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EFFECT OF S*ACCHAROMYCES CEREVISIAE* STRAIN CNCM I-1077 ON THE RUMINAL DEGRADABILITY OF FORAGES FROM SOUTH AMERICA

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“Ain't about how fast I get there,
Ain't about what's waiting on the other side,
It's the climb”
Jon Mabe and Jessi Alexander

To my mother, who always struggled to give me the best education

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BIOGRAFY

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In March of 2017 she joined the Graduate Program in Animal Science at the State University of Maringá. In April of 2019 she submitted to the examining board in order to receive the title of Master’s in Animal Science.

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ABSTRACT

The effect of live yeast *Saccharomyces cerevisiae* strain CNCM I-1077 (SC) on the ruminal degradability of forages commonly found in dairy diets in South America was evaluated. Four non-lactating rumen-cannulated Holstein cows were housed in a tie-stall barn and randomly assigned to two treatment sequences: Control-SC-Control or SC-Control-SC, in a switchback design, with three 30-d periods. Cows in the SC treatment were supplied with 1×10^{10} cfu of yeast daily via rumen cannula. The in situ degradability of DM and NDF was measured in 16 forage samples collected in Brazil, Argentina, Colombia and Peru, and included corn silage (n = 5), tropical grass silage (n = 2), sugarcane silage (n = 2), oat silage (n = 2), ryegrass silage (n = 2), alfalfa silage (n = 1), alfalfa hay (n = 1) and kikuyu (n = 1). Each forage was incubated in the rumen for 0, 12, 24, 36 and 120 h after feeding. Rumen fluid was collected from the ventral sac for measuring yeast count, pH, ammonia and VFA. Cows supplemented with SC had higher counts of live yeasts in rumen fluid, showed a trend of higher ruminal pH and lower ruminal ammonia concentration. Acetate to propionate ratio was higher in the rumen fluid of animals receiving SC. Live yeast supplementation decreased the pool size of fraction C (undegradable) and increased fraction B (potentially degradable) of DM and NDF. Furthermore, SC accelerated the DM and NDF degradation, as noticed by higher disappearance of DM and NDF at 12 and 24 h of incubation. Live yeast supplementation is a strategy to improve rumen function and increase the nutritive value of forages grown in tropical and subtropical areas.

I. INTRODUCTION

Feeding is the costliest factor of animal production and might represent almost 80% of the total production costs in dairy and beef operations (USDA, 2018). Hence, the efficiency of converting feedstuffs in human foods, such as milk and meet, have a high impact on animal production systems. Since ruminant diets typically contain a certain amount of forage, fiber digestibility is a crucial point in ruminant nutrition.

Cellulose and other structural polysaccharides present in the plant cell wall are the major source of energy for herbivorous animals fed forage-based diets, due to the symbiosis between these animals and microorganisms present in the rumen (Weimer, 1992). The main fermentation products of these components are volatile fatty acids (VFA), mainly acetate, propionate and butyrate, as well as gases, carbon dioxide and methane. In addition, the protein deamination process performed by some microorganisms can produce ammonia, microbial protein, VFA and carbon dioxide (Bergman, 1990).

The action of the microorganisms on plant degradation is dependent on the quality and accessibility to the plant cell wall matrix. These factors are related to the maturity, genetics, chemical and physical composition of tissues (Akin, 1989). Thus, lower quality plants have lower ruminal degradability and are not used efficiently for animal production. In this way, the use of feed additives such as probiotics may be a strategy to enhance feed efficiency, animal performance and health (Chaucheyras-Durand et al., 2008).

1. Literature Review

1.1. Forage Quality

Forage quality is a relative term to describe the degree to which forage meets the nutritional requirements of a specific kind and class of animal (Allen et al., 2011). Hence, quality is associated to animal response and, for instance, can be measured by weight gain and milk yield. Since animal performance is strongly related to intake of digestible nutrients, forage quality is mainly a function of intake and digestibility (Paterson et al., 1994).

Because cell wall is the single largest component of forages, fiber content and digestibility are primary determinants of forage quality. The plant cell wall is a complex matrix of polymers that surrounds every plant cell. Walls provide the physical support required for plants to grow and serve as a barrier to attack by pathogens and insects. While all cell walls share basic chemical characteristics, marked differences exist among plant tissues in terms of cell wall concentration, composition, and structural organization (Jung, 2012).

1.1.2. Factors affecting ruminal digestibility

There are several factors that affect the structure and quality of the forage plant, which may be due to environmental factors and factors inherent in the plant itself. Factors such as soil quality, temperature, solar radiation, water availability, cultivars and maturity are related to affect the characteristics of the same plant species (Ball et al., 2001).

1.1.2.1 Chemical Composition

Lignin and polysaccharides (cellulose, hemicellulose and pectin) are the main compounds of the plant cell wall matrix, in addition to proteins, phenolic compounds, water and minerals (Åman, 1993). Those polymers can be divided into two categories based on their associations with other compounds and availability to the animal: those that have some covalent attachment to core lignin and are incompletely digested in the rumen and those that are poorly covalently attached to core lignin and largely fermentable in the rumen (Van Soest, 1994).

1.1.2.1.1. Cellulose

Cellulose is a homopolymer formed by β-D-glucose 1 → 4 bonds which build long chains with high degree of polymerization and high molecular weight. These chains can bind through hydrogen bonds forming cellulose microfibrils, which has great value for the availability of this molecule to microbial enzymatic hydrolysis during ruminal degradation (Iiyama et al., 1993; Delmer and Amor, 1995). Cellulose, in its majority, is found in combination with other components of the plant wall, such as hemicellulose and lignin. Cellulose can be separated into two fractions, the potentially digestible and the indigestible, can be found in several plant constituents and their amount varies between them and between species (Giger-Reverdin, 1995; Pereira, 2013).

1.1.2.1.2. Hemicellulose

Hemicellulose is a heteropolysaccharide that is found in the cell wall. Hemicellulose is characterized by several units of amorphous sugars linked by different types of bonds. Their chains have a lower degree of polymerization when compared to cellulose (and not as resistant to solubilization and hydrolysis) but are commonly found associated to lignin by covalent bonds. They occur in various structural types and are divided into four subgroups: xylan, γ-glycan, xyloglycan, and mannan, being named according to the predominant monosaccharide (Giger-Reverdin, 1995; Ebringerová et al., 2005).

1.1.2.1.3. Pectin

Pectin is a polymer formed by complex polysaccharides, found in the middle lamella, and has the function of hydrating and cellular adhesion. In addition, pectin can play a role on the firmness of the cell, but it depends on the orientation, proprieties and connections among cellulose and pectic substances. Its content decreases from the primary to secondary wall, in the direction of plasma membrane. Grasses have a low pectin content when compared to legumes. It is one of the components of the cell wall that has low molecular weight and is highly digestible. Pectin is a non-fiber carbohydrate,

due to its solubility in neutral detergent (Van Soest, 1994; Thakur et al., 1997; Lempp, 2013).

1.1.2.1.4. Lignin

Lignin is a phenolic polymer composed of highly branched phenylpropanoids, unique to vascular land plants (Adler, 1977). Lignin is deposited on the cell wall during the secondary wall formation to confer thickening and protection, it is generally related to the indigestible fraction of the forages (Jung and Deetz, 1993). The denomination is used to describe groups of polymers with three aromatic alcohols (p-coumaril, coniferil and synapil). The terms "core" and "non-core" are used to differentiate the types of lignin found in forages (Jung, 1989; Susmel and Stefanon, 1993).

Core lignin generally has two or more bonds between phenolic monomers units, has high molecular weight and it is highly condensed. On the other hand, non-core lignin has a low molecular weight, a covalent bond on the phenolic compound and is generally bound to the hemicellulose fraction in the secondary cell wall (Jung, 1989; Van Soest, 1994). According to Hartley (1972) the p-coumaric acid, generally related to less digestible materials, has a higher concentration in non-core lignin, which possibly demonstrates that this type of lignin has a greater effect on animal nutrition. However, Wilson (1994) believes that this division presents little importance for the study of digestibility since both types have an effect on fiber degradability.

