DANNYLO OLIVEIRA DE SOUSA

Alteration of fiber digestibility for ruminants: Effects on intake, performance, and ruminal ecosystem

Pirassununga

2017

DANNYLO OLIVEIRA DE SOUSA

Alteration of fiber digestibility for ruminants: Effects on intake, performance, and ruminal ecosystem

Tese apresentada ao Programa de Pós-Graduação em Nutrição e Produção Animal da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo para obtenção do título de Doutor em Ciências

Departamento:

Nutrição e Produção Animal

Área de concentração:

Nutrição e Produção Animal

Orientador:

Prof. Dr. Luis Felipe Prada e Silva

Pirassununga

2017

CERTIFICADO

Certificamos que a proposta intitulada "Efeito da alteração da digestibilidade da fibra pela época de corte e pelo genótipo sobre o desempenho, consumo, cinética e ecossistema ruminal de novilhos alimentados com silagem de cana-de-açúcar ", protocolada sob o CEUA nº 3602270215, sob a responsabilidade de **Luís Felipe Prada E Silva** - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (CEUA/FMVZ) na reunião de 10/05/2017.

We certify that the proposal "Effect of altering fiber digestibility by time of harvest and by genotype on performance, intake, rumen kinetics and ecosystem of steers fed sugarcane silage", utilizing 56 Bovines (56 males), protocol number CEUA 3602270215, under the responsibility of **Luís Felipe Prada E Silva** - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the School of Veterinary Medicine and Animal Science (University of São Paulo) (CEUA/FMVZ) in the meeting of 05/10/2017.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 09/2014 a 12/2014 Área: Nutrição E Produção Animal

Origem:	Animais de proprietários						
Espécie:	Bovinos	sexo:	Machos	idade:	24 a 30 meses	N:	56
Linhagem:	Nelore			Peso:	280 a 400 kg		

Resumo: A digestibilidade da parede celular é o principal fator limitante do valor nutritivo de forragens. A identificação de um parâmetro de avaliação da qualidade da fibra que efetivamente tenha impacto sobre o desempenho animal é de grande importância para os programas de melhoramento genético da cana-de-açúcar. Objetiva-se com este estudo avaliar o efeito da alteração da digestibilidade da fibra pela época de corte e pelo genótipo sobre o desempenho, consumo, cinética e ecossistema ruminal de novilhos alimentados com silagem de cana-de-açúcar, sobre os seguintes parâmetros: 1) consumo de matéria seca; 2) ganho de peso e variação da condição corporal; 3) taxa de digestão e passagem ruminal da FDN; 4) parâmetros de fermentação ruminal; 5) quantificação de bactérias ruminais celulolíticas, amilolíticas e fermentadoras de lactato. Serão utilizados cinquenta e seis novilhos da raça Nelore com aproximadamente 350 kg, sendo que oito novilhos serão fistulados para colocação de cânulas ruminais. As rações experimentais serão compostas de 40% de volumoso na matéria seca. O delineamento estatístico será o de blocos casualizados com 12 repetições (desempenho) e quadrado latino 4X4 com 2 repetições (cinética e metabolismo ruminal). O volume e a massa total do conteúdo ruminal serão determinados. Amostras serão retiradas de ambas as fases, sólida e líquida, para determinação do tamanho do compartimento ruminal de componentes da digesta. A dieta, sobras e digesta ruminal serão analisados para conteúdo de nutrientes. Serão realizados os cálculos de taxa de passagem do FDN pelo rúmen (kp), taxa de digestão ruminal de FDN (kd) e da digestibilidade ruminal aparente do FDN. Será feita uma quantificação relativa das principais bactérias, por PCR em tempo real, em relação ao total de bactérias da amostra de conteúdo ruminal.

Local do experimento: Laboratótio de Pesquisa em Bovinos de Corte/FMVZ/USP

São Paulo, 10 de maio de 2017

Profa. Dra. Denise Tabacchi Fantoni
Presidente da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

Roseli da Costa Gomes
Secretaria Executiva da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

CERTIFICADO

Certificamos que a proposta intitulada "Efeito da suplementação com Saccharomyces cerevisiae CNCM | 1077 sobre a digestibilidade da fibra e do ecossistema ruminal de gado a pasto e em confinamento com diferentes qualidades de forragem.", protocolada sob o CEUA nº 5488241113, sob a responsabilidade de **Luís Felipe Prada E Silva** *e equipe; Dannylo Oliveira de Sousa* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (CEUA/FMVZ) na reunião de 18/03/2015.

We certify that the proposal "título em inglês", utilizing 8 Bovines (8 males), protocol number CEUA 5488241113, under the responsibility of **Luís Felipe Prada E Silva** *and team; Dannylo Oliveira de Sousa* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the School of Veterinary Medicine and Animal Science (University of São Paulo) (CEUA/FMVZ) in the meeting of 03/18/2015.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 11/2013 a 11/2014 Área: Nutrição E Produção Animal

Resumo: O presente estudo irá avaliar o efeito da suplementação com S. cerevisiae sobre a digestibilidade da fibra e ecossistema ruminal de bovinos nelore em pastejo durante as quatro estações do ano, e em bovinos confinados recebendo dieta a base de silagem de cana-de-açúcar. Serão utilizados 16 novilhos Nelore castrados e canulados no rúmen, com aproximadamente 300 kg e 20 meses de idade. No experimento 1: 8 animais serão mantidos em pastejo durante as quatro estações do ano. No experimento 2: 8 animais serão confinados em baias individuais. O delineamento experimental será: Experimento 1: Fatorial 2x4. Tratamento (com ou sem a levedura) e estação do ano (primavera, verão, outono e inverno). Experimento 2: Quadrado latino 4x4 duplicado. Tratamento (com ou sem a levedura) e nível de concentrado na dieta (60 ou 80% da matéria seca). Os animais tratados receberão uma capsula diária, através da cânula ruminal, contendo 10 gramas da levedura em questão. Amostras do conteúdo ruminal serão coletadas a cada 45 dias para analise do perfio dos ácidos graxos de cadeia curta, quantificação da população de bactérias celulolíticas e pH ruminal.

Local do experimento:

São Paulo, 10 de maio de 2017

Profa. Dra. Denise Tabacchi Fantoni
Presidente da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

Roseli da Costa Gomes
Secretaria Executiva da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

FOLHA DE AVALIAÇÃO

Autor: SOUSA, Dannylo Oliveira

Título: **Alteration of fiber digestibility for ruminants: Effects on intake, performance, and ruminal ecosystem**

Tese apresentada ao Programa de Pós-Graduação em Nutrição e Produção Animal da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo para obtenção do título de Doutor em Ciências

Data: ____/____/____.

Banca Examinadora

Prof. Dr._____

Instituição:_____Julgamento:_____

Prof. Dr._____

Instituição:_____Julgamento:_____

Prof. Dr._____

Instituição:_____Julgamento:_____

Prof. Dr._____

Instituição:_____Julgamento:_____

Prof. Dr._____

Instituição:_____Julgamento:_____

DEDICATÓRIA

Aos meus pais, **Ercio** e **Edileuza**, pelo amor, apoio e dedicação em todas as fases da minha vida, pela presença constante e pelos ensinamentos.

Aos meus irmãos, **Danniel** e **Thays**, pelo apoio e por todos os bons momentos vividos juntos.

À minha avó, **Maria Aparecida**, por todo amor, dedicação e pelas orações.

À minha namorada, **Larissa**, por todo amor, carinho e dedicação durante essa fase.

AGRADECIMENTOS

Agradeço primeiramente a Deus por tudo.

À minha família por ser a minha base sólida para encarar a vida com coragem e dignidade. Minha eterna gratidão.

À Larissa e sua família, por todo amor e carinho dedicado a mim.

Ao meu amigo, professor e orientador Prof. Luis Felipe Prada e Silva, por todos os ensinamentos e pela confiança. Um exemplo de caráter e profissionalismo.

A todos os professores do Departamento de Nutrição e Produção Animal, em especial, Romualdo Fukushima, Alexandre Vaz Pires, Marcos Veiga dos Santos, Francisco Palma Rennó, Augusto Gameiro, Alexandre Gobesso, Júlio Balieiro e Paulo Mazza pela amizade e pelo conhecimento compartilhado.

Aos meus amigos e irmãos Alejandro Vargas, Tiago Tomazi, Juliane Diniz, Cassiele e Johnny. Estes, não apenas por estarem ao meu lado nos momentos bons, mas principalmente, por dividirem fardos pesados em momentos difíceis. Obrigado sempre!

Aos Professores Mike Allen e Mike VandeHaar da Michigan State University que me acolheram nos EUA durante o meu estágio em pesquisa.

Aos amigos da Michigan State University, Jim, Dave, Laura, Jonas, Rodrigo, Gabriela, Roni, Kate, Rodrigo Albornoz, Amy, Irene, Alex, Jared e Lauren, pelos ensinamentos e bons momentos vividos.

A todos os funcionários do VNP. Em especial, ao João Paulo, Alessandra e Fábia. A vocês meu sincero agradecimento.

À Faculdade de Medicina Veterinária e Zootecnia (FMVZ/USP) pela oportunidade de realização deste trabalho.

À Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) pelas concessões das bolsas de Doutorado (processo n° 2013/15751-1) e BEPE (processo n° 2015/02435-0).

À Lallemand Animal Nutrition pelo apoio à pesquisa, através do financiamento e contribuições intelectuais, em especial ao Lucas Mari e Eric Chevaux.

A todos que aqui não citei, mas que de alguma forma contribuíram para a realização deste sonho.

MUITO OBRIGADO!

EPÍGRAFE

“O segredo do sucesso é a constância no propósito”.

Benjamin Disraeli

RESUMO

SOUSA, D. O. **Alteração da digestibilidade da fibra para ruminantes: Efeitos sobre consumo, desempenho e ecossistema ruminal.** [Alteration of fiber digestibility for ruminants: Effects on intake, performance, and ruminal ecosystem]. 2017. 63 f. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Pirassununga, 2017.

O efeito do aumento da digestibilidade da fibra detergente neutro (DFDN) da silagem de cana-de-açúcar sobre consumo, cinética e metabolismo ruminal, população microbiana, desempenho e características de carcaça de bovinos de corte foram avaliados através de dois experimentos. No experimento de metabolismo, oito novilhos Nelore canulados no rúmen foram utilizados em delineamento experimental com dois quadrados latinos 4×4 contemporâneos. No experimento de desempenho, quarenta e oito novilhos nelore foram usados em delineamento em blocos inteiramente casualizados com arranjo de tratamento em fatorial 2×2. Dois genótipos de cana-de-açúcar com alta e baixa-DFDN colhidos para ensilagem em dois estágios de maturidade (2 genótipos e 2 estágios de maturidade). As dietas foram formuladas contendo 40% de silagem de cana-de-açúcar na matéria seca. O consumo de matéria seca (CMS) foi limitado pela via metabólica. O CMS dos novilhos canulados no rúmen foi reduzido quando foram alimentados com a cana-de-açúcar colhida na maturidade tardia, mas somente para o genótipo de baixa-DFDN. A taxa de passagem e o turnover de FDN foram acelerados quando o genótipo de alta-DFDN foi fornecido. O pool ruminal de MS, FDN e FDNi foram maiores para as dietas contendo o genótipo de baixa-DFDN. Houve tendência para maior população relativa de *F. succinogenes* e *R. albus* quando os animais foram alimentado com dietas contendo o genótipo de baixa-DFDN. O desempenho e as características de carcaça não foram afetados pelos tratamentos. O excesso de carboidratos não fibrosos (CNF) e os produtos de fermentação das silagens podem limitar o consumo pela via metabólica. O genótipo de alta-DFDN pode acelerar a taxa de passagem e o turnover de FDN no rúmen. O CMS pode ser reduzido quando o genótipo de baixa-DFDN é colhido para ensilagem com maturidade tardia. O feito da suplementação com levedura viva (LV - *Saccharomyces cerevisiae* CNCM I-1077) sobre a digestibilidade *in situ*, fermentação ruminal e população de bactérias celulolíticas no rúmen de bovinos Nelore em pastejo foi avaliado ao longo de um ano. Oito novilhos canulados no rúmen foram usados em arranjo fatorial 2×4:

com ou sem LV e quantro estações do ano. O tratamento com LV foi realizado diariamente para promover uma suplementação de $8x10^9$ UFC por animal. Os animais foram mantidos em sistema de pastejo rotativo com suplementação mineral. A cada 45 dias, foi realizada a degradação de FDN *in situ* de 5 forragens referencia, determinada após 24 e 48h de incubação ruminal. O fluido ruminal foi coletado para avaliar a contagem de leveduras e os parâmetros de fermentação, e ainda para a quantificação de bactérias celulolíticas por PCR. Mesmo com grande variação da qualidade do pasto ao longo do ano, não foi observado interação entre LV*estação do ano. A suplementação com LV aumentou a degradabilidade da FDN em todas as estações do ano. Entre as quatro bactérias ruminais avaliadas, a *R. flavefaciens* foi a mais prevalente e o tratamento com LV aumentou sua população relativa no verão e na primavera. A suplementação com LV pode beneficiar bactérias degradadoras de FDN e aumentar a digestibilidade ruminal de fibra em animais em pastejo, independentemente da estação do ano.

Palavras-chave: Consumo. Levedura viva. Digestibilidade da FDN. Taxa de passagem. Bactéria ruminal.

ABSTRACT

SOUSA, D. O. **Alteration of fiber digestibility for ruminants: Effects on intake, performance, and ruminal ecosystem.** [Alteração da digestibilidade da fibra para ruminantes: Efeitos sobre consumo, desempenho e ecossistema ruminal]. 2017. 63 f. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Pirassununga, 2017.

The effect of increased neutral detergent fibre digestibility (NDFD) of sugarcane silage on intake, rumen kinetics and metabolism, microbial population, performance, and body fatness of beef cattle was evaluated through two trials. In the metabolism trial, eight ruminal-cannulated Nellore steers were used in a replicated 4×4 Latin square design. In the performance trial, forty-eight Nellore young bulls were used in a complete randomized block design with a 2×2 factorial arrangement of treatments. Two sugarcane genotypes with high or low NDFD harvested for silage in two maturity stages were used (2 genotypes and 2 maturity stages). Diets were formulated with 40% of sugarcane on dry matter (DM) basis. Intake of DM was likely limited by metabolic feedback. The DM intake (DMI) of cannulated steers was reduced when late-maturity sugarcane was fed, but only with low-NDFD genotype. Turnover and passage rate of NDF was accelerated when high-NDFD genotype was fed. Rumen DM, NDF and iNDF pools were greater when diets with the low-NDFD genotype were fed. There was a tendency for greater relative populations of *F. succinogenes* and *R. albus* when animals were fed with diets containing low-NDFD genotype. Performance and carcass characteristics were not affected by treatments. Excess of NFC and fermentation end products of sugarcane silage can limit intake by metabolic pathway. High-NDFD genotype can accelerated NDF turnover and passage rate in the rumen. Late-maturity ensiled sugarcane can reduce DMI of beef steers fed with low-NDFD genotype. The effect of live yeast (LY - *Saccharomyces cerevisiae* CNCM I-1077) on *in situ* fiber digestibility, rumen fermentation, and rumen cellulolytic bacteria population of grazing Nellore cattle was evaluated throughout the year. Eight rumen cannulated steers were used in a 2x4 factorial arrangement of treatments: with or without LY, and the four seasons of the year. Yeast was given daily to provide $8x10^9$ CFU per animal. Animals were kept on a rotational grazing system with mineral supplementation. Every 45 d, *in situ* rumen NDF degradability of 5 reference forages was determined after 24 and 48h incubation. Rumen fluid was collected to measure yeast count and fermentation

parameters, and rumen contents collected for quantitative PCR quantification of cellulolytic bacteria. Although pasture composition varied greatly throughout the year, there was no LY*season interaction. Yeast supplementation increased NDF degradability in all seasons. Among the four rumen bacteria evaluated, *R. flavefaciens* was the most prevalent and LY increased *R. flavefaciens* population in the summer and spring. Supplementing LY can benefit fibre-degrading bacteria and increase fibre degradability of grazing animals, independently of the season.

Keywords: Intake. Live yeast. NDF digestibility. Passage rate. Rumen bacteria.

SUMÁRIO

1 INTRODUÇÃO

Feed supply represents the greater cost of the ruminant production system (Archer et al., 1999). Still, it is predicted that grain price will increase in the next years due the competition with human diets (Guyomard et al., 2013). However, the inclusion of forages in ruminant diets should be maximized to improve the economic efficiency of the production systems, even more in a tropical condition such is Brazil, where harvest roughage is cheaper than grains, although, this roughage has to have high fiber quality to do not limit feed intake and performance, and consequently compromise profitability. Indeed, the main factor limiting greater inclusion of forages in the diets of high producing ruminants is the low fiber digestibility (Van Soest, 1994), that limits voluntary feed intake by rumen fill (Dado e Allen, 1995).

