Effects of feeding a live yeast on rumen fermentation and fiber degradability of tropical and subtropical forages

Amanda Camila Poppi, Gustavo Lazzari, Ana Luiza Mendonça Gomes, Rodolpho Martin Prado, Rafaela Takako Ribeiro Almeida, Daniele Maria Zanzarin, Eduardo Jorge Pilau, Clóves Cabreira Jobim, Lucas José Mari, Eric Chevaux, et al.

Research Article

Received: 5 December 2020 Revised: 21 April 2021 Accepted article published: 29 April 2021 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/jsfa.11273

Effects of feeding a live yeast on rumen fermentation and fiber degradability of tropical and subtropical forages

Amanda Camila de Poppi,[a] Gustavo Lazzari,[a] Ana Luiza Mendonça Gomes,[a] Rodolpho Martin do Prado,[a] Rafaela Takako Ribeiro de Almeida,[a] Daniele Maria Zanzarin,[a] Eduardo Jorge Pilau,[a] Clóves Cabreira Jobim,[a] Lucas José Mari,[b] Eric Chevaux,[c] Frédérique Chaucheyras-Durand,[c] Adegbola Tolulope Adesogan[d] and João Luiz Pratti Daniel[a]*

Abstract

BACKGROUND: The effect of live yeast *Saccharomyces cerevisiae* strain CNCM I-1077 (SC) on the ruminal degradability of different forages commonly found in dairy diets in South America was evaluated. We also assessed if SC supplementation interacts with forage group to affect ruminal fiber degradability. Four non-lactating rumen-cannulated Holstein cows were randomly assigned to two treatment sequences: Control-SC-Control or SC-Control-SC, in a switchback design, with three 30-day periods. Cows in the SC treatment were supplied with 1×10^{10} colony-forming units of yeast daily via rumen cannula. *In situ* degradability of dry matter (DM) and neutral detergent fiber (aNDF) was measured in 15 forages collected in South America. Forages were assigned to one of three groups: corn silages; tropical grasses (sugarcane silages and tropical grass silages); and temperate grasses and alfalfa (oat silages, ryegrass silages, alfalfa silage, and alfalfa hay).

RESULTS: Cows supplemented with SC had higher ($P = 0.05$) counts of yeasts and lower ($P = 0.03$) concentration of lactate in rumen fluid. There was no interaction between forage group and yeast supplementation ($P > 0.10$) on *in situ* degradability. The SC increased DM (by 4.6%) and aNDF degradation (by 10.3%) at 24 h of incubation ($P < 0.05$). Metabolomics revealed that a chemical entity ($C_{17}H_{29}N_6O_3$, *m/z* 365.2284 $[M + H]^+$) from the family of lipids and related molecules was suppressed in the rumen fluid of cows supplemented with SC.

CONCLUSION: The SC supplementation improved DM and aNDF degradability regardless of the forage group.

Keywords: cell wall; lactic acid; rumen metabolomics; *Saccharomyces cerevisiae*

INTRODUCTION

In diets of high-producing ruminants, forages are included to provide physically effective fiber, to enhance ruminal function, and to maintain animal health.[1] Forages are also important sources of nutrients, depending on their quality, which is mainly determined by its fiber content and digestibility.[2] The content and digestibility of fiber in diets influences feed intake due to physical filling of the rumen, which can influence dry matter (DM) intake and animal performance.[3,4]

Forage species, genotype, maturity and harvest management can affect forage composition and digestibility. Crop growth environment may also have an effect on forage quality. Several authors have reported that warmer temperatures increase lignin deposition and decrease *in vitro* digestibility compared with forage grown at lower temperatures.[5–8] Hence, growth strategies capable of enhancing fiber digestibility would benefit dairy systems in tropical and subtropical areas.

Post-harvest strategies can also be used to improve forage digestibility, such as the application of exogenous fibrolytic enzymes[9] and chemicals (e.g., sodium hydroxide, anhydrous ammonia, or calcium oxide).[10,11] Moreover, manipulation of ruminal fermentation with probiotics can improve forage digestibility.[12–14]

Several studies with diets based on forages cultivated in temperate conditions have indicated that supplementation with live yeasts, such as *Saccharomyces cerevisiae* strains, increases the total number of cellulolytic bacteria, stimulates lactate utilization, and

* *Correspondence to: JLP Daniel, State University of Maringá, Maringá 87020900, Brazil. E-mail: jlpdaniel@uem.br*

a *State University of Maringá, Maringá, Brazil*

b *Lallemand Brasil Ltda, Aparecida de Goiânia, Brazil*

c *Lallemand SAS, Blagnac, France*

d *Department of Animal Sciences, IFAS, University of Florida, Gainesville, FL, USA*

decreases lactate production, increases rumen pH and reduces oxygen concentration in the rumen fluid, resulting in higher fiber degradation, higher synthesis of microbial protein and improved animal performance.[15–19] De Ondarza *et al*. showed increased milk yield and feed efficiency in animals supplemented with live yeasts, but their meta-analysis was mainly based on diets with forage cultivated in temperate climates.[16] Meanwhile, potential benefits of live yeast supplementation of the diet on ruminal environment and degradability of different forages grown in tropical and subtropical areas is seldom reported and thus is not well established.[20,21] Nonetheless, field nutritionists have claimed in dairy farms that tropical forages would have a greater improvement of digestibility than forages grown in temperate zones.

Thus, the aim was to evaluate effects of live yeast *Saccharomyces cerevisiae* strain CNCM I-1077 on ruminal environment and degradability of DM and neutral detergent fiber (aNDF) of several forages commonly found in diets of dairy cows in Brazil and other countries in South America, and especially to determine if a potential improvement in DM and aNDF degradation varies by forage group.