1.1.2.2. Morphology

Forages are complex organisms that consist of leaf, stem, inflorescence, and root and its cell walls differentiated structurally and chemically according to their functions within the plant. Thus, densely clustered, thick-walled and lignin-rich cells can be found in tissues that have function linked to lift, whereas thin-walled and lignin-free cells may be related to biochemical processes of carbon assimilation (Wilson, 1994; Paciullo, 2002).

Three forms of vegetal cell wall are found: primary, secondary and tertiary. The primary wall has a thickness of approximately 0.2 μm and its development occurs during the cell growth, and may be the only wall to develop, as in the parenchyma. The

secondary wall develops internally to the primary wall after complete cellular expansion and gives the cell protection to tension and compression due to its lignification, being able to reach a thickness of 5 μm. Finally, the tertiary wall is located internally of the secondary wall and is characterized as being membranous and thin (Wilson, 1993).

According to Akin (1989), tissues can be classified as: quickly digested, partially or slowly digested and nondigestible tissues. Some plant tissues can be rapidly degraded by ruminants as result of no physical barrier to digestion. Other tissues can vary in digestibility, showing partial resistance or not to ruminal microorganisms and this difference may be a result of stressful situation or even maturity (e.g. high temperature and hydric stress) increasing lignin and phenolic complexes. Forages with large proportions of sclerenchyma and xylem cells in leaf blades, and epidermis, sclerenchyma ring (grasses) or interbundular cells (legumes), and xylem in stems has generally low rates of digestion, showing that this tissues generally form structural barriers, being nondigestible for ruminants (Akin, 1989).

In tropical forage leaves, the tissues that have fast digestion are mesophilic and phloem, the epidermis and parenchymatic sheath of the bundles have an intermediate digestibility, and the xylem and sclerenchyma are not accessible. In temperate forage leaves, in addition to the mesophyll and the phloem, the epidermis has a high rate of digestion, while the parenchymatic sheath of the bundles can be rapidly digested depending on its species, and as in the tropics, the xylem and the inner sheath of the bundles are indigestible. For grasses, the epidermis and ring of sclerenchyma are nondigestible, the parenchyma can be rapidly degraded or depending on its maturity and the phloem is rapidly degraded. Finally, in legumes the mesophyll is rapidly degraded in leaflets and vascular tissues in general are indigestible. In legume steam, the digestibility of the parenchyma is dependent on its maturity, and xylem is not accessible for ruminant digestibility (Akin, 1989).

Strongly related, the anatomical characteristics of the plant and its nutritional value are shown as good indicators of food quality, where the proportion of tissues and thickness of the cell wall are the main characteristics that affect animal use. The lignified and highly fibrous tissues have low digestibility (Allinson and Osbourn, 1970; Carvalho and Pires, 2008). The difficulty of lignin degradation can be related to several factors, such as the physical impediment caused by the binding of lignin with polysaccharides that may hind the access of the enzymes, hydrophobicity caused by lignin polymers that

limit the action of fibrolytic enzymes, and a possible toxic effect of lignin components on ruminal microorganisms (Jung and Deetz; Susmel and Stefanon, 1993). Jung (1989) reported that there was a negative correlation between lignin core and in vitro fermentation. The p-coumaric acid is esterified in the core-lignin, where in experiments using its free form, its presence reduced activities of cellulolytic microorganisms, decreased bacterial growth rate and reduced fungal activity. Beyond that, the ferulic acid is primarily esterified in hemicellulose, and at experimental levels it was correlated with decrease in degradation in vitro. It was also observed that cinnamic acids had a significant reduction in digestibility. However, the toxicity caused by these acids is unlikely due to their low concentration in forage and ruminal environment and the bacteria have detoxification mechanisms (Paciullo, 2002).

The biggest limitation of forage lignification apparently is due to its physical impediment to the action of the hydrolytic enzymes at the carbohydrate center of reaction, where the concentration, ramification and association with other carbohydrates of the lignin causing negative effects on its degradation (Jung and Deetz, 1993). Moreover, the thickness of the cell wall is a physical factor inhibiting to digestion, where the largest thickness of the secondary wall, the smaller is the access of the microorganisms and the longer the time necessary for its complete digestion (Carvalho and Pires, 2008).

Other characteristics that may be related to forage quality are the anatomical characteristics that show the proportion and disposition of lignified and non-lignified tissues within the plant, as well as physiological characteristics such as efficiency in the carbon cycle. With increasing forage age, the most are lignified components and there are lost in the nutritive value within foliar sheaths and stems, as they increase the parenchyma tissue, and can be affected by the environment and the species (Lempp, 2013).

Epidermall cells, such as cell rich of silica and bulliform cells, have negative effects on cell degradation. Silica confers stiffness to the cell and bulliform cells are more resistant to ruminal degradation and occupy large space in the leaf blade. In addition, the epidermis may present cuticle and cutin that resist colonization of the microorganisms (Wilson, 1993; Paciullo, 2002; Lempp, 2013).

Although grasses have a lower lignin content, they have a lower rate of degradability when compared to other species. One of the plausible explanations is that there are lignin binds through xylose and arabinose covalently to the hemicellulose, hampering its ruminal degradation (Jung, 1989). Compared to the C3 and C4 plants, the

first one has a greater advantage in relation to its qualitative potential, because it has a lower elongation of stem coarseness, slides with lower proportion of lignifiable tissues, lower levels of neutral detergent fiber (NDF) and lignin. In addition, C4 plants exhibit Girder cells, which cause thickening of well-developed veins and parenchymal cells, thereby decreasing their rate of degradation (Paciullo, 2002; Lempp, 2013).

1.1.3. Ways to Improve Forage Degradability

Although there are intrinsic factors in plants that hinder access and degradability by the ruminal microorganisms, there are ways to reverse them such as the use of different genotypes of forages, use of plants with different maturities, use of exogenous substances capable of cleaving cell walls (e.g. chemicals, enzymes), and supply of additives able to enhance the ruminal environment and potentialize the action of fibrolytic microorganisms.

Several studies have been carried out with the aim of improving the forage composition through genetic selection and manipulation. The composition can be altered by modifying the concentration and composition of lignin, by the quality of the protein, decreasing anti-nutritional factors and thereby increasing its nutritional value (Casler, 2004). In addition, with the advancement of maturity the fiber content in the plant is increased, making it less digestible (Raymond, 1969). Salazar et al. (2010), in an experiment carried out at the Agronomic Institute in Campinas-SP, evaluating the effect of 15 maize hybrids at different maturity stages (harvested with 90, 120 and 150 days post-germination), observed that there was an increase in lignin deposition at maturity, and there was a difference between the hybrids used, suggesting a great variability among the genetic groups and maturity.

Exogenous substances may also be used to improve forage digestibility. Exogenous enzymes can be used at the time of feeding or during the ensiling process, hydrolyzing the cell wall in readily fermentable sugars for silo and rumen microorganisms (Adesogan, 2005). Alkalizing agents (sodium hydroxide (NaOH), calcium hydroxide ($Ca(OH)_2$), anhydrous ammonia (NH_3) and calcium oxide (CaO)) partially solubilize the hemicellulose and damage the hydrogen bonds, increasing fiber digestion (Oliveira et al., 2002; Andrade et al., 2007; Mota et al., 2010).

Another way of changing forage degradability is by manipulating the ruminal environment. Due to the importance of ruminal digestion, the manipulation of fermentation is a tool that allows making the system more efficient, for instance by increasing the transformation of fibrous compounds into nutrients for the synthesis of meat and milk (Wallace, 1994; Arcuri and Mantovani, 2006; Mantovani and Bento, 2008).

Among additives used for ruminants, pre- and probiotics, which normally contain live strains of microorganisms, inactivated microorganisms or microbial cell fractions, potentially may benefit the indigenous microbiota (Martin and Nisbet, 1992). Benefits on gut bacteria population and animal immune response has been reported (Rose, 1987). In addition, biological additives do not generate residues into the final products, being an interesting alternative to the traditional additives.

1.2.Yeast effect on the Ruminal Environment

1.2.1. Yeast Characterization

Yeasts are eukaryotic cells, belonging to the *Fungi* kingdom with nuclear membrane and cell walls. Measuring between 3 and 10 μm, they have the capacity to produce energy and soluble forms of nutrients from any organic matter source, being denominated heterotrophic (Bennett, 1998). Through enzymes, yeasts digest proteins, glucose and lipids, and can absorb amino acids and monosaccharides from their cell membrane. They are considered facultative anaerobes, where, in the presence of oxygen converts sugars into carbon dioxide and energy and in its absence produce ethanol (Walker and White, 2005).

