), 2 mL of NaOH (10 M) were added to each bottles and gas pressure was determined immediately. Mixing of the contents with NaOH allowed absorption of CO_2, with the gas volume remaining in the head space of bottles considered to be CH_4 (Demeyer *et al*., 1988).

Degradability and sample analysis

At the end of incubation (*i.e*. 72 h), the contents of each serum bottle were filtered under vacuum through glass crucibles with a sintered filter (coarse porosity no. 1, pore size 100 to 160 µm; Pyrex, Stone, UK). Fermentation residues were dried at 105°C overnight to estimate DM disappearance with loss in weight after drying being the measure of undegradable DM. The NDF and ADF were calculated in the residues after DM degradability (DMD) determinations for establishing NDF and ADF degradability. Neutral detergent fibre was assayed without use of an alpha amylase but with sodium sulfite in the NDF. Both NDF and ADF are expressed without residual ash. Neutral detergent fibre and ADF were also determined in the residues samples after incubations for NDF and ADF degradability. Samples of the feeds were analysed for DM (#934.01), ash (#942.05), nitrogen (#954.01) and ether extract (#920.39) according to AOAC (1997). The NDF (Van Soest *et al*., 1991), ADF, and lignin (AOAC, 1997; #973.18) analyses used an ANKOM200 Fibre Analyser Unit (ANKOM Technology Corp., Macedon, NY, USA).

Calculations and statistical analyses

All the calculations were mentioned and descripted before in Salem (2012) as in the following.

Kinetic parameters of GP were estimated (mL/g DM) by fitted data in the NLIN option of SAS (2002) according to France *et al*. (2000) 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 gas production.

Metabolisable energy (ME; MJ/kg DM) and *in vitro* organic matter digestibility (OMD; g/kg OM) were estimated according to Menke *et al*. (1979) as:

ME=2.20+0.136 GP (mL/0.5 g DM)+0.057 CP (g/kg DM) OMD=148.8+8.89 GP+4.5 CP (g/kg DM)+0.651 ash (g/kg DM)

where GP is net GP in mL from 200 mg of dry sample after 24 h of incubation.

The partitioning factor at 24 h of incubation (PF_{24}; a measure of fermentation efficiency) was calculated as the ratio of DMD *in vitro* (mg) to the volume (mL) of GP at 72 h (*i.e*., DMD/total gas production (GP_{96}) according to Blümmel *et al*. (1997). Gas yield (GY_{24}) was calculated as the volume of gas (mL gas/g DM) produced after 24 h of incubation divided by the amount of DMD (g) as:

Gas yield (GY_{24})=mL gas/g DM/g DMD

Short chain fatty acid (SCFA) concentrations were calculated according to Getachew *et al*. (2002) as:

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

where GP is the 24 h net gas production (mL/200 mg DM).

The experimental design for the *in vitro* ruminal GP, degradability and fermentation parameters analysis was a completely random design considering, as fixed factors, type of forage (S) and *S. cerevisiae* level (C) in the linear model (Steel *et al*., 1997) within each method (M) of application (direct or pre-incubation). Data of each of the three runs within the same sample were averaged prior to statistical analysis. Mean values of each individual sample within each species (three samples of each) were used as the experimental unit. The statistical model was:

$$Y_{ijklm}=\mu+ S_j+C_k+M_l+(S\times C)_{jk}+(S\times M)_{jl}+(M\times C)_{lk}+(S\times M\times C)_{jkl}+E_{ijklm}$$

where Y_{ijk}=is every observation of the i^{th} fibrous species (S_i) when incubated in the j^{th} yeast (C_j; *S. cerevisiae*); µ is the general mean; S_i (i=1-4) is the feed effect; C_j is the yeast dose effect (j=1-4); M_j is the application method (j=1-2), $(S*C)_{ij}$ is the interaction between feed and yeast dose; $(S*M)_{jl}$ is the interaction between feed and application methods; $(S*M*C)_{jkl}$ is the interaction between the three variable study (feed, yeast and application method); and E_{ijklm} is experimental error. Linear and quadratic polynomial contrasts were used to examine responses of feeds to increasing addition levels of the *S. cerevisiae*.