Maximizing forage fiber digestibility is an important management goal in nutrition of growing animals fed with high neutral detergent fiber (NDF) diets, because feed intake regulation by filling results from the slow removal rate of rumen content by digestion, absorption, and passage of the fiber, but increasing forage NDF digestibility (NDFD) increases the clearance rate of forage NDF from the rumen, decreasing its filling effect over time (Oba and Allen, 2000). Digestion of fiber in the rumen is a dynamic process that involves microbial attachment and fermentation of plants cell-wall (Dado e Allen, 1995). The extent of fiber digestion is a function of the proportion of fiber that is potentially digestible, the rate of fiber digestion in the rumen, and the rate of fiber passage from the rumen (Allen, 2000). Thus, any alteration that increases microbial attachment or extent fiber digestion would be imperative to maximize animal efficiency. Evaluating corn hybrid for silage, Ferraretto and Shaver (2015) observed that dry matter intake (DMI), milk yield, and fat milk increased when high producing dairy cows were fed with corn silage hybrid with high-digestibility, compared to the conventional hybrid. As feed intake and performance increase fiber digestion become more important (Chase and Grant, 2013). Increasing fiber digestibility in forages may reduce the need for high grain diets, decreasing diet cost, improving animal health and longevity (Ferraretto et al., 2015).

Previous studies reported a potential benefits when fiber digestibility is increased by genotype or hybrid on feed intake and performance of ruminants (Tine et al., 2001; Weiss and Wyatt, 2002; Sousa et al., 2014; Ferraretto and Shaver, 2015; Lim et al., 2015). However,

others have found positive effects when increasing NDF digestibility by harvesting the crop at early-maturity (Rinne et al., 1997; Rinne et al., 2002; Kung et al., 2008; Kuoppala et al., 2009; Vanhatalo et al., 2009; Kuoppala et al., 2010; Kammes e Allen, 2012; Randby et al., 2012). And yet, another alternative found was to increase fiber degradability inside the rumen, supplementing animals with live yeast to improve ruminal environment that would enhance cellulolytic bacteria growth, and thus increase fiber digestion (Hristov et al., 2010; Allen e Ying, 2012; Patra, 2012; Dehghan-Banadaky et al., 2013). With that, our objectives were to evaluate the alteration of fiber digestibility and its effects on intake, performance, and ruminal ecosystem of beef cattle.

2 EFFECT OF GENOTYPE AND MATURITY STAGE ALTERING FIBER DIGESTIBILITY ON FEED INTAKE AND RUMINAL PARAMETERS OF GROWING STEERS

2.1 INTRODUCTION

Intake of high-fiber, low-energy, and less digestible diets is controlled by rumen fill limitation, whereas intake of highly digestible, high-energy diets is controlled by metabolic regulation (Allen, 2000; NRC, 2016). Advance in maturity of fresh sugarcane during the harvest season increases sugar accumulation and reduces fiber digestibility (Sousa et al., 2014), resulting in a particular roughage source with two factors capable of limiting intake: high soluble sugar content and low fibre digestibility (Allen, 2014). Therefore, ensiling of sugarcane has been used as a conservation method that, apart from other advantages, can increase NDF digestibility by allowing harvest to be done at the beginning of the harvest season and decreases sugar content, but it also increases fiber content and promotes accumulation of organic acids and ethanol (Kung and Stanley, 1982; Daniel et al., 2013).

Intake limitation by rumen fill results from the slow removal of rumen contents by digestion and passage of the fiber. Therefore, increasing forage neutral detergent fiber digestibility increases the clearance rate of NDF from the rumen, decreasing its filling effect (Allen, 2014). Soluble sugars, such as sucrose, have been used as an alternative energy source, with different fermentation characteristics than starch. Oba (2011) reported that when sugars were used to partially replace dietary starch, there was no reduction in rumen pH. In fact, rumen pH was increased, even though sugars have faster fermentation rate than starch (Weisbjerg et al., 1998). Replacing starch with sugars also increases molar proportion of butyrate in the rumen and also intake of dairy cows (Broderick et al., 2008; Gao and Oba, 2016).

Forage breeding for greater NDF digestibility has been an obvious target to increase nutritional quality of cattle forage sources (Casler, 2001). However, it is imperative to understand weather the ensiling process alters the animal response to greater NDFD. Because sugarcane combines poor fiber digestibility with high levels of soluble sugars, it makes a unique model to study mechanisms of intake control in cattle. Therefore, the objectives of this study were to evaluate the effects of increased NDFD of ensiled sugarcane on intake, rumen kinetics and metabolism, microbial population, performance, and body fatness of beef cattle.

2.2 MATERIALS AND METHODS

All experimental procedures were in agreement with the Guide for Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999), with all animal procedures approved by the University of São Paulo Animal Bioethics Committee (protocol number 3602270215).

2.2.1 Sugarcane Genotypes

Two sugarcane genotypes (IAC86-2480 and IACSP93-3046) with high in vitro NDFD were selected and planted together with 2 other genotypes with low in vitro NDFD (SP91-1049 and IAC82-2120) in December of 2010. An area of 4 ha at the University of São Paulo Beef Cattle Research Laboratory (Pirassununga, SP, Brazil) was prepared for several experiments testing sugarcanes of divergent fiber digestibility to increase knowledge in ruminant nutrition. The agronomic conduction and monitoring of the four sugarcane genotypes have been described in detail in Sousa et al. (2014).

Nine months after regrowth, the four plots were sampled weekly and subsampled for apparent sucrose and NDFD determination. Apparent sucrose was determined by polarimetric determination after juice extraction of 500-g samples crushed in a hydraulic press to determine the maturation stage and consequently the first time of harvest. In vitro NDFD analysis was performed to select the most divergent genotypes among the four sugarcane varieties. How it was stated in Sousa et al. (2014) there are 4 distinct growth stages in sugarcane (i.e., germination, tillering, grand growth, and maturity), and maturation has been defined as the accumulation of sucrose, and sugarcane is usually considered mature and fit for harvest when it has attained a minimum of 16% apparent sucrose in the juice (Lingle, 1999). The two genotypes most divergent for NDFD selected for this study were: IAC86-2480, with high NDFD, and IAC82-2120, with low NDFD.

2.2.2 Silage Confection

Ten months after regrowth, both genotypes reached 16% of apparent sucrose and were partially harvested (half the area – 50 ton of original matter) on June of 2014 for silage manufacturing, and the remaining half was collected three months after the first harvest time

on September of 2014 to obtain two maturity stages (early and late maturity). Forage harvest was performed with a silage harvester (Colhiflex; Menta Mit, Cajuru, SP, Brazil) adjusted for 10 to 20 mm forage particle length. Microbial inoculant containing *Lactobacillus buchneri* (LalSil Cana, Lallemand, Brazil) was applied at a dose of 2g of inoculant per metric ton of silage mass to achieve a standard rate of 4×10^5 cfu/g of fresh forage to manipulate fermentation and reduce alcoholic fermentation (Pedroso et al., 2008). The product was uniformly spread onto the freshly chopped forage by a calibrated inoculant applicator fitted at the forage harvester. Both sugarcane genotypes in both harvest time were individually ensiled in 1.83-m-diameter long tube silage bag. Silages were kept closed until 30 days after the second harvest time before opening.

2.2.3 Animals and Diets

Two experiments were conducted to evaluate the effect of altering fiber digestibility by genotype and maturity stage of sugarcane silage on ruminal fermentation, ruminal kinetics, and ruminal bacterial populations in the metabolism trial, and on feed intake, performance, and carcass characteristics in the performance trial. In the metabolism trial, eight ruminal-cannulated Nellore steers (374 ± 14 kg BW and 18 months of age) were used, and housed in in tie stalls with concrete floors, individual feeders, and water bunks. In the performance trial, forty-eight Nellore young bulls (432 ± 27 kg of BW and 22 months of age) were used, and grouped in collective pens, with three animals per pen, with concrete floors, partial cover, feeders and water bunks. Stalls and bunks were cleaned daily.

Diets were fed at 0700 and 1300 h daily and, to ensure ad libitum intake, between 5 and 10% orts were allowed. Experimental diets were formulated with 40% of sugarcane on DM basis (Table 1), and offered diet and orts were weighed daily for intake measurement. The concentrate was composed of ground corn, soybean meal, urea, limestone, salt and a commercial mineral supplement. Two genotypes with high or low NDFD harvested for silage in two maturity stages were used, making up 4 experimental diets in a 2 × 2 factorial arrangement of treatments (2 genotypes and 2 maturity stages). Diets were formulated according to the NRC (2000) to provide BW gain of 1.2 kg/d. The offered diet and orts were analyzed for particle size distribution using the Penn State Particle Separator (Nasco, Fort Atkinson, WI), as described by Heinrichs and Kononoff (2002).

Both experiments started together, although, the performance trial had duration of 63 d, divided in three periods of 21 d and the metabolism trial had duration of 84 d, with four periods of 21 d, with the last 4 d used for determination of intake, performance, ruminal evacuation, and ruminal fluid collection.

Table 1. Composition of the experimental diets and sugarcane silages

	Early maturity		Late maturity	
Item[1]	Low[2]	High[3]	Low	High
Ingredient composition of the experimental diets				
Sugarcane silage	40	40	40	40
Ground corn	49	49	49	49
Soybean meal	7.8	7.8	7.8	7.8
Urea	1.2	1.2	1.2	1.2
Limestone	0.5	0.5	0.5	0.5
Salt	0.2	0.2	0.2	0.2
Mineral Mixture[4]	1.3	1.3	1.3	1.3
Chemical composition of the sugarcane silages				
DM, %	23.2	21.7	24.3	24.5
Ash, % DM	5.3	4.9	4.7	5.3
CP, % DM	1.5	2.2	1.2	1.6
NFC, % DM	20.7	21.3	21.7	25.8
NDF, % DM	72.1	71,2	72.0	66.9
ADF, % DM	52.5	50.3	50.8	46.3
iNDF, % DM	43.6	36.4	43.3	34.6
DMD, % DM	71.2	75.8	75.4	77.5
ADL, % NDF	12.4	10.1	11.9	9.8
NDFD, % NDF	32.5	39.2	11.0	38.4

[1]DM=dry matter, CP=Crude Protein, NFC=non-fibre carbohydrate, NDF=neutral detergent fibre, ADF=acid detergent fibre, iNDF=indigestible NDF, DMD=dry matter digestibility, ADL=acid detergent lignin, NDFD=neutral detergent fibre digestibility.
[2]IAC82-2120 sugarcane genotype.
[3]IAC86-2480 sugarcane genotype.
[4]The trace mineral mixture contained per kilogram: 302 g of calcium, 30 mg of cobalt, 1,008 mg of copper, 80 g of sulphur, 390 mg of fluorine, 39 g of phosphorus, 60 mg of iodine, 20 g of magnesium, 2,998 mg of manganese, 30 mg of selenium, 4,032 mg of zinc, 400,000 IU of vitamin A, 40,000 IU of vitamin D3, and 1,450 IU of vitamin E.

2.2.4 In Vitro Digestibility, and Chemical Composition

Total diet, each roughage source, concentrate mix, and orts from each stall were sampled on the last 3 days of each experimental period of each study. Samples from the 3 d were combined in equal amounts, mixed, and subsampled for determination of chemical

composition. The pooled samples were dried in a forced-ventilation oven at 55°C for 72 h and ground at a Willey mill (Tecnal, Piracicaba, Brazil) to pass a 1-mm screen.

In vitro DM digestibility (DMD) and NDFD were determined on composited roughage samples. Samples (0.25 g) were digested for 48 h in triplicates in F-57 bags with a DaisyII incubator (ANKOM Technology Corp., Fairpoint, NY). Ruminal fluid was collected from a ruminal cannulated crossbred cow fed a diet with 50% fresh sugarcane and 50% concentrate mix based on corn, soybean meal, and mineral supplement. After collection, ruminal fluid was combined with in vitro buffer as in Holden (1999).

Total DM and ash were analyzed according to AOAC (2000) methods 930.15 and 942.05, respectively. Concentration of CP was determined by combustion (Leco protein/N analyzer, model FP-528; Leco Corp., St. Joseph, MI), and concentration of NDF was determined using the method described by Vansoest et al. (1991) using 8 M urea and heat stable α-amylase (Sigma A3306; Sigma Chemical Co., St. Louis, MO) in an ANKOM A200 Fiber Analyzer (ANKOM Technology Corp.). Acid detergent lignin and ADF were analyzed according to Van Soest and Robertson (1985). Indigestible NDF (iNDF) was determined by the NDF content in samples after 288 h of in situ incubation, as described by Rinne et al. (2002).

2.2.5 Ruminal Kinetics

In the metabolism trial, total ruminal contents were manually evacuated through the ruminal cannula at 0900 h (2 h post-feeding) on d 19 of each period and at 0500 h (2 h before feeding) on d 20 of each period (Dado and Allen, 1995). Total rumen content mass was weighed and volume was determined using graduated plastic containers. To aid in proper subsampling, 10% of the total rumen contents were squeezed by hand through a nylon screen to separate into solid and liquid phases. Samples were taken from both phases for determination of nutrient pool size. The NDF ruminal kinetics was calculated based on iNDF rumen pool size (Dado and Allen, 1995). The NDF turnover rate was defined as NDFI divided by total mass of ruminal NDF, and the NDF passage rate (kp) was calculated by dividing iNDF intake by the total mass of ruminal iNDF.

Ruminal liquid volume and liquid turnover rates were measured by using polyethylene glycol-4000 (PEG) as a marker. On d 21 of each period, 100 mL of a 10% PEG (Synth, São Paulo, Brazil) solution was infused at 0700 h in the rumen and manually mixed with the

ruminal contents. Samples of ruminal contents were obtained at 0, 1, 3, 6, 9, 12, and 24 h after PEG infusion. The sample at time 0 was collected before PEG infusion. Ruminal samples were collected from the dorsal, ventral, and anterior sac of the rumen using a vacuum pump. The concentration of PEG was determined by the turbidimetric method of Hyden (1956). The natural logarithm of PEG concentrations was regressed with time to calculate fluid dilution rate (%/h), and ruminal liquid volume was calculated from the antilogarithm of the intercept.

2.2.6 Ruminal Fermentation

In the metabolism trial, on d 21 of each period, ruminal fluid samples were collected at 6 times: 0, 1, 3, 6, 9, and 12 h after feeding. At each time point, samples were taken from cranial, ventral, and caudal areas of the rumen. After mixing, ruminal fluid samples were filtered through a 1-mm nylon mesh (Albercan Group, Itajubá, Brazil) and 100 mL of ruminal fluid was divided into two 50-mL aliquots, one for measuring ruminal pH and another for analysis of short chain fatty acids (SCFA) and ammonia. Ruminal fluid pH was immediately measured using a portable pH meter (Tec- 3MP, Tecnal, Brazil) calibrated with pH 4.0 and 7.0 buffer solutions (Fisher Scientific, Fairlawn, NJ). After centrifuging the remaining 50 mL of ruminal fluid at 6,500 × g at 25°C for 15 min, a 2-mL subsample of the supernatant was taken, mixed with 0.4 mL of formic acid, and stored at –20°C for SCFA determination. One milliliter of 1 N sulfuric acid was added to another 2 mL of the supernatant and stored at –20°C for ammonia-N determination by the phenol-hypochlorite method (Kangmeznarich and Broderick, 1980).

Ruminal fluid concentration of SCFA was measured by gas chromatography with a capillary column (Stabilwax; Restek, Bellefonte, PA) using the method described by Erwin et al. (1961) and adapted by Getachew et al. (2002). Frozen acidified ruminal fluid samples were thawed at room temperature and centrifuged at 14,500 × g at 4°C for 10 min. Supernatant (1 mL) was transferred into a clean dry vial with 100 μL of internal standard (2-ethylbutyric acid 100 mM; Chem Service, West Chester, PA) and capped. Concentrations of SCFA were determined using a gas chromatography (GC-2014; Shimadzu, Kyoto, Japan), with split injector and dual flame ionization detector temperatures at 250°C and column temperature at 145°C. External standards were prepared with acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids (Chem Service). The software GCSolution (Shimadzu) was used

for separation and integration of chromatographic peaks and for calculation of SCFA concentrations.

2.2.7 Ruminal Bacteria Population

In the metabolism trial, two ruminal bacteria important for fiber degradation (*Fibrobacter succinogenes* and *Ruminococcus albus*), 1 important for starch digestion and lactic acid production (*Streptococcus bovis*) and 1 ruminal bacteria important for lactate utilization and propionic production (*Megasphaera elsdenii*) had their population quantified by quantitative PCR for determining the effect of diets on ruminal population. During evacuation of the ruminal contents 2 h before and after feeding, a sample of approximately 600 mL, proportionally representing the liquid and solid phases of the ruminal content, was collected and brought to the laboratory. For each sample, 25 mL of fluid and 25 g of solids were used for processing, as described by Stevenson and Weimer (2007), and the resulting bacteria pellet was dissolved in 700 μL of buffer (100 m*M* Tris/HCl, 10 m*M* EDTA, and 0.15 *M* NaCl, pH 8.0) and stored at –80°C until DNA extraction.