A very widespread use of yeast in animal production is in the form of active dry yeast products (ADY), which preserves the viability and metabolic activity of the cell and have a high concentration of viable cells (> 10 billion cfu/g). There are about 500 different yeast species with morphological, metabolic and reproductive differences. *Saccharomyces cerevisiae* stands out in the production of beverages, food and animal use, being the most common strain currently in use (Chaucheyras-Durand et al., 2008).

1.2.2. Yeast Effects on Ruminal Environment

Studies have shown that the use of *Saccharomyces cerevisiae* assists in ruminal metabolism, increase the total number of viable bacteria and cellulolytic bacteria, besides stimulating lactate-consuming bacteria in the rumen, resulting in a greater degradation of fiber, greater synthesis of microbial protein and higher animal performance (Rose, 1987; Chaucheyras-Durand et al., 2008).

1.2.2.1 Ruminal pH

Diets of high-producing ruminant animals often contain a high proportion of concentrate, low proportion of forages and physically effective NDF and smaller particle size, causing a low chewing rate A reduced chewing activity and diets with high content of readily fermentable substrates can cause an accumulation of acids (e.g. VFA and lactic acid) produced by ruminal microorganisms and a reduction in ruminal buffering capacity, causing a drop in pH (Plaizier et al., 2008). Prolonged ruminal acidity causes detrimental in consumption and nutrient degradation. In addition, some microorganism's species, such as cellulolytic microorganisms, are sensitive to ruminal acidity. Low ruminal pH is associated with lower fiber degradability and diseases such as ruminites, liver abscess, lameness, inflammations, diarrheas and milk-fat depression (Russell et al., 1979; Dijkstra et al., 2012).

In a study carried out by Bach et al. (2007), daily supplementation of *Saccharomyces cerevisiae* strain CNCM I-1077 at 10^{10} CFU/d, led to higher ruminal pH (6.05 vs. 5.49). Thrune et al. (2009) reported that the same yeast strain resulted in a shorter time in subacute acidosis. Similar results were found by Nocek et al. (2002) and Chung et al. (2011). In contrast, McGinn et al. (2004) evaluating ruminal parameters in addition to commercial yeasts (1g/d) did not find differences for ruminal pH.. Possenti et al. (2008) comparing the inclusion of yeast in cattle's diet (10 g/d) did not find significative differences for ammonia concentration among the treatments and pH was more stable in the control treatment (without yeast).

However, it is suggested that the effect of yeast on the maintenance of ruminal pH generally occurs with a decrease in lactate concentration, which may be related to substrate competition with lactate-producing bacteria, as well as to stimulate the growth of lactate-consuming microorganisms, as summarized by Chaucheyras-Durand et al. (2008). Although there is a tendency to improve ruminal fermentation and pH

stabilization, there is still no consensus on the use of yeast in ruminant production, and there are studies with different responses to this additive (Desnoyers et al., 2009).

The increase in ruminal bacterial cells is often observed with the use of live yeast, which diverts N ruminal to microbial protein synthesis, changing volatile fatty acids production and consequently raising the pH (Chaucheyras-Durand et al., 2008). Another effect that may be related to the action of living yeast is the stimulation of Entodiniomorphid protozoa, which competes with amylolytic bacteria per substrate, have a lower rate of starch fermentation and consumes lactate. As facultative anaerobic organisms, yeast can consumes the oxygen present in the rumen, benefiting the ruminal metabolism, beyond providing nutrients for these other microorganisms (Brassard et al., 2006; Chaucheyras-Durand et al., 2008; Vohra et al., 2016).

1.2.2.2. Fiber digestibility

Ruminants have the ability to degrade forage cell wall components by symbiosis with ruminal microorganisms, which hydrolyze these molecules and produce energy, volatile fatty acids, gases, microbial protein, among other compounds (Weimer, 1998). However, in some situations, such as in different species, maturation and plant parts this degradation is hampered by complex and not accessible structures, diminishing the use by the animal.

Chaucheyras-Durand et al. (2010) found out that the supplementation of yeast in resulted in higher ruminal in situ degradation of DM and NDF in alfalfa hay, associated to a stimulation on anaerobic fungi and *B. fibrisolvens* growth. Similar results were found by Guedes et al. (2008) evaluating the supplementation of yeast on fiber degradation in corn silage samples with different quality (high and low degradability). Yeast supplied at 1 g/d had a greater benefit on the ruminal degradability of lower quality silage. Williams et al. (1991) evaluated the effects of live yeast for heifers and verified an increase of DM degradation with the inclusion of yeast, mainly at 12 h of incubation. The same results were reported by Bitencourt et al. (2011). On the other hand, Hadjipanayiotou (1997) evaluated the degradability of five feedstuffs (barley grain, soybean meal, barley straw, barley hay, alfalfa hay) in three rumen-fistulated goats, and concluded that the use of yeast did not affect diet digestibility and animal performance. Hristov et al. (2010) measured the ruminal degradation and fermentation in dairy cows, and also did not observe differences with the use of the yeast.

The increase in fiber degradability has been not consistence among experiments. However, when observed, the higher degradability in the presence of yeasts may be due to its influence on the activity of fiber-degrading microorganisms in the rumen. Apparently, live yeasts may increase fungal colonization, polysaccharidase and glycoside-hydrolase activities, besides increasing and accelerating the proliferation of fibrolytic bacteria (Chaucheyras-Durand et al., 2008). The increase of these microorganisms may be due to growth factors related to these additives, in addition the oxygen consumption carried out by the yeasts and a higher rumen pH (Desnoyers et al., 2009; Vohra et al., 2016; Shurson, 2018).

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494

II Effect of *Saccharomyces cerevisiae* strain CNCM I-1077 on the ruminal degradability of forages from South America

(Manuscript style and form consistent with the Instructions for Authors of the Journal of Animal Science)

ABSTRACT

The effect of live yeast *Saccharomyces cerevisiae* strain CNCM I-1077 (SC) on the ruminal degradability of forages commonly found in dairy diets in South America was evaluated. Four non-lactating rumen-cannulated Holstein cows were housed in a tie-stall barn and randomly assigned to two treatment sequences: Control-SC-Control or SC-Control-SC, in a switchback design, with three 30-d periods. Cows in the SC treatment were supplied with 1×10^{10} cfu of yeast daily via rumen cannula. The in situ degradability of DM and NDF was measured in 16 forage samples collected in Brazil, Argentina, Colombia and Peru, and included corn silage (n = 5), tropical grass silage (n = 2), sugarcane silage (n = 2), oat silage (n = 2), ryegrass silage (n = 2), alfalfa silage (n = 1), alfalfa hay (n = 1) and kikuyu (n = 1). Each forage was incubated in the rumen for 0, 12, 24, 36 and 120 h after feeding. Rumen fluid was collected from the ventral sac for measuring yeast count, pH, ammonia and VFA. Cows supplemented with SC had higher counts of live yeasts in rumen fluid, showed a trend of higher ruminal pH and lower ruminal ammonia concentration. Acetate to propionate ratio was higher in the rumen fluid of animals receiving SC. Live yeast supplementation decreased the pool size of fraction C (undegradable) and increased fraction B (potentially degradable) of DM and NDF. Furthermore, SC accelerated the DM and NDF degradation, as noticed by higher disappearance of DM and NDF at 12 and 24 h of incubation. Live yeast

519 supplementation is a strategy to improve rumen function and increase the nutritive value
520 of forages grown in tropical and subtropical areas.
521
522 **Key words**: cell wall, feed additive, fermentation, rumen, yeast
523

INTRODUCTION

In high-producing ruminant diets, forages are included to provide physically effective fiber, to keep ruminal function and animal health (Mertens, 1997). Nevertheless, forages are also important source of nutrients, depending on their quality, which is mainly defined by the content of neutral detergent fiber (NDF) and its digestibility (NDFD) (Huhtanen et al., 2006). Moreover, the content and digestibility of NDF in diet may regulate feed intake, due to the physical filling of digestive compartments, and in turn, constrain the animal performance (Mertens, 1994; Allen, 2000).

