UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"
FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS
CAMPUS DE JABOTICABAL

IMPACTO DA SUPLEMENTAÇÃO COM BLOCOS MULTIFUNCIONAIS SOBRE OS PARÂMETROS NUTRICIONAIS E DIVERSIDADE BACTERIANA RUMINAL DE NOVILHOS NELORE

Rayanne Viana Costa
Zootecnista

2021

UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"
FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS
CAMPUS DE JABOTICABAL

IMPACTO DA SUPLEMENTAÇÃO COM BLOCOS MULTIFUNCIONAIS SOBRE OS PARÂMETROS NUTRICIONAIS E DIVERSIDADE BACTERIANA RUMINAL DE NOVILHOS NELORE

Rayanne Viana Costa

Orientadora: Profa. Dra. Jane Maria Bertocco Ezequiel

Co-orientadora: Dra. Yury Tatiana Granja-Salcedo

Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias - Unesp, Campus de Jaboticabal, como parte das exigências para obtenção do título de Doutor em Zootecnia.

2021

C837i

Costa, Rayanne Viana
Impacto da suplementação com blocos multifuncionais sobre os parâmetros nutricionais e diversidade bacteriana ruminal de novilhos Nelore / Rayanne Viana Costa. -- Jaboticabal, 2021
105 f.

Tese (doutorado) - Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal
Orientadora: Jane Maria Bertocco Ezequiel
Coorientadora: Yury Tatiana Granja-Salcedo

1. Nutrição Animal. 2. Bovino de corte. 3. Rúmen Microbiologia. 4. Nitrogênio na nutrição animal. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca da Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal. Dados fornecidos pelo autor(a).

Essa ficha não pode ser modificada.

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Jaboticabal

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: IMPACTO DA SUPLEMENTAÇÃO COM BLOCOS MULTIFUNCIONAIS SOBRE OS PARÂMETROS NUTRICIONAIS E DIVERSIDADE BACTERIANA RUMINAL DE NOVILHOS NELORE

AUTORA: RAYANNE VIANA COSTA
ORIENTADORA: JANE MARIA BERTOCCO EZEQUIEL
COORIENTADORA: YURY TATIANA GRANJA SALCEDO

Aprovada como parte das exigências para obtenção do Título de Doutora em ZOOTECNIA, pela Comissão Examinadora:

Dra. YURY TATIANA GRANJA-SALCEDO (Participaçao Virtual)
Corporação Colombiana de Pesquisa Agropecuária / Medelín/Colômbia

Dra. LAURA FRANCO PRADOS (Participaçao Virtual)
APTA - Gabiente da Coordenadoria/Conselho Técnico Científico / Colina/SP

Prof. Dr. MARCO TÚLIO COSTA ALMEIDA (Participaçao Virtual)
Universidade Federal do Espírito Santo-UFES / Alegre/ES

Pós-doutoranda JULIANA AKAMINE TORRECILHAS (Participaçao Virtual)
Departamento de Zootecnia / FCAV / UNESP - Jaboticabal

Pós-doutoranda ANA REBECA CASTRO LIMA (Participaçao Virtual)
Departamento de Zootecnia / FCAV / UNESP - Jaboticabal

Jaboticabal, 03 de agosto de 2021

DADOS CURRICULARES DA AUTORA

Rayanne Viana Costa, nascida no dia 05 de agosto de 1992, em Tangará da Serra, no estado de Mato Grosso, filha de Edivaldo Ferreira Costa e Vilma Viana da Silva Costa. No ano de 2010, ingressou no curso de graduação em Zootecnia pela Faculdade de Agronomia e Zootecnia (FAAZ) da Universidade Federal de Mato Grosso - UFMT, campus Cuiabá, concluindo em dezembro de 2014. Em março de 2015 ingressou Mestrado em Ciência Animal na área de produção de ruminantes na mesma instituição, sob orientação do Prof. Dr. Luciano da Silva Cabral, concluindo em fevereiro de 2017. Neste mesmo ano, ingressou no doutorado em Zootecnia na área de nutrição e alimentação de ruminantes pela Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP) – Campus de Jaboticabal, sob orientação da Profa. Dra. Jane Maria Bertocco Ezequiel. Participou do Programa Doutorado Sanduíche no Exterior (PDSE – CAPES) atuando como pesquisadora visitante no Commonwealth Scientific and Industrial Research Organisation (CSIRO – Brisbane, Austrália), sob orientação do Dr. Christopher Mcsweeney.