For each ruminal sample, 200 μL was submitted to DNA extraction with QIAamp DNA Stool Mini Kit (Qiagen, Valencia, CA) and used according to the manufacturer's instructions. Briefly, ruminal samples were homogenized in ASL buffer (Qiagen) and heated at 95°C for 5 min to lyse bacterial cells. After removal of potential inhibitors by incubation with an InhibitEx tablet (Qiagen), the lysates were treated with proteinase K (Qiagen) and AL buffer (Qiagen) at 70°C for 10 min to remove protein and polysaccharides. The DNA was precipitated by ethanol, applied to a column provided in the kit followed by washes with AW1 and AW2 buffers (Qiagen), and then dissolved in AE buffer (Qiagen) and followed with ethanol precipitation, as described by Sambrook and Russell (2001).

Individual real-time PCR reactions were performed in 48-well plates on a StepOne Real Time PCR System containing 10 μL of 2x SYBR Green Master Mix (Life Technologies, Foster City, CA), 0.30 μ*M* concentration of each primer (Table 2), and 1 μL of DNA template in a final volume of 20 μL per reaction. A universal primer was used for quantification of total bacteria (Table 2), to standardize the amount of DNA added to the reactions. The PCR reactions of all bacteria were run in duplicate. The PCR amplification protocol was as follows: an initial denaturation step at 95°C for 10 min, then 44 cycles of heating and cooling

at 95°C for 15 s and 60°C for 30 s, and extension at 72°C for 30 s. Melting curves were analyzed at the end of the reactions to verify the specificity of each amplification.

Table 2. Oligonucleotides used in PCR quantification of rumen microorganisms

Target organism	Sequence (5'→3')[1]	Reference
F. Succinogenes	F: GGTATGGGATGAGCTTGC R: GCCTGCCCCTGAACTATC	Tajima et al. (2001)
R. albus	F: CCCTAAAAGCAGTCTTAGTTCG R: CCTCCTTGCGGTTAGAACA	Koike and Kobayashi (2001)
S. bovis	F: CTAATACCGCATAACAGCAT R: AGAAACTTCCTATCTCTAGG	Stevenson and Weimer (2007)
M. elsdenii	F: GACCGAAACTGCGATGCTAGA R: CGCCTCAGCGTCAGTTGTC	Ouwerkerk et al. (2002)
Eubacteria	F: CCTACGGGAGGCAGCAG R: ATTACCGCGGCTGCTGG	Muyzer et al. (1993)

[1]F=Forward; R=Reverse.

2.2.8 Performance and carcass traits

In the performance trial, animals were weighted after 16-h of solid fasting and also had their carcasses evaluated at the beginning of the experiment and on d 21 of each period, for better monitoring of changes in BW and in carcass characteristics. Carcasses were evaluated for *Longissimus dorsi* muscle area (LMA) between the 12th to 13th ribs, fat thickness at three-quarters of the lateral distance across the *Longissimus dorsi* between the 12th to 13th ribs (BFT), and fat thickness over the termination point of the *Biceps femoris* muscle in the rump (RFT) by ultrasound, using an Aloka 500V system equipped with a 3.5-MHz, 17-cm transducer (Aloka USA, Inc., Wallingford, CT). Images were analyzed using the Lince software.

Animals were slaughtered at a commercial slaughterhouse in accordance with current guidelines after 16-h fast from solids and fluids. The animals were stunned by cerebral concussion, suspended, and exsanguinated through the jugular vein, and weighted to obtain hot carcass weight (HCW). Carcass dressing percentage was obtained by dividing HCW by final BW.

2.2.9 Statistical Analysis

In both experiments, statistical analyses were conducted using the MIXED procedure of SAS version 9.2 (SAS Inst. Inc., Cary, NC). In the metabolism trial, intake, ruminal kinetics, and ruminal bacteria population data were analyzed as a replicated 4 × 4 Latin square design, with a 2 × 2 factorial arrangement of treatments. The model included the fixed effects of sugarcane genotype (Gen), the maturity stage (Mat) and the Gen × Mat interaction. It also included the random effects of square, animal within square, and periods. For analysis of ruminal pH and SCFA, time of sample collection (TIME; repeated measure) and time × treatments interactions were included as fixed effects in the model.

In the performance trial, data were analyzed as a complete randomized block design, with a 2 × 2 factorial arrangement of treatments. The model included the fixed effects of sugarcane genotype, the maturity stage and the Gen × Mat interaction. Block was included in the model as a random effect, and pen was considered as the experimental unit.

The degrees of freedom and tests were adjusted by the Kenward-Roger option, and significance of main effects was declared at $P \leq 0.05$ and tendency at $P \leq 0.10$. The effects of moderate interactions ($P < 0.15$) were investigated with the SLICE option of PROC MIXED to determine whether these interactions affect main-effect comparisons (Lowry, 1992).

2.3 RESULTS

2.3.1 Roughages and Diets Characteristics

As expected, the genotype IAC86-2480 had greater NDFD and DMD in both maturity stages ($P < 0.01$, Table 1), when compared to IAC82-2120 genotype. There was no Gen × Mat interaction for any chemical component of the total diet ($P > 0.15$, Table 3). There was greater ADF ($P = 0.04$), iNDF ($P < 0.01$) and ADL ($P < 0.01$) and a tendency for greater DM ($P = 0.1$) content for the diets containing the low-NDFD genotype (Table 3). There was greater DM ($P = 0.02$) for the diets with late-maturity sugarcane, and greater ADF ($P = 0.02$) and a tendency for greater iNDF ($P = 0.07$) for the diets with early-maturity sugarcane (Table 3). However, ash, CP, NFC and NDF of the diets were not affected by treatments ($P > 0.15$, Table 3).

Table 3. Chemical composition of the experimental diets

	Early maturity		Late maturity			*P*-value		
Item[1]	Low[2]	High[3]	Low	High	SEM	Gen	Mat	Gen × Mat
DM	60.0	59.1	60.7	60.3	0.4	0.10	0.02	0.50
	---------- % of DM ----------							
Ash	4.8	4.6	4.6	4.8	0.2	0.86	0.78	0.15
CP	12.2	12.4	12.2	12.2	0.2	0.41	0.31	0.38
NFC	44.1	44.1	44.6	46.0	1.0	0.36	0.14	0.38
NDF	37.5	37.4	37.2	35.6	0.8	0.28	0.19	0.36
ADF	26.4	25.7	25.5	24.1	0.6	0.04	0.02	0.39
iNDF	22.4	19.7	22.1	18.9	0.7	<0.01	0.07	0.40
	---------- % of NDF ----------							
ADL	6.5	5.6	6.3	5.5	0.2	<0.01	0.34	0.70

[1]DM=dry matter; CP=Crude Protein; NFC=non-fibre carbohydrate; NDF=neutral detergent fibre; ADF=acid detergent fibre; iNDF=indigestible NDF; ADL=acid detergent lignin.
[2]IAC82-2120 sugarcane genotype.
[3]IAC86-2480 sugarcane genotype.

2.3.2 Feed Intake, Rumen Pool, and Fibre and Liquid Kinetics (Metabolism trial)

In the metabolism trial, intake of DM and iNDF were affected by Gen × Mat interaction ($P < 0.05$, Table 4). Intake of DMI was greater ($P = 0.05$) when the cannulated animals were fed with early-maturity sugarcane silage, but only with low-NDFD genotype (Table 4). Greater ($P = 0.1$) intake of iNDF was observed when feeding the low-NDFD genotype, but only with early-maturity (Table 4). Intake of NDF was influenced by maturity, with greater ($P = 0.04$) NDF intake for early-maturity sugarcane diets (Table 4). There was no either Gen × Mat interaction or simple effect of maturity on rumen pool parameters ($P > 0.15$, Table 4). Rumen DM, NDF and iNDF pools were greater ($P < 0.01$) when diets with the low-NDFD genotype were fed (Table 4).

Turnover of NDF and dNDF, and NDF passage rate were greater ($P < 0.01$) when animals were fed with high-NDFD sugarcane silage in the diet (Table 4). Passage rate of NDF was also greater ($P = 0.05$) when early-maturity sugarcane silage was given (Table 4). There was no Gen × Mat interaction on rumen fiber kinetics ($P > 0.15$, Table 4). Liquid turnover rate in the rumen, expressed as liters per day was affected by Gen × Mat interaction, where the greater ($P = 0.13$) turnover rate was for diets containing early-maturity sugarcane silages, but only with low-NDFD genotype (Table 4). Volume of ruminal liquid was greater ($P = 0.04$) for diets with early-maturity silages and tended ($P = 0.1$) to be greater for diets with

low-NDFD genotypes (Table 4). There was no effect of treatments on liquid turnover rate in the rumen, expressed as percent per hour ($P > 0.15$, Table 4).

Table 4. Effects of treatments on intake, rumen pool size, and fibre and liquid kinetics of rumen cannulated steers

Item[1]	Early maturity		Late maturity		SEM	*P*-value		
	Low[2]	High[3]	Low	High		Gen	Mat	Gen × Mat
	---------- Intake, kg/d ----------							
DM	6.91	6.45	6.20	6.58	0.29	0.86	0.16	0.05
NDF	2.59	2.42	2.31	2.34	0.12	0.42	0.04	0.24
iNDF	1.55[A]	1.26[B]	1.37	1.25	0.06	<0.01	0.05	0.10
	---------- Rumen pool, kg ----------							
DM	3.4	3.0	3.4	3.0	0.3	<0.01	0.96	0.88
NDF	2.3	2.0	2.3	2.0	0.2	<0.01	0.99	0.95
iNDF	1.5	1.1	1.5	1.1	0.1	<0.01	0.79	0.92
	---------- Fiber kinetics, %/h ---------							
Turnover NDF	4.8	5.4	4.4	5.2	0.4	<0.01	0.03	0.38
Turnover dNDF	5.3	6.1	5.0	5.8	0.7	<0.01	0.16	0.83
k_p - NDF	4.5	4.9	4.1	4.8	0.3	<0.01	0.05	0.21
	---------- Ruminal liquid ----------							
Volume, L	31.6	29.5	28.9	27.3	1.8	0.10	0.04	0.82
Turnover rate, %/h	9.9	10.0	9.3	10.3	0.8	0.29	0.79	0.39
Turnover rate, L/d	72.7	68.8	64.3	66.1	3.6	0.57	<0.01	0.13

[1]k_p=iNDF passage rate.
[2]IAC82-2120 sugarcane genotype.
[3]IAC86-2480 sugarcane genotype.
[A-B]Means within a row with different superscripts differ within maturity stage ($P < 0.15$).

2.3.3 Rumen Fermentation (Metabolism trial)

There was Gen × Mat interaction ($P \leq 0.15$, Table 5) on ruminal pH and total SCFA. Ruminal pH was greater ($P = 0.04$) when early-maturity silages were fed, but only with high-NDFD genotype (Table 5). Total SCFA concentration in the rumen was greater ($P = 0.15$) when early-maturity silages were fed, but only with low-NDFD genotype (Table 5). There was no effect ($P > 0.15$) of treatments and its interaction on ruminal ammonia (Table 5). Proportion of propionate was greater ($P = 0.04$) in the rumen of animals fed with high-NDFD sugarcane genotype (Table 5). Diets containing low-NDFD genotype resulted in greater proportion of BCFA ($P > 0.01$) and greater A:P ration ($P = 0.02$) in the rumen content (Table 5). Proportion of butyrate was grater ($P = 0.02$) when late-maturity sugarcane silages were given to the animals (Table 5). There was no effect ($P > 0.1$) of treatments and its interaction on proportion of acetate and valerate (Table 5). However, analysing the effect of treatments

on rumen fermentation throughout the day, there was a Gen × Time interaction for acetate (*P* = 0.02), propionate (*P* = 0.02) and A:P ratio (*P* = 0.02), and a Mat × Time interaction for butyrate (*P* = 0.02). Proportion of acetate and A:P ratio were greater for diets containing low-NDFD genotype, but only 3 hours post-feeding (Figures 1 and 4). Proportion of propionate was greater for diets containing high-NDFD genotype, but only 3 hours post-feeding (Figure 2). Proportion of butyrate was greater for diets containing late-maturity sugarcane silages, but only at 1, 6 and 9 hours post-feeding (Figure 3).

Table 5. Effects of treatments on rumen fermentation parameters of cannulated steers

	Early maturity		Late maturity			*P*-value		
Item[1]	Low[2]	High[3]	Low	High	SEM	Gen	Mat	Gen × Mat
Ruminal pH	6.6	6.7	6.7	6.6	0.1	0.56	0.26	0.04
Ammonia, mg/dL	13.8	12.9	13.0	12.3	1.2	0.42	0.49	0.93
Total SCFA, m*M*	60.4	56.3	51.1	54.7	3.4	0.94	0.04	0.15
Percent of total SCFA								
Acetate	65.7	64.5	64.3	64.2	0.7	0.22	0.12	0.28
Propionate	19.7	20.9	20.0	20.7	0.8	0.04	0.88	0.67
Butyrate	10.5	11.0	11.4	11.4	0.5	0.30	0.02	0.43
Valerate	1.3	1.16	1.3	1.3	0.8	0.17	0.16	0.47
BCFA	2.9	2.54	3.1	2.4	0.2	<0.01	0.88	0.30
A:P ratio	3.4	3.11	3.3	3.1	0.2	0.02	0.65	0.37

[1]SCFA=short chain fatty acids; BCFA=branched-chain fatty acids; A:P ratio=acetate:propionate ratio.
[2]IAC82-2120 sugarcane genotype.
[3]IAC86-2480 sugarcane genotype.

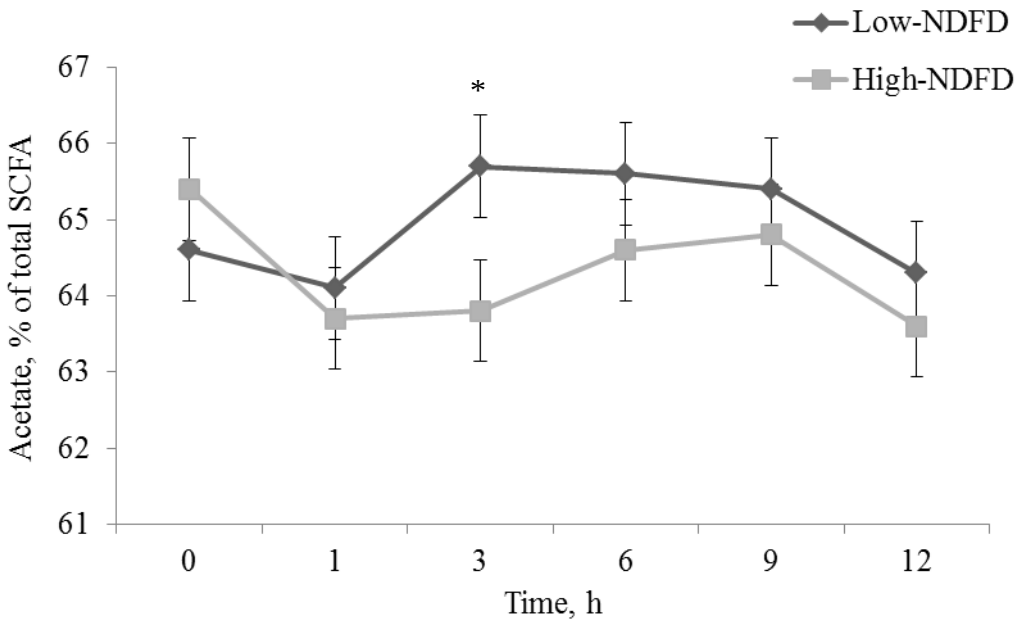

Figure 1: Proportion of acetate (% of total) relative to time of treatment diets. Gent × Time interaction (*P* = 0.06) was significant. *Means within the same time period differ (*P* < 0.05).

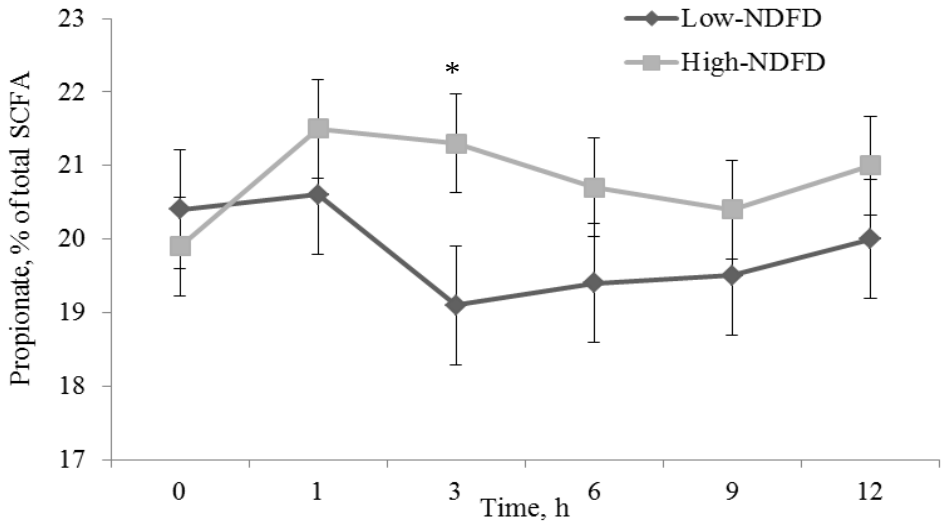

Figure 2: Proportion of propionate (% of total) relative to time of treatment diets. Gent × Time interaction (P = 0.03) was significant. *Means within the same time period differ (P < 0.05).