Forage species, genotypes, growing environment, maturity and harvesting management affect forage composition and digestibility. Meanwhile, different strategies can be used to improve forage digestibility, such as the application of exogenous fibrolytic enzymes (Adesogan, 2005) and chemicals (e.g. sodium hydroxide, anhydrous ammonia, calcium oxide) (Klopfenstein, 1978), as well as the manipulation of the ruminal fermentation (Wallace, 1994; Arcuri and Mantovani, 2006). The use of pre- and probiotics in ruminant diets is an alternative to improve forage degradability via amelioration of rumen fermentation, in addition to the benefits to animal health (Shurson, 2018).

The supplementation of live yeasts, such as *Saccharomyces cerevisiae* strains, may increase the total number of cellulolytic bacteria, stimulate lactate consumption and decrease lactate production, increase rumen pH and reduce oxygen concentration in the rumen fluid, resulting in higher fiber degradation, greater synthesis of microbial protein and improved animal performance (Rose, 1987; Chaucheyras-Durand et al., 2008; Ondarza et al., 2010). However, the benefits of live yeast supplementation on ruminal

549 degradability of forages grown in tropical and subtropical areas in seldom reported
550 (Sousa et al., 2018).

551 The aim of this study was to evaluate the effect of live yeast *Saccharomyces*
552 *cerevisiae* strain CNCM I-1077 on the ruminal degradability of DM and NDF of several
553 forage samples commonly found in diets of dairy cows in South America and verify if
554 the magnitude of improvement in DM and NDF degradation is dependent on forage
555 quality.

556

557 **MATERIAL AND METHODS**

558 ***Forage Samples***

559 Sixteen forage samples, including corn silage, tropical grass silage, sugarcane
560 silage, alfalfa haylage and hay, ryegrass haylage, oat haylage and kikuyu, were collected
561 across the South America (Brazil, Argentina, Colombia and Peru). Samples were dried
562 in a forced-air oven at 55ºC during 72 h at sampling site, packed in polyethylene bags
563 and sent to the State University of Maringá. Information about collection sites and
564 forage composition is shown in Tables 1 and 2.

565

566 ***Cows, Facilities and Experimental Design***

567 Animal care and handling procedures were approved by the Ethics Committee
568 for Animal Use of the Maringa State University (protocol number 8208090218 –
569 CEUA/UEM). Four non-lactating rumen-cannulated Holstein cows (average 545 kg of
570 BW) were housed in a tie-stall barn with rubber beds, individual feedbunks and water
571 bowls. The diet offered to the cows consisted of 65% of corn silage and 35% of
572 concentrates (corn grain ground, soybean meal, wheat bran and mineral-vitamin mix)
573 and contained 12% of CP and 38% of NDF (DM basis). Every morning, diet ingredients

were mixed and fed as a total mixed ration (TMR) at 08:00 h, after removing the refusals from the previous day. The amount of TMR was adjusted daily to allows at least 10% as orts.

The experimental treatments were: 1) control and 2) live yeast (*Saccharomyces cerevisiae* strain CNCM I-1077; Lallemand Animal Nutrition, Aparecida de Goiânia, GO) supplemented at 1×10^{10} cfu/d per cow (SC). The live yeast was diluted in 250 mL of distilled water at 40°C and dosed directly into the rumen, through the rumen cannula, every morning immediately before TMR distribution. Cows receiving the control treatment were also dosed with 250 mL of distilled water at 40°C to avoid ruminal oxygen stress bias between treatments. The treatments were compared in a switchback design, with three 30-d periods, being 19 d of adaptation and the last 11 d of sampling. There were two treatment sequences: Ctrl-SC-Ctrl or SC-Ctrl-SC. Two cows were randomly assigned to each treatment sequence.

In Situ Degradability

From d 20 to d 30 of each period, two 5-d runs were performed for measuring the in situ disappearance of DM and NDF of the 16 forage samples (8 forages per run). Dry forage samples were ground in a Wiley mill with a 5-mm screen and weighed in woven in situ bags (10 × 20 cm; 50 µm porosity; Ankon Technology, Macedon, USA). Approximately 5 g was placed in each bag. Each feed was incubated in triplicate for 0, 12, 24, 36 and 120 h after feeding. Two blank bags were included in each time point. Before the incubation, the bags were soaked in warm water (39°C) for 20 min. Bags were inserted in reverse order and recovered all together. Immediately after removing, bags were submerged in cold water (0°C) for 5 min and washed in a washing machine (three cycles, followed by a final spin). Washed bags were dried in an air-forced oven at

599 55°C for 72 h, weighted, and its contents were ground through a 1-mm screen using a
600 Wiley Mill for measuring NDF concentration.
601 Fraction A (solubles and particles of smaller size than the porosity of the bags)
602 of DM or NDF was estimated as the proportion of mass that disappear after washing
603 unincubated bags (time 0 h). Fraction C (undegraded fraction) was calculated as the
604 proportion of residual mass in bags at 120 h of incubation. Fraction B (potentially
605 degradable fraction) was computed by difference (B = 100 – A – C). Fractional
606 degradation rate of B (kd) of DM and NDF was calculated using a 1-pool exponential
607 model, without lag (Ørskov and McDonald, 1979), using the NLIN procedure of SAS
608 (version 9.4). Effective ruminal degradability (ED) of forage DM and NDF were
609 estimated at specific fractional passage rates (kp) (for DM 2%/h, 4%/h and 6%/h and for
610 NDF 1.5%/h, 3%/h and 4.5%/h), as DE (%) = 100 × kd / (kd + kp).
611
612 ***Sampling of Feed, Feces and Rumen Fluid***
613 Samples of diet ingredients were collected from d-20 to d-30 of each period and
614 subsequently composed by period. The apparent digestibility of DM, NDF and NDS
615 were determined using indigestible NDF (iNDF) as internal marker (Huhtanen et al.,
616 1994). Fecal grab samples were collected every 8 h, from d-20 to d-24 in each period
617 and composed by cow. Samples were oven-dried at 55°C for 72 h and ground (1-mm
618 screen; Wiley mill) for analyzes of DM, ash, NDF and iNDF.
619 On d-30 of each period, rumen fluid was collected from the ventral sac at 0, 2, 4,
620 8 and 12 h after feeding for measuring pH (pH meter model Tec5, Tecnal® Piracicaba,
621 Brazil), ammonia and VFA. Yeast count was measured in samples collected at 0, 2 and
622 8 h.
623

Laboratorial Analyses

Samples of forages, ration and feces were analyzed for DM (method 934.01; AOAC, 1990), aNDF, assayed with a heat stable amylase and expressed inclusive of residual ash (Mertens, 2002), ash (method 942.05; AOAC, 1990) and iNDF, by in situ incubation for 288 h (Huhtanen et al., 1994). Neutral detergent solubles was calculated as NDS = 100 – ash – NDF. Ration was also analyzed for CP by Kjeldahl procedure (method 984.13; AOAC, 1990). Forage samples were additionally analyzed for CP, ADF, assayed sequentially and expressed inclusive of residual ash, and lignin, determined by solubilization of cellulose with sulphuric acid and expressed inclusive of residual ash (Van Soest, 1967).

Ruminal volatile fatty acids were determined by gas chromatography (GCMS QP 2010 plus, Shimadzu, Kyoto, Japan) using a capillary column (Stabilwax, Restek, Bellefonte, PA; 60 m, 0.25 mm ø, 0.25 μm crossbond carbowax polyethylene glycol). Ammonia concentration was determined by a colorimetric method (Chaney and Marbach, 1962). Yeast was enumerated in malt extract agar (M137, Himedia®, Mumbai, India) acidified to pH 3.5 with lactic acid. The plates were incubated aerobically for 2 d at 30ºC. The number of colony forming units (cfu) was expressed as $\log_{10}$ cfu/mL.

Statistical Analysis

Statistical analysis was performed using the MIXED procedure of SAS (version 9.4). The DM intake and apparent digestibility were compared using a model that included fixed effects of treatment, period, treatment × period and random effects of cow and cow × treatment. An AR(1) covariance structure was defined and the effect of cow was the subject. Rumen fluid parameters (yeast count, ammonia, pH and VFA)

were analyzed with the same model including the fixed effect of time and its interactions.