Results

The chemical composition varied between

different species. Corn stover had higher (P<0.05) CP content with low (P<0.05) content of ADF and NDF fractions. Oat and sorghum straws had the highest (P<0.05) ADF and NDF fractions contents with low (P<0.05) content of CP (Table 1).

Fibrous species×method of application×*S. cerevisiae* interaction occurred (P<0.0001) for *b*, *c*, *L*, GP, CH_4 production, ME, SCFA, GY_{24} and PF_{24} (Tables 2 and 3).

Effect of fibrous species

Gas production parameters were varied (P<0.001) between different fibrous species. Corn stover and oat straw improved the asymptotic gas production with increasing of the initial delay before GP beginning followed by sorghum straw and sugarcane baggas which had the rate of GP increased. During the first 14 h of incubation, sugarcane baggas improved (P<0.001) *in vitro* GP followed by corn stover where oat straw had the lowest (P<0.001). After 14 h of incubation, corn stover and sugarcane bagass improved (P<0.001) *in vitro* GP followed by oat straw, while sorghum straw had the lowest (P<0.001). After 24 h of incubation only corn stover showed a gas production higher (P<0.001) than oat and sorghum straw (P<0.001), while sugarcane baggas did not improve gas production compared to the straws (Table 2). Oat straw, sugarcane baggas, and sorghum straw had both ruminal pH (P=0.006) and GY_{24} (P<0.001) increased with lowering (P<0.001) PF_{24} value compared to corn stover. Corn stover had DMD, OMD, ME, SCFA, and PF_{24} values improved (P<0.001). On the contrary, sorghum straw had the highest (P<0.001) values of CH_4, NDFD, and ADFD compared to the other feeds. Sugarcane baggas had the lowest values (P<0.001) of CH_4, NDFD, OMD, and ME, where oat straw had the lowest (P<0.001) SCFA production compared to other feeds (Table 3).

Effect of application methods

Direct addition of *S. cerevisiae* improved (P<0.001) the rate of GP with lowering (P<0.001) the initial delay before GP beginning. The *in vitro* GP was also improved (P<0.001) compared to the 72 h pre-incubation method (Table 2).

The direct addition of *S. cerevisiae* also improved CH_4 (P=0.047), DMD (P=0.005), NDFD (P=0.005), ADFD (P=0.020), (P<0.001)

Table 1. Chemical composition of the four fibrous feeds.

Species	OM, g/kg DM	CP, g/kg DM	ADF, g/kg DM	NDF, g/kg DM
Corn stover	959.7	62.9^{a}	274.4^{c}	476.7^{b}
Oat straw	923.6	37.2^{b}	380.0^{a}	537.8^{a}
Sugarcane bagasse	982.0	25.7^{c}	324.4^{b}	458.9^{b}
Sorghum straw	944.3	40.0^{b}	377.8^{a}	556.7^{a}
SEM	42.3	9.3	28.1	37.1

OM, organic matter; DM, dry matter; CP, crude protein; ADF, acid detergent fibre; NDF, neutral detergent fibre. $^{a-c}$Different superscripts following means in the same row indicate differences at P<0.05.

Table 2. *In vitro* rumen gas kinetics of four low quality roughages as affected by the direct addition or 72 h pre-incubation with different levels of *Saccharomyces cerevisiae*.