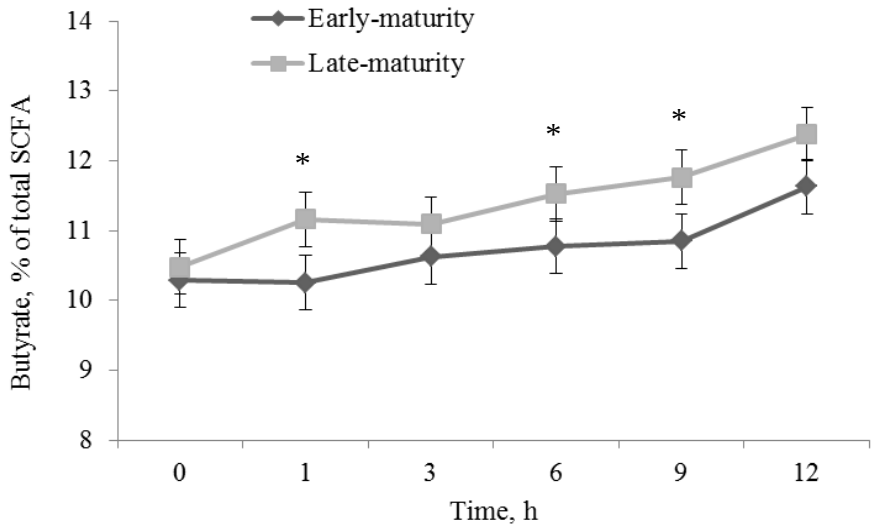

Figure 3: Proportion of butyrate (% of total) relative to time of treatment diets. Mat × Time interaction (P = 0.14) was significant. *Means within the same time period differ (P < 0.05).

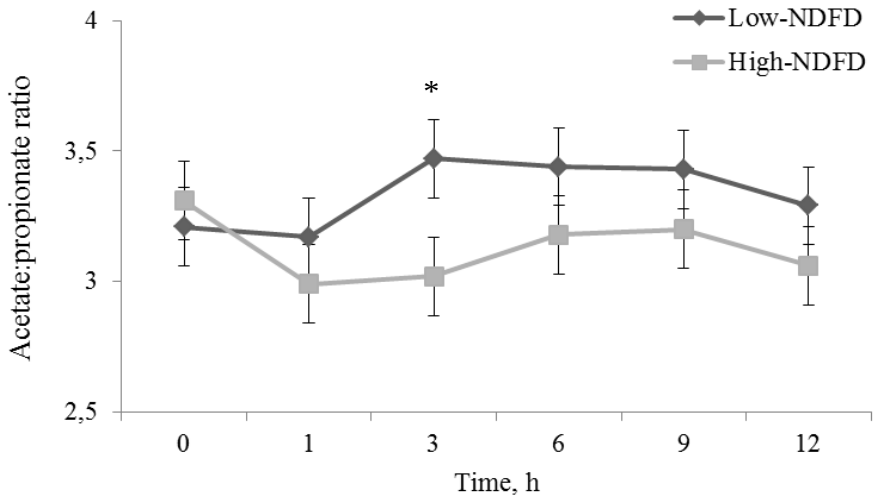

Figure 4: Acetate:propionate ratio relative to time of treatment diets. Gent × Time interaction (P = 0.02) was significant. *Means within the same time period differ (P < 0.05).

2.3.4 Ruminal Bacteria Population (Metabolism trial)

Relative populations of ruminal bacteria were not altered by Gen × Mat interaction (P > 0.15, Table 6). *Megasphaera elsdenii* was the only ruminal bacteria affected by maturity, where the relative population was greater (P = 0.02) when diets with late-maturity sugarcane silages were fed (Table 6). There was a tendency for greater relative populations of *Fibrobacter succinogenes* (P = 0.07) and *Ruminococcus albus* (P = 0.1) when animals were fed with diets containing low-NDFD genotype (Table 6). Relative population of *Streptococcus bovis* was not affected by treatments and its interaction (P > 0.15, Table 6). However, both *F. succinogenes* and *S. bovis* populations were affected by time of sample collection, where the relative populations were greater ($P \leq 0.01$) 2 h before than 2 h after feeding (Figures 5 and 6).

Table 6. Effects of treatments on relative[1] population of ruminal bacteria of cannulated steers

	Early maturity		Late maturity			*P*-value		
Species	Low[2]	High[3]	Low	High	SEM	Gen	Mat	Gen × Mat
F. succinogenes	3.19	2.22	2.60	1.84	1.04	0.07	0.31	0.83
R. albus	0.073	0.044	0.070	0.052	0.03	0.10	0.87	0.74
S. bovis	0.00064	0.00094	0.00071	0.00077	0.00023	0.14	0.66	0.30
M. elsdenii	0.00005	0.00008	0.00013	0.00011	0.00003	0.97	0.02	0.34

[1]The relative population size is presented as percentage of total microbial population.
[2]IAC82-2120 sugarcane genotype.
[3]IAC86-2480 sugarcane genotype.

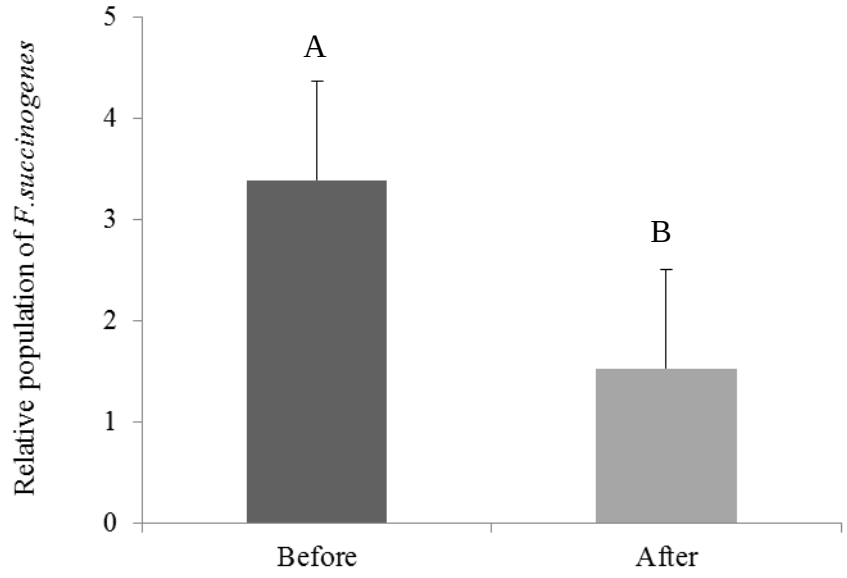

Figure 5: Relative population of *Fibrobacter succinogenes* to time of sample collection. Time effect ($P < 0.01$) was significant. [A-B]Means with different superscripts differ.

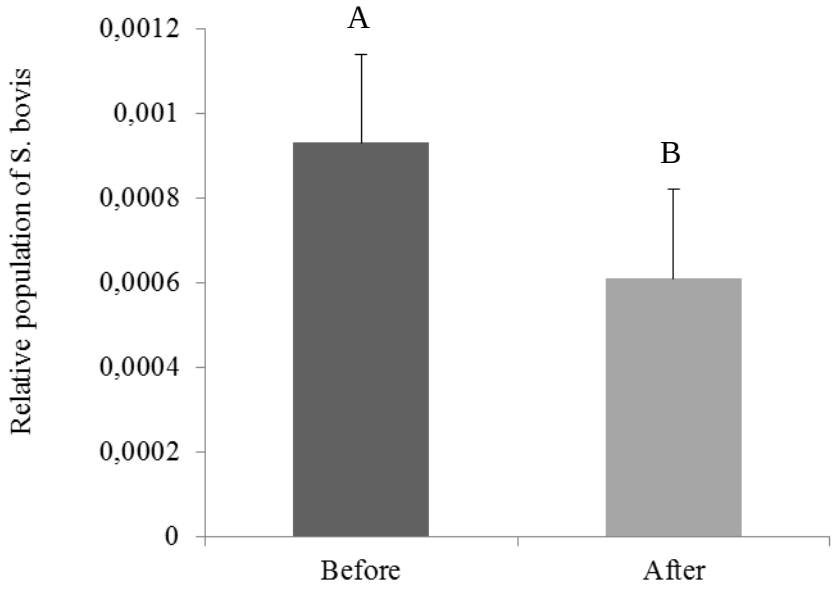

Figure 6: Relative population of *Streptococcus bovis* to time of sample collection. Time effect ($P = 0.01$) was significant. [A-B]Means with different superscripts differ.

2.3.5 Feed Intake, Performance and Carcass Characteristics (Performance trial)

There was no Gen × Mat interaction ($P > 0.15$) or simple effect of maturity ($P > 0.1$) on intake of DM, NDF and iNDF (Table 7). Intake of iNDF was greater ($P < 0.01$) when animals received diets with low-NDFD sugarcane genotype (Table 7). There was no Gen × Mat interaction ($P > 0.15$) or simple effects of either genotype or maturity ($P > 0.1$) on initial and

final BW, ADG, and G:F ratio (Table 7). Carcass characteristics were not affected by Gen × Mat interaction ($P > 0.15$; Table 7), however, there was a tendency for greater ($P = 0.09$) BFT when diets with low-NDFD genotype were fed, and was also a tendency for greater ($P = 0.09$) BFT for animals receiving late-maturity sugarcane silages (Table 7). There was no effect ($P > 0.1$) of either genotype or maturity on HCW, dressing, LM area and FBF (Table 7).

Table 7. Effects of treatments on intake, performance and carcass characteristics of beef cattle

	Early maturity		Late maturity			*P*-value		
Item[1]	Low[2]	High[3]	Low	High	SEM	Gen	Mat	Gen × Mat
---------- Intake, kg/d ----------								
DM	10.6	9.88	10.3	10.4	0.34	0.37	0.64	0.18
NDF	3.85	3.61	3.61	3.67	0.13	0.50	0.48	0.27
iNDF	2.36	1.99	2.23	2.04	0.07	<0.01	0.50	0.16
---------- Performance ----------								
Initial BW	434	434	430	431	22	0.99	0.68	0.95
Final BW	534	526	540	532	22	0.45	0.58	0.98
ADG, kg	1.93	1.84	1.95	1.87	0.12	0.49	0.83	0.98
G:F, g/kg	0.17	0.15	0.18	0.17	0.01	0.23	0.16	0.61
----- Carcass characteristics -----								
HCW, kg	294	286	292	292	10.6	0.51	0.74	0.52
Dressing, %	55.1	54.7	54.1	55.0	0.6	0.68	0.45	0.19
LMA, cm²	75.8	74.1	74.3	73.8	0.9	0.22	0.29	0.53
BFT, mm	3.9	3.3	4.5	4.9	0.3	0.09	0.09	0.99
RFT, mm	6.7	5.8	6.1	6.6	0.5	0.76	0.91	0.17

[1]BW=body weight; ADG=average daily gain; G:F=gain to feed ratio; HCW=Hot carcass weight; LMA=loin muscle area; BFT=back-fat thickness; RFT=rump fat thickness.
[2]IAC82-2120 sugarcane genotype.
[3]IAC86-2480 sugarcane genotype.

2.3.6 Diet Selection (Metabolism and Performance trials)

In the metabolism trial, diet selection was affected by Gen × Time interaction ($P = 0.11$), where lower proportion of large particles was observed on orts of diets with high-NDFD genotype, indicating that animals selected in favour of large particles (Table 8). In the performance trial, there was Gen × Mat × Time interaction ($P = 0.08$) on diet selection (Table 8). There was greater proportion of large particles on orts of diets containing low-NDFD genotype, but only for late-maturity silages, indicating that animals selected in favour of small particles (Table 8). There was no either Gen × Mat or Mat × Time interactions ($P > 0.15$) and because of that these results are not presented in Table 8.

Table 8. Effects of treatments on diet selection of rumen cannulated steers and beef cattle

	Early maturity				Late maturity					*P*-value[5]				
	Low[1]		High[2]		Low		High							
Trial	Offered[3]	Orts[4]	Offered	Orts	Offered	Orts	Offered	Orts	SEM	Gen	Mat	Time	G × T	G × M × T
	---------- % retained on 8.0 mm sieve ----------													
Metabolism	35.7	32.9	41.4^{A}	34.5^{B}	35.8	38.5	39.9^{A}	32.9^{B}	4.6	0.49	0.76	0.11	0.11	0.49
Performance	38.0	34.6	39.7	37.7	36.1^{B}	45.4^{A}	38.8	35.6	3.1	0.77	0.44	0.94	0.14	0.08

[1]IAC82-2120 sugarcane genotype.
[2]IAC86-2480 sugarcane genotype.
[3]Total diet evaluated before feeding.
[4]Diet orts evaluated after feeding.
[5]G=effect of genotype; T=effect of time; M=effect of maturity.

2.4 DISCUSSION

Mechanisms of intake regulation (e.g. rumen distention, osmotic effects, metabolic feedback) change with diet characteristics and physiological state of animals, which is highly variable among ruminants (Allen, 2014). Ruminal distention might dominate control of feed intake when ruminants consume low-energy diets or when energy requirements are high, but fuel-sensing by tissues is likely to dominate control of feed intake when energy supply is in excess of that required (Allen et al., 2009; Mertens, 2009). The predicted DMI for growing and finishing cattle weighting 400 kg of BW and fed a 70% TND diet is 9.49 kg/d (NRC, 2016). However, in the present study the DMI of cannulated steers was lower (average 6.54 kg/d) than predicted by NRC (2016), indicating that feed intake was limited before maximum rumen filling. Mertens (1994) estimated maximum rumen fill with NDF intake of 12.5 g kg^{-1} of BW per day, and Huhtanen et al. (2016) estimated a maximum rumen fill of 13.4 g rumen NDF kg^{-1} of BW. In the current study, NDF intake was much lower (average 6.03 g kg^{-1} BW) than the limiting number predicted by Mertens (1994), and rumen pool of NDF was also much lower (average 5.4 g rumen NDF kg^{-1} of BW) than the maximum predicted by Huhtanen et al. (2016), suggesting that intake was not limited by rumen fill.

We hypothesized that using sugarcane silage with high inclusion (40% DM basis) in the diet would limit feed intake by rumen filling, and the increased NDFD would affect DMI and ruminal kinetics positively. However, the turnover and passage rate of NDF was accelerated when high-NDFD genotype was fed, but with no difference on DMI, indicating that feed intake was limited by metabolic pathway before greater fiber digestibility could stimulate intake by faster clearance of NDF content from the rumen. Sugarcane silage-based-diets had great NFC content from sucrose of the sugarcane and from concentrate of the diet, which could explain the lack of genotype effect on intake and performance parameters among these diets. One accepted theory is that excess ruminal fermentation would lead to excess of hepatic oxidation of propionic acid, and the accumulation of hepatic ATP would thus lead to reduce feed intake (Allen et al., 2009). And also, an additive effect of end products of silage fermentation could have cooperated to DMI limitation (Huhtanen et al., 2007), once is known that approximately 21% (DM basis) of sugarcane silage is volatile compounds (Daniel et al., 2013).

Furthermore, there was a great decrease of NDF digestibility for low-NDFD genotype harvested at late-maturity stage, and it affected DMI negatively when compared to the same genotype harvested at early-maturity in the metabolism trial, suggesting that DMI can be

reduced when low-NDFD genotype is harvested for silage at late-maturity stage. Low feed intake of sugarcane based-diets is generally attributed to low-NDFD (Sousa et al., 2014; Daniel et al., 2016), which is correct to assume, however sugarcane crop has relatively high energy levels (2.0 to 2.4 Mcal/kg; Correa et al., 2003) that should be also considerate as a potential feed intake inhibitor due the rapid rates of hydrolysis (248 to 1,404 %/h) and fermentation (264 to 738 %/h) of sugars (Weisbjerg et al., 1998) in the rumen. Indeed, previous studies indicated that partial substitution of dietary starch with sugars often increased DMI and performance (Broderick et al., 2008; Penner and Oba, 2009; Martel et al., 2011). Although, when high energy diet ingredients are not replaced but combined, excessively fermentable diets might further suppress feed intake, because of greater delivery of fatty acids to the liver stimulating complete oxidation (Allen, 2009). Similar results were observed before (Sousa et al., 2014; Sousa et al., 2017), where DMI was also likely limited by metabolic feedback when growing beef cattle was fed with sugarcane silage or freshly cut as roughage source with either 60 or 80% corn-based concentrate (DM basis) in the diet.