Epígrafe

“Não fui eu que lhe ordenei? Seja forte e corajoso! Não se apavore, nem se desanime, pois o Senhor, o seu Deus, estará com você por onde você andar". (Josué 1:9)

Dedicatória

Aos meus pais, ***Edivaldo e Vilma*** *pelos exemplos de dignidade e amor, por acreditar em mim e ser a razão de meus esforços.*

Ao meu irmão, ***Rafael,*** *e minhas sobrinhas,* ***Sophia e Anna Samara****, pelo apoio e amor incondicional.*

A minha avó ***Ana Alves da Costa*** *(in memoriam), exemplo de perseverança, me fazendo acreditar que meus sonhos poderiam ser concretizados.*

Agradecimentos

Agradeço primeiramente a Deus, por ter me iluminado e me sustentado durante esse período.

A minha família, meus pais Edivaldo e Vilma, meu irmão Rafael e minhas sobrinhas Sophia e Anna Samara. O apoio e o amor incondicional de vocês me dão forças para seguir a diante quando eu penso que não consigo mais.

A minha orientadora profa Dra Jane Maria Bertocco Ezequiel, por ter aceitado me orientar e por todas as portas que me foram abertas durante o doutorado. A minha coorientadora e mentora Dra Yury Tatiana Granja-Salcedo, que me acolheu e me conduziu durante o doutorado. Sou muito grata por ter te conhecido e ter tido a oportunidade de trabalhar com você e crescer como pessoa e profissional.

A De Heus, pelo apoio financeiro para condução do experimento e ao CNPq pela bolsa de doutorado sanduíche.

Aos Drs Chris Mcsweeney e Stuart Denman pela acolhida durante minha estadia no CSIRO e pela ajuda com a manipulação dos dados de bioinformática, tudo isso foi fundamental para o resultado da minha tese.

Aos meus amigos australianos que foram minha família fora de casa: Bianca Bee, Cécile Godde, Alessandra Albuquerque, Rita Andrade e as minhas housemates Kristina e Jane. Obrigada por cada morning tea e trivia! Em especial ao James Broadbent, por cada sessão de cinema e observações da lua, os dias na Austrália ficaram muito mais leves com você!

Aos meus irmãozinhos Janinos: Sérgio, Edivilson Filho, Ari, Maria Carolina e aos estagiários (agora companheiros de profissão) Andresa, João Pedro, Lívia e Julia por toda ajuda na condução do experimento.

Aos amigos conquistados, pelas conversas, pelos ensinamentos, pelas risadas, pelas comidas, pela parceria, pelos colos, pelo companheirismo e ajuda na hora do desespero! Carol, Juliana Akamine, Débora, Laura, Paloma, mil vezes obrigada!

Às melhores amigas que a vida poderia me presentear, Karine Dalla Vecchia e Kênia Alves. Obrigada por toda compreensão, paciência e parceria. Esse doutorado não

teria saído sem a sua ajuda de vocês. Pela amizade, por ter dividido e participado ativamente das minhas conquistas e decepções. Por ter me ensinado, sempre me acolhendo e me apoiando em tudo e em todos os momentos!

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq – processo nº 141882/2017-4

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.

A todos que contribuíram direta e indiretamente para execução desse projeto.