MATERIAL AND METHODS

Forage samples

Fifteen forage samples were collected from dairy farms (one composite sample obtained by mixing 8 to 10 subsamples of each forage in each farm) in different regions of Brazil (13), Argentina (1), and Peru (1), and assigned to one of three groups: tropical grasses (sugarcane silage and tropical grass silage), corn silages, and temperate grasses and alfalfa (oat silage, ryegrass silage, alfalfa silage, and alfalfa hay). The groups were designed to create C4 grasses with low starch content and expected low fiber digestibility (i.e., tropical grasses), C4 grasses with high starch content (i.e., corn silages) and forage with low starch content but expected high fiber digestibility (i.e., temperate grasses and alfalfas). Information about the forages, collection sites, and composition are presented in Tables 1 and 2. Immediately after sampling, the forages were dried in a forced-air oven at 55 °C for 72 h at the sampling site, packed into polyethylene bags, and sent to the State University of Maringá, Maringá – PR, Brazil.

Cows, facilities and experimental design

Animal care and handling procedures were approved by the ethics committee for animal use of the Maringa State University (protocol number 8208090218 – CEUA/UEM). Four non-lactating rumen-cannulated Holstein cows (two primiparous and two multiparous); weighing 545 ± 177 kg were housed in a tie-stall barn with rubber bedding, individual feedbunks, and a water trough. The proportion of ingredients and the chemical composition of the diet offered are presented in Table 3. Every morning, ingredients were mixed and fed as a total mixed ration (TMR) at 08:00 h, after removing refusals from the previous day. The amount of TMR was adjusted daily to allow 50 to 100 g·kg^{-1} of orts (*ad libitum* diet access).

The experimental treatments were (i) control and (ii) live yeast supplemented at 1×10^{10} colony forming units (CFU day^{-1} per cow) (SC; *Saccharomyces cerevisiae* strain CNCM I-1077; Lallemand Animal Nutrition, Aparecida de Goiânia, GO, Brazil). The dose offered to animals was established after yeast plating in laboratory, as described below for the rumen fluid. Immediately before supplementing, the live yeast (10 g) was diluted in 250 mL of distilled water at 40 °C and dosed directly into the rumen, through the rumen cannula, every morning before TMR feeding. The application through the rumen cannula ensured the target yeast amount was supplied daily. Cows receiving the control treatment

Table 1. Forages sampled

ID	Forage	Assigned group	Type	Scientific name	Conservation	Locality	Country
A	Corn	Corn silage	C4 grass	*Zea mays*	Silage	Saladillo	Argentina
B	Corn	Corn silage	C4 grass	*Zea mays*	Silage	Castro-PR	Brazil
C	Corn	Corn silage	C4 grass	*Zea mays*	Silage	Bela Vista de Goiás-GO	Brazil
D	Corn	Corn silage	C4 grass	*Zea mays*	Silage	Mandaguaçu-PR	Brazil
E	Corn	Corn silage	C4 grass	*Zea mays*	Silage	Arequipa	Peru
F	Alfalfa	Temperate/ Alfalfa	Legume	*Medicago sativa*	Hay	Lunardelli-PR	Brazil
G	Alfalfa	Temperate/ Alfalfa	Legume	*Medicago sativa*	Silage	Castro-PR	Brazil
H	Oat	Temperate/ Alfalfa	C3 grass	*Avena sativa*	Silage	Arapoti-PR	Brazil
I	Oat	Temperate/ Alfalfa	C3 grass	*Avena sativa*	Silage	Castro-PR	Brazil
J	Ryegrass	Temperate/ Alfalfa	C3 grass	*Lolium multiflorum*	Silage	Castro-PR	Brazil
K	Ryegrass	Temperate/ Alfalfa	C3 grass	*Lolium multiflorum*	Silage	Castro-PR	Brazil
L	Sugarcane	Tropical grass	C4 grass	*Saccharum officinarum*	Silage	Nova Andradina-MS	Brazil
M	Sugarcane	Tropical grass	C4 grass	*Saccharum officinarum*	Silage	Agudos-SP	Brazil
N	Tropical grass	Tropical grass	C4 grass	*Panicum maximum* cv. Mombaça	Silage	São Miguel do Araguaia-GO	Brazil
O	Tropical grass	Tropical grass	C4 grass	*Panicum maximum* cv. Mombaça	Silage	Terenos-MS	Brazil

Table 2. Chemical composition of the forage samples (g·kg^{-1} dry matter, unless otherwise stated)

Forage group	Dry matter (g kg^{-1} as fed)	Crude protein	Ash	aNDF[a]	Hemicellulose	ADF[b]	Cellulose	Lignin(sa)	iNDF[c]
A-Corn silage	272	77.7	59.4	536	260	275	238	37.2	177
B-Corn silage	340	74.5	39.3	431	253	177	156	21.1	133
C-Corn silage	255	45.8	25.8	590	256	334	284	49.6	205
D-Corn silage	293	76.0	34.3	403	200	203	177	25.9	138
E-Corn silage	323	89.6	104	601	246	354	296	58.1	167
F-Alfalfa hay	907	140	71.8	720	190	530	390	140	473
G-Alfalfa silage	536	158	87.5	542	162	380	288	92.9	296
H-Oat silage	217	71.2	77.2	611	247	364	303	59.8	262
I-Oat silage	290	94.4	87.7	661	266	395	355	38.4	175
J-Ryegrass silage	490	143	108	595	248	344	298	46.0	188
K-Ryegrass silage	545	169	121	519	216	303	271	31.9	122
L-Sugarcane silage	331	25.8	22.5	767	298	448	340	109	373
M-Sugarcane silage	243	24.9	24.3	805	311	494	389	105	404
N-Tropical grass silage	285	30.9	83.4	837	285	552	466	85.9	428
O-Tropical grass silage	390	42.9	72.4	813	325	488	421	66.6	382

[a] Neutral detergent fiber.
[b] Acid detergent fiber.
[c] Indigestible aNDF.