Even with high NFC in the diets, the average of ruminal pH was high and concentration of total SCFA in the rumen was considered low among treatments that can be related to high forage NDF in the diet. However, Vallimont et al. (2004) indicated that feeding sucrose could result in increased molar proportion of butyrate in the rumen, and Malhi et al. (2013) reported that ruminal butyrate infusion increased the ruminal epithelia growth and absorption capacity of fatty acids in the rumen. With increased capacity of ruminal epithelia to uptake volatile fatty acids might have maintained lower total SCFA concentration and thus rumen pH above critical level. In addition, Oba (2011) showed that rumen pH did not decrease (McCormick et al., 2001; DeFrain et al., 2004; Broderick et al., 2008), but even increased (Chamberlain et al., 1993; Heldt et al., 1999), when sugar was used.

Most cellulolytic bacteria in the rumen prefer pH near neutrality for growth, none of the predominant fiber degrading species (*Fibrobacter succinogenes, Ruminococcus flauefaciens,* and *Ruminococcus albus*) grow at pH <6.0 (Russell and Wilson, 1996). The slow rates (5 to 8 %/h) of cellulose digestion involves adherence of cellulolytic bacteria to the fibers (Weimer, 1996; Miron et al., 2001). Due the greater NDF rumen pool and the slower removal of NDF from the rumen of animals fed with low-NDFD genotype, the relative population of the two cellulolytic bacteria evaluated tended to be greater and consequently, BCFA and A:P ratio were greater and propionate concentration was lower for these treatments. The lactate utilizing bacteria measured was *Megasphaera elsdenii*, which population increased when late-maturity sugarcane was fed, and also butyrate proportion was greater in these treatments.

Ruminal *M. elsdenii* population is able to grow on sucrose or glucose, and from these substrates the end product is butyrate, which comes from lactate (Hino et al., 1994; de Souza et al., 2017).

In conclusion, animals fed with sugarcane silage based-diets had their DMI likely limited by metabolic pathway that can be attributed to fermentation of sugars and accumulation of fermentation end products in the silages. With DMI limited by excess fermentation of fuels the lack of treatments effect on performance and carcass characteristics is not unexpected. However, the DMI of cannulated steers was reduced when low-NDFD genotype was harvested for silage at late-maturity stage, due the great decrease of NDF digestibility between maturities within this genotype. Besides DMI results, rumen pool of nutrients and fiber kinetics were positively affected by high-NDFD genotype, suggesting that a potential effect of increased NDFD forages. Better understanding of intake regulation of sugarcane based-diets is critical to formulate diets to increase health, productivity, and efficiency of nutrient utilization in ruminants.

REFERENCES

Allen, M.S. (2000). Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J Dairy Sci 83, 1598-1624.

Allen, M.S. (2014). Drives and limits to feed intake in ruminants. Animal Production Science 54, 1513-1524.

Allen, M.S., Bradford, B.J., Oba, M. (2009). Board Invited Review: The hepatic oxidation theory of the control of feed intake and its application to ruminants. J Anim Sci 87, 3317-3334.

AOAC. (2000). Official methods of analysis. 17th ed. AOAC Int., Arlington, VA.

Broderick, G.A., Luchini, N.D., Reynal, S.M., Varga, G.A., Ishler, V.A. (2008). Effect on Production of Replacing Dietary Starch with Sucrose in Lactating Dairy Cows. Journal of Dairy Science 91, 4801-4810.

Casler, M.D. (2001). Breeding forage crops for increased nutritional value. Advances in Agronomy, Vol 71 71, 51-107.

Chamberlain, D.G., Robertson, S., Choung, J.J. (1993). Sugars versus starch as supplements to grass-silage - effects on ruminal fermentation and the supply of microbial protein to the small-intestine, estimated from the urinary-excretion of purine derivatives, in sheep. Journal of the Science of Food and Agriculture 63, 189-194.

Dado, R.G., Allen, M.S. (1995). Intake limitations, feeding behavior, and rumen function of cows challenged with rumen fill from dietary fiber or inert bulk. J Dairy Sci 78, 118-133.

Daniel, J.L.P., Amaral, R.C., Goulart, R.S., Zopollatto, M., Santos, V.P., Toledo, S.G., Cabezas-Garcia, E.H., Lima, J.R., Santos, M.C., Nussio, L.G. (2013). Short-term effects of silage volatile compounds on feed intake and digestion in beef cattle. Journal of Animal Science 91, 2321-2331.

Daniel, J. L. P., Jacovaci, F. A., Junges, D., Santos, M. C., Lima, J. R., Anjos, I. A., Landell, M. G. A., Huhtanen, P. and Nussio, L. G. (2016). Fibre digestibility and its relationships with chemical and morphological traits in thirty-two sugarcane varieties. Grass Forage Sci. doi:10.1111/gfs.12254

de Souza, J.M., de Sousa, D.O., de Mesquita, B.S., Mesquita, L.G., Silva, L.F.P. (2017). Effect of sugarcane fiber digestibility, conservation method and concentrate level on the ruminal ecosystem of beef cattle. AMB Express 7, 55.

DeFrain, J.M., Hippen, A.R., Kalscheur, K.F., Schingoethe, D.J. (2004). Feeding lactose increases ruminal butyrate and plasma beta-hydroxybutyrate in lactating dairy cows. Journal of Dairy Science 87, 2486-2494.

Erwin, E. S., Marco, G. J., Emery, E. M. (1961). Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. Journal of Dairy Science 44:1768–1771.

FASS. (1999). Guide for the care and use of agricultural animals in agricultural research and teaching. 1st rev. ed. Federation of Animal Sciences Societies, Savoy, IL.

Gao, X., Oba, M. (2016). Effect of increasing dietary nonfiber carbohydrate with starch, sucrose, or lactose on rumen fermentation and productivity of lactating dairy cows. Journal of Dairy Science 99, 291-300.

Getachew, G., Makkar, H.P.S., Becker, K. (2002). Tropical browses: contents of phenolic compounds, in vitro gas production and stoichiometric relationship between short chain fatty acid and in vitro gas production. Journal of Agricultural Science 139, 341-352.

Heinrichs, J., and Kononoff, P. (2002). Evaluating particle size of forages and TMRs using the new Penn State Forage Particle Separator. Pennsylvania State University, College of Agricultural Sciences, Cooperative Extension DAS, 02-42

Heldt, J.S., Cochran, R.C., Stokka, G.L., Farmer, C.G., Mathis, C.P., Titgemeyer, E.C., Nagaraja, T.G. (1999). Effects of different supplemental sugars and starch fed in combination with degradable intake protein on low-quality forage use by beef steers. Journal of Animal Science 77, 2793-2802.

Hino, T., Shimada, K., Maruyama, T. (1994). Substrate preference in a strain of megasphaera-elsdenii, a ruminal bacterium, and its implications in propionate production and growth competition. Applied and Environmental Microbiology 60, 1827-1831.

Holden, L.A. (1999). Comparison of methods of in vitro dry matter digestibility for ten feeds. Journal of Dairy Science 82, 1791-1794.

Huhtanen, P., Detmann, E., Krizsan, S.J. (2016). Prediction of rumen fiber pool in cattle from dietary, fecal, and animal variables. Journal of Dairy Science 99, 5345-5357.

Huhtanen, P., Rinne, M., Nousiainen, J. (2007). Evaluation of the factors affecting silage intake of dairy cows: a revision of the relative silage dry-matter intake index. Animal 1, 758-770.

Hyden, S.A. (1956). Turbidimetric method for the determination of higher polyethyleneglycol in biological meterial. Kgl. Lantbruks-Hogskol. Ann Uppsala 22:139-145.

Kangmeznarich, J.H., Broderick, G.A. (1980). Effects of incremental urea supplementation on ruminal ammonia concentration and bacterial protein-formation. Journal of Animal Science 51, 422-431.

Koike, S., & Kobayashi, Y. (2001). Development and use of competitive PCR assays for the rumen cellulolytic bacteria: *Fibrobacter succinogenes*, *Ruminococus albus* and *Ruminococcus flavefaciens*. FEMS Microbiol Lett 204:361–366. doi:10.1111/j.1574-6968.2001.tb10911.x

Kung, L., Stanley, R.W. (1982). Effect of stage of maturity on the nutritive-value of whole-plant sugarcane preserved as silage. Journal of Animal Science 54, 689-696.

Lowry, S.R. (1992). Use and misuse of multiple comparisons in animal-experiments. Journal of Animal Science 70, 1971-1977.

Malhi, M., Gui, H.B., Yao, L., Aschenbach, J.R., Gabel, G., Shen, Z.M. (2013). Increased papillae growth and enhanced short-chain fatty acid absorption in the rumen of goats are associated with transient increases in cyclin D1 expression after ruminal butyrate infusion. Journal of Dairy Science 96, 7603-7616.

Martel, C.A., Titgemeyer, E.C., Mamedova, L.K., Bradford, B.J. (2011). Dietary molasses increases ruminal pH and enhances ruminal biohydrogenation during milk fat depression. Journal of Dairy Science 94, 3995-4004.

McCormick, M.E., Redfearn, D.D., Ward, J.D., Blouin, D.C. (2001). Effect of protein source and soluble carbohydrate addition on rumen fermentation and lactation performance of Holstein cows. Journal of Dairy Science 84, 1686-1697.

Mertens, D.R. (1994). Regulation of forage intake. Forage Quality, Evaluation, and Utilization, 450-493.

Mertens, D.R. (2009). Impact of NDF Content and Digestibility on Dairy Cow Performance. Advances in Dairy Technology, Vol 21 21, 191-201.

Miron, J., Ben-Ghedalla, D., Morrison, M. (2001). Invited review: Adhesion mechanisms of rumen cellulolytic bacteria. Journal of Dairy Science 84, 1294-1309.

Muyzer, G., Dewaal, E.C., Uitterlinden, A.G. (1993). Profiling of complex microbialpopulations by denaturing gradient gel-electrophoresis analysis of polymerase chain reaction-amplified genes-coding for 16 s ribosomal- RNA. Appl Environ Microbiol 59:695–700

NRC (2000). Nutrient Requirements of Beef Cattle. 7 th Edition. National Research Council. National Academy of Sciences, Washington, DC, USA.

NRC (2016). Nutrient Requirements of Beef Cattle. Eighth Revised Edition. Washington, DC: The National Academies Press. doi: 10.17226/19014.

Oba, M. (2011). Review: Effects of feeding sugars on productivity of lactating dairy cows. Canadian Journal of Animal Science 91, 37-46.

Ouwerkerk D, Klieve AV, Forster RJ (2002) Enumeration of *Megasphaera elsdenii* in rumen contents by real-time Taq nuclease assay. J Appl Microbiol 92:753–758. doi:10.1046/j.1365-2672.2002.01580.x

Pedroso, A., Nussio, L., Loures, D., Paziani, S., Ribeiro, J., Mari, L., Zopollatto, M., Schmidt, P., Mattos, W., Horii, J. (2008). Fermentation, losses, and aerobic stability of sugarcane silages treated with chemical or bacterial additives. Scientia Agricola 65, 589-594.

Penner, G.B., Oba, M. (2009). Increasing dietary sugar concentration may improve dry matter intake, ruminal fermentation, and productivity of dairy cows in the postpartum phase of the transition period. Journal of Dairy Science 92, 3341-3353.

Rinne, M., Huhtanen, P., Jaakkola, S. (2002). Digestive processes of dairy cows fed silages harvested at four stages of grass maturity. J Anim Sci 80, 1986-1998.

Russell, J.B., Wilson, D.B. (1996). Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? Journal of Dairy Science 79, 1503-1509.

Sambrook, J., Russell, D.W. (2001). Molecular Cloning - A Laboratory Manual. 3rd ed. Cold Spring Harbor Press, Woodbury, NY.

Sousa, D.O., Mesquita, B.S., Diniz-Magalhaes, J., Bueno, I.C.S., Mesquita, L.G., Silva, L.F.P. (2014). Effect of fiber digestibility and conservation method on feed intake and the ruminal ecosystem of growing steers. Journal of Animal Science 92, 5622-5634.

Sousa, D.O., Mesquita, B.S., Pires, A.V., Santana, M.H.A., Silva, L.F.P. (2017). Effects of fibre digestibility and level of roughage on performance and ruminal fermentation of finishing beef cattle (not published).

Stevenson, D.M., Weimer, P.J. (2007). Dominance of Prevotella and low abundance of classical ruminal bacterial species in the bovine rumen revealed by relative quantification real-time PCR. Appl Microbiol Biotechnol 75, 165-174.

Tajima, K., Aminov, R.I., Nagamine, T., Matsui, H., Nakamura, M., Benno. Y. (2001). Diet-dependent shifts in the bacterial population of the rumen revealed with real-time PCR. Appl Environ Microbiol 67:2766–2774. doi:10.1128/AEM.67.6.2766-2774.2001

Vallimont, J.E., Bargo, F., Cassidy, T.W., Luchini, N.D., Broderick, G.A., Varga, G.A. (2004). Effects of replacing dietary starch with sucrose on ruminal fermentation and nitrogen metabolism in continuous culture. Journal of Dairy Science 87, 4221-4229.

Van Soest, P.J., and Robertson, J. (1985). Analysis of forages and fibrous foods, (Cornell University, Ithaca)

Van Soest, P.J., Robertson, J.B., Lewis, B.A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74, 3583-3597.

Weimer, P.J. (1996). Why don't ruminal bacteria digest cellulose faster? Journal of Dairy Science 79, 1496-1502.

Weisbjerg, M.R., Hvelplund, T., Bibby, B.M. (1998). Hydrolysis and fermentation rate of glucose, sucrose and lactose in the rumen. Acta Agriculturae Scandinavica Section a-Animal Science 48, 12-18.

3 LIVE YEAST SUPPLEMENTATION IMPROVES RUMEN FIBRE DEGRADATION IN CATTLE GRAZING TROPICAL PASTURES THROUGHOUT THE YEAR

3.1 INTRODUCTION

Greater forage NDF digestibility has been associated with enhanced NDF digestion, resulting in higher rumen passage rate and increased capacity for intake (Kramer-Schmid et al., 2016). Feed intake and performance of grazing animals is dependent of pasture quality, which vary greatly throughout the seasons of the year (Holden et al., 1994). Even in systems with poor fibre digestibility, the potential benefit of improved rumen fermentation can be significant. Working in northern Australia, Ash et al. (2015) simulated that increasing rumen digestibility of poor quality forages by only three percentage points would result in large gains in productivity and financial performance, leading to an increase of $20 per animal in gross margin, and an increase of 57% in annual net profit of extensive beef cattle systems.

With growing concern over the use of antibiotics and other growth promoters in the animal feed industry, ruminant nutritionists and rumen microbiologists have renewed interest in exploring alternative solutions to improve rumen efficiency of fermentation (Patra, 2012). Active dry yeasts are increasingly used in ruminant nutrition as feed additives to improve feed efficiency and performance and, at the same time, to prevent health disorders (Chaucheyras-Durand et al., 2008).

The primary mechanisms by which live yeasts affect animal performance appears to be related to changes on rumen bacterial populations mainly focused on optimizing fibre digestion (Chaucheyras-Durand et al., 2008). The rumen microbial ecosystem is a critical factor that links diets to bovine physiology and productivity; therefore, information about dietary effects on microbial populations has enormous importance to animal nutrition (Mullins et al., 2013). However, there is a lack of information about the effect of long term live yeast supplementation on rumen cellulolytic bacteria and fibre degradation of grazing ruminants.

Therefore, the objectives of this study were to evaluate the effects of long term live yeast supplementation on fibre degradability, rumen cellulolytic bacteria population, and rumen fermentation of grazing beef cattle in all seasons of the year.

3.2 MATERIALS AND METHODS

All experimental procedures were in agreement with the Guide for Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999), with all animal procedures approved by the University of São Paulo Animal Bioethics Committee (protocol number 5488241113). The experiment was conducted at the Beef Cattle Research Laboratory at the Department of Animal Science – School of Veterinary Medicine and Animal Science – University of São Paulo, during the period from December 2013 to December 2014.

3.2.1 Animals, Management, and Treatments

Eight rumen cannulated Nellore steers (298 ± 19 kg of BW and 18 months of age) were assigned randomly to treatment sequence in a 2 × 4 factorial block design experiment with eight 45-d experimental periods. Throughout the experiment, steers were kept on an intensive rotational grazing system, receiving similar mineral supplementation and free access to water bunks. This research was conducted over a year on 4-ha marandu palisade grass [*Brachiaria brizantha* cv. Marandu] pastures, subdivided into 12 paddocks (0.33-ha each) by double strands of electrified fencing.

