Effects of types and doses of yeast on gas production and *in vitro* digestibility of diets containing maize *(Zea mays)* and lucerne *(Medicago sativa)* or oat hay

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Abstract

Two yeast products formulated with *Saccharomyces cerevisiae* were evaluated at the same colony-forming units (CFUs) per gram of substrate. Samples of maize, lucerne and oat hays were mixed (0.5 kg) to a proportion of 80% forage (lucerne or oat) with 20% maize (DM basis) and combined with each yeast to obtain 1.5 x 10^7 or 3.0 x 10^7 CFU/g DM. There was also a control without yeast. *In vitro* gas production was measured at 0, 2, 4, 6, 8, 10, 14, 18, 24, 30, 36, 42, 48, 60, and 72 h incubation. There was no forage/yeast interaction. Both yeast products tended to reduce the maximum volume produced quadratically and lag time linearly, while *in vitro* dry matter digestibility (IVDMD) increased linearly. Ruminal ammonia N and lactic acid were not affected, whereas methane and carbon dioxide tended to be reduced with the intermediate dose of yeast. When the mixture included oat hay, the total volume of gas increased, the lag time decreased, and there was higher IVDMD than in the lucerne-based mixtures, which were associated with lower methane production. Ammonia and lactic acid remained unchanged. The two yeast products showed the same effects on the dynamics of gas production and *in vitro* digestibility when dosed at the same number of viable cells or CFUs, and there was no interaction with forage quality.

Keywords: forages, ruminal fermentation *in vitro*, *Saccharomyces cerevisiae*
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Introduction

Yeast products for ruminants based on *Saccharomyces cerevisiae* increase the number of cellulolytic bacteria (Wallace & Newbold, 1993; Alzahal *et al*., 2014), and are associated with a higher rumen pH promoted by the yeast, which favours the growth of fibrolytic bacteria *(Fibrobacter and Ruminococcus)* and lactate-utilising bacteria (*Megasphaera* and *Selenomonas*; Pinloche *et al*., 2013). They have thus been regarded as rumen pH stabilisers (Chaucheyras-Durand *et al*., 2008; Desnoyers *et al*., 2009). In most *in vivo* evaluations of commercial products that contain *Saccharomyces cerevisiae*, researchers confirmed that the amounts of live cells were described by the commercial manufactures (Crosby *et al*., 2004; Pinloche *et al*., 2013; Ahmed *et al*., 2015; Pienaar *et al*., 2012).

In a few experiments, the colony-forming units (CFUs) were corroborated (Bitencourt *et al*., 2011; Vyas *et al*., 2014; Emmanuel *et al*., 2007). In contrast, data from Arcos-García *et al*. (2000) showed that the CFU value determined in the laboratory differed from that reported on the yeast product packaging. Opsi *et al*. (2012) demonstrated that live yeast affects ruminal fermentation slightly more than inactivated yeast.

Several studies have been conducted to evaluate neutral detergent fibre (NDF) levels with yeast (Plata *et al*., 1994; Miranda *et al*., 1996; Wang *et al*., 2001), but information that compares forage sources is scarce. Roa *et al*. (1997) compared lucerne and coffee hull and cornstalk with or without *Saccharomyces cerevisiae* on *in situ* digestion and rumen fermentation, and did not find forage/yeast interactions with differences among forages. However, a legume and a lignocellulosic residue differ greatly in nutritional value and the response to yeast addition in digestibility can be different. Therefore, the objective of this study was to evaluate the effects of two commercial yeast products on *in vitro* fermentation kinetic parameters, as

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determined by gas production, of lucerne- and oat-based diets, dosed at the same CFU levels of *Saccharomyces cerevisiae*.

Materials and Methods

The products that were evaluated were Procreatin 7 (7.53 x 10^9 CFU/g) and Biosaf SC 47 (1.18 x 10^9 CFU/g) (Safmex S.A. de C.V Mexico), both of which are formulated with *Saccharomyces cerevisiae.* They were dosed at the same CFUs per gram of substrate, based on the viable yeast concentration determined in the laboratory (Camacho *et al*., 2009).