Outcomes from the in situ assay were analyzed with the same model described above including the fixed effects of forage and interaction between forage and treatment. For outcomes with a significant effect of interaction between treatment and forage ($P \leq 0.15$), a linear regression between the means obtained for SC against the control were performed using the REG procedure of SAS. Differences between treatment was declared if $P \leq 0.05$ and trends considered if $0.05 < P \leq 0.15$.

RESULTS

The SC did not affect the DM intake (average 10.45 kg/d) and apparent digestibility of nutrients (Table 3). Cows supplemented with SC had higher counts of yeast in rumen fluid (Figure 1) and showed a trend of lower ruminal ammonia concentration (Figure 2). There was a tendency for higher ruminal pH ($P = 0.12$) in cows fed SC, specially at 8 and 12 h after feeding (Figure 3).

Ruminal VFA concentrations are shown in Figure 4. Animals treated with SC had higher acetate:propionate ratio, and there was a trend for lower concentrations of propionate ($P = 0.12$) and valerate ($P = 0.15$) in the rumen fluid. The concentrations of acetate, iso-butyrate, butyrate, iso-valerate and total VFA did not differ across treatments.

The pool size of fraction A of DM and NDF is presented in Table 4. The soluble fraction of DM ranged from 11.6 to 49.2%, whereas the fraction A of NDF ranged from 1.5 to 8.51%. Live yeast supplementation decreased the pool size of fraction C and increased fraction B of DM and NDF (Tables 5 and 6). No difference was detected for the modeled kd and ED of DM and NDF. Meanwhile, the SC increased the actual in situ

674 degradability of DM at 12 h and tended to increase the actual in situ degradability of
675 NDF at 12 h. There was a trend (P = 0.11) of interaction between SC and forage source
676 for the actual in situ degradability of DM at 24 h. Yeast supplementation increased DM
677 degradability at 24 h of incubation, especially for forages with a higher ruminal
678 degradability (Figure 5). The actual in situ degradability of NDF at 24 h was higher in
679 cows receiving SC. No difference was observed for the actual in situ degradability of
680 DM and NDF at 36 h of incubation.
681

682 **DISCUSSION**

683 Active dry yeasts have been widely used as feed additive to improve animal
684 performance and health (Chaucheyras-Durand et al., 2008). Cattle responses attributed
685 to live yeast supplementation are often associated with improved rumen function.
686 Reduced redox potential (by oxygen scavenging) (Marden et al., 2008), higher pH (by
687 decreasing lactic acid production and increasing utilization of lactic acid) (Williams et
688 al 1991; Chaucheyras et al., 1996; Chaucheyras-Durand et al. 2005; Guedes et al, 2008)
689 and greater availability of growth factors (e.g. organic acids and vitamins) (Jouany,
690 2006; Chaucheyras-Durand et al., 2008) have been associated with stimulation of rumen
691 bacteria (Newbold et al., 1996; Mosoni et al., 2007; Sousa et al., 2018), increased
692 microbial protein synthesis (Moya et al., 2007) and enhanced fiber degradation in the
693 rumen of animals fed live yeasts (Chaucheyras-Durand and Fonty, 2001; Guedes et al,
694 2008; Sousa et al., 2018). In the current trial, the most notable response was the greater
695 in situ degradability of NDF in tropical and subtropical forages incubated in cows
696 receiving the SC.

697 In our study, animals fed SC had higher counts of yeasts, tended to have higher
698 pH values and lower ammonia concentrations at a comparable concentration of VFA in

699 the rumen fluid. These founds indicate that SC might have stimulated the growth of
700 bacteria in the rumen (Harrison et al., 1988; Erasmus et al., 1992). Usually, the increase
701 in rumen pH in animals supplemented with SC is related to a lower concentration of
702 lactate and an increased activity of cellulolytic bacteria in the ruminal digesta
703 (Chaucheyras-Durand and Fonty, 2001; Desnoyers et al., 2009). Although there was no
704 difference in the content of total VFA and most individual VFA, cows fed SC had a
705 higher acetate:propionate ratio, due to a trend of lower propionate concentration. In the
706 rumen, propionate is synthetized via succinyl-CoA and acrylyl-CoA pathways (Russell
707 and Wallace, 1988). Lactic acid ingested with fermented feedstuffs or produced by
708 rumen bacteria can be converted to propionate via acrylyl-CoA pathway by lactate-
709 fermenting bacteria, such as *Veillonella alcalescens, Megasphaera elsdenii* and
710 *Selenomonas ruminantium* (Mackie et al., 1984). Yeast supplementation has been
711 associated with either a decreased in production and an increased in utilization of lactic
712 acid (Williams et al 1991; Chaucheyras et al., 1996; Chaucheyras-Durand et al. 2005;
713 Guedes et al, 2008). In our study, SC likely decreased lactic acid formation as an
714 intermediate of ruminal fermentation, which may have led to a lower concentration of
715 propionate and a higher acetate:propionate ratio. Moreover, the greater fiber degradation
716 might have contributed to the greater acetate:propionate ratio in cows receiving SC.
717 Compared with species capable of fermenting non-fiber carbohydrates, ruminal
718 fibrolytic microorganisms generally leads to a higher proportion of acetate among their
719 fermentation end-products (Russell and Wallace, 1988; Wolin and Miller, 1988).
720 It has been claimed that forage quality can influence the SC effect on ruminal
721 degradation. Guedes et al. (2008) described a larger response to SC supplementation in
722 corn silages of lower NDF degradability than in corn silages with higher NDF
723 degradability in situ. Recently, Sousa et al. (2018) reported a higher relative benefit of

SC on NDF degradability in tropical forages of lower NDF degradability. However, the absolute increase in NDF degradability (g/kg) reported by the authors was higher in forages with higher quality, as such a higher increase in NDF degradability in Palisade grass (+25 g/kg), Guineagrass (+ 23 g/kg) and corn silage (+ 26 g/kg) than in sugarcane silage (+ 17 g/kg) and Bermudagrass hay (+ 19 g/kg). Since the SC benefits are mainly based on the stimulation of fibrolytic microorganisms (Chaucheyras-Durand et al., 2008), it is expected that plant tissues with lower lignification and easier access to the rumen microorganism (Jung and Deetz, 1993) should reveals a potentiated response to SC supplementation.

In this trial, SC supplementation increased the in situ degradability of DM at 12 and 24 h of incubation by 1.1%-unit (+2.5%) and 2.4%-units (+4.6%), whereas increased the in situ degradability of NDF at 12 and 24 h of incubation by 1.4%-unit (+9.7) and 2.6%-units (+10.3%), respectively. Overall, the improvement in DM degradability was a response to the greater NDF degradability. However, there was a trend of interaction between SC and forage source for DM degradability at 24 h, indicating that forages with higher quality tended to have a larger improvement in DM degradation when SC was supplied. Since the response in NDF degradation was similar across forage sources, the SC may have also enhanced the degradation of NDS in high quality forages.

Previous reports have suggested that SC supplementation could accelerate the rate of fiber degradation, with a small or no SC effect for longer incubation times (William et al. 1991; Girard and Dawson, 1995; Callaway and Martin, 1997; Sousa et al., 2018). In the present study, it is likely that the degradation rate of NDF of the forage sources was faster when SC was fed. Meanwhile, no difference between control and SC were observed when the forage samples were incubated for 36 h. Since the fractional

749 rate of degradation was modeled with all incubation time-points, no effect of SC was
750 observed for kd and estimates of effective degradability (ED) of DM and NDF.
751 In addition to the improvement in the ruminal degradability at shorter incubation
752 times, the extension of degradation was increased by SC supplementation, as noticed by
753 lower proportion of undegraded fraction of NDF (-2.1%-unit) and DM (-1.4%-unit)
754 (fraction C). The mechanism by which SC improved the extent of NDF and DM
755 degradability (proportion degraded at 120 h) is not clear, but the size of this benefit is
756 perhaps of less impact on animal performance than the acceleration in NDF
757 degradability.
758

759 **CONCLUSION**

760 *Saccharomyces cerevisiae* strain CNCM I-1077 improved rumen function,
761 decreased the pool size of the undegradable fraction and increased fiber and dry matter
762 degradability. Live yeast supplementation is a strategy to improve the nutritive value of
763 forages grown in tropical and subtropical areas.
764