The paddocks were fertilized with 50 kg N/ha, divided into 3 applications, first at the beginning of the summer and the other two immediately after each grazing event. Three paddocks were grazed once and reserved to allow forage accumulation to be used during the winter. Steers grazed each paddock for 4 days before rotating to the next in the sequence, allowing for 32 days of resting for each paddock before the next grazing event. Pasture samples were taken at the beginning of each grazing event in each paddock throughout the experiment and analysed as a pool of samples by season of the year. The chemical composition of the pasture is presented in Table 1.

Table 1. Chemical composition of the pasture throughout the year

Item	Summer	Autumn	Winter	Spring	SEM	P-value
DM, g kg^{-1}	252^{b}	293^{b}	574^{a}	247^{b}	64	0.05
	---------- g kg^{-1} DM ----------					
CP	95	87	57	121	17	0.22
NDF	591^{bc}	646^{ab}	720^{a}	535^{c}	28	0.04
ADF	329^{b}	356^{b}	432^{a}	277^{c}	12	<0.01
ADL	56^{c}	63^{b}	85^{a}	54^{c}	2.0	<0.01
	---------- g kg^{-1} NDF ----------					
ADL	95	98	118	102	7.0	0.19

SEM, standard error mean; DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin.
[a-c]Means in rows with different superscripts differ ($P < 0.05$).

Treatments were live yeast (LY) containing the specific *Saccharomyces cerevisiae* strain CNCM I-1077 (Lallemand Animal Nutrition, Aparecida de Goiás, GO) and control (CON) during the four seasons of the year (summer, autumn, winter, and spring). Treatments were applied in capsules, every morning, at 07:00 via rumen cannula to promote a daily minimum intake of 8 x 10^{9} CFU of LY per animal in the treated group, or empty capsule for the control animals in order to introduce the same oxygen stress to the rumen.

Samples were collected from d 43 to d 45 during each experimental period, making two sample collections for each animal in each season of the year. Rumen fluid (300 mL) was collected on d 43 of each experimental period, at two times: immediately before and 5 h after treatment, to measure pH, concentration of short chain fat acids, and yeast quantification. Rumen digesta (50 g) was collected 5 h after treatment on d 43 of each period, for quantification of relative rumen population of fibre degrading bacteria. At the same time, *in situ* degradability of fibrous feedstuffs was also determined. Feedstuff samples were introduced simultaneously in the rumen at 12:00 and removed sequentially after 24 h (d 44) and 48 h (d 45) of incubation.

3.2.2 Rumen pH, Short Chain Fatty Acids (SCFA), and Yeast quantification

Rumen fluid samples were collected through the rumen cannula with a vacuum pump for pH measurement. Approximately 300 mL of rumen fluid were collected from cranial, ventral, and caudal areas of the rumen. After the collection of samples, the remaining fluid was returned to the rumen. Immediately after the collection, 100 mL of rumen fluid was used for pH determination with a portable digital pH meter (HANNA Instruments Limited HI8424, Bedfordshire, UK) calibrated with solutions of pH 4.0 and 7.0.

Rumen fluid concentration of SCFA was measured by gas chromatography with a capillary column (Stabilwax; Restek, Bellefonte, PA) using the method described by Erwin et al. (1961) and adapted by Getachew et al. (2002). Frozen acidified rumen fluid samples were thawed at room temperature and centrifuged at 14,500 × g at 4°C for 10 min. Supernatant (1 mL) was transferred into a clean dry vial with 100 μL of internal standard (2-ethylbutyric acid 100 mM; Chem Service, West Chester, PA) and capped. Concentrations of SCFA were determined using a gas chromatography (GC-2014; Shimadzu, Kyoto, Japan), with split injector and dual flame ionization detector temperatures at 250°C and column temperature at 145°C. External standards were prepared with acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids (Chem Service). The software GCSolution (Shimadzu) was used for separation and integration of chromatographic peaks and for calculation of SCFA concentrations.

To verify the effectiveness of the treatment on increasing the population of yeast inside the rumen, rumen yeast count was performed using the Petrifilm™ Yeast and Mold Count Plate (3M Company, Medical Products Division, St. Paul, MN). Samples (50 mL) of rumen fluid were collected before and 5 hours after treatment and were serially diluted before depositing 1-mL aliquots in the centre of Petrifilm™ plates, distributed with a spreader, and incubated in an upright position at 25°C for 3 and 5 d. Yeast growth was then calculated as recommended by the manufacturer. The results of the rumen yeast counts measured before and after treatment are presented in Figure 1.

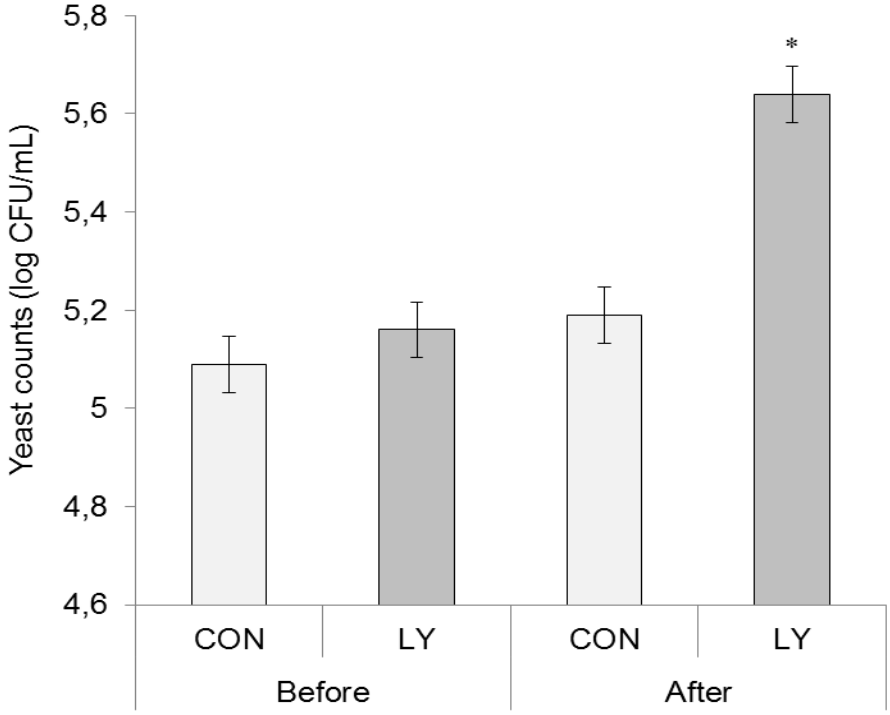

Figure 1. Rumen yeast counts immediately before and five hours after treatment.

3.2.3 *In situ* rumen degradability

In situ NDF degradability was determined in five reference roughages: 1) corn silage, 2) bermudagrass [*Cynodon dactylon (L.) Pers.*], 3) sugarcane silage, 4) Marandu palisade grass, and 5) Mombaça guineagrass [*Panicum maximum* cv. Mombaça] to evaluate the cellulolytic activities of the rumen environment. The chemical composition of the five reference roughages used for rumen fibre degradability evaluation is presented in Table 2.

Table 2. Chemical composition of the five roughages used for determination of *in situ* NDF degradability

Roughage	DM (g kg^{-1})	NDF	ADF	ADL	CP	Days of regrowth	Height, cm
		-------- g kg^{-1} DM ---------					
Bermudagrass hay	825	661	351	103	136	60	40
Palisade grass[†]	148	567	300	71	166	25	30
Guineagrass[‡]	161	660	400	82	157	25	80
Corn silage	190	647	408	104	57	~100	--
Sugarcane silage	323	636	445	172	40	~300	--

DM, dry matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; CP, crude protein.
[†]*Brachiaria brizantha* cv. Marandu.
[‡]*Panicum maximum* cv. Mombaça.

Roughages were dried in a forced-air oven for 72h at 55^{o}C, and ground at a Wiley mill to pass a 1-mm screen. Polyfilament polyester bags (ANKOM R510; size 5 cm×10 cm; mean pore size 50 µm) containing 0.9 g of DM (18–20mg/cm^{2}) were introduced simultaneously in the rumen on d 43 at 12:00 h (in triplicates), and removed sequentially at 24 and 48 h of incubation. Blank bags were also inserted in the rumen to correct for bacterial contamination. Before rumen incubation, bags were submerged (15') in warm water (39^{o}C) and after collection from rumen, were soaked in cold water, and stored at −20^{o}C.

Once thawed, bags were washed (three times) with automatic machine, dried in a forced air oven (60^{o}C, 72 h), and weighed. Dry matter losses were computed as the difference in weight of the pre- and post-incubated bags, and expressed as proportion of initial weight. Residues of replicates per time within steers were pooled prior to analysis. For determination of NDF degradability, the residues were analysed for NDF content.

3.2.4 Chemical analysis

Total DM was analysed according to AOAC (2000) method 930.15, concentration of CP was determined by combustion (Leco protein/N analyser, model FP-528; Leco Corp., St. Joseph, MI), and concentration of NDF was determined using the method described by Van Soest et al. (1991) using 8M urea and heat stable α-amylase (Sigma A3306; Sigma Chemical Co., St. Louis, MO) in an ANKOM A200 Fibre Analyzer (ANKOM Technology Corp.). Acid detergent lignin and ADF were analysed according to Van Soest and Robertson (1985).

3.2.5 Rumen population of cellulolytic bacteria

The relative populations of four rumen bacteria important for fibre degradation (*Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *Ruminococcus albus*) were quantified by quantitative PCR. At the 12:00 rumen collection, approximately equal volumes of solids and liquid totalling 1.8 l was collected and transported to the laboratory in sealed, pre-warmed thermos bottles. Within 10 min of collection, rumen samples were tightly squeezed through four layers of cheesecloth into a vessel continually spared with CO_2 to yield rumen fluid and squeezed solids. For each sample, 25 ml of fluid and 25 g of solids were used for DNA extraction. Samples were processed as in Stevenson and Weimer (2007) to extraction of total bacteria DNA. Extracted DNA was used as template

in qPCR reactions using specific primers (Table 3) for quantification of relative rumen population of cellulolytic bacteria.

Table 3. Oligonucleotides used in PCR quantification of rumen microorganisms

Target organism	Sequence (5'→3')	Reference
F. succinogenes	F:GGTATGGGATGAGCTTGC R:GCCTGCCCCTGAACTATC	Tajima et al. (2001)
R. albus	F:CCCTAAAAGCAGTCTTAGTTCG R:CCTCCTTGCGGTTAGAACA	Koike and Kobayashi (2001)
R. flavefaciens	F:TCTGGAAACGGATGGTA R:CCTTTAAGACAGGAGTTTACAA	Koike and Kobayashi (2001)
B. fibrisolvens	F:ACCGCATAAGCGCACGGA R:CGGGTCCATCTTGTACCGATAAAT	Stevenson and Weimer (2007)
Eubacteria	F:CCTACGGGAGGCAGCAG R:ATTACCGCGGCTGCTGG	Muyzer et al. (1993)

F. succinogenes, Fibrobacter succinogenes; R. albus, Ruminococcus albus; R. flavefaciens, Ruminococcus flavefaciens; B. fibrisolvens, Butyrivibrio fibrisolvens; F, forward; R, reverse; G, guanine; T, thiamine; A, adenine; C, cytosine.

3.2.6 Statistical Analysis

Data were analysed as a completely randomized design in a 2 × 4 factorial arrangement of treatments using the MIXED procedure of SAS version 9.2. Model included the fixed effects of treatment (with or without LY; 1 DF), season of the year (spring, summer, fall and winter; 3 DF), and interaction (3 DF); and the random effects of animals within season (3 DF). The effect of LY was compared using Fisher's least significant difference (option DIFF from LSMEANS). The degrees of freedom and tests were adjusted by the Kenward-Roger option, and significance of main effects was declared at $P \leq 0.05$ and tendency at $P \leq 0.10$. The effects of moderate interactions ($P < 0.15$) were investigated with the SLICE option of PROC MIXED to determine whether these interactions affect main-effect comparisons (Lowry, 1992).

3.3 RESULTS

3.3.1 Pasture Characteristics

The mean chemical composition of the pasture grazed by the experimental animals throughout the four seasons of the year is presented in Table 1. Pasture quality changes

according to the season of the year, where the lowest quality pasture with greater DM (P = 0.06) and greater fibre compounds NDF (P = 0.04), ADF (P < 0.01), and ADL (P < 0.01) was found during the winter. The spring pasture presented the highest quality with the lowest contents of ADF (P < 0.01), NDF (P = 0.04), and ADL (P < 0.01), compared to autumn and winter pastures. The last two factors (NDF and ADL) were similar to the summer pasture. There was no difference (P > 0.10) for CP or ADL (g kg^{-1} NDF) of the pasture among seasons (Table 1).

3.3.2 *In Situ* NDF Degradability

There was no three-way interaction of Trt × T × Ssn or simple interaction of Trt × Ssn (P > 0.2) on *in situ* NDF degradability. Therefore, the results are presented in Table 4 and Figure 2 with the simple interactions (Ssn × T and Trt × T) that had statistical significance. There was a Trt × T interaction for all forages analysed (P < 0.15) and the effect of LY supplementation on forage NDF degradability was dependent on the time (T) of rumen incubation (Table 4). The LY supplementation increased NDF degradability only when measured after 24 h of incubation (Table 4). No effect of LY (P > 0.2) was observed when the NDF degradability was determined at 48 h of incubation (Table 4). When LY supplementation was applied, NDF degradability increased 6.3% for bermudagrass hay (P = 0.02), 4.1% for palisade grass (P = 0.01), 4.9% for guineagrass (P = 0.03), 13% for sugarcane silage (P = 0.02), and 6.9% for corn silage (P = 0.04) when compared to the CON group at 24 h of rumen incubation (Table 4).

Table 4. Forage NDF degradability incubated for 24 or 48 h in the rumen of steers supplemented or not with live yeast

	24		48		SEM	P-value		
Forage	CON	LY	CON	LY		Trt	T	Trt × T
	---------- g kg^{-1} NDF ----------							
Bermudagrass hay	302^{b}	321^{a}	444	440	58	0.26	<0.01	0.02
Palisade grass†	609^{b}	634^{a}	745	748	64	0.04	<0.01	0.11
Guineagrass‡	466^{b}	489^{a}	649	651	71	0.14	<0.01	0.08
Corn silage	374^{b}	400^{a}	581	585	84	0.12	<0.01	0.14
Sugarcane silage	130^{b}	147^{a}	238	239	49	0.09	<0.01	0.11

24, 24 h of ruminal incubation; 48, 48 h of ruminal incubation; CON, control; LY, live yeast; SEM, standard error mean; Trt, effect of treatment; T, effect of ruminal incubation time.
†*Brachiaria brizantha* cv. Marandu.
‡*Panicum maximum* cv. Mombaça.
$^{a-b}$Means in rows with different superscripts differ within time of incubation (P < 0.05).

For sugarcane silage, there was no Ssn × T interaction on NDF degradability ($P = 0.24$). An effect of Season on sugarcane silage NDF digestibility was observed ($P < 0.01$), in which the greater NDF digestibility was during winter and spring (207 and 208 g kg^{-1} NDF, respectively) and the lower degradability was at summer and autumn (163 and 177 g kg^{-1} NDF, respectively).

For the other four forages, the effect of Time of rumen incubation, Season of the year, and its interaction on forage NDF degradability is presented in Figure 2. A significant Ssn × Time interaction was observed for NDF degradability when bermudagrass hay ($P < 0.01$; Figure 2-A), corn silage ($P = 0.05$; Figure 2-B), guineagrass ($P = 0.02$; Figure 2-C), and palisade grass ($P < 0.01$; Figure 2-D) were analysed. Fibre digestibility was altered by seasons of the year, with more pronounced season effects when NDF degradability was measured after 24h on rumen incubation than when measured after 48h of rumen incubation (Figure 2). In general, NDF degradability of the four forages was lower during the summer and increased through the year until the end of the experiment in the spring (Figure 2).