Table 3. Composition of basal diet (g kg^{-1} DM, unless otherwise stated)

Item	Composition
Ingredients	
Corn silage	650
Concentrate[a]	350
Nutrients	
Dry matter, g·kg^{-1} as fed	473 ± 5.7
Crude protein	124 ± 1.5
Ash	54 ± 0.1
Starch	283 ± 2.4
Neutral detergent fiber	419 ± 3.1
Acid detergent fiber	221 ± 1.2

[a] Concentrate based on ground corn, soybean meal and mineral-vitamin mix.

were also dosed with 250 mL of distilled water at 40 °C to avoid bias between treatments.

The treatments were compared in a switchback design, with three periods of 30 days including 24 days of adaptation and 6 days of sampling. Cows were paired according to parity and randomly assigned to each treatment sequence. There were two treatment sequences within the parity pair: Control-SC-Control or SC-Control-SC.

In situ degradability

From Day 25 to 28 of each period, two 2 day runs were used to measure the *in situ* disappearance of DM and aNDF of the 15 forage samples (7 or 8 forages randomly allocated in each run). Dry forage samples were ground in a Wiley mill (Marconi MA340, Piracicaba, SP, Brazil) with a 5 mm screen and 5 g were weighed into polyfilament polyester *in situ* bags (10 × 20 cm; 50 µm porosity; Ankom Technology, Macedon, NY, USA). Each forage was incubated in triplicate in each cow for 12, 24 and 36 h after feeding. Such times were intended to represent a range of retention times in dairy cows, considering fractional passage rates of 0.027 to 0.083 h^{-1}.[22]

Before incubation, bags were soaked in warm water (39 °C) for 20 min to simulate rumen temperature and remove the soluble fraction. Two blank bags were included for each time point. Bags were inserted in reverse order and recovered together. Immediately after removal, all bags were submerged in cold water (0 °C) for 5 min and washed with water only in a washing machine (three cycles with water at room temperature, followed by a final spin). Washed bags were dried in a forced-air oven at 55 °C for 72 h, weighed, and their contents were ground through a 1 mm screen using a Wiley mill.

Sampling of feed and rumen fluid

Approximately 500 g of dietary ingredients were collected from day 25 to day 30 of each period and subsequently composited by period (n = 3). Samples were oven-dried at 55 °C for 72 h and ground (1 mm screen; Wiley mill) prior to chemical analyses.

On day 30 of each period, rumen fluid was collected from the ventral sac at 0, 2, 4, 8, and 12 h after feeding to measure pH, ammonia, lactate, volatile fatty acids (VFA) and for metabolomics. Yeast counts were also measured in samples collected at 0, 2, and 8 h after feeding. The pH was measured immediately after sampling using a pH meter (Tec5, Tecnal, Piracicaba, Brazil). After measuring the pH, a sub-sample was used for enumeration of yeasts as described below (only in samples collected at 0, 2, and 8 h). Another sub-sample (100 mL) was used for metabolomics analysis, as described below. A third sub-sample was centrifuged at 10 000×g for 15 min at 4 °C and the supernatant was frozen at −80 °C for further analyses of ammonia, lactate, and VFA.

Laboratory analyses

Forage samples were analyzed for DM (method 934.01),[23] crude protein (CP) by the Kjeldahl procedure (method 984.13),[23] ash (method 942.05),[23] aNDF with a heat stable amylase and expressed inclusive of residual ash,[24] indigestible NDF (iNDF) by *in situ* incubation for 288 h,[25] acid detergent fiber (ADF; assayed sequentially and expressed inclusive of residual ash) and lignin(sa) (expressed inclusive of residual ash).[26] Hemicellulose was calculated as aNDF minus ADF and cellulose as ADF minus lignin(sa). Samples of TMR were analyzed for starch,[27] and DM, CP, ash, aNDF, and ADF as described for forage samples.

Samples of rumen fluid and SC product were used to enumerate yeasts in malt extract agar (M137, Himedia®, Mumbai, India) acidified to pH 3.5 with lactic acid. Plates were incubated aerobically for 2 days at 30 °C. The number of colony forming units (CFU) was expressed as $\log_{10}$ CFU mL^{-1}.

After thawing the supernatant of rumen fluid samples, VFA were determined by gas chromatography (GCMS QP 2010 plus, Shimadzu, Kyoto, Japan) using a capillary column (Stabilwax, Restek, Bellefonte, PA, USA; 60 m, 0.25 mm ø, 0.25 µm crossbond carbowax polyethylene glycol). Ammonia and lactate were determined by colorimetric methods as described by Chaney and Marbach and Pryce respectively.[28–29]

Liquid–liquid extraction of rumen fluid metabolites

The fresh rumen fluid samples for metabolomics were first centrifuged at 1000×*g* for 30 min at 4 °C. Then, the supernatant was centrifuged at 13 000×*g* for 60 min at 4 °C. The clarified rumen fluid was filtered using a 0.45 and 0.22 µm pore size membrane filter (Merck Millipore; Burlington, MA, USA).

An aliquot (10 mL) was mixed with 10 mL of ethyl acetate and 1.0 g of NaCl for the liquid–liquid extraction (LLE). The solution was vortexed for 2 min and allowed to separate into two phases. The upper organic layer was decanted, and the bottom layer was mixed with another 10 mL of ethyl acetate and 1.0 g of NaCl. This procedure was repeated a third time. The organic phases were combined and concentrated under nitrogen flow. Similar procedures were repeated, but using ethyl acetate with 1% acetic acid (v:v); and ethyl acetate with 1% ammonium hydroxide (v:v). Extracts were kept at −20 °C until untargeted metabolomics analysis was performed.

