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Research article

Effects of live yeast and starch supplementation on in vitro fermentation and methane production of tropical grass

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ABSTRACT: This study aimed to evaluate the effects of supplementation of live yeast (LY) associated with starch levels (SL) on the *in vitro* kinetic parameters of the gas production (GP), *in vitro* neutral detergent fiber digestibility (IVNDFD), volatile fatty acid (VFA) and methane (CH_4) production of *Urochloa brizantha* cv. Marandu grass harvested in both the rainy and dry seasons. The study was conducted in a 2 × 3 factorial arrangement, whereby LY supplementation (with and without LY) was associated with 0, 10, and 20 % of starch supplementation. The variables were analyzed using PROC MIXED in SAS (Statistical Analysis System, version 9.3). LY and SL had no interaction effects on any of the variables tested. LY supplementation increased total GP at 72 h of incubation in both forage seasons by approximately 25 %. Additionally, LY supplementation decreased lag time; however, the GP rate was unaffected. The LY also caused a slight drop in IVNDFD at 48 h in both forage seasons. LY supplementation decreased total VFA production by 25.96 % for the rainy season and 11.32 % for the dry season and caused a 40 and 46 % drop in CH_4 for the rainy and dry season forage, respectively. The assumption that combining LY and starch would enhance LY activity was not confirmed in this study, as most analyzed variables showed no interaction between the two factors. Consequently, no beneficial effects on increased SL could be attributed to LY supplementation.

Keywords: VFA, digestibility, gas production, probiotic

Introduction

Forage cell wall constituents are the major components of tropical grass, which present slowly and incompletely digested and occupy space in the digestive tract of ruminants (Mertens, 1996), which, according to the author, generally limits the intake of dry matter (DM) and nutrients of grazing animals. There is a need to develop technologies to improve the neutral detergent fiber digestibility (NDFD), which is approximately 55 %, in animals fed a diet based on tropical forage (Cabral et al., 2006). Furthermore, approximately 4 to 14 % of the energy consumed by animals fed with tropical grass is lost in the form of methane (CH_4), which is also a greenhouse gas (Johnson and Johnson, 1995), justifying the use of technology that aims to increase NDFD and decrease CH_4 emissions (Nussio et al., 2006).

Live yeast (LY), *Saccharomyces cerevisiae* Meyen ex E.C. Hansen, has been shown to stabilize ruminal fermentation, prevent ruminal disorders, and improve ruminal ammonia efficiency. In addition, it stimulates the growth of fibrolytic fungi and bacteria to provide nutrients and vitamins to the environment. This process increases DM, NDFD, and reduces CH_4 emissions by stimulating the activity of acetogenic bacteria (Elghandour et al., 2016).

Previous studies have shown that the effect of LY is dependent on the proportion of concentrate in dairy cattle diets (Desnoyers et al., 2009). Thus, considering the mechanisms of the action of LY, we hypothesized about supplementing LY in forage-based diets. This study aimed to evaluate the effect of *S. cerevisiae* strain 1026 (LY) supplementation associated with starch levels (SL) on the kinetic parameters of gas production (GP), NDFD, volatile fatty acid (VFA) and CH_4 production of Marandu grass *in vitro*.

Materials and Methods

The experiment was carried out at the Farm Experimental at the Universidade Federal de Mato Grosso in Santo Antônio do Leverger, in the Mato Grosso state, located at 15°47′5″ S, 56°04′00″ W, altitude 140 m, the central-south mesoregion of Mato Grosso, in the microregion of Cuiabá. The climate, according to Köppen classification, is type Aw, that is, a tropical climate, megathermal, characterized by two well-defined seasons: dry (Apr to Sept) and rainy (Oct to Mar), according to Alvares et al. (2013).

The local Committee of Ethics on the Use of Animals of Universidade Federal do Mato Grosso approved this research (protocol number 23108.193858/2017-62).