Sumário

CERTIFICADO DO COMITÊ DE ÉTICA NO USO DE ANIMAIS

unesp UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO" Câmpus de Jaboticabal

CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o projeto intitulado **"Desempenho nutricional de bovinos_ suplementados com mistura de umidade baixa (MUB®)"**, protocolo nº 010216/17, sob a responsabilidade da Prof.ª Dr.ª Jane Maria Bertocco Ezequiel, 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) - encontra-se de acordo com os preceitos da lei nº 11.794, de 08 de outubro de 2008, no decreto 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), da FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS, UNESP - CÂMPUS DE JABOTICABAL-SP, em reunião ordinária de 03 de Agosto de 2017.

Vigência do Projeto	10/08/2017 a 20/04/2018
Espécie / Linhagem	*Bos taurus*
Nº de animais	06
Peso / Idade	350 Kg / 22 meses
Sexo	Macho
Origem	Unidade Animal de Estudos Digestivos e Metabólicos – FCAV/Unesp

Jaboticabal, 03 de Agosto de 2017.

Profª Drª Lizandra Amoroso
Coordenadora – CEUA

Faculdade de Ciências Agrárias e Veterinárias
Via de Acesso Prof. Paulo Donato Castellane, s/n - 14884-900 - Jaboticabal - SP - Brasil
tel 16 3209 2600 fax 3202 4275 www.fcav.unesp.br

IMPACTO DA SUPLEMENTAÇÃO COM BLOCOS MULTIFUNCIONAIS SOBRE OS PARÂMETROS NUTRICIONAIS E DIVERSIDADE BACTERIANA RUMINAL DE NOVILHOS NELORE

RESUMO: Objetivou-se com este estudo avaliar os parâmetros de fermentação ruminal, balanço de nitrogênio, parâmetros da degradabilidade ruminal da matéria seca (MS) e fibra em detergente neutro (FDN), comportamento ingestivo, os parâmetros metabólicos e a diversidade bacteriana ruminal de novilhos Nelore em regime de alimentação com forragem tropical de baixa qualidade recebendo diferentes fontes de suplementação. Foram utilizados seis novilhos Nelore (350 ±10 kg peso corporal inicial e 24 meses de idade), castrados, canulados permanentemente no rúmen, distribuídos aleatoriamente em um Quadrado Latino duplo 3×3, sendo três tratamentos e três períodos experimentais. Os animais receberam feno de Urochloa brizantha (Hochst.) Stapf, "Marandu" *ad libitum* como dieta basal e foram suplementados com sal mineral ureado (SMU), concentrado proteico-energético comercial (SPE) ou bloco multifuncional (BMF). O consumo de MS, suplemento, MO, FDNcp e FDA foi superior para os animais suplementados com SPE (P<0,05). Entretanto, o uso do BMF aumentou o consumo e a digestibilidade de PB (P<0,001). Maiores coeficientes de digestibilidade da FDNcp foram observados em animais suplementados com SMU (P=0,032). Houve interação tempo × tratamento para a concentração ruminal de N-NH_3 onde animais suplementados com SPE apresentaram maior concentração de amônia as 2, 4 e 6 horas após a alimentação, quando comparado a SMU e BMF (P<0,001). A proporção de propionato foi maior quando BMF foi suplementado (P=0,016). Animais suplementados com BMF e SPE tiveram similar retenção de nitrogênio (RN, g/d) em função do nitrogênio consumido e digerido e eficiência de uso do nitrogênio, porém maiores que os animais suplementados com SMU (P<0,007). A suplementação com concentrado proteico-energético proporcionou maior abundância relativa ruminal de *Mogibacterium.spp*, Ruminococcaceae UCG-004 e *Papilibacter.spp* e tendência a maior população relativa de Eubacterium grupo coprostanoligenes quando comparado ao SMU (P<0,05). O Clostridium sp. CAG-352, Lachnoclostridium 10, Prevotellaceae UCG-004, Ruminococcaceae-UCG-011 e *Succinivibrio.spp* foram identificados apenas no rúmen de novilhos alimentados com SPE. Novilhos suplementados com SMU apresentaram maior abundância relativa ruminal de *Fretibacterium.spp* e do grupo Lachnospiraceae ND3007 Em relação ao comportamento alimentar, animais suplementados com SPE e BMF despenderam maior tempo na interação com o feno (P=0,004), enquanto animais suplementados com SMU e BMF apresentaram menor tempo de interação com o suplemento (P=0,002). O tempo de alimentação foi maior em animais suplementados com SPE (P<0,001). As concentrações sanguíneas de glicose, insulina, proteínas totais não foram influenciadas pelos tratamentos (P>0,05). Entretanto, a concentração de ureia foi superior em animais suplementados com BMF (P=0,05) e houve efeito de tratamento e tempo de coleta (P<0,05) na concentração de triglicerídeos; o uso do BMF resultou em maiores concentrações de triglicerídeos e houve uma diminuição linear das