Untargeted metabolomic analysis

Extracts were analyzed using an ultra-high performance liquid chromatograph (UHPLC) (Shimadzu, Nexera X2, Japan) coupled to a hybrid quadrupole time-of-fight high resolution mass spectrometry (Impact II, Bruker Daltonics Corporation, Germany) equipped with an electrospray ionization source. Chromatographic separation was performed with an Acquity UHPLC CSH C18 packed with 135 Å pore size, 1.7 µm particle size, 2.1 × 100 mm column (Waters, UK), at a flow rate of 0.2 mL·min^{-1}. The gradient mixture of solvents A (H_2O with 0.1% formic acid; v:v) and B (acetonitrile with 0.1% formic acid; v-v) was as follows: 5% B 0–1 min, 50% B 1–5 min, 95% B 5–10 min, maintained at 95% B 10–16 min, 5% B 16–18 min, and maintained at 5% B 18–21 min at 40 °C. The capillary voltage was operated in positive ionization mode, set at 4500 and 3000 V, respectively; with an end plate offset potential of −500 V. The dry gas parameters were set to 8 L·min^{-1} at 180 °C with a nebulization gas pressure of 4 bar. Data were collected from *m/z* 50 to 1300 with an acquisition rate of 5 Hz, and the four ions of interest were selected by auto MS/MS scan fragmentation. Molecular networking approach required the conversion of mass spectrometry raw data into mzXML file format followed by upload to the Global Natural Products Social Molecular Networking (GNPS) to generate the molecular networking.[30]

Statistical analysis

Statistical analysis were performed using the mixed procedure of the SAS 9.4 software (SAS Institute Inc., Cary, NC, USA, 2015).[31] Data regarding the DM intake determined on days 24 to 30 of each period were averaged per cow and analyzed using a model that included fixed effects of treatment, period, treatment × period, and random effects of cow and cow × treatment. Rumen fluid parameters (yeast count, ammonia, pH, VFA) were analyzed with the same model, including fixed effects of time and treatment × time. For the *in situ* assay, forages were assigned to one of three groups: corn silages, tropical grasses (sugarcane silage and tropical grass silage) and temperate grasses and alfalfa (oat

Table 4. Yeast count, pH, ammonia and VFA concentrations in the rumen fluid of non-lactating cows supplemented with or without live yeast

	Treatment			*P*-value[a]		
	Control	SC[b]	SEM	T	H	T × H
Yeast count ($\log_{10}$ CFU mL^{-1})	4.99	5.40	0.084	0.05	<0.01	0.37
Ammonia (mg dL^{-1})	11.0	9.39	0.50	0.10	<0.01	0.72
pH	6.13	6.24	0.031	0.09	<0.01	0.91
Acetate (mM)	72.4	69.4	2.33	0.44	0.01	0.96
Propionate (mM)	26.2	23.3	0.94	0.12	<0.01	0.98
Butyrate (mM)	13.1	12.2	0.41	0.23	<0.01	0.88
i-Butyrate (mM)	1.49	1.37	0.127	0.55	0.99	0.97
i-Valerate (mM)	0.53	0.56	0.021	0.38	<0.01	0.79
Valerate (mM)	1.30	1.16	0.050	0.15	<0.01	0.63
Total VFA (mM)	115	109	3.4	0.27	<0.01	0.99
Acetate: Propionate	2.82	3.02	0.045	0.05	<0.01	0.42
Lactate (mM)	0.64	0.47	0.027	0.03	<0.01	0.04

[a] T: effect of yeast supplementation, H: effect of hour after feeding, T × H: interaction between yeast and hour after feeding.
[b] *Saccharomyces cerevisiae* strain CNCM I-1077 supplied at 1 × 10^{10} CFU day^{-1}.

silage, ryegrass silage, alfalfa silage and alfalfa hay). Outcomes were analyzed with the model described for DM intake but including fixed effects of the forage group and the interaction between the forage group and treatment. The fixed effect of treatment × period was not significant for any variable ($P \geq 0.22$), and then removed from the model. Differences between treatments were declared if $P \leq 0.05$ and trends accepted if $0.05 < P \leq 0.10$. Metabolomics data were evaluated qualitatively for the presence or absence of putative compounds. Each spectrum was manually verified to check precursors and product ions.

RESULTS

The SC addition did not affect ($P = 0.53$) DM intake (10.1 versus 10.8 kg day^{-1} for control and SC, respectively). Cows supplemented with SC had higher ($P = 0.05$) counts of yeast in rumen fluid (Table 4). There was an interaction ($P = 0.04$) between yeast supplementation and time after feeding for lactate concentration (Fig. 1). At 8 h after feeding, cows receiving SC had a lower lactate concentration in rumen fluid ($P < 0.01$).

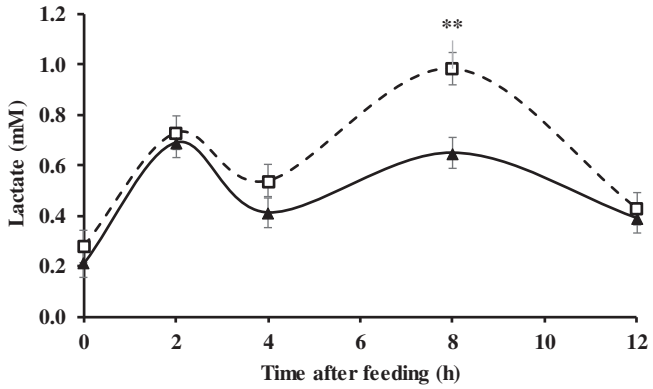

Figure 1. Ruminal lactate concentration in cows supplemented with (▲) or without (□) with live yeast. $P = 0.03$ for treatment, $P < 0.01$ for time, $P = 0.04$ for treatment × time. **$P < 0.01$ for control versus yeast supplementation at 8 h after feeding.