Substrate and treatments

The study considered a 2 × 3 factorial arrangement, where the presence or absence of LY was associated with three SL (0, 10 and 20 % on the DM basis of incubated substrate). The LY strain evaluated was *S. cerevisiae* 1026 (5×10^6 CFU kg^{-1} of product) supplemented *in vitro,* and considered a dose recommended by the manufacturer company of 1 g 100 kg^{-1} of body weight (BW) per day, assuming an animal weighing 400 kg consuming 10 kg of DM daily, equivalent to 0.1 g of product kg^{-1} of DM. The substrate used in *in vitro* incubation was *Urochloa brizantha* cv. Marandu grass which was collected over two seasons (rainy and dry seasons), and was obtained by manual grazing simulation.

In vitro incubation

Two simultaneous incubations were set up. The first one used the Ankom® automatic pressure transducer system to obtain cumulative GP curves for 96 h. In contrast, the second one used 100 mL amber flasks to measure accumulated GP at 24 and 48 h, with the aim of obtaining carbon dioxide (CO_2) and CH_4 production so as to evaluate VFA production and NDFD (48 and 96 h). In each incubation system, three consecutive runs were performed.

In both incubation systems, McDougal's buffer (McDougal, 1949) was previously reduced with oxygen-free CO_2 flushing and reducing solution (HCl-cysteine, sodium sulfide, and NaOH 1 N).

For the incubations set up in the Ankom® system, approximately 1 g of the diet was weighed, which was incubated in duplicate and placed in vials with a capacity of 310, and 80 mL of buffer and 20 mL of ruminal inoculum liquid were added. All procedures used in this system were carried out according to the manufacturer's manual, and pressure readings were recorded every 10 min for 96 h, which were then applied to building a GP profile used to estimate the kinetic parameters of GP.

For 100 mL flasks, two sets of incubation flasks were used, in duplicate, one for 48 h and the other for 96 h of incubation, wherein both sets of 500 mg samples of the substrate were weighed. Subsequently, 40 mL of McDougal's buffer containing or not yeast solution and 10 mL of ruminal inoculum were added. Immediately after, flaks were capped with rubber stoppers and aluminum seals and placed in a water bath at 39 °C. Four blank flasks were used in each incubation. For 48 h flasks, gas samples were collected to determine CH_4 production, fluid was collected to measure total VFA production, and the residue was filtered for neutral detergent fiber (NDF) evaluation. In 96 h, residues were also evaluated for NDF, as was done for 48 h flasks to determine NDFD.

The ruminal inoculum was collected from two castrated and cannulated cattle (400 kg of BW) supplemented with salt mineral and kept in pasture formed by *Urochloa brizantha* cv. Marandu grass. Immediately after collecting the ruminal inoculum, it was filtered through gauze, packed in thermoses at 39 °C and transported to the laboratory.

Analysis

All samples of the substrates were analyzed in the Laboratório de Nutrição Animal of Universidade Federal do Mato Grosso for DM, ash, crude protein (CP), NDF and neutral detergent fiber corrected for ash and protein (NDFap) according to Detmann et al. (2012).

The concentrations of CH_4 at 48 h of incubation were analyzed by gas chromatography (Shimadzu® GC-2014 model) with an automatic injector. This chromatograph was equipped with flame ionization detectors (FID) for measuring CH_4 concentrations. The FID and electron capture detector temperatures were maintained at 250 and 325 °C, respectively, and the chromatographic column at 75 °C in the isothermal system. The columns were in the Hayesep series (1.0, 4.0, 1.5, 1.5, 0.7 M). The carrier gas was N_2. The gas solution containing CH_4 standards (White Martins®) was used to determine the standard curve.