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882

Table 1. List of forages sampled in the South America

ID	Family	C3/C4	Forage	Scientific name	Conservation	Local	State	Country
A	Legume	C4	Alfalfa	*Medicago sativa*	Hay	Lunardelli	PR	Brazil
B	Legume	C4	Alfalfa	*Medicago sativa*	Silage	Castro	PR	Brazil
C	Grass	C4	Corn	*Zea mays*	Silage	Saladillo	Buenos Aires	Argentina
D	Grass	C4	Corn	*Zea mays*	Silage	Castro	PR	Brazil
E	Grass	C4	Corn	*Zea mays*	Silage	Bela Vista de Goiás	GO	Brazil
F	Grass	C4	Corn	*Zea mays*	Silage	Mandaguaçu	PR	Brazil
G	Grass	C4	Corn	*Zea mays*	Silage	Arequipa	Arequipa	Peru
H	Grass	C4	Kikuyu	*Pennisetum clandestinum*		Ubaté	Cundinamarca	Colombia
I	Grass	C3	Oat	*Avena sativa*	Silage	Arapoti	PR	Brazil
J	Grass	C3	Oat	*Avena sativa*	Silage	Castro	PR	Brazil
K	Grass	C3	Ryegrass	*Lolium multiflorum*	Silage	Castro	PR	Brazil
L	Grass	C3	Ryegrass 2	*Lolium multiflorum*	Silage	Castro	PR	Brazil
M	Grass	C4	Sugarcane	*Saccharum officinarum*	Silage	Nova Andradina	MS	Brazil
N	Grass	C4	Sugarcane	*Saccharum officinarum*	Silage	Agudos	SP	Brazil
O	Grass	C4	Tropical grass	*Panicum maximum* cv. Mombaça	Silage	São Miguel do Aragaia	GO	Brazil
P	Grass	C4	Tropical grass	*Panicum maximum* cv. Mombaça	Silage	Terenos	MS	Brazil

Table 2. Chemical composition of the forage samples (% DM)

Forage	CP	Ash	aNDF	HEMI	ADF	CEL	LIG	iNDF
A (Alfalfa hay)	14.0	7.18	72.0	19.0	53.0	39.0	14.0	47.3
B (Alfalfa silage)	15.8	8.75	54.2	16.2	38.0	28.8	9.29	29.6
C (Corn silage)	7.77	5.94	53.6	26.0	27.5	23.8	3.72	17.7
D (Corn silage)	7.45	3.93	43.1	25.3	17.7	15.6	2.11	13.3
E (Corn silage)	4.58	2.58	59.0	25.6	33.4	28.4	4.96	20.5
F (Corn silage)	7.60	3.43	40.3	20.0	20.3	17.7	2.59	13.8
G (Corn silage)	8.96	10.4	60.1	24.6	35.4	29.6	5.81	16.7
H (Kikuyu)	18.2	11.7	60.5	32.7	27.7	24.2	3.69	17.3
I (Oat silage)	7.12	7.72	61.1	24.7	36.4	30.3	5.98	26.2
J (Oat silage)	9.44	8.77	66.1	26.6	39.5	35.5	3.84	17.5
K (Ryegrass silage)	14.3	10.8	59.5	24.8	34.4	29.8	4.60	18.8
L (Ryegrass silage)	16.86	12.14	51.92	21.59	30.33	27.14	3.19	12.24
M (Sugarcane silage)	2.58	2.25	76.65	29.82	44.83	33.97	10.87	37.30
N (Sugarcane silage)	2.49	2.43	80.45	31.05	49.39	38.93	10.46	40.36
O (Tropical grass silage)	3.09	8.34	83.73	28.50	55.23	46.64	8.59	42.81
P (Tropical grass silage)	4.29	7.24	81.28	32.51	48.78	42.12	6.66	38.18
Mean	9.04	7.11	62.7	25.6	37.0	30.8	6.27	25.6

Table 3. Dry matter intake and apparent digestibility of nutrients in non-lactating cows supplemented or not with live yeast

	Treatment			
Item	Control	Yeast	SEM	P-value
DM intake (kg/d)	10.1	10.8	1.03	0.53
DM digestibility (%)	63.1	63.2	1.22	0.95
NDF digestibility (%)	43.7	44.9	2.27	0.72
NDS[1] digestibility (%)	82.1	82.8	1.03	0.65

[1]NDS: neutral detergent solubles.

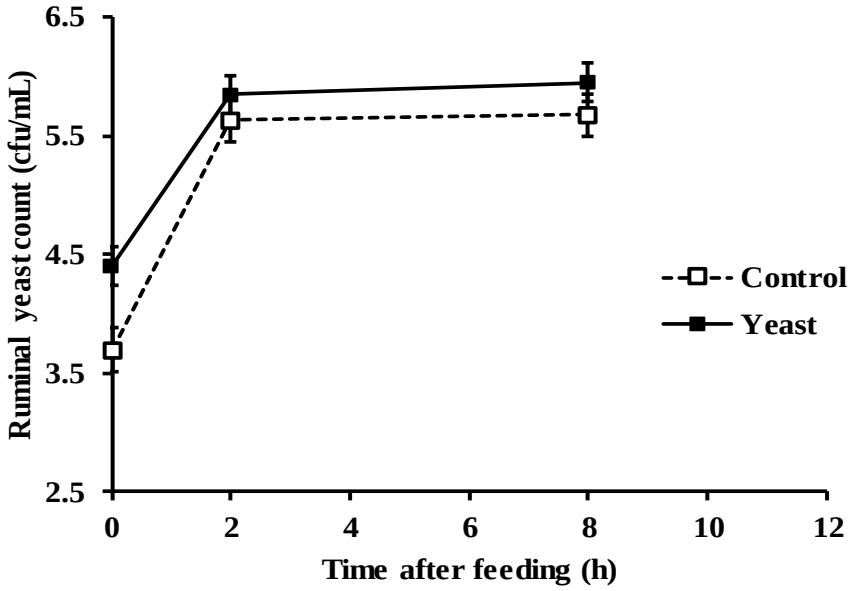

Figure 1. Yeast counts in the rumen fluid of non-lactating cows supplemented or not with live yeast. P = 0.05 for treatment, P < 0.01 for time, P = 0.37 for treatment × time.

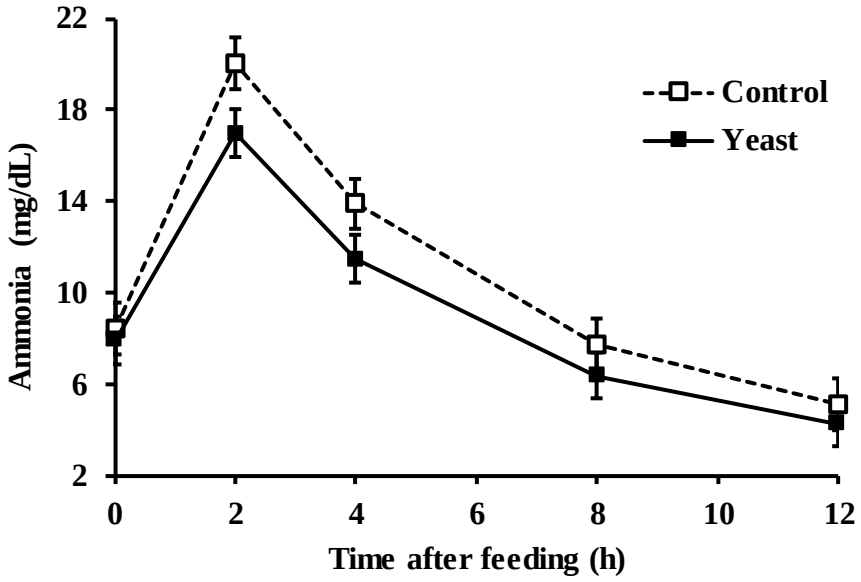

Figure 2. Ruminal ammonia concentration in non-lactating cows supplemented or not with live yeast. P = 0.10 for treatment, P < 0.01 for time, P = 0.72 for treatment ×time.