Cows fed SC had a higher ($P = 0.05$) acetate: propionate ratio in the rumen fluid, but concentrations of acetate, propionate, butyrate, iso-butyrate, iso-valerate, valerate, and total VFA did not differ between treatments ($P > 0.10$). There was a tendency for there to be lower ammonia ($P = 0.10$) and higher pH ($P = 0.09$) when SC were fed to cows.

There was no interaction ($P > 0.10$) between forage group and yeast supplementation for the *in situ* degradability of DM and aNDF (Table 5), but SC feeding increased aNDF ($P = 0.04$) and DM degradability ($P = 0.03$) at 24 h. There was a trend for higher DM ($P = 0.09$) and aNDF degradability ($P = 0.08$) at 12 h for SC. No differences occurred for *in situ* degradability of DM and aNDF at 36 h of incubation ($P > 0.10$).

Untargeted metabolomics revealed that a chemical entity with the putative molecular formula of $C_{17}H_{29}N_6O_3$ with *m/z* 365.2284 $[M + H]^+$ was completely suppressed (relative abundance zero) in the rumen fluid of all cows supplemented with SC. This compound was annotated in the family of lipids and related molecules. Chemical entities with *m/z* 198.0394 $[M + H]^+$ and 276.0283 $[M + H]^+$ from the organoheterocyclic compound family, *m/z* 355.2578 $[M + H]^+$ from the benzenoid family, *m/z* 373.2217 $[M + H]^+$, 359.2412 $[M + H]^+$ and 267.1942 from the lipids and related molecules, and *m/z* 253.1412 $[M + H]^+$ from the phenylpropanoids and polyketides were also exclusively observed in control cows.

DISCUSSION

Supplementation of live *Saccharomyces cerevisiae* strains has been reported to increase fiber degradation and animal performance.[15,16] Benefits of live yeast supplementation are often associated with improved rumen function due to factors such as reduced redox potential by oxygen scavenging,[32] higher ruminal

Table 5. Effect of live yeast and forage group on the ruminal degradability

	Treatment									
	Control			SC[a]				*P*-value[b]		
	Temp/ Leg[c]	Corn silage[c]	Trop. grass[c]	Temp/ Leg[c]	Corn silage[c]	Trop. grass[c]	SEM	T	G	T × G
DM[d] degradability (g kg^{-1} DM)										
12 h	476	494	249	496	516	271	15.1	0.09	<0.01	0.99
24 h	560	567	310	594	597	329	15.3	0.03	<0.01	0.85
36 h	666	652	391	669	661	391	17.8	0.77	<0.01	0.97
aNDF[e] degradability (g kg^{-1} NDF)										
12 h	194	108	89	218	127	105	14.9	0.08	<0.01	0.89
24 h	314	210	162	350	250	178	19.0	0.04	<0.01	0.70
36 h	461	355	260	473	366	252	23.4	0.78	<0.01	0.87

[a] *Saccharomyces cerevisiae* strain CNCM I-1077 supplied at 1×10^{10} CFU day^{-1}.
[b] T: effect of yeast supplementation, G: effect of forage group, T × G: interaction between yeast supplementation and forage group.
[c] Forage group: Temp/Leg – oat silages, ryegrass silages, alfalfa silage and alfalfa hay; Trop. grass – sugarcane silages and tropical grass silages; Corn silage – corn silages.
[d] Dry matter.
[e] Neutral detergent fiber.

pH due to decreased lactic acid production and increased utilization of lactic acid,[33–36] higher availability of growth factors (e.g., organic acids and vitamins)[15,37–39] and stimulation of rumen microbial growth.[21,40–41] However, potential benefits of live yeast supplementation on ruminal degradability of forages grown in tropical and subtropical areas are seldom reported and there is no information on the potential improvement in DM, and aNDF degradation varies by forage group in dairy cows, whether they are lactating or not.

An important result in this study was the higher rumen *in situ* degradability of aNDF in cows fed SC, especially in early incubation times (i.e., 24 h), which is in line with the modifications in the rumen environment. Cows fed SC had higher counts of yeasts, tended to have higher pH values, and lower concentrations of ammonia and lactate at comparable concentrations of VFA in the rumen fluid. These findings indicate that SC improved the rumen environment and might have stimulated the growth of cellulolytic and anaerobic bacteria and hastened their colonization onto fiber particles.[42,43] The increase in rumen pH in animals supplemented with SC is attributable at least partly to the lower concentration of lactate and may have increased activity of fibrolytic bacteria and fungi in the ruminal digesta,[14,41,42] resulting in the greater aNDF degradability in such cows. The concomitant reduction of ammonia nitrogen suggests a greater uptake of the available nitrogen pool in the rumen by the fibrolytic bacterial populations, which are known to utilize ammonia as an important nitrogen source.[19]

Although there was no difference in the concentration of total VFA and most individuals' VFA, cows fed SC had a lower concentration of lactate and higher acetate: propionate ratio due to a numerical reduction in propionate concentration. In the rumen, propionate is synthetized *via* succinyl-CoA and acrylyl-CoA pathways.[44] In the latter pathway, lactic acid produced by rumen bacteria or ingested with fermented feedstuffs can be converted to propionate by lactate-fermenting bacteria.[45] Yeast supplementation has been associated with either a decreased production or increased utilization of lactic acid.[33–36] In our study, although lactate concentrations were relatively low (<1 mM), indicating non-acidotic conditions in our non-lactating cows, two lactate peaks were detected in the rumen fluid. The first occurred immediately after feeding, reflecting the intake of lactic acid present in the corn silage. At 8 h after feeding, lactate concentration increased again, likely as an intermediate of ruminal fermentation, which coincided with the highest concentrations of VFA and the pH nadir (not shown). Though the size of the first peak immediately after feeding did not differ across treatment, that for SC cows was much lower at 8 h than the peak for control cows, in support of previous studies in which SC reduced lactate concentration in cows. Hence, the lower lactate concentration at the second peak associated with the numerical trend of lower propionate concentration suggest that decrease in lactate might have occurred due to a lower production of lactate rather than a stimulation of lactate utilizer microorganisms. The higher acetate: propionate ratio might have been a consequence of this pattern. The numerically lower concentration of valerate is in line with the numerically lower concentration of propionate, due to fact that valerate is partially formed by the condensation of propionate and acetate.[46] Moreover, the greater fiber degradation in cows fed SC may have contributed to their greater acetate: propionate ratio. This is likely due to stimulation of ruminal fibrolytic microorganisms, which generally increases the molar proportion of acetate to a greater extent than non-fiber fermenting ruminal microorganisms.[44,47] In our study, these benefits were observed even in a non-acidogenic diet. Thus, SC supplementation is likely to assist the ruminal fermentation in dairy cows having high DM intake of diets with great proportion of rumen fermentable carbohydrates. De Ondarza *et al*. reported greater milk yield for cows supplemented with live yeast than non-supplemented cows and with the effect being greater for cows <100 days in milk than for cows >100 days in milk.[16]