The VFA concentration in the ruminal fluid was quantified by a gas chromatograph (C.G. H.P. 7890A; Injetor HP 7683B; Agilent Technologies) equipped with an HP-FFAP capillary column (19091F-112; 25 m; 0.320 mm; 0.50 µm; L & W Technologies). Injection was administered automatically, the carrier gas was H_2, which were maintained at a flow rate of 34.35 mL min^{-1}. The temperature of the injector and detector was 260 °C. The total time of the chromatographic run was 16.5 min divided into three heating ramps, as follows: 80 °C (1 min); 120 °C (20 °C min^{-1}, 3 min); 205 °C (10 °C min^{-1}, 2 min). The VFA (mM) concentration was determined on an external calibration curve. The solution was prepared with individual solutions of acetic, propionic, and butyric acid, obtaining a standard VFA solution with a known concentration of 92.76, 40.77, and 20.77 mMol mL^{-1}, respectively. Some replicates per sample (between X and Y) was needed to make the difference between readings less than 5 %. The standard solution was injected after each sample injection for possible distortion of the readings due to possible contamination of the needle and spine. VFA concentrations were calculated using the VFA concentration in the standard solution.

For analysis of the *in vitro* digestion of NDF, data were obtained by weight difference of the samples before and after incubation at 48 and 96 h. The fermentation residues were obtained by filtering the contents of the vials in 2 µ porosity crucibles (Pirex -Vidrotec®) after digestion with the neutral detergent solution for 1 h in the autoclave at 105 °C. The crucibles were dried for 24 h in an oven at 105 °C and weighed to calculate NDF digestion values (Detmann et al., 2012).

Statistical analysis

The kinetic parameters of cumulative GP as a function of time were estimated using the NLIN procedure of

SAS (Statistical Analysis System, version 9.3) and the unicompartmental logistic model (Schofield et al., 1994):

$$V(t) = (Vf / (1 + exp(2 - 4 \times kd \times (T - L)))) \quad (1)$$

where: $V(t)$ is the cumulative volume in time; Vf is the final volume of gas; kd (h^{-1}) is the digestion rate; L is the latency or colonization time in hours, and T the time (h).

After checking whether the data presented a normal distribution, the variables were analyzed using PROC MIXED in SAS (Statistical Analysis System, version 9.3). The statistical model considers LY addition (with and without yeast), starch effect (0, 10 and 20 % inclusion), interaction LY addition × SL as a fixed factor, and a week as a random factor. Only the major factors (LY addition and SL) were presented. Using the SAS CONTRAST statement, orthogonal contrasts were used for specific partitioning of the SL in linear and quadratic. The level starch effect was evaluated within each yeast (with and without) using the same contrast method. The LSMEANS was used to generate the individual means for each treatment. In all analyses, significance was considered when $p < 0.05$.

Results

Chemical composition of substrates

Dry season forage presented higher levels of DM, NDF, NDFap, and organic matter (OM) than rainy season forage. In contrast, the rainy forage season presented a higher content of CP, soluble fraction in neutral detergent fiber and ash compared to the dry season forage (Table 1).

Kinetic parameter of gas production

There was no interaction between LY and SL on kinetic parameters of GP was not observed for most of the kinetic parameters estimated for GP (Table 2), except for GP at 6 h (GP_6) of incubation ($p < 0.01$) and lag time ($p < 0.01$) for dry season forage, where SL increased GP_6 for control ($p < 0.01$) and did not affect GP_6 in LY ($p = 0.0394$). At the same time, SL caused a drop on lag time in the control ($p < 0.01$) and did not affect lag time in LY.

For the rainy season forage, SL caused a quadratic effect ($p < 0.01$) on total GP (GP_{48} and GP_{96}) as well as a linear increase ($p < 0.01$) on the digestion rate (kd) and GP at 24 and 72 h ($p < 0.01$) (Table 2, Figure 1A and B). For the dry season forage, SL promoted a linear increase ($p < 0.01$) on kd ($p < 0.01$) and GP at 12, 24, 48, 72 and 96 h ($p < 0.01$) (Table 2).

For rainy season forage, LY supplementation caused a decrease ($p < 0.01$) on lag time by 19.72 % and an increase of GP at 6, 12, 24, ($p < 0.01$) and 48 h ($p = 0.02$) of *in vitro* incubation. For dry season forage LY also increased ($p < 0.01$) GP at 12, 24, 48, and 72 h of incubation (Figure 2A and B) but did not influence kd ($p > 0.05$) (Table 2). On average, LY increased GP until 72 h for both the rainy and dry forage seasons by 25.94 and 24.78 %, respectively, compared to the control.