	GP parameters			*In vitro* GP, mL/g DM										
	b, mL/g DM	c, /h	L, /h	Gas2	Gas4	Gas6	Gas8	Gas10	Gas12	Gas14	Gas24	Gas30	Gas48	Gas54	Gas72
Effect of S															
Corn stover	329.5^{a}	0.029^{c}	0.74^{b}	18.2^{b}	35.4^{b}	51.6^{b}	66.9^{b}	81.3^{b}	94.9^{b}	107.8^{b}	162.0^{a}	187.9^{a}	243.4^{a}	256.5^{a}	284.8^{a}
Oat straw	273.9^{b}	0.029^{c}	1.14^{a}	14.5^{c}	28.2^{c}	41.0^{c}	53.1^{d}	64.5^{d}	75.2^{d}	85.4^{d}	128.1^{c}	148.7^{b}	193.8^{b}	204.7^{b}	229.1^{b}
Sugarcane baggas	159.0^{d}	0.099^{a}	0.36^{c}	28.4^{a}	51.1^{a}	69.4^{a}	84.2^{a}	96.3^{a}	106.2^{a}	114.4^{a}	138.8^{b}	146.1^{b}	155.4^{c}	156.6^{c}	158.3^{c}
Sorghum straw	257.4^{c}	0.041^{b}	0.45^{c}	17.5^{b}	33.4^{b}	48.1^{b}	61.6^{c}	74.0^{c}	85.4^{c}	96.0^{c}	138.1^{b}	156.9^{c}	195.4^{b}	204.2^{b}	223.2^{b}
LSD	15.27	0.0064	0.153	1.56	2.71	3.54	4.16	4.62	4.97	5.25	6.11	6.54	8.02	8.53	9.99
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Effect of application M															
Direct	256.1	0.056^{a}	0.51^{b}	22.3^{a}	41.6^{a}	58.4^{a}	73.3^{a}	86.6^{a}	98.4^{a}	109.2^{a}	150.3^{a}	168.1^{a}	203.8^{a}	211.8^{a}	228.8^{a}
Pre- incubation	253.8	0.043^{b}	0.84^{a}	17.0^{b}	32.5^{b}	46.7^{b}	59.6^{b}	71.5^{b}	82.5^{b}	92.6^{b}	133.2^{b}	151.7^{b}	190.2^{b}	199.2^{b}	218.8^{b}
LSD	8.19	0.0034	0.082	0.84	1.45	1.90	2.23	2.48	2.67	2.82	3.28	3.51	4.30	4.58	5.36
P	0.5895	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Effect of Y, mg/g DM															
0	243.1^{b}	0.041^{b}	0.55^{b}	16.5^{c}	31.7^{c}	45.7^{c}	58.5^{c}	70.3^{c}	81.2^{c}	91.2^{c}	131.5^{c}	149.6^{c}	186.3^{c}	194.7^{c}	212.6^{c}
4	259.4^{a}	0.045^{b}	0.66^{ab}	19.0^{b}	36.1^{b}	51.6^{b}	65.8^{b}	78.6^{b}	90.4^{b}	101.2^{b}	143.7^{ab}	162.4^{ab}	200.4^{ab}	209.0^{ab}	227.5^{ab}
8	251.9^{ab}	0.056^{a}	0.74^{a}	21.0^{a}	39.2^{a}	55.1^{ab}	69.2^{ab}	81.6^{ab}	92.8^{ab}	103.0^{ab}	142.1^{b}	159.3^{b}	194.8^{b}	203.1^{bc}	221.1^{bc}
12	265.4^{a}	0.056^{a}	0.75^{a}	22.1^{a}	41.1^{a}	57.7^{a}	72.4^{a}	85.6^{a}	97.4^{a}	108.1^{a}	149.8^{a}	168.3^{a}	206.4^{a}	215.2^{a}	234.1^{a}
LSD	15.27	0.0064	0.153	1.56	2.71	3.54	4.16	4.62	4.97	5.25	6.11	6.54	8.02	8.53	9.99
Linear	0.0065	0.1165	0.076	0.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0002
Quadratic	0.006	<0.0001	0.0066	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Interactions															
S×M	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0024
S×Y	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
M×Y	0.0989	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
S×M×Y	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0002

GP, gas production; DM, dry matter; b, asymptotic gas production; c, rate of gas production; L, initial delay before gas production begins; LSD, least significant difference; S, species; M, method; Y, yeast product. $^{a-d}$Different superscripts following means in the same row indicate differences at P<0.05.

OMD, SCFA, and GY_{24}, with lowering ($P<0.001$) PF_{24} compared to the 72 h pre-incubation method. The method of application did not affect ($P>0.05$) both of the asymptotic gas production and ruminal pH (Table 3).