It has been claimed that forage type can influence the SC effect on ruminal fiber degradation. Guedes *et al*.[36] described a larger response to SC supplementation in corn silages of lower fiber degradability than in corn silages with higher fiber degradability *in situ*. Recently, Sousa *et al*.[21] reported a higher relative benefit of SC for tropical forages of lower fiber degradability. However, the absolute increase in fiber degradability ($g \cdot kg^{-1}$) reported by these authors was higher in forages with increased quality, with higher increase in fiber degradability in Palisade grass (+25 $g \cdot kg^{-1}$), Guineagrass (+ 23 $g \cdot kg^{-1}$), and corn silage (+ 26 $g \cdot kg^{-1}$), than in sugarcane silage (+ 17 $g \cdot kg^{-1}$) and bermudagrass hay (+ 19 $g \cdot kg^{-1}$). In fact, Chaucheyras-Durand *et al*.[14] showed a higher benefit for SC after 24 h on fiber degradation for soybean hulls (+ 32.8 $g \cdot kg^{-1}$) with a higher fiber digestibility than in wheat bran (+ 20.7 $g \cdot kg^{-1}$), alfalfa hay (+17.6 $g \cdot kg^{-1}$), or wheat silage (+14.0 $g \cdot kg^{-1}$) with lower digestibility coefficients. As SC benefits are likely based on increased fibrolytic activity by stimulation of bacteria and fungi, acting synergistically,[14] it seems likely that plant tissues with greater recalcitrance would be more affected than those with a less lignified cell wall by SC supplementation, at least under realistic digesta retention times.

In the current trial, there was no interaction between forage group and yeast supplementation for *in situ* degradability; meanwhile, the SC supplementation increased *in situ* degradability of DM by 4.6% at 24 h of incubation, mainly due to an increase of aNDF degradability by 10.3% at 24 h of incubation. Previous studies have indicated that fiber degradation is better predicted assuming that fiber is the sum of iNDF and potentially digestible neutral detergent fiber, and that potential digestible fiber is represented by two digestible fractions, with rapidly degradable and slowly degradable fractions.[48–50] In our study, it is likely that the degradation rate of aNDF of the forage sources was faster when SC was fed. Several reports have suggested that SC supplementation increases the rate of fiber degradation, with a more pronounced effect at shorter incubation times.[21,33,51,52] The lack of difference between control and SC that was observed when the forage samples were incubated for 36 h suggests that SC acted on the degradation of the potential digestible fiber fast pool.

Non-targeted metabolomics was used as an exploratory approach to prospect ruminal chemical entities affected by SC supplementation. Interestingly, some compounds were suppressed in the rumen of cows that received SC. Among those was one having the molecular formula of $C_{17}H_{29}N_6O_3$ and *m/z* 365.2284 $[M + H]^+$, which was putatively annotated as lipid and related molecules. This opens a new window of opportunity to understand the mechanisms of improved ruminal metabolism upon SC supplementation. Recently, rumen metabolites involved in linoleic and alpha-linolenic metabolism were linked to feed efficiency in beef[53] and dairy cattle.[54] De Ondarza *et al*.[16] reported in a multi-study analysis greater milk fat yield and higher feed efficiency for cows supplemented with SC compared with cows not supplemented. Collectively, those findings may suggest that the ruminal lipid metabolism, including the biohydrogenation pathways, may be involved on the improved rumen function in cows

receiving SC. In future studies, the integration of rumen metabolomics with other omics techniques is expected to assist describing key metabolic pathways that ultimately improve the ruminal function.

CONCLUSIONS

For dairy cows fed a corn silage based TMR, *Saccharomyces cerevisiae* strain CNCM I-1077 supplementation decreased ruminal lactate concentrations and increased DM and aNDF degradability after 24 h of incubation regardless of forage group.

ACKNOWLEDGEMENTS

The authors thank the GESF team and the staff of the Iguatemi Experimental Farm (FEI-UEM) for assistance with the study. We gratefully acknowledge financial support from Lallemand SAS (Blagnac, France).

CONFLICT OF INTEREST

The authors declare no real or perceived conflicts of interest. The co-authors from the live yeast supplying company (L.J.M, E.C. and F.C.D.) have only been associated with the design of the study.