In vitro neutral detergent fiber digestibility (IVNDFD)

There was no interaction between LY and SL on IVNDFD (Table 3), but LY caused a drop in IVNDFD of 6.55 and 12.26 % ($p < 0.01$) at 48 h of incubation for forage obtained in both the rainy and dry seasons,

Table 1 – Chemical composition of Marandu grass obtained by grazing simulation during rainy and dry seasons.

Variable	Rainy season forage	Dry season forage
DM[1]	23.49	36.59
CP[2]	10.63	6.18
NDF[2]	65.74	70.19
NDFap[2]	60.40	66.14
SND[2]	34.26	29.81
OM[2]	91.95	92.94
ash[2]	8.05	7.06

[1]% in natural matter; [2]% in dry matter. DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; NDFap = neutral detergent fiber corrected for ash and protein; SND = soluble fraction in neutral detergent fiber; OM = organic matter.

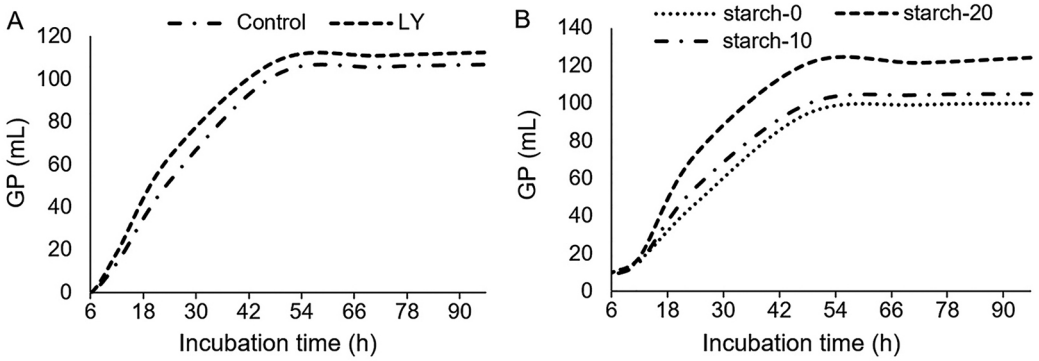

Figure 1 – A) Effects of live yeast (LY) and B) starch levels on cumulative gas production (GP) of Marandu grass incubated *in vitro* (rainy forage season).

Table 2 – Effects of live yeast and starch levels on kinetic parameters of Marandu grass incubated *in vitro*.

Item	Control			Yeast			SEM	*p*-value		
	Starch levels			Starch levels				Yeast	Starch	Y × S
	0	10	20	0	10	20				
					Rainy season forage					
$GP_{6\,h}$	8.1	6.2	9.0	11.4	10.4	10.5	0.94	< 0.01	0.086	0.138
$GP_{12\,h}$	13.4	12.1	16.5	19.3	18.7	19.5	3.63	< 0.01	0.241	0.469
$GP_{24\,h}$	39.3	48.8	68.5	53.6	61.3	76.8	7.99	< 0.01	$< 0.01^{L}$	0.709
$GP_{48\,h}$	90.1	96.7	118.2	98.6	102.6	123.9	6.11	0.028	$< 0.01^{Q}$	0.907
$GP_{72\,h}$	94.7	103.6	118.2	103.3	105.3	124.7	6.45	0.148	$< 0.01^{L}$	0.755
$Gp_{96\,h}$	95.4	103.2	121.5	104.1	106.8	127.0	5.83	0.059	$< 0.01^{Q}$	0.781
kd	3.3	3.6	3.8	3.13	3.6	3.9	0.26	0.943	$< 0.01^{L}$	0.687
Lag time	10.9	11.7	10.1	7.9	8.7	9.5	1.13	< 0.01	0.394	0.058
					Dry season forage					
$GP_{6\,h}$	5.7	6.6	9.3	10.1	10.6	10.5	0.97	< 0.01	$< 0.01^{L}$	0.025
$GP_{12\,h}$	9.1	11.9	16.1	15.7	17.8	18.7	3.10	< 0.01	$< 0.01^{L}$	0.182
$GP_{24\,h}$	24.5	39.4	57.3	35.5	48.5	64.5	6.62	< 0.01	$< 0.01^{L}$	0.705
$GP_{48\,h}$	66.1	79.6	96.8	73.9	88.3	102.7	6.60	< 0.01	$< 0.01^{L}$	0.836
$GP_{72\,h}$	78.0	84.4	100.0	83.4	92.5	105.7	5.75	< 0.01	$< 0.01^{L}$	0.842
$Gp_{96\,h}$	80.2	85.8	105.2	82.5	94.2	105.4	5.54	0.160	$< 0.01^{L}$	0.406
kd	2.4	2.9	3.2	2.4	2.9	3.5	0.15	0.667	$< 0.01^{L}$	0.196
Lag time	12.2	9.6	8.5	7.9	7.1	7.6	1.60	< 0.01	$< 0.01^{L}$	0.017