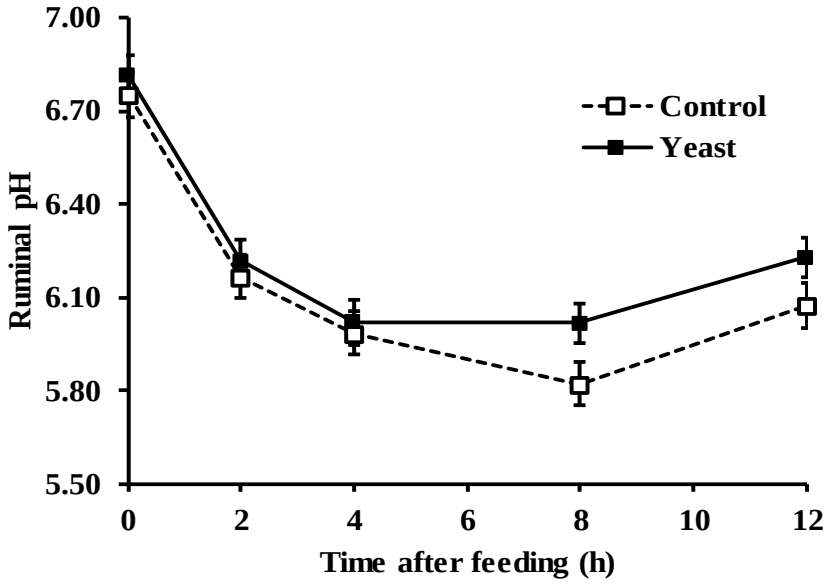

Figure 3. Ruminal pH in non-lactating cows supplemented or not with live yeast. P = 0.12 for treatment, P < 0.01 for time, P = 0.71 for treatment ×time.

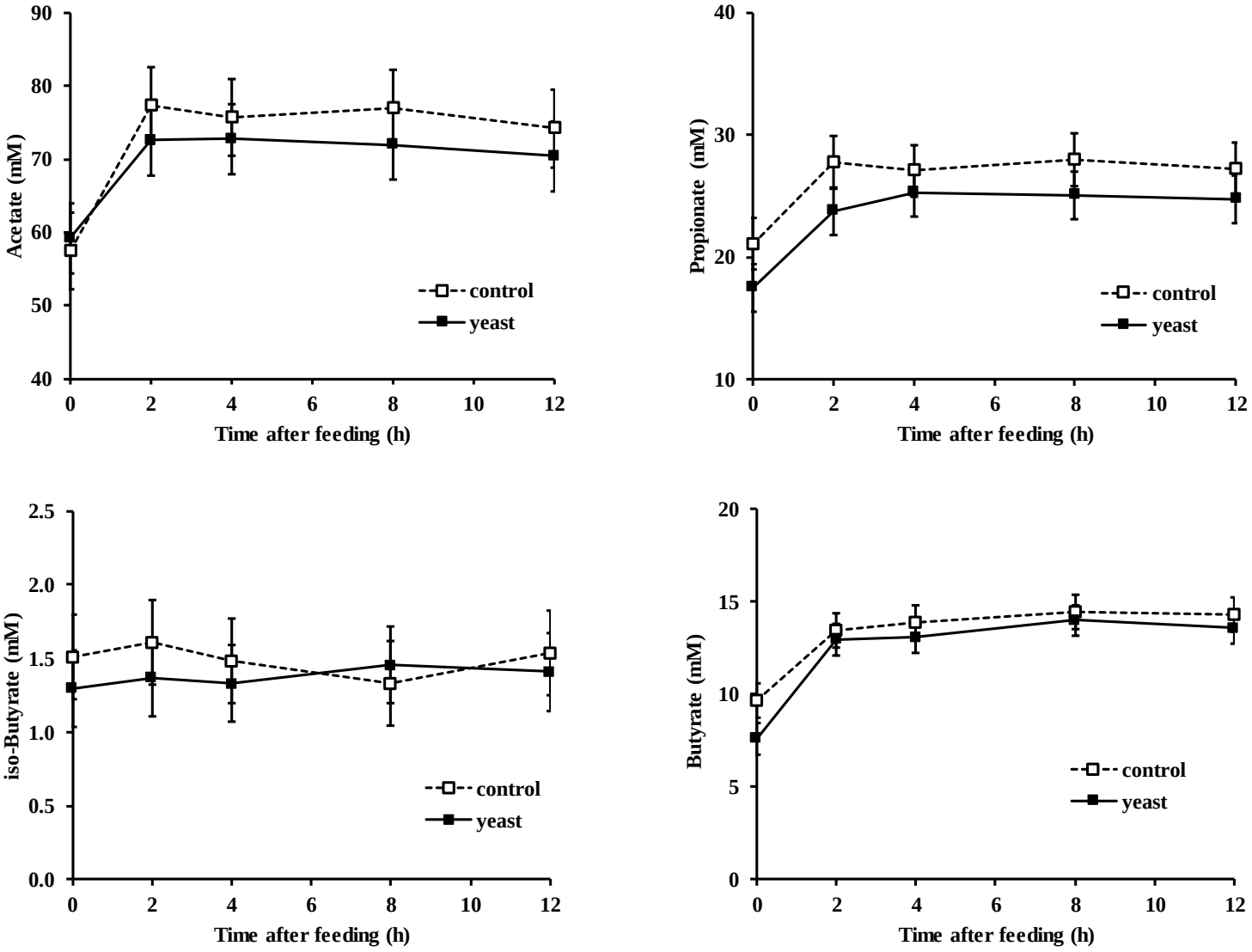

Figure 4. Ruminal volatile fatty acids in non-lactating cows supplemented or not with live yeast. For acetate: P = 0.43 for treatment, P = 0.01 for time, P = 0.95 for treatment × time; For Propionate: P = 0.12 for treatment, P < 0.01 for time, P = 0.97 for treatment × time; For iso-Butyrate: P = 0.55 for treatment, P = 0.99 for time, P = 0.97 for treatment × time and for butyrate: P = 0.23 for treatment, P < 0.01 for time, P = 0.88 for treatment × time.