REFERENCES

1 Mertens DR, Creating a system for meeting the fiber requirements of dairy cows. *J Dairy Sci* **80**:1463–1481 (1997).
2 Huhtanen P, Nousiainen J and Rinne M, Recent developments in forage evaluation with special reference to practical applications. *Agric Food Sci* **15**:293–323 (2006).
3 Mertens DR, Regulation of forage intake, in *Forage Quality, Evaluation, and Utilization*, ed. by Fahey GC Jr. American Society of Agronomy, Madison, WI, pp. 450–493 (1994).
4 Allen MS, Effects of diet on short-term regulation of feed intake by lactating dairy cattle. *J Dairy Sci* **83**:1598–1624 (2000).
5 Wilson JR, Deinum B and Engels FM, Temperature effects on anatomy and digestibility of leaf and stem of tropical and temperate forage species. *Neth J Agr Sci* **39**:31–48 (1991).
6 Moore KJ and Jung HJG, Lignin and fiber digestion. *J Range Manage* **54**: 420–430 (2001).
7 Crasta OR and Cox WJ, Temperature and soil water effects on maize growth, development yield, and forage quality. *Crop Sci* **36**:341–348 (1996).
8 Cone JW and Engels FM, Influence of growth temperature on anatomy and in vitro digestibility of maize tissues. *J Agric Sci* **114**:207–212 (1990).
9 Arriola KG, Oliveira AS, Ma ZX, Lean IJ, Giurcanu MC and Adesogan AT, A meta-analysis on the effect of dietary application of exogenous fibrolytic enzymes on the performance of dairy cows. *J Dairy Sci* **100**:4513–4527 (2017).
10 Klopfenstein T, Chemical treatment of crop residues. *J Anim Sci* **46**: 841–848 (1978).
11 Chaudhry A, Rumen degradation *in sacco* in sheep of wheat straw treated with calcium oxide, sodium hydroxide and sodium hydroxide plus hydrogen peroxide. *Anim Feed Sci Technol* **83**:313–323 (2000).
12 Wallace RJ, Ruminal microbiology, biotechnology and ruminant nutrition: progress and problems. *J Anim Sci* **72**:2992–3003 (1994).
13 Chaucheyras-Durand F, Ameilbonne A, Walker ND, Mosoni P and Forano E, Effect of a live yeast *Saccharomyces cerevisiae* I-1077 on *in situ* ruminal degradation of alfalfa hay and fibre-associated microorganisms. *J Anim Sci* **88**:145 (2010).
14 Chaucheyras-Durand F, Ameilbonne A, Bichat A, Mosoni P, Ossa F and Forano E, Live yeasts enhance fibre degradation in the cow rumen through an increase in plant substrate colonisation by fibrolytic bacteria and fungi. *J Appl Microbiol* **120**:560–570 (2016).
15 Chaucheyras-Durand F, Walker ND and Bach A, Effects of active dry yeasts on the rumen microbial ecosystem: past, present and future. *Anim Feed Sci Technol* **145**:5–26 (2008).
16 De Ondarza MB, Sniffen CJ, Dussert L, Chevaux E, Sullivan J and Walker N, Case study: mutiple-study analysis of the effect of live yeast on milk yield, milk component content and yield and feed efficiency. *Prof Anim Sci* **26**:661–666 (2010).
17 Mosoni P, Chaucheyras-Durand F, Béra-Maillet C and Forano E, Quantification by real-time PCR of cellulolytic bacteria in the rumen of sheep after supplementation of a forage diet with readily fermentable carbohydrates: effect of a yeast additive. *J Appl Microbiol* **103**: 2676–2685 (2007).
18 Desnoyers M, Giger-Reverdin S, Bertin G, Duvaux-Ponter C and Sauvant D, Meta-analysis of the influence of *Saccharomyces cerevisiae* supplementation on ruminal parameters and milk production of ruminants. *J Dairy Sci* **92**:1620–1632 (2009).
19 Chaucheyras-Durand F and Fonty G, Establishment of cellulolytic bacteria and development of fermentative activities in the rumen of gnotobiotically-reared lambs receiving the microbial additive *Saccharomyces cerevisiae* CNCM I-1077. *Reprod Nutr Dev* **41**:57–68 (2001).
20 Cabrera EJI, Mendoza MGD, Aranda IE, Garcia-Bojalil C, Bárcena GR and Ramos JJA, *Saccharomyces cerevisiae* and nitrogenous supplementation in growing steers grazing tropical pastures. *Anim Feed Sci Technol* **83**:49–55 (2000).
21 Sousa DO, Oliveira CA, Mari LJ, Chevaux E, Velasquez AV, Souza JM *et al*., Live yeast supplementation improves rumen fibre degradation in cattle grazing tropical pastures throughout the year. *Anim Feed Sci Technol* **236**:149–158 (2018).
22 Krizsan SJ, Ahvenjarvi S and Huhtanen P, A meta-analysis of passage rate estimated by rumen evacuation with cattle and evaluation of passage rate prediction models. *J Dairy Sci* **93**:5890–5901 (2010).
23 Association of Official Analytical Chemists – AOAC, *Official Methods of Analysis*, 15th edn. Association of Official Analytical Chemists, Inc, Arlington, VA (1990).
24 Mertens DR, Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. *J AOAC Int* **85**:1217–1240 (2002).
25 Huhtanen P, Kaustell K and Jaakkola S, The use of internal markers to predict total digestibility and duodenal flow of nutrients in cattle given six different diets. *Anim Feed Sci Technol* **48**:211–227 (1994).
26 Van Soest PJ, Collaborative study of acid-detergent fiber and lignin. *J AOAC Int* **56**:781–784 (1973).
27 Hall MB, Determination of dietary starch in animal feeds and pet food by an enzymatic-colorimetric method: collaborative study. *J AOAC Int* **98**:397–409 (2015).
28 Chaney AL and Marbach EP, Modified reagents for determination of urea and ammonia. *Clin Chem* **8**:130–137 (1962).
29 Pryce JD, A modification of Barker-Summerson method for the determination of lactic acid. *Analyst* **94**:1151–1152 (1969).
30 Wang M, Carver J, Phelan V, Sanchez LM, Garg N, Peng Y *et al*., Sharing and community curation of mass spectrometry data with global natural products social molecular networking. *Nat Biotechnol* **34**:828–837 (2016).
31 SAS Institute Inc, *Base SAS® 9.4 Procedures Guide*, 5th edn. SAS Institute Inc, Cary, NC (2015).
32 Marden JP, Julien C, Monteils V, Auclair E, Moncoulon R and Bayourthe C, How does live yeast differ from sodium bicarbonate to stabilize ruminal pH in high-yielding dairy cows? *J Dairy Sci* **91**: 3528–3535 (2008).
33 Williams PE, Tait CA, Innes GM and Newbold CJ, Effects of the inclusion of yeast culture (*Saccharomyces cerevisiae* plus growth medium) in the diet of dairy cows on milk yield and forage degradation and fermentation patterns in the rumen of steers. *J Anim Sci* **69**:3016–3026 (1991).
34 Chaucheyras F, Fonty G, Gouet P, Bertin G and Salmon JM, Effects of a strain of *Saccharomyces cerevisiae* (Levucell® SC), a microbial additive for ruminants, on lactate metabolism in vitro. *J Anim Sci* **42**:927–933 (1996).
35 Chaucheyras-Durand F, Masséglia S and Fonty G, Effect of the microbial feed additive *Saccharomyces cerevisiae* CNCM I-1077 on protein and peptide degrading activities of rumen bacteria grown in vitro. *Curr Microbiol* **50**:96–101 (2005).
36 Guedes CM, Gonçalves D, Rodrigues MAM and Dias-da-Silva A, Effects of a *Saccharomyces cerevisiae* yeast on ruminal fermentation and fiber degradation of maize silages in cows. *Anim Feed Sci Technol* **145**:27–40 (2008).
37 Jouany JP, Optimizing rumen functions in the close-up transition period and early lactation to drive dry matter intake and energy balance in cows. *Anim Reprod Sci* **96**:250–264 (2006).