^{L}Linear effect of starch level ($p < 0.05$); ^{Q}Quadratic effect of starch levels ($p < 0.05$). Y × S = interaction among yeast × starch; GP = gas production (mL); *kd* = digestion rate (% h^{-1}); Lag time (h) = colonization time; SEM = standard error mean.

Table 3 – Effects of live yeast and starch levels on neutral detergent fiber digestibility and liquid pH of Marandu grass incubated *in vitro*.

Item	Control			Yeast			SEM	*p*-value		
	Starch levels			Starch levels				Yeast	Starch	Y × S
	0	10	20	0	10	20				
					Rainy season forage					
$IVNDFD_{48\,h}$	57.5	59.7	58.5	54.0	55.8	54.4	2.14	< 0.01	0.281	0.967
$IVNDFD_{96\,h}$	78.9	80.3	79.2	78.4	81.5	78.8	1.37	0.916	0.196	0.754
					Dry season forage					
$IVNDFD_{48\,h}$	37.9	38.7	38.4	35.8	32.2	32.9	2.20	< 0.01	0.560	0.290
$IVNDFD_{96\,h}$	63.8	64.1	64.0	64.1	64.8	65.5	1.81	0.013	0.121	0.343

Y × S = interaction yeast × starch; IVNDFD = *in vitro* neutral detergent fiber digestibility; SEM = standard error mean.

respectively. On the other hand, the $IVNDFD_{96}$ for dry season forage increased ($p = 0.013$) by 1.28 % with the addition of LY (Table 3), while $IVNDFD_{96}$ for rainy forage season was not affected by LY.

VFA and methane production

LY and SL had no interaction effect ($p = 0.140$) of on total VFA production and VFA profile for rainy season forage (Table 4). However, an interaction effect ($p = 0.014$) between LY and SL was observed for dry season forage in the total of VFA, where SL had a quadratic effect on the total of VFA for control but did not affect the total of VFA for LY.

LY caused a drop of 25.96 % in total production of VFA ($p < 0.01$) for the rainy forage season and by 11.32 % for the dry forage season, although there was no effect on the molar ratios of the VFA (Table 4).

SL caused a linear drop in the molar proportion of acetate and a linear increase in the molar proportion of butyrate for rainy season forage. For dry season forage, SL caused a quadratic effect on the total production of VFA, a linear decrease in the molar proportion of acetate and acetate:propionate (A:P) ratio, and a linear increase in the molar proportion of propionate and butyrate (Table 4).

There was no interaction ($p = 0.591$) between LY and SL in CH_4 production for both forage samples. However, LY caused a drop ($p < 0.01$) in CH_4 production of 40 and 46 % for both rainy and dry season forage when compared to the control, respectively (Figure 3A). Furthermore, SL caused a quadratic effect ($p = 0.026$) on CH_4 production for rainy season forage (Figure 3B), but there was no effect ($p > 0.05$) when the dry season forage was supplemented with starch.