Effect of yeast doses

Both low and high doses of *S. cerevisiae* improved the asymptotic GP, with increasing *S. cerevisiae* doses, the rate of GP (linear, P=0.007; quadratic, P=0.006), the initial delay before GP beginning (quadratic, $P<0.001$), and *in vitro* GP (linear, quadratic, $P\leq0.001$) during the period before the first 24 h. After 24 h and up to 72 h of incubation, the highest dose of *S. cerevisiae* had the *in vitro* GP increased (linear, quadratic, $P<0.001$) where the lowest dose had the lowest *in vitro* GP compared to other doses (Table 2).

The lowest dose of *S. cerevisiae* improved DMD (linear, P=0.005; quadratic, $P<0.001$), and PF_{24} (linear, $P<0.001$; quadratic, P=0.011) with lowering (linear, quadratic, $P<0.001$) ME, OMD, SCFA, and (linear, $P<0.001$; quadratic, P=0.005) GY_{24} compared to the other doses. In contrary, the highest dose of *S. cerevisiae* had the highest values of DMD, ME, OMD, and SCFA (Table 3). No effects ($P>0.05$) of *S. cerevisiae* doses on ruminal pH, CH_4, and ADFD; however, addition of yeast caused a lowered (linear, P=0.005) values for NDFD (Table 3).

Discussion

Chemical composition

The chemical composition varied between the four fibrous feeds used in our study. These variations arise from variation in the genotype of the crops, differences between production environments, and from the interaction between environment and genotypes (Welch, 1995; Denčić *et al*., 2011). Environmental differences will include variation in climate, the soil and agronomic practice, together with variations raised from different harvesting conditions, and post harvesting treatments (Welch, 1995; Elghandour *et al*. 2013). There is usually an inverse relationship between the CP and crude fibre content in a given forage species, and this has been revalidated in this study.

Gas production, rumen fermentation and degradability

The responses to *S. cerevisiae* are fibrous species type, forage composition, application methods and dose-dependent in addition to interactions among yeast and diet (Patra, 2012). Gas production from different fibrous species depends on its chemical composition. In our study, during the first 24 h of incubation, sugarcane bagasse and corn stover produced more GP than sorghum and oat straw compared to the period after 24 h up to 72 where they produced more gases. The production of gases from tested roughages depends on portentous and fibrous contents of feeds (Paya *et al*., 2007). Higher GP during the first period of fermentation of both sugarcane bagasse and corn stover refers to high content of highly fermentable constituents than sorghum and oat straw. Conversely, the fermentation process of sorghum and oat straw refers to their content of low fermentable constituents. Gas production depends on nutrient availability for rumen microorganisms (Mahala and Fadel Elseed, 2007). Fermenta-

Table 3. *In vitro* rumen fermentation profile of four low quality roughages as affected by the direct addition or 72 h pre-incubation with different levels of *Saccharomyces cerevisiae*.