38 Salvati GG, Morais Júnior NN, Melo AC, Vilela RR, Cardoso FF, Aronovich M *et al*., Response of lactating cows to live yeast supplementation during summer. *J Dairy Sci* **98**:4062–4073 (2015).
39 Dias JDL, Silva RB, Fernandes T, Barbosa EF, Graças LEC, Araujo RC *et al*., Yeast culture increased plasma niacin concentration, evaporative heat loss, and feed efficiency of dairy cows in a hot environment. *J Dairy Sci* **101**:5924–5936 (2018).
40 Newbold CJ, Wallace RJ and McIntosh FM, Mode of action of the yeast *Saccharomyces cerevisiae* as a feed additive for ruminants. *Br J Nutr* **76**:249–261 (1996).
41 Moya D, Ferret A, Blanch M, Fuentes MC, Fandiño JL and Calsamiglia S, Effects of live yeast (*Saccharomyces cerevisiae*) and type of cereal on rumen microbial fermentation in a dual flow continuous culture fermentation system. *J Anim Physiol Anim Nutr* **102**: 1488–1496 (2018).
42 Harrison GA, Dawson KA, Barker KB, Harmon RJ and Hemken RW, Influence of addition of yeast culture supplement to diets of lactating cows on ruminal fermentation and microbial populations. *J Dairy Sci* **71**:2967–2975 (1988).
43 Erasmus LJ, Botha PM and Kistner A, Effect of yeast culture supplement on production, rumen fermentation, and duodenal nitrogen flow in dairy cows. *J Dairy Sci* **75**:3056–3065 (1992).
44 Russell JB and Wallace RJ, Energy yielding and consuming reactions, in *The Rumen Microbial Ecosystem*. Elsevier Science Publishers, New York, NY, pp. 185–215 (1988).
45 Mackie RI, Gilchrist FM and Heath S, An *in vivo* study of ruminal microorganisms influencing lactate turnover and its contribution to volatile fatty acid production. *J Agric Sci* **103**:37–51 (1984).
46 Gray FV, Pilgrim AF, Rodda HJ and Weller RA, The nature and origin of the volatile fatty acids in the rumen of the sheep. *J Exp Biol* **29**:57–68 (1952).
47 Wolin MJ and Miller TL, Microbe-microbe interaction, in *The Rumen Microbial Ecosystem*. Elsevier Science Publishers, New York, NY, pp. 343–361 (1988).
48 Ellis WC, Mahlooji M and Matis JH, Models for estimating parameters of neutral detergent fiber digestion by ruminal microorganisms. *J Anim Sci* **83**:1591–1601 (2005).
49 Huhtanen P, Seppälä A, Ahvenjärvi S and Rinne M, Prediction of in vivo neutral detergent fiber digestibility and digestion rate of potentially digestible neutral detergent fiber: comparison of models. *J Anim Sci* **86**:2657–2669 (2008).
50 Raffrenato E, Nicholson CF and Van Amburgh ME, Development of a mathematical model to predict pool sizes and rates of digestion of 2 pools of digestible neutral detergent fiber and an undigested neutral detergent fiber fraction within various forages. *J Dairy Sci* **102**: 351–364 (2019).
51 Girard ID and Dawson KA, Stimulation of ruminal bacteria by different fractions derived from cultures of *Saccharomyces cerevisiae* strain 1026. *J Anim Sci* **73**:264 (1995).
52 Callaway ES and Martin SA, Effects of a *Saccharomyces cerevisiae* culture on ruminal bacteria that utilize lactate and digest cellulose. *J Dairy Sci* **80**:2035–2044 (1997).
53 Artegoitia VM, Foote AP, Lewis RM and Freetly HC, Rumen fluid metabolomics analysis associated with feed efficiency on crossbred steers. *Sci Rep* **7**:2864 (2017).
54 Li YQ, Xi YM, Wang ZD, Zeng HF and Han ZY, Combined signature of rumen microbiome and metabolome in dairy cows with different feed intake levels. *J Anim Sci* **98**:skaa070 (2020).