Discussion

In this way, the effectiveness of LY in improving animal performance is caused by the strain, dose, and

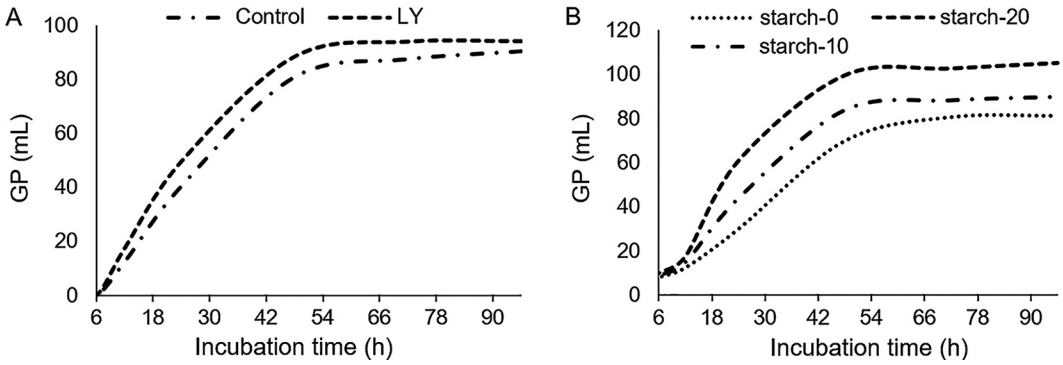

Figure 2 – A) Effects of live yeast (LY) and (B) starch levels on cumulative gas production (GP) of Marandu grass incubated *in vitro* (dry forage season).

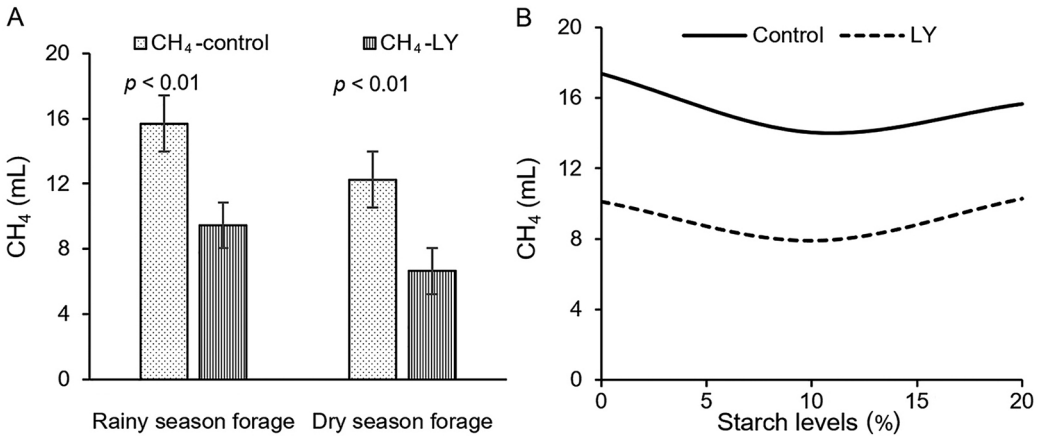

Figure 3 – A) Effect of methane (CH_4) live yeast (LY) on methane production of Marandu grass collected in both the rainy and dry seasons incubated *in vitro* and B) the effect of starch levels on methane production on rainy season forage.

Table 4 – Effects of live yeast and starch levels on volatile fatty acid profile of Marandu grass incubated *in vitro* for 48 h.

Item	Control			Yeast			SEM	*p*-value		
	Starch levels			Starch levels				Yeast	Starch	Y × S
	0	10	20	0	10	20				
Rainy season forage										
VFA	34.1	30.1	29.4	23.0	24.0	22.3	2.06	< 0.01	0.119	0.140
Acet (%)	69.1	66.8	65.9	68.3	67.2	66.8	0.84	0.706	< 0.01^{L}	0.393
Prop (%)	21.2	22.0	21.8	20.6	21.4	21.1	0.93	0.113	0.217	0.990
But (%)	9.7	11.1	12.3	11.0	11.3	12.1	0.36	0.127	< 0.01^{L}	0.087
A:P	3.3	3.0	3.0	3.3	3.1	3.2	0.17	0.234	0.082	0.907
Dry season forage										
VFA	25.4	20.7	30.7	22.9	22.9	22.3	1.63	0.046	0.030^{Q}	0.014
Acet (%)	68.6	67.4	66.4	68.5	67.1	65.9	0.74	0.435	< 0.01^{L}	0.918
Prop (%)	20.5	21.1	21.8	20.7	21.5	21.4	0.78	0.734	< 0.01^{L}	0.404
But (%)	10.9	11.5	11.8	10.8	11.4	12.7	0.30	0.377	< 0.01^{L}	0.200
A:P	3.4	3.2	3.0	3.3	3.1	3.1	0.15	0.526	< 0.01^{L}	0.681