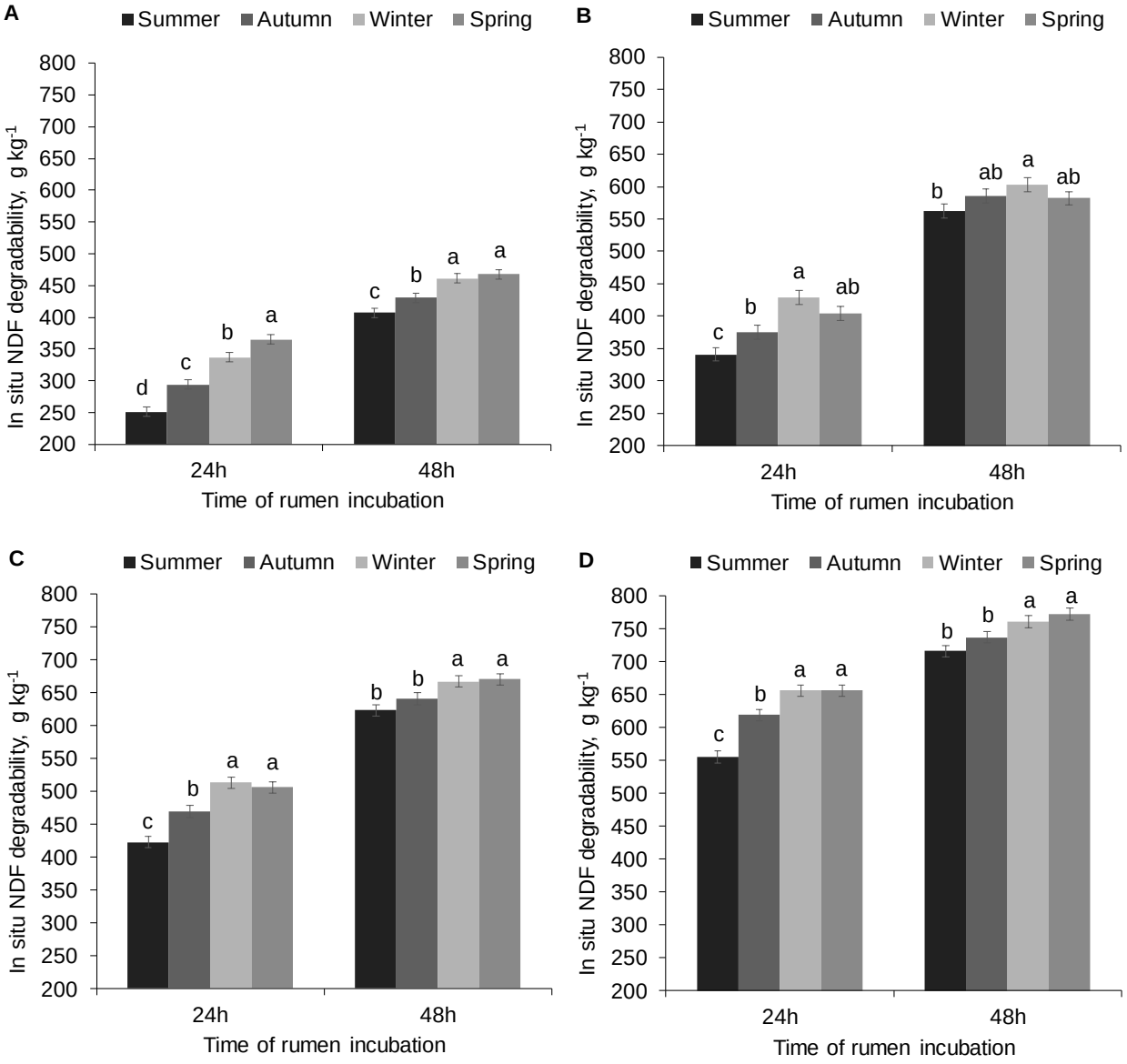

Figure 2. In situ NDF degradability of forages incubated for 24 h or 48 h in the rumen of steers throughout the year. A) Bermudagrass hay, B) Corn silage, C) Guineagrass, and D) Palisade grass. [a-c]Means in bars with different superscripts differ within time of incubation ($P < 0.05$).

3.3.3 Rumen pH and Short Chain Fatty Acids

Because there was no three-way interaction of Trt × T × Ssn or of the simple interaction of Trt × Time ($P > 0.2$) on rumen pH and SCFA data, the results were divided into two tables (Tables 5 and 6) with the simple interactions Trt × Ssn and T × Ssn, respectively that had statistical significance for most rumen fermentation parameters. The effect of Treatment, Season of the year, and its interaction on rumen pH and SCFA is presented in Table 5. No significant effect ($P > 0.3$) of Treatment, Season, and its interaction on rumen pH, butyric acid, and branched-chain fatty acids was observed (Table 5). There was a Trt × Ssn

interaction for total SCFA (P = 0.12), acetic acid (P < 0.01), propionic acid (P = 0.05), and A:P ratio (P = 0.05). Greater total SCFA was observed for animals fed with LY compared to the CON group, but only in the autumn (Table 5). During the summer, LY supplementation decreased acetic acid in the rumen and increased propionic acid concentration, with no difference within the other seasons of the year (Table 5). Therefore, there was a greater A:P ratio for animals in the CON group only in the summer (Table 5). There was a tendency for greater valeric acid (P = 0.09) concentration in the rumen when steers were fed LY compared to the CON group (6.2 vs. 5.0 ± 1.8 mmol/mol; Table 5).

The effect of Time of sampling, Season of the year, and its interaction on pH and SCFA is showed in Table 6. There was a significant T × Ssn interaction for rumen pH (P < 0.01), total SCFA (P = 0.01), acetic acid (P = 0.11), propionic acid (P = 0.03), valeric acid (P < 0.01), and A:P ratio (P = 0.02). The rumen pH was greater after treatment in the summer, autumn, and spring (Table 6). Greater total SCFA and A:P ratio was observed before treatment, but only in the summer and autumn (Table 6) with no difference of sampling time during the winter and spring. The concentration of acetic acid was greater before treatment only in the autumn, although propionic acid in the rumen was greater after treatment during summer and autumn only (Table 6). Greater rumen concentration of valeric acid was observed after treatment in the winter with no significant difference in other season of the year (Table 6). No significant effect (P > 0.2) of Time of sampling and Season of the year, and its interaction on butyric acid and branched-chain fatty acids concentrations was observed (Table 6).

Table 5. Ruminal pH and short chain fatty acids of steers supplemented or not with live yeast throughout the year

	Summer		Autumn		Winter		Spring		SEM	P-value		
Item	CON	LY	CON	LY	CON	LY	CON	LY		Trt	Ssn	Trt × Ssn
Ruminal pH	6.35	6.30	6.31	6.27	6.57	6.59	6.41	6.43	0.11	0.80	0.34	0.82
Total SCFA, m*M*	50.7	48.0	36.8[b]	46.6[a]	28.5	33.8	41.2	43.8	5.80	0.19	0.29	0.12
Proportion of total SCFA	---------- mmol/mol ----------											
Acetate	703[a]	691[b]	689	695	680	684	677	674	12	0.69	0.67	<0.01
Propionate	185[b]	193[a]	196	191	210	203	197	196	4.4	0.53	0.17	0.05
Butyrate	90	93	93	91	92	94	105	106	7.8	0.78	0.74	0.31
Valerate	5.5	6.1	5.7	5.6	5.6	7.0	3.2	5.9	3.2	0.09	0.99	0.21
BCFA	15.0	16.2	16.8	17.5	12.5	13.0	16.8	19.0	2.7	0.46	0.61	0.91
A:P ratio	3.81[a]	3.59[b]	3.54	3.65	3.25	3.38	3.46	3.46	0.13	0.96	0.31	0.02

CON, control; LY, live yeast; SEM, standard error mean; Trt, effect of treatment; Ssn, effect of season of the year; SCFA, short chain fatty acids; m*M*, millimolar; BCFA, branched-chain fatty acids; A:P, acetate:propionate.

[a-b]Means in rows with different superscripts differ within season of the year ($P < 0.05$).

Table 6. Ruminal pH and short chain fatty acids of steers sampled before or after treatment throughout the year

Item	Summer		Autumn		Winter		Spring		SEM	P-value		
	0h[†]	5h[‡]	0h	5h	0h	5h	0h	5h		T	Ssn	T × Ssn
Ruminal pH	6.21[b]	6.42[a]	6.17[b]	6.43[a]	6.59	6.55	6.31[b]	6.52[a]	0.11	<0.01	0.34	<0.01
Total SCFA, m*M*	53.9[a]	44.8[b]	49.0[a]	34.4[b]	29.9	32.5	44.6	40.3	8.75	<0.01	0.29	0.01
Proportion of total SCFA	---------- mmol/mol ----------											
Acetate	702	692	698[a]	685[b]	685	678	673	678	16.7	<0.01	0.67	0.11
Propionate	184[b]	194[a]	187[b]	200[a]	204	209	199	193	7.2	0.01	0.17	0.03
Butyrate	93	91	93	91	93	93	105	106	11.1	0.47	0.74	0.31
Valerate	5.6	6.1	6.2	5.0	4.6[b]	8.1[a]	4.9	4.3	4.5	0.21	0.99	<0.01
BCFA	15.0	16.2	16.3	17.9	13.0	12.3	17.3	18.4	3.9	0.29	0.61	0.54
A:P ratio	3.83[a]	3.56[b]	3.74[a]	3.44[b]	3.37	3.26	3.39	3.53	0.20	<0.01	0.31	0.02

SEM, standard error mean; T, effect of sampling time; Ssn, effect of season of the year; SCFA, short chain fatty acids; m*M*, millimolar; BCFA, branched-chain fatty acids; A:P, acetate:propionate.
[†]Sampling collection immediately before treatment.
[‡]Sampling collection 5 h after treatment.
[a-b]Means in rows with different superscripts differ within season of the year ($P < 0.05$).

Table 7. Relative population[†] of ruminal cellulolytic bacteria of steers supplemented or not with live yeast throughout the year

Item	Summer		Autumn		Winter		Spring		SEM	P-value		
	CON	LY	CON	LY	CON	LY	CON	LY		Trt	Ssn	Trt × Ssn
R. flavefaciens	7.39[b]	12.61[a]	4.45	6.04	6.45	8.77	1.28[b]	4.48[a]	0.95	<0.01	0.52	0.10
F. succinogenes	4.32	4.05	5.57[a]	2.66[b]	1.63	1.21	0.14[a]	0.04[b]	0.15	0.02	<0.01	0.08
R. albus	0.46	0.29	0.23	0.25	1.61	1.37	0.75	0.65	0.07	0.47	0.26	0.57
B. fibrisolvens	0.66	0.38	0.47	0.43	0.45	0.38	0.44	0.52	0.03	0.12	0.97	0.18

CON, control; LY, live yeast; SEM, standard error mean; Trt, effect of treatment; Ssn, effect of season of the year; *F. succinogenes*, *Fibrobacter succinogenes; R. albus*, *Ruminococcus albus; R. flavefaciens*, *Ruminococcus flavefaciens; B. fibrisolvens*, *Butyrivibrio fibrisolvens*.
[†]The relative population size is presented as g kg^{-1} of total microbial population.
[a-b]Means in rows with different superscripts differ within season of the year ($P < 0.05$).

3.3.4 Rumen Cellulolytic Bacteria Population

The effect of yeast supplementation, Season of the year and its interaction on relative population of rumen cellulolytic bacteria of steers is presented in Table 7. For the relative population of *R. flavefaciens*, *F. Succinogenes*, and *B. fibrisolvens*, there was interaction between Treatment and Season of the year ($P < 0.2$). Live yeast supplementation increased *R. flavefaciens* population, but only in the summer and spring (Table 7) with no difference between CON and LY during autumn and winter. However, LY treatment decreased *F. Succinogenes* population but only in the autumn and spring (Table 7). There was no effect of Treatment, Season and its interaction on *R. albus* and *B. fibrisolvens* populations ($P > 0.1$; Table 7).

3.4 DISCUSSION

The most notable response observed in this experiment was the greater *in situ* NDF degradability for all forages when LY was fed to grazing beef cattle, independently of the season of the year, and the related increase in the relative population of most predominant cellulolytic bacteria studied. *R. flavefaciens* was the most prevalent cellulolytic bacteria in the rumen of the experimental animals and its greater population suggest that the cellulolysis process was more efficient when *S. cerevisiae* was supplemented, explaining the positive effect on NDF degradability throughout the year.

Increased bacterial numbers leading to improved fibre degradability has been one of the beneficial effects of yeast supplementation in ruminants (Dehghan-Banadaky et al., 2013). Chaucheyras-Durand et al. (2008) in review suggested that yeast, as a facultative anaerobe organism, may consume available oxygen on the surface of freshly ingested feeds to maintain metabolic activity, consequently decreasing redox potential in the rumen. The oxygen removal creates better conditions for the growth of strict anaerobic cellulolytic bacteria, stimulating their attachment to forage particles and decreasing lag time in the cellulolysis process (Chaucheyras-Durand et al., 2008). Furthermore, *S. cerevisiae* may provide growth factors, such as organic acids or vitamins, which could also stimulate rumen populations of cellulolytic bacteria and fungal colonization (Chaucheyras et al., 1995; Jouany, 2006; Chaucheyras-Durand et al., 2008).

We hypothesized that because of differences in the rumen environment, the effect of LY on NDF degradability would vary among seasons of the year. However, even with very

different pasture composition and availability throughout the year, the increase in the cellulolytic activity in the rumen of animals receiving LY did not vary. Supplementing with LY increased *in situ* NDF degradability uniformly throughout the year.

Although there was a positive effect of LY on NDF degradability for all tested roughages, the effect was significant only when measured after 24 h of incubation, with no difference between control and LY groups when samples of the five forages were incubated for 48 h. According to Callaway and Martin (1997), *S. cerevisiae* supplementation could accelerate the rate, but not the extent of fibre degradation, because *S. cerevisiae* in the rumen reduced the lag time for growth of *R. albus*, *R. flavefaciens*, and *B. fibrisolvens* (Girard and Dawson, 1995), increasing the rate of NDF degradation. Therefore, in the present study, it is possible to conclude that the digestion rate of NDF of the five roughage sources was accelerated when LY was fed, although the extension of digestion was similar between treatments when samples were retained in the rumen for 48 h.

Working with corn silages with different NDF digestibilities, Guedes et al. (2008) reported a larger response to LY supplementation in corn silages of lower initial NDF digestibility (measured after 36h of incubation). In the current experiment, the magnitude of LY effect on NDF degradability was also apparently inversely related with the initial degradability of the forage source. One possibility would be that forages with easily accessible and faster degrading NDF (such as leaf blades from young and tender palisade grass and guineagrass in the current experiment) would benefit less from LY stimulating effects on rumen fermentation than forages with slower degrading NDF (such as corn silage and sugarcane silage in the current experiment).

Considering that NDF degradability is better described when assuming two fractions with different degradation rates (fast and slow degrading NDF - Ellis et al., 2005; Raffrenato and Van Amburgh, 2010), and considering that LY has larger effects on slower degrading forages, it is possible to speculate that LY supplementation could have more of an effect on forages rich in the slow degrading NDF fraction than on forages rich in the fast degrading NDF fraction.

The relative population of *R. flavefaciens* was significantly enhanced by LY supplementation only at the summer and spring. However, the positive effect on rumen cellulolytic activity during autumn and winter could have been attributed to others rumen microorganisms. Ding et al. (2014), evaluating *in situ* fibre degradability in rumen cannulated beef cattle supplemented with *S. cerevisiae* observed that the greater fibre degradation was related to increased total rumen fungi and protozoa, and relative population of *S.*

ruminantium. It is important to observe that other rumen microorganisms that were not quantified in the present study would have been stimulated by LY supplementation and played important roles in the fibre degradation, mostly during the seasons with lower pasture quality.

Yeasts are naturally found in the rumen, but the rumen temperature does not promote their growth, which is optimal at 25ºC (Lund, 1974). Therefore, using yeast as feed additive, requires continuous daily supplementation (Bitencourt et al., 2011) to maintain the yeast population in a proper range that is necessary to manipulate the microbial ecosystem. The beneficial effect of continuous daily yeast supplementation was confirmed in this study where the population of *R. flavefaciens* was greater in all seasons of the year when LY was supplemented for one year long.

Some studies have identified significant effects of yeast supplementation on NDF degradability (Plata et al., 1994; Miranda et al., 1996; Schwartz and Ettle, 2002; Marden et al., 2008; Lascano et al., 2009; Dehghan-Banadaky et al., 2013; Ding et al., 2014); while others reported only a trend (Chaucheyras-Durand and Fonty, 2001; Bitencourt et al., 2011) or no significant differences (Harrison et al., 1988; Angeles et al., 1998; Mir and Mir, 1994). However, only a few studies have shown the effect of yeast supplementation on rumen cellulolytic bacteria (Harrison et al., 1988; Chaucheyras-Durand and Fonty, 2001; Ding et al., 2014) to justify the responses on NDF degradability. To our knowledge, there are no other report in the literature that have tested the effect of long term live yeast supplementation on grazing animals.

The greater proportion of acetic acid in total SCFA is due to the type of diet offered to the animals, i.e., only pasture. During the summer, there was a lower relative proportion of acetate and increased propionate for the group of animals supplemented with LY; while in autumn, animals supplemented with LY had greater total SCFA without altering the rumen pH. Yeasts are able to increase total rumen production of SCFA and also to limit the decrease in rumen pH that is usually linked to an increase in total SCFA (Desnoyers et al., 2009).

In an *in vitro* trial, Miller-Webster et al. (2002) observed that yeast supplementation increased total SCFA, propionic and valeric acid, and reduced acetate and A:P ratio when compared with control. Dawson et al. (1990) testing the effect of a yeast supplement containing *Saccharomyces cerevisiae in vitro* reported a similar occurrence, where the total SCFA and molar proportion of propionic acid increased and acetic acid decreased. Furthermore, Harrison et al. (1988) using Holstein cows fed a diet with 40% corn silage and 60% concentrate (DM basis) reported decreased molar proportion of acetic acid and A:P ratio,

and increased molar proportion of propionic and valeric acid in the rumen fluid of cows supplemented with *S. cerevisiae*. And yet, the total SCFA concentration did not differ between control and treated groups. The responses to yeast supplementation on rumen fermentation is not constant and it might be attributed to dose, type of diets, yeast strains, physiological stage of the animals, and feeding systems (Patra, 2012).