	pH	CH_4, mL/g DM	DMD, mg/g DM	NDFD, mg/g DM	ADFD mg/g DM	ME, MJ/kg DM	OMD, g/kg DM	SCFA, mmol/g DM	PF_{24}, mg DMD:mL gas	GY_{24}, mL gas/g DMD
Effect of S										
Corn stover	6.78^{b}	18.7^{b}	326.1^{a}	256.7^{c}	264.6^{c}	6.84^{a}	459^{a}	3.58^{a}	2.03^{a}	497.4^{b}
Oat straw	6.98^{a}	17.1^{b}	285.0^{b}	276.6^{b}	363.0^{a}	6.12^{bc}	413^{bc}	2.82^{c}	2.24^{b}	450.1^{a}
Sugarcane baggas	6.97^{a}	6.8^{c}	272.4^{c}	217.2^{d}	310.1^{b}	6.08^{c}	405^{c}	3.06^{b}	2.00^{b}	511.4^{a}
Sorghum straw	7.00^{a}	25.2^{a}	270.9^{c}	291.1^{a}	360.7^{a}	6.27^{b}	422^{b}	3.04^{b}	1.99^{b}	517.1^{a}
LSD	0.153	2.12	4.69	7.00	4.78	0.166	10.9	0.136	0.117	24.42
P	0.0006	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Effect of application M										
Direct	6.91	17.5^{a}	290.4^{a}	263.2^{a}	326.1	6.56^{a}	440^{a}	3.32^{a}	1.96^{b}	522.1^{a}
Pre-incubation	6.96	16.4^{b}	286.7^{b}	257.7^{b}	323.1	6.09^{b}	410^{b}	2.94^{b}	2.18^{a}	465.9^{b}
SEM	0.082	1.14	2.52	3.75	2.60	0.089	5.83	0.073	0.063	13.11
P	0.267	0.0467	0.0046	0.0045	0.0204	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Effect of Y product, mg/g DM										
0	6.92	17.6	290.4^{a}	263.3^{a}	325.4	6.05^{c}	407^{c}	2.90^{c}	2.23^{a}	454.5^{b}
4	6.97	17.0	283.9^{b}	255.4^{b}	325.3	6.38^{ab}	428^{ab}	3.17^{ab}	2.00^{b}	513.9^{a}
8	6.92	17.0	285.3^{b}	261.4^{ab}	323.7	6.34^{b}	426^{b}	3.13^{b}	2.03^{b}	500.0^{a}
12	6.93	16.3	294.8^{a}	261.6^{ab}	324.0	6.55^{a}	439^{a}	3.30^{a}	2.01^{b}	507.7^{a}
LSD	0.153	2.12	4.69	6.70	4.78	0.166	10.9	0.136	0.117	24.42
Linear	0.3417	0.4311	0.0005	0.0045	0.9362	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Quadratic	0.7076	0.1493	<0.0001	0.3303	0.3932	<0.0001	<0.0001	<0.0001	0.011	0.0046
Interactions										
S×M	0.1869	0.2509	<0.0001	<0.0001	0.6104	<0.0001	<0.0001	<0.0001	0.0011	<0.0001
S×Y	0.8837	<0.0001	<0.0001	<0.0001	0.0005	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
M×Y	0.8066	0.4184	0.1636	0.0001	0.4479	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
S×M×Y	0.9481	<0.0001	<0.0001	<0.0001	0.0152	<0.0001	<0.0001	<0.0001	0.0032	<0.0001

CH_4, methane emission; DM, dry matter; DMD, dry matter degraded substrate; NDFD, neutral detergent fibre degradability; ADFD, acid detergent fibre; ME, metabolisable energy; OMD, organic matter digestibility; SCFA, short chain fatty acids; PF24, partitioning factor at 24 h of incubation; GY_{24}, gas yield at 24 h; S, species; LSD, least significant difference; M, method; Y, yeast product. $^{a-d}$Different superscripts following means in the same row indicate differences at $P<0.05$.

tion of dietary carbohydrates to acetate, propionate and butyrate produces gases in the rumen which are mainly composed of hydrogen, carbon dioxide and methane. However, fermentability of protein produces relatively small GP compared to carbohydrate fermentation (Makkar *et al*., 1995). This can explain how *S. cerevisiae* addition could improve GP at the time it reduced NDFD. All depends on the chemical composition of fermented feeds. Availability of nutrients for rumen microorganisms will stimulate the degradability of different nutrients (Paya *et al*., 2007). It is very important to stress that not only has the addition of *S. cerevisiae* the ability to improve GP, but it can also make qualitative changes in GP thereby reducing its negative effect on the environment. *S. cerevisiae* has the ability to decrease methane and ammonia production, and to improve fermentation efficiency to contribute to a reduction in greenhouse gas emissions (Hristov *et al*., 2013). Moreover, decreasing protein degradation and ammonia production in the rumen (Mao *et al*., 2013) has the ability to decrease the overall nitrogen excretion by the animal, which would contribute to decreased ammonia emissions from cattle manure.

There are a few data in the literature regarding the effect of *S. cerevisiae* method of application on *in vitro* gas kinetics and fermentation profile. It has been found that the method of the *S. cerevisiae* product application depends on number of live or metabolically active *S. cerevisiae* that will stimulate rumen fermentation (Dawson *et al*., 1990). Direct application of *S. cerevisiae* ensures the viability of *S. cerevisiae* cells so an improvement occur for *in vitro* GP and fermentation kinetics and profile compared with pre-incubation method. Pre-incubation of *S. cerevisiae* with different fibrous feeds may negatively affect the fermentation process which reflected on low fermentability for all fibrous species. Doreau and Jouany (1998) stated that direct-fed microbial are often recommended in various European countries than other administration methods. Elam *et al*. (2003) hypothesised that the initial advantages of direct-fed microbial involve a favourable alteration of the gastrointestinal micro-flora and that over time that innate immunological mechanisms of control animals provide this same function.