^{L}Linear effect of starch level ($p < 0.05$); ^{Q}Quadratic effect of starch level ($p < 0.05$). Y × S = interaction yeast × starch; VFA = volatile fatty acid (mMol L^{-1}); Acet = acetate; Prop = propionate; But = butyrate; A:P = acetate:propionate.

concentration of cells of LY in commercial products, as well as interactions of these factors with the diet fed. The interaction between LY and diets is the major source of variation of results, where the effect of yeast supplementation increases when high-concentrate diets are fed (Desnoyers et al., 2009).

The mechanisms by which LY acts in the rumen are reported to be associated with its capacity to consume oxygen as a faculty anaerobe, and such action might be beneficial to strict anaerobe organisms such as fibrolytic bacteria and fungi. Additionally, LY supplies nutrients that stimulate bacteria growth, such as organic acids,

complex B vitamins, amino acids, and peptides, which could improve the digestion of polymers in the rumen, especially those components of the plant cell wall (Durand-Chaucheyras et al., 1998).

Thus, considering these mechanisms of the action of LY, we hypothesized that the supplementation of LY in forage-based diets would improve forage digestion *in vitro*, which was confirmed by the increase in GP in most of the reading times compared to the control, as well as by a decrease in lag time (Table 2, Figure 1A and B). These results may be explained by the increase in microbial growth and activity stimulated by supplementation with LY, according to Durand-Chaucheyras et al. (1998).

However, assuming that LY would be used in farms where technology is being applied, such as the supplementation of grazing animals to improve animal performance, it was supposed that the response to LY supplementation would be related to SL combined with forage-based diets. Desnoyers et al. (2009), who highlighted a stronger response to LY supplementation with increasing levels of concentrate in the diet, as LY may be more active in the rumen and have access to a higher amount of soluble sugars for growth, given that LY cannot degrade cellulose or hemicellulose.

Thus, in diets with a low concentration of soluble sugars, such as those used in this work, the action of LY in the first hours of incubation may, instead of symbiotic, be competitive for nutrients with rumen microbes, which would result in the reduction of colonization efficiency and the beginning of degradation of the NDF by fibrolytic bacteria. This statement is evidenced by the fact that, in the treatments with LY supplementation, there was a decrease in NDFD at 48 h of *in vitro* incubation for both forages (from both the rainy and dry seasons). A similar result was found by Elghandour et al. (2014) when evaluating increasing doses of yeast associated with four fibrous foods by *in vitro* incubations, where the inclusion of yeast caused a decrease in NDF degradation values.

The decrease in lag time in the presence of LY could have been the result of LY being more efficient in free sugar fermentation than the fibrolytic bacteria (Russell, 2003). Therefore, the fermentation and the GP began faster after inoculation with ruminal liquid. Latency time may also have been decreased by the respiratory activity of yeast, which contributed to the elimination of oxygen from the environment, which is toxic to the anaerobic bacteria of the rumen, especially to the fibrolytic bacteria, making its activity difficult (Newbold et al., 1996).

Since the addition of yeast, there has been a decrease in the latency time. As observed in the present study, GP was also expected to increase. Although this production was higher in the first hours, it was observed that there was no difference in the final volume of gas, indicating that the effect of LY supplementation is restricted to the first hours of incubation. This behavior may be due, in large part, to the fact that yeasts in the ruminal environment remain active for approximately 30 h (Durand-Chaucheyras et al., 1998), thus indicating the need for daily supplementation.