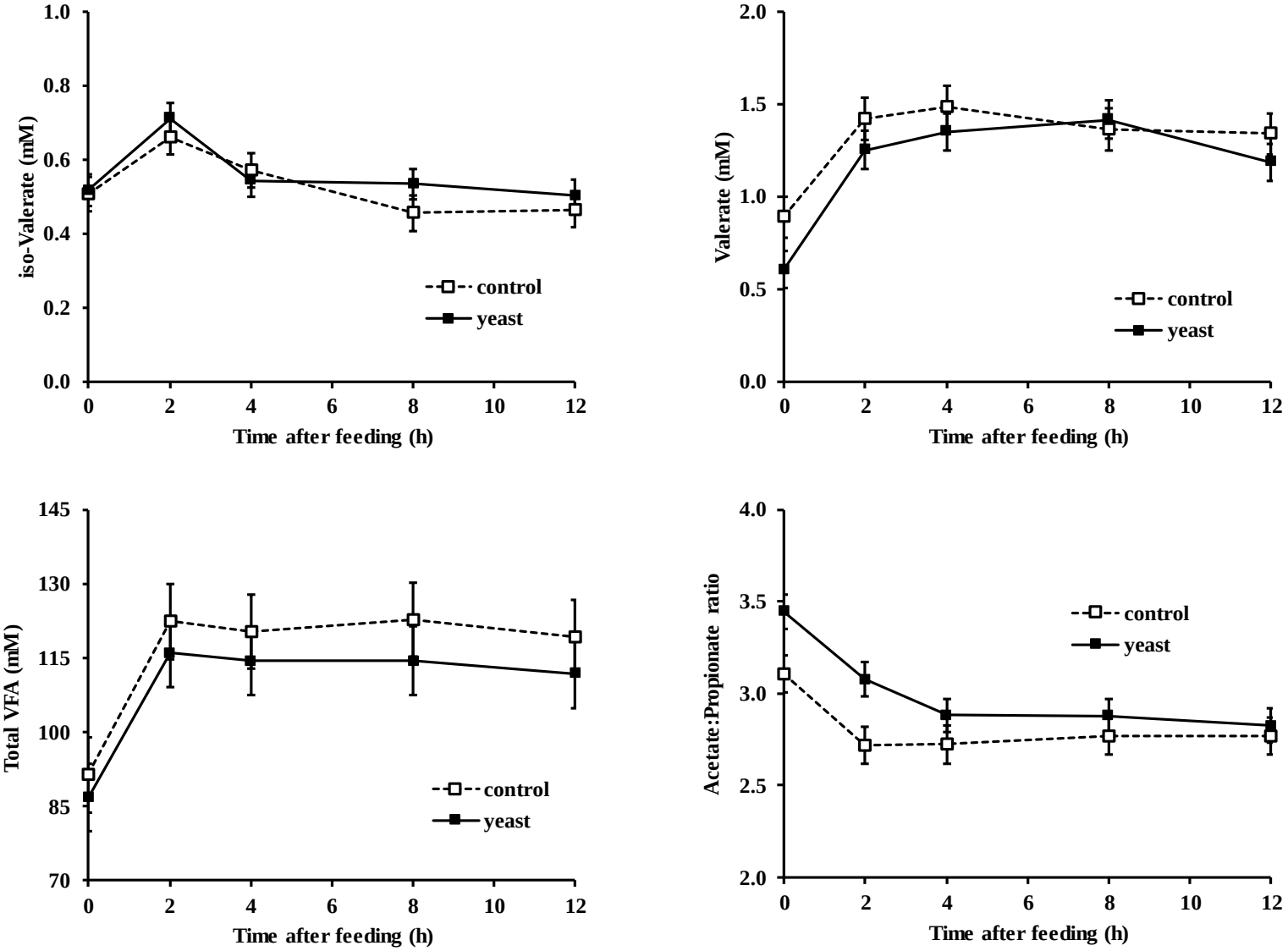

Figure 4 (continuation). Ruminal volatile fatty acids in non-lactating cows supplemented or not with live yeast. For iso-Valerate: P = 0.37 for treatment, P < 0.01 for time, P = 0.78 for treatment × time; For Valerate: P = 0.15 for treatment, P < 0.01 for time, P = 0.62 for treatment × time; For total VFA: P = 0.27 for treatment, P < 0.01 for time, P = 0.99 for treatment × time and for acetate:propionate ratio: P = 0.04 for treatment, P < 0.01 for time, P = 0.42 for treatment × time.

Table 4. Fraction A of DM and NDF in the forage samples

	DM fraction A		NDF fraction A	
Forage	Mean	SD	Mean	SD
A (Alfalfa hay)	23.1	3.54	8.51	2.724
B (Alfalfa silage)	31.7	2.42	4.45	2.780
C (Corn silage)	31.8	4.23	2.80	0.715
D (Corn silage)	46.4	6.37	3.20	1.539
E (Corn silage)	32.5	3.41	2.54	1.177
F (Corn silage)	49.2	6.52	2.89	1.664
G (Corn silage)	37.0	1.14	2.60	0.895
H (Kikuyu)	33.6	0.65	3.17	1.274
I (Oat silage)	31.9	2.84	2.20	0.649
J (Oat silage)	32.7	2.79	2.56	1.406
K (Ryegrass silage)	36.2	1.57	1.51	0.489
L (Ryegrass silage)	42.1	1.45	1.97	0.828
M (Sugarcane silage)	29.3	1.47	4.19	2.098
N (Sugarcane silage)	21.2	0.87	2.58	1.017
O (Tropical grass silage)	11.6	1.94	3.01	1.012
P (Tropical grass silage)	18.1	1.50	2.74	1.005

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

	Treatment			P-value[3]		
Item[1]	Control	Yeast	SEM[2]	T	F	T × F
Fraction C of DM (%)	27.7	26.3	0.41	<0.01	<0.01	0.95
Fraction B of DM (%)	40.6	41.9	0.60	0.02	<0.01	0.96
kd of fraction B of DM (%/h)	3.38	3.25	0.20	0.38	<0.01	0.56
ED2 of DM (%)	56.7	57.2	0.64	0.28	<0.01	0.94
ED4 of DM (%)	49.9	50.3	0.59	0.48	<0.01	0.91
ED6 of DM (%)	46.1	46.4	0.52	0.57	<0.01	0.92
DegDM12 (%)	43.2	44.3	0.59	0.03	<0.01	0.99
DegDM24 (%)	50.5	52.9	0.79	<0.01	<0.01	0.11
DegDM36 (%)	59.7	60.0	1.05	0.75	<0.01	0.81

[1]ED2: Effective degradability of DM at kp = 2%/h; ED4: Effective degradability of DM at kp = 4%/h; ED6: Effective degradability of DM at kp = 6%/h; DegDM12: Actual ruminal degradability of DM at 12 h of incubation; Actual ruminal degradability of DM at 24 h of incubation; DegDM36: Actual ruminal degradability of DM at 36 h of incubation.

[2]Standard error of the mean.

[3]T: effect of yeast supplementation, F: effect of forage source, T × F: interaction between yeast and forage source.

Table 6. Effect of live yeast on the ruminal degradability of forage NDF

	Treatment			P-value[3]		
Item[1]	Control	Yeast	SEM[2]	T	F	T × F
Fraction C of NDF (%)	41.0	38.8	0.51	<0.01	<0.01	0.97
Fraction B of NDF (%)	56.1	57.9	0.61	<0.01	<0.01	0.49
kd of fraction B of NDF (%/h)	2.84	2.80	0.17	0.73	<0.01	0.41
ED1.5 of NDF (%)	39.1	40.1	1.10	0.22	<0.01	0.96
ED3 of NDF (%)	29.9	30.6	1.05	0.34	<0.01	0.91
ED4.5 of NDF (%)	24.5	25.1	0.95	0.38	<0.01	0.86
DegNDF12 (%)	14.4	15.8	0.99	0.08	<0.01	0.99
DegNDF24 (%)	25.2	27.8	1.61	0.03	<0.01	0.94
DegNDF36 (%)	38.7	38.6	1.88	0.96	<0.01	0.35

[1]ED1.5: Effective degradability of NDF at kp = 1.5%/h; ED3: Effective degradability of NDF at kp = 3%/h; ED4.5: Effective degradability of NDF at kp = 4.5%/h; DegNDF12: Actual ruminal degradability of NDF at 12 h of incubation; DegNDF24: Actual ruminal degradability of NDF at 24 h of incubation; DegNDF36: Actual ruminal degradability of NDF at 36 h of incubation.

[2]Standard error of the mean.

[3]T: effect of yeast supplementation, F: effect of forage source, T × F: interaction between yeast and forage source.

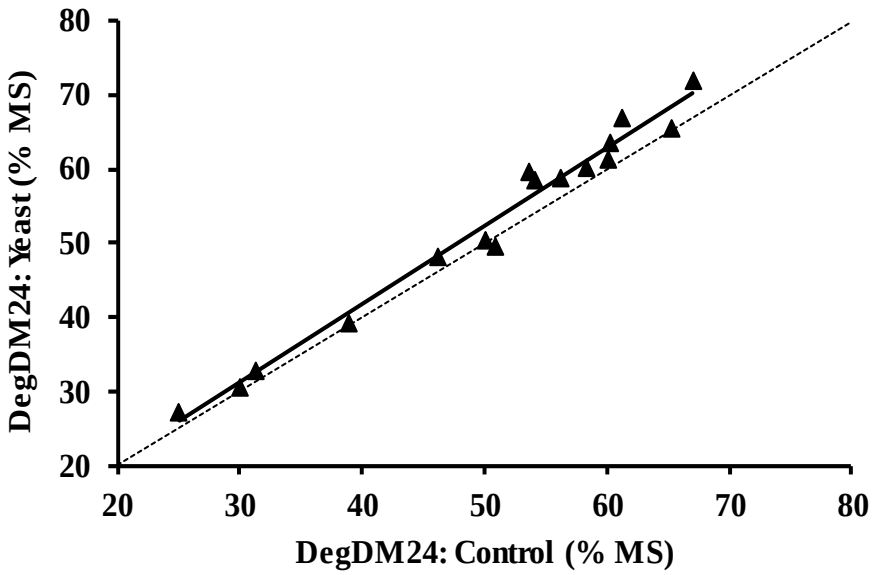

Figure 5. Influence of live yeast supplementation on actual DM in situ degradability at 24 h of incubation (DegDM24) in forages sampled in the South America. $y = 1.056 x - 0.466$ $R^2 = 0.98$, $P = 0.83$ for intercept = 0, $P < 0.01$ for slope = 0. Dashed line represents equality ($y = x$).