Improved GP with increasing *S. cerevisiae* doses reflects the enhanced ruminal environment. Paulus *et al*. (2012) and Mao *et al*. (2013) documented the positive effects of *S. cerevisiae* on ruminal fermentation and microbial activities. A number of specific hypothetical biochemical mechanisms have been developed to explain the stimulatory effects of *S. cerevisiae* in the rumen (Chevaux and Fabre, 2007). Some of these mechanisms have been based on the ability of yeast to provide important nutrients or nutritional cofactors that stimulate microbial activities (Callaway and Martin, 1997). Another suggested the ability of *S. cerevisiae* to scavenge excess oxygen creating a more optimal environment for rumen anaerobic bacteria (Newbold *et al*., 1996; Jouany, 2001). Others studies suggested that *S. cerevisiae* supplementation could provide vitamins such as biotin and thiamine, which are reported to be required for microbial growth and activity (Akin and Borneman, 1990). In addition, others suggested that *S. cerevisiae* can provide a focal point for the development of a stable microbial consortium (Jouany, 2001). In this model, the *S. cerevisiae* cells provide a site for metabolic exchanges and an environment that promotes the growth of beneficial microorganisms around substrates.

One possible explanation for the varied response with a different level of *S. cerevisiae* in this study is at least partially due to the nature of the *in vitro* procedure. For the *in vitro* model, the substrate amount relative to the rumen liquid volume is much less than in the rumen of a cow (<1 *vs* 12%). Therefore, when a rumen modulator like *S. cerevisiae* is supplemented at a different rate, it could change the fermentation rate and cause different substrate depletion, resulting in different response as the fermentation length is changed (Mao *et al*., 2013). Lila *et al*. (2004) found variable effects of *S. cerevisiae* on ruminal fermentation when different substrates were used *in vitro*.

Decreased lag time with *S. cerevisiae* addition can be illustrated based on two basic mechanisms. The first mode of yeast action reported by Newbold *et al*. (1996) is the respiratory activity that scavenges O_2, which is toxic to anaerobic bacteria and causes inhibition of adhesion of cellulolytic bacteria to cellulose, and this peak in O_2 concentration occurs at approximately the time of feeding (initial time). The second mode is that *S. cerevisiae* contains small peptides and other nutrients that required to predominant ruminal cellulolytic bacteria to initiate growth (Callaway and Martin, 1997).

Addition of *S. cerevisiae* increased SCFA production on forage substrates (Mao *et al*., 2013). Increased SCFA production and ME are associated with high activities of microbes in the rumen. *S. cerevisiae* produces growth factors for microbial growth that can stimulate rumen microbial growth and activity (Chiquette, 2009). In addition to the ability of *S. cerevisiae* to provide conducive conditions to microbial growth in a way that is capable of using O_2 in the rumen so that the conditions of an aerobic rumen awake (Mosoni *et al*., 2007). *S. cerevisiae*. Newbold *et al*. (1996), for example, used this mode of action to explain a 35% increase in total bacterial counts with *S. cerevisiae in vitro*.

Addition of *S. cerevisiae* lowered PF_{24} values. A lower PF_{24} would reflect lower conversion of degraded substrate into microbial biomass and vice versa (Harikrishna *et al*., 2012). Ruminal pH was not changed during fermentation processes. Several studies have suggested that *S. cerevisiae* moderate the ruminal pH by increasing lactate utilisation making pH relatively more stable and meet the needs of rumen microbes to perform its activity (Paulus *et al*., 2012).

Conclusions

The responses to supplemental *S. cerevisiae* varied among the fibrous species tested, the results of this study suggest that the addition of *S. cerevisiae* can support ruminal fermentation of low-quality forages. In general, *S. cerevisiae* added at 4 to 12 g/kg DM showed the greatest responses in most variables tested.

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