Composite representative samples (n = 3) of maize, lucerne and oat hay were obtained from the experimental dairy farm at the University of Chapingo, oven dried at 55 °C, and ground to 1 mm. After this, 0.5 kg each of forage and maize grain were mixed in a proportion of 80% forage (lucerne or oat) with 20% maize (DM basis), and combined with each yeast product to obtain 1.5 x 10^7 or 3.0 x 10^7 CFU/g DM. There was also a control without yeast. The treatments were allotted in a completely randomized design with a 2 x 3 factorial arrangement, in which the factors were forage source (lucerne and oat) and yeast product (Procreatin 7 and Biosaf SC 47), evaluated at three concentrations (0.0, 1.5 x 10^7 and 3.0 x 10^7 CFU/g). Forage and maize samples were analysed for dry matter (DM), organic matter (OM) and ether extract (EE), according to AOAC (1990), and neutral detergent fibre (NDF) and acid detergent fibre (ADF), according to Van Soest *et al*. (1991). Starch in the maize was measured enzymatically from the glucose that was released, as described by MacRae & Armstrong (1968) and modified by Wester *et al*. (1992). The compositions of the forages and maize are shown in Table 1.

Table 1 Chemical compositions of feeds on a dry matter basis used in the experiment (n = 3)

	Lucerne	Oat	Maize
Dry matter %	23.59	19.40	91.8011
Ash %	8.60	8.04	1.12
Crude protein %	18.22	10.51	8.80
Neutral detergent fibre %	46.00	47.67	33.30
Acid detergent fibre %	36.83	23.00	3.68
Ether extract %	3.93	3.51	4.86
Starch %	-	-	77.79

Amber flasks (100 ml) were prepared with 500 mg DM from each treatment, with four tubes per treatment. The inoculums consisted of rumen liquor obtained as described by Mendoza-Martínez *et al*. (2015) using an oesophageal probe from two sheep (34 ± 1.6 kg bodyweight) fed a 50:50 forage : concentrate ratio. The inoculum was obtained before the morning feeding, and was mixed and strained through eight layers of cheesecloth into a flask flushed with carbon dioxide (CO_2). Then 10 ml strained ruminal fluid was added to each bottle, and 80 ml of the buffer solution described by Goering & Van Soest (1970) was added under a continuous flow of carbon dioxide to maintain anaerobic conditions. Each flask was closed tightly with a rubber stopper and aluminium crimp. The flasks were incubated in a water bath at 38 ºC. Gas pressure was measured with a pressure gauge (Metron, Mode: 63100, Mexico) at 0, 2, 4, 6, 8, 10, 14, 18, 24, 30, 36, 42, 48, 60 and 72 h of incubation (Blümmel & Lebzien, 2001). Head space pressure values were transformed to gas volumes by a linear regression equation:

$$V= (P+0.0186) (0.0237)^{-1}$$

At each time fraction, three parameters of the kinetics of gas production were estimated: lag phase (*h*); maximal volume (Vm; mL g^{-1} DM of substrate); and rate (S; h^{-1}) of gas production, using the model proposed by Menke & Steingass (1988), Vo= Vm /(1+e(2-4*s*(t-L))). At the end of incubation, the residuals from each bottle were filtered using a flask Buchner with a sintered filter (filter paper F/ fast MOD.617 Code P.V.NO.1034) to estimate DM digestibility. The fermentation residues were dried at 65 °C overnight before being weighed.

Lactate and N-NH_3 were determined by spectrophotometry using samples of residual fluid collected at 36 h incubation. Fluid samples were centrifuged (25,200 x g for 10 min) and subsamples of supernatant were used to analyse lactate (Taylor, 1996) and ammonia N (McCullough, 1967).