Although LY supplementation did not positively affect IVNDFD at 48 h, it did at 96 h dry season forage, showing potential as an additive for improving fiber digestion by rumen organisms. It is supposed that at the end of its life cycle, LY loses viability and lyse releases soluble nutrients and growth factors such as organic acids, vitamins, and amino acids to the rumen microbial population, including fibrolytic organisms.

The decrease in total VFA production with LY supplementation is likely explained by the increased use of energy sources for microbial growth rather than VFA production. The carbohydrates used by rumen microbes between VFA production and microbial growth can be affected and changed by supplying limiting nutrients, such as N (NH_3, amino acids, and peptides) and vitamins (Russell 2003; Mao et al., 2013), which are cited as being supplied by LY (Durand-Chaucheyras et al., 1998).

However, VFA production is highly correlated to GP, which is not the case in this study because GP increased until 48 h while VFA decreased. However, LY supplementation can increase GP *in vitro* by directly metabolizing the sugars from the substrate incubated or indirectly stimulating rumen microbial growth and metabolism. Additionally, when LY ferments glucose under anaerobic conditions, such as in incubation flasks, it contributes to an increase in total GP directly but does not increase VFA production, as LY tends to produce ethanol instead of VFAs (Russell, 2003). This helps explain the decline in VFA production observed alongside an increase in total GP when LY was supplemented.

LY supplementation did not affect the VFA profile, which was also found in the meta-analysis by Desnoyers et al. (2009) using data from 157 experiments evaluating the effects of yeasts on ruminant diets, where it was verified that the yeast supplementation did not present any influence on the proportions of A:P.

The decrease in CH_4 production observed in this study may be explained by the effect of LY in stimulating the growth of rumen bacteria that produce less hydrogen and acetogenic bacteria (Hristov et al., 2013), as well as by LY's direct effect in fermenting soluble sugars to ethanol, which consumes hydrogen. This reduces the hydrogen supply available to methanogens for CH_4 production (Nussio et al., 2006).

Overall, SL caused an increase in total GP and digestion rate. A decrease in lag time did not affect NDFD and total VFA production, causing a drop in acetate proportion, A:P ratio and increased butyrate proportion. It also caused a quadratic effect on CH_4 for rainy season forage. These effects were expected since starch is a non-fiber carbohydrate, which presents fast and almost total availability in the digestive tract compared to fiber carbohydrates (Van Soest, 1994; Mertens, 1996).

LY supplementation to animals fed forage-based diets might help manipulate rumen fermentation. This

would increase the use of dietary energy by animals and reduce their environmental impact since CH_4 production would be reduced by up to 40 %. LY does not decrease forage digestibility, which is significant from a productive perspective.

The assumption that combining LY and starch would enhance LY activity was not confirmed in this study, as most analyzed variables showed no interaction between the two factors. Consequently, no beneficial effects on increased SL could be attributed to LY supplementation.

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Authors' Contributions

Conceptualization: Fabian EC, Pedreira BC, Paula NF, Cabral LS, Pacheco RDL. **Data curation:** Fabian EC, Costa AC, Fonseca ASR, Vieira KPN, Petter FA, Negrão FM. **Formal analysis:** Fabian EC, Pedreira BC. **Funding acquisition:** Cabral LS. **Investigation:** Paula NF, Cabral LS. **Methodology:** Fabian EC, Pedreira BC, Paula NF, Cabral LS, Pacheco RDL. **Project administration:** Fabian EC. **Resources:** Cabral LS. **Supervision:** Cabral LS. **Writing-original draft:** Fabian EC, Cabral LS, Pedreira BC, Costa AC, Fonseca ASR, Vieira KPN, Petter FA, Tavares JR, Negrão FM. **Writing-review & editing:** Fabian EC, Cabral LS, Pedreira BC, Negrão FM.

Conflict of interest

The authors declare no conflict of interest.

Data availability statement

All data that support the findings of this study are included within the article.

Declaration of use of AI Technologies

The authors did not use.

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