In conclusion, grazing animals supplemented throughout the year with LY containing *S. cerevisiae* had greater rumen NDF degradability of different fibre sources, with greater extension of degradability for forages with lower fibre digestibility, and greater relative population of *R. flavefaciens* in the rumen regardless the season. Supplementing LY also increased total rumen SCFA concentration and the proportion of propionic and valeric acid, without decreasing rumen pH.

REFERENCES

Angeles, S.C., Mendoza, G.D., Cobos, M.A., Crosby, M.M., Castrejon, F.A. (1998). Comparison of two commercial yeast cultures (Saccharomyces cerevisiae) on ruminal fermentation and digestion in sheep fed on corn-stover diet. Small Ruminant Research 31, 45-50.

AOAC. (2000). Official methods of analysis. 17th ed. AOAC Int., Arlington, VA.

Ash, A., Hunt, L., McDonald, C., Scanlan, J., Bell, L., Cowley, R., Watson, I., McIvor, J., MacLeod, N. (2015). Boosting the productivity and profitability of northern Australian beef enterprises: Exploring innovation options using simulation modelling and systems analysis. Agricultural Systems 139, 50-65. doi:10.1016/j.agsy.2015.06.001

Bitencourt, L.L., Silva, J.R.M., de Oliveira, B.M.L., Dias, G.S., Lopes, F., Siecola, S., Zacaroni, O.D., Pereira, M.N. (2011). Diet digestibility and performance of dairy cows supplemented with live yeast. Scientia Agricola 68, 301-307.

Callaway, E.S., & Martin, S.A. (1997). Effects of a Saccharomyces cerevisiae culture on ruminal bacteria that utilize lactate and digest cellulose. Journal Dairy Science 80, 2035-2044.

Chaucheyras, F., Fonty, G., Bertin, G., Gouet, P. (1995). Effects of live Saccharomyces cerevisiae cells on zoospore germination, growth, and cellulolytic activity of the rumen anaerobic fungus, Neocallimastix frontalis MCH3. Current Microbiology 31, 201-205.

Chaucheyras-Durand, F., & Fonty, G. (2001). 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. Reproduction Nutrition Development 41, 57-68.

Chaucheyras-Durand, F., Walker, N.D., Bach, A. (2008). Effects of active dry yeasts on the rumen microbial ecosystem: Past, present and future. Animal Feed Science and Technology 145, 5-26.

Dawson, K.A., Newman, K.E., Boling, J.A. (1990). Effects of microbial supplements containing yeast and lactobacilli on roughage-fed ruminal microbial activities. Journal Animal Science 68, 3392-3398.

Dehghan-Banadaky, M., Ebrahimi, M., Motameny, R., Heidari, S.R. (2013). Effects of live yeast supplementation on mid-lactation dairy cows performances, milk composition, rumen digestion and plasma metabolites during hot season. Journal of Applied Animal Research 41, 137-142. doi:10.1080/09712119.2012.739085

Desnoyers, M., Giger-Reverdin, S., Bertin, G., Duvaux-Ponter, C., Sauvant, D. (2009). Meta-analysis of the influence of Saccharomyces cerevisiae supplementation on ruminal parameters and milk production of ruminants. Journal of Dairy Science 92, 1620-1632.

Ding, G.Z., Chang, Y., Zhao, L.P., Zhou, Z.M., Ren, L.P., Meng, Q.X. (2014). Effect of Saccharomyces cerevisiae on alfalfa nutrient degradation characteristics and rumen microbial populations of steers fed diets with different concentrate-to-forage ratios. Journal of Animal Science and Biotechnology 5, 9. doi: 10.1186/2049-1891-5-24

Ellis, W.C., Mahlooji, M., Matis, J.H. (2005). Models for estimating parameters of neutral detergent fiber digestion by ruminal microorganisms. Journal of Animal Science 83, 1591-1601.

Erwin, E. S., Marco, G. J., Emery, E. M. (1961). Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. Journal of Dairy Science 44:1768–1771.

FASS. (1999). Guide for the care and use of agricultural animals in agricultural research and teaching. 1st rev. ed. Federation of Animal Sciences Societies, Savoy, IL.

Getachew, G., Makkar, H.P.S., Becker, K. (2002). Tropical browses: contents of phenolic compounds, in vitro gas production and stoichiometric relationship between short chain fatty acid and in vitro gas production. Journal of Agricultural Science 139, 341-352.

Girard, I.D., Dawson, K.A. (1995). Stimulation of ruminal bacteria by different fractions derived from cultures of Saccharomyces cerevisiae strain 1026. Journal of Animal Science 73, 264-264.

Harrison, G.A., Hemken, R.W., Dawson, K.A., Harmon, R.J., Barker, K.B. (1988). Influence of addition of yeast culture supplement to diets of lactating cows on ruminal fermentation and microbial populations. Journal of Dairy Science 71, 2967-2975.

Holden, L.A., Muller, L.D., Fales, S.L. (1994). Estimation of intake in high producing holstein cows grazing grass pasture. Journal of Dairy Science 77, 2332-2340.

Jouany, J.P. (2006). Optimizing rumen functions in the close-up transition period and early lactation to drive dry matter intake and energy balance in cows. Animal Reproduction Science 96, 250-264.

Kramer-Schmid, M., Lund, P., Weisbjerg, M.R. (2016). Importance of NDF digestibility of whole crop maize silage for dry matter intake and milk production in dairy cows. Animal Feed Science and Technology 219, 68-76. doi: 10.1016/j.anifeedsci.2016.06.007

Koike, S., & Kobayashi, Y. (2001). Development and use of competitive PCR assays for the rumen cellulolytic bacteria: *Fibrobacter succinogenes*, *Ruminococus albus* and *Ruminococcus flavefaciens*. FEMS Microbiol Lett 204:361–366. doi:10.1111/j.1574-6968.2001.tb10911.x

Lascano, G.J., Zanton, G.I., Suarez-Mena, F.X., Heinrichs, A.J. (2009). Effect of limit feeding high- and low-concentrate diets with Saccharomyces cerevisiae on digestibility and on dairy heifer growth and first-lactation performance. Journal of Dairy Science 92, 5100-5110. doi:10.3168/jds.2011-5047

Lowry, S.R. (1992). Use and misuse of multiple comparisons in animal-experiments. Journal of Animal Science 70, 1971-1977.

Lund, A. (1974). Yeasts and molds in bovine rumen. Journal of General Microbiology 81, 453-462.

Marden, J.P., Julien, C., Monteils, V., Auclair, E., Moncoulon, R., Bayourthe, C. (2008). How does live yeast differ from sodium bicarbonate to stabilize ruminal pH in high-yielding dairy cows? Journal of Dairy Science 91, 3528-3535. doi:10.3168/jds.2007-0889

Miller-Webster, T., Hoover, W.H., Holt, M., Nocek, J.E. (2002). Influence of yeast culture on ruminal microbial metabolism in continuous culture. Journal of Dairy Science 85, 2009-2014. doi:10.3168/jds.S0022-0302(02)74277-X

Mir, Z., & Mir, P.S. (1994). Effect of the addition of live yeast (saccharomyces-cerevisiae) on growth and carcass quality of steers fed high-forage or high-grain diets and on feed digestibility and in-situ degradability. Journal of Animal Science 72, 537-545.

Miranda, R.L.A., Mendoza, M.G.D., BarcenaGama, J.R., Gonzalez, M.S.S., Ferrara, R., Ortega, C.M.E., Cobos, P.M.A. (1996). Effect of Saccharomyces cerevisiae or Aspergillus oryzae cultures and NDF level on parameters of ruminal fermentation. Animal Feed Science and Technology 63, 289-296.

Mullins, C.R., Mamedova, L.K., Carpenter, A.J., Ying, Y., Allen, M.S., Yoon, I., Bradford, B.J. (2013). Analysis of rumen microbial populations in lactating dairy cattle fed diets varying in carbohydrate profiles and Saccharomyces cerevisiae fermentation product. Journal of Dairy Science 96, 5872-5881. doi:10.3168/jds.2013-6775

Muyzer, G., Dewaal, E.C., Uitterlinden, A.G. (1993). Profiling of complex microbialpopulations by denaturing gradient gel-electrophoresis analysis of polymerase chain reaction-amplified genes-coding for 16 s ribosomal- RNA. Appl Environ Microbiol 59:695–700

Patra, A.K. (2012). The use of live yeast products as microbial feed additives in ruminant nutrition. Asian Journal of Animal and Veterinary Advances 7, 366-375.

Plata, F., Mendoza, G.D., Barcenagama, J.R., Gonzalez, S. (1994). Effect of a yeast culture (saccharomyces-cerevisiae) on neutral detergent fiber digestion in steers fed oat straw based diets. Animal Feed Science and Technology 49, 203-210.

Raffrenato, E., Van Amburgh, M.E., Cudas. (2010). Development of a mathematical model to predict sizes and rates of digestion of a fast and slow degrading pool and the indigestible NDF fraction. Proceedings 2010 Cornell Nutrition Conference for Feed Manufacturers, 72nd Meeting, 52-65.

Schwartz, F.J., & Ettle, T. (2002). Effect of live yeast (*Saccharomyces cerevisiae*, Levucell SC CNCM I-1077) on performance of beef and dairy cattle. Proceedings of the Society of Nutrition Physiology Band, 11.

Stevenson, D.M., & Weimer, P.J. (2007). Dominance of Prevotella and low abundance of classical ruminal bacterial species in the bovine rumen revealed by relative quantification real-time PCR. Applied Microbiol Biotechnology 75, 165-174. doi:10.1007/s00253-006-0802-y

Tajima, K., Aminov, R.I., Nagamine, T., Matsui, H., Nakamura, M., Benno. Y. (2001). Diet-dependent shifts in the bacterial population of the rumen revealed with real-time PCR. Appl Environ Microbiol 67:2766–2774. doi:10.1128/AEM.67.6.2766-2774.2001

Van Soest, P. J., & Robertson, J. B. (1985). Analysis of forages and fibrous foods. Laboratory manual for animal science 613. Ithaca, NY, (Cornell University Press).

Van Soest, P.J., Robertson, J.B., Lewis, B.A. (1991). Methods for dietary fibre, neutral detergent fibre, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74, 3583-3597.

4 CONCLUSION

Besides the intake limitation by excess of fuels, using sugarcane genotype to increase fiber digestibility accelerated NDF turnover and passage rate, and also reduced rumen pool of DM and fiber contents, indicating a potential benefit of high-NDFD when DMI is limited by rumen fill. Using maturity stage to increase fiber digestibility of sugarcane functioned only for the low-NDFD genotype, where at early-maturity presented greater NDFD than late-maturity stage, and it reflected on DMI of cannulated steers, where the DMI was greater for animals fed with low-NDFD genotype harvested at early-maturity. Supplementing grazing beef cattle with live yeast containing *Saccharomyces cerevisiae* increased the relative population of *Ruminococcus flavefaciens* in the rumen, increasing rumen NDF degradability of different fibre sources, with greater extension of degradability for forages with lower fibre digestibility. The methods of fiber digestibility alteration applied in these studies have proven that effectiveness in increase NDF digestibility for ruminants that depending on the diet characteristics and energy status of the animal has the potential to improve DMI, performance and ruminal parameters.

REFERENCES

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598–1624.

Allen, M. S., and Y. Ying. 2012. Effects of Saccharomyces cerevisiae fermentation product on ruminal starch digestion are dependent upon dry matter intake for lactating cows. J Dairy Sci 95: 6591-6605.

Archer, J. A., E. C. Richardson, R. M. Herd, and P. F. Arthur. 1999. Potential for selection to improve efficiency of feed use in beef cattle: A review. Aust. J. Agric. Res. 50:147–162.

Chase, L. E., and R. J. Grant. 2013. High forage rations—What do we know? Pages 203–209 in Proc. Cornell Nutrition Conf. Cornell University, Ithaca, NY.

Dado, R. G., and M. S. Allen. 1995. Intake limitations, feeding behavior, and rumen function of cows challenged with rumen fill from dietary fiber or inert bulk. J Dairy Sci 78: 118-133.

Dehghan-Banadaky, M., M. Ebrahimi, R. Motameny, and S. R. Heidari. 2013. Effects of live yeast supplementation on mid-lactation dairy cows performances, milk composition, rumen digestion and plasma metabolites during hot season. Journal of Applied Animal Research 41: 137-142.

Ferraretto, L. F., A. C. Fonseca, C. J. Sniffen, A. Formigoni, and R. D. Shaver. 2015. Effect of corn silage hybrids differing in starch and neutral detergent fiber digestibility on lactation performance and total-tract nutrient digestibility by dairy cows. J Dairy Sci 98: 395-405.

Ferraretto, L. F., and R. D. Shaver. 2015. Effects of whole-plant corn silage hybrid type on intake, digestion, ruminal fermentation, and lactation performance by dairy cows through a meta-analysis. J Dairy Sci 98: 2662-2675.

Guyomard, H., S. Manceron, and J.-L. Peyraud. 2013. Trade in feed grains, animals, and animal products: Current trends, future prospects, and main issues. Anim. Front. 3:14–18.

Hristov, A. N. et al. 2010. Effect of Saccharomyces cerevisiae fermentation product on ruminal fermentation and nutrient utilization in dairy cows. J Dairy Sci 93: 682-692.

Kammes, K. L., and M. S. Allen. 2012. Nutrient demand interacts with grass maturity to affect milk fat concentration and digestion responses in dairy cows. J Dairy Sci 95: 5133-5148.

Kung, L., B. M. Moulder, C. M. Mulrooney, R. S. Teller, and R. J. Schmidt. 2008. The effect of silage cutting height on the nutritive value of a normal corn silage hybrid compared with brown midrib corn silage fed to lactating cows. J Dairy Sci 91: 1451-1457.

Kuoppala, K., S. Ahvenjärvi, M. Rinne, and A. Vanhatalo. 2009. Effects of feeding grass or red clover silage cut at two maturity stages in dairy cows. 2. Dry matter intake and cell wall digestion kinetics. J Dairy Sci 92: 5634-5644.

Kuoppala, K., M. Rinne, S. Ahvenjärvi, J. Nousiainen, and P. Huhtanen. 2010. The effect of harvesting strategy of grass silage on digestion and nutrient supply in dairy cows. J Dairy Sci 93: 3253-3263.

Lim, J. M., K. E. Nestor, and L. Kung. 2015. The effect of hybrid type and dietary proportions of corn silage on the lactation performance of high-producing dairy cows. J Dairy Sci 98: 1195-1203.

Oba, M., and M. S. Allen. 2000. Effects of brown midrib 3 mutation in corn silage on productivity of dairy cows fed two concentrations of dietary neutral detergent fiber: 3. Digestibility and microbial efficiency. J. Dairy Sci. 83: 1350-1358.

Patra, A. K. 2012. The Use of Live Yeast Products as Microbial Feed Additives in Ruminant Nutrition. Asian Journal of Animal and Veterinary Advances 7: 366-375.

Randby, A. T., M. R. Weisbjerg, P. Nørgaard, and B. Heringstad. 2012. Early lactation feed intake and milk yield responses of dairy cows offered grass silages harvested at early maturity stages. J Dairy Sci 95: 304-317.

Rinne, M., P. Huhtanen, and S. Jaakkola. 2002. Digestive processes of dairy cows fed silages harvested at four stages of grass maturity. J Anim Sci 80: 1986-1998.

Rinne, M., S. Jaakkola, and P. Huhtanen. 1997. Grass maturity effects on cattle fed silage-based diets .1. Organic matter digestion, rumen fermentation and nitrogen utilization. Animal Feed Science and Technology 67: 1-17.

Sousa, D. O. et al. 2014. Effect of fiber digestibility and conservation method on feed intake and the ruminal ecosystem of growing steers. J Anim Sci 92: 5622-5634.

Tine, M. A., K. R. Mcleod, R. A. Erdman, and R. L. Baldwin. 2001. Effects of brown midrib corn silage on the energy balance of dairy cattle. J Dairy Sci 84: 885-895.

Vanhatalo, A., K. Kuoppala, S. Ahvenjärvi, and M. Rinne. 2009. Effects of feeding grass or red clover silage cut at two maturity stages in dairy cows. 1. Nitrogen metabolism and supply of amino acids. J Dairy Sci 92: 5620-5633.

Van Soest, P. J. 1994. Nutritional Ecology of the Ruminant. 2nd ed. Cornell University Press, Ithaca, NY.

Weiss, W. P., and D. J. Wyatt. 2002. Effects of feeding diets based on silage from corn hybrids that differed in concentration and in vitro digestibility of neutral detergent fiber to dairy cows. J Dairy Sci 85: 3462-3469.