The results were analysed according to a completely randomized design in which treatments were regarded as fixed effects, testing linear and quadratic effects for grain level (Steel *et al*. 1997). Means were compared using a Tukey's test. Differences among treatments were declared at P <0.05 and a tendency at P <0.10. Data were analysed with JMP7 software (Sall *et al*. 2012).

Results

There was no forage/yeast interaction. Therefore, the main effects of yeast and forage are presented separately in Tables 2 and 3. Both yeast products tended to reduce the maximum volume of gas produced quadratically, while Procreatin 7 reduced lag time linearly, but they increased *in vitro* DM digestibility linearly (P <0.001). Ruminal ammonia N and lactic acid were not affected, whereas methane tended to be reduced (P <0.11) at the intermediate dose of yeast for Procreatin 7, while the carbon dioxide (CO_2) was increased (P <0.05). Biosaf had no effect on either gas (Table 2). In Table 3 shows that oats increased the total volume of gas produced, decreased lag time, and increased *in vitro* digestibility (P <0.0001), compared with the lucerne

Table 2 Main effects of two commercial products dosed at two colony forming unit levels of *Saccharomyces cerevisiae* on *in vitro* gas production parameters, digestibility and fermentation gases incubated with maize and forages (20 : 80)

	Control	Biosaf SC 47		*P*-value		Procreatin 7		*P*-value		
	CFU/g DM x 10^7					CFU/g DM x 10^7				
	0	1.5	3.0	L	Q	1.5	3.0	L	Q	SEM
Vmax ml	415	384	398	0.22	0.07	384	399	0.26	0.07	17.0
S h^{-1}	0.034	0.035	0.035	0.41	0.57	0.035	0.034	0.77	0.20	0.0007
Lag h	2.23	2.06	2.11	0.42	0.36	2.03	1.98	0.09	0.57	0.750
IVDMD %	61.65	70.05	70.23	0.0003	0.02	70.27	71.27	0.0001	0.03	2.629
CH_4 ml	29.83	35.00	32.33	0.59	0.34	26.00	35.33	0.25	0.11	4.368
CO_2 ml	42.33	40.08	42.83	0.92	0.57	48.92	38.00	0.40	0.05	3.724
N-NH_3 mg h^{-1}	6.66	7.34	7.47	0.50	0.79	6.46	7.99	0.27	0.41	1.014
Lactic acid µg^{-1}	15.41	19.62	25.44	0.16	0.89	20.40	24.66	0.19	0.95	4.768

CFUs: colony forming units; DM: dry matter; L: linear; Q: quadratic; SEM: standard error of the mean; VMax: maximum volume, S: rate of gas production, Lag: lag time, and IVDMD: *in vitro* dry matter digestibility

Table 3 Main effects of forage source incubated with maize and commercial yeast products on *in vitro* gas production parameters, digestibility and fermentation gases

	Lucerne	Oat	SEM	*P*-value
Vmax ml	363^b	429^a	6.0	0.0001
S h^{-1}	0.035	0.034	0.0004	0.31
Lag h	3.74^a	0.42^b	0.065	0.0001
IVDMD %	63.62^b	73.76^a	0.887	0.0001
CH_4 ml	37.86^a	25.53^b	2.458	0.03
CO_2 ml	45.50	39.37	2.540	0.49
N-NH_3 mg dl^{-1}	8.34^a	6.03^b	0.604	0.16
Lactic acid µg ml^{-1}	18.87	23.35	3.036	0.30

SEM: standard error of the mean; VMax: maximum volume, S: rate of gas production, Lag: lag time, and IVDMD: *in vitro* dry matter digestibility

-based mixtures. It is postulated that these effects could be associated with lower methane production (P <0.05) for oats, as ammonia and lactic acid remained unchanged.

Discussion

The results from this experiment indicated that the use of *Saccharomyces cerevisiae,* dosed at similar CFU levels, had the same effect on the dynamics of fermentation in two diets based on oat or lucerne. Therefore, some of the variability in the results reported in the literature, described as a yeast/diet interaction (Patra 2012; Lascano & Heinrichs, 2007), may be explained by differences in the number of viable cells that were used. Different substrate combinations, however, cannot be disregarded. Elghandour *et al*. (2016) for instance compared three commercial yeast products and observed that one strain was more effective in the stimulation of gas production. They suggested that the difference could be related to the number of active cells and other factors, such as nutrients and carrier materials. The variation in viability is then a concern. Wallace & Newbold (1995) reported that the viability of preparations can vary from $10^9 – 10^{10}$ live cells/g to 2 x 10^7 live cells/g. Opsi *et al*. (2012) showed higher gas production with live yeast than with inactivated yeast, and concluded that live yeast affects ruminal fermentation slightly more than inactivated yeast. Since one of the mechanisms of action of yeast is oxygen consumption, related to the yeast high respiratory rate (Newbold *et al*., 1996), the numbers of viable cells tested should be reported in yeast evaluations, so that various strains that differ in oxygen consumption ability and metabolic activity can be identified (Kutasi *et al*., 2004).

Although it has been reported that yeast produces metabolites, such as malate, which stimulate lactate-using bacteria (Nisbet & Martin, 1991; Martin & Nisbet, 1992; Nisbet & Martin, 1993), no changes were detected in this metabolite, possibly because grain levels were low in the current substrate mix. Other studies with 62% forage found no yeast effect in ruminal lactate, even when the average ruminal pH in the control diet was significantly higher (Křížová *et al*., 2011).

The positive effects on digestibility have been confirmed in meta-analyses (Desnoyers *et al*., 2009; Poppy *et al*., 2012) and other studies that showed dose responses with increasing levels of CFUs in straw-based diets (Ganai *et al*., 2015). The higher digestibility values could be explained by a higher population of cellulolytic bacteria, which is one of the most consistent effects of yeast (Martin & Nisbet, 1992; Wallace & Newbold, 1993). However, the positive effects are not consistent, even in experiments with increasing doses of yeast (Crosby *et al*., 2004) where cell viability was not certified.

In terms of the comparison of diets based on grasses (oat) and legumes (lucerne), Doran *et al*. (2007) observed lower digestibilities with lucerne diets compared with oats, which were associated with a higher lignin cellulose ratio in the lucerne legume than in the oats. Ghasemi *et al*. (2012) compared 0 or 5 g SC 47 (8 x 10^9 CFU/g) with lucerne hay or maize silage and detected only an improvement in the NDF *in situ* digestion measured after three hours' incubation. In another study, which compared several straws with increasing doses of *Saccharomyces cerevisiae*, Tang *et al*. (2008) observed that supplementation with yeast cultures increased cumulative gas production, but digestibility was not affected. This may be explained by the lignocellulosic characteristic of the substrates, because the doses used by Tang *et al* (2008) were higher than in the current experiment. Several studies have confirmed that substrates with low digestibility do not respond to yeast supplementation *in vivo* (Roa *et al.*, 1997; Arcos-García *et al.*, 2000; Crosby *et al.*, 2004). It is possible that the variability in response to yeast supplementation in terms of forage quality is a function of the potentially digestible fraction, as has been suggested for the response to fibrolytic enzymes (Mendoza *et al.*, 2014), which is another factor that needs to be considered in yeast evaluation assays.

Conclusions

The results indicate that in order to conduct a proper comparison of yeast products, it is necessary to evaluate the number of CFUs to incubate products with the same number of viable cells. This will allow to elucidate the effects among forage quality x yeast source x dose in *in vitro* evaluations. These results show the importance of checking the CFUs of *Saccharomyces cerevisiae* in products used as feed additives for ruminants.

Authors' Contributions

AMAE, LAM, GV and GDM conceived and designed the experiment. AMAE conducted the experiment. PAH and AZMS participated in statistical analyses and all authors participated in interpretation of results and writing and integration of the manuscript.

Conflict of Interest Declaration

The authors declare that they have no conflict of interest between the authors and other people or organizations that could inappropriately bias their results.

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