concentrações em função do tempo de coleta (P<0,05). A degradabilidade ruminal da fração “a” da MS e a degradabilidade efetiva estimada a 2,5 e 8%$^{h-1}$ foi similar para animais suplementados com SPE e BMF (P<0,05). Em contraste, a degradabilidade ruminal da fração “b” foi similar entre SMU e SPE (P=0,013) e a degradabilidade potencial foi superior para animais suplementados com SPE (P=0,015). A fração “b” foi correlacionada positivamente com o filo Fibrobacter (P=0,034; r=0,50) e negativamente com a classe Bacilli, as ordens MVP 15, Lactobacillales, os gêneros *Lachnospiraceae bacterium FD2005* e *Succinimonas.spp* (P<0,016; r<-0,56). A fração “b” da FDN foi correlacionada positivamente com as ordens Aeromonadales, Desulfuromonadales, os gêneros *Eubacterium hallii group, Coprococcus 1, Ruminiclostridium 5, Ruminobacter* e a espécie *Lachnospiraceae UCG,002* (P<0,001, r>0,70). A ureia plasmática se correlacionou positivamente com a classe Gammaproteobacteria, a família Ruminococcaceae, os gêneros *Ruminobacter.spp* e *Succinivibrio.spp* e a OTU *Family XI 2II* (P<0,007; r>0,63) e negativamente com a família Prevotellaceae e o gênero *Fretibacterium.spp* (P<0,036; r<-0,52). O uso dos blocos multifuncionais influencia a composição do ecossistema bacteriano ruminal em favor da família *Neisseriaceae* e melhora a retenção e a eficiência de uso do nitrogênio. Entretanto, estas mudanças foram semelhantes as geradas pelo suplemento proteico-energético comercial.

Palavras-chave: bactérias, estacionalidade forrageira, feno de baixa qualidade, retenção de nitrogênio, rumen, suplementação alimentar

IMPACT OF LOW-MOISTURE, SUGARCANE MOLASSES-BASED BLOCK SUPPLEMENTATION ON NUTRITIONAL PARAMETERS AND RUMINAL BACTERIAL DIVERSITY OF NELORE STEERS

ABSTRACT: The objective of this study was to evaluate the parameters of ruminal fermentation, nitrogen balance, parameters of ruminal dry matter (DM) and neutral detergent fiber (NDF) degradability, ingestive behavior, metabolic parameters and ruminal bacterial diversity of Nellore steers fed with low quality tropical forage, receiving different sources of supplementation. Six Nellore steers (350 ±10 kg initial body weight and 24 months of age), castrated, permanently cannulated in the rumen, randomly distributed in a 3×3 double Latin Square, with three treatments and three experimental periods, were used. The animals received Urochloa brizantha (Hochst.) Stapf hay, "Marandu" ad libitum as basal diet and were supplemented with: urea mineral salt (SMU), commercial protein-energy concentrate (SPE) or low-moisture, sugarcane molasses-based block (BMF). The consumption of DM, supplement, MO, NDFcp and FDA was higher for animals supplemented with SPE ($P<0.05$). However, the use of BMF increased CP intake and digestibility ($P<0.001$). Higher coefficients of digestibility of NDFcp were observed in animals supplemented with SMU ($P=0.032$). There was an interaction time × treatment for the ruminal concentration of N-NH3 where animals supplemented with SPE had higher ammonia concentration at 2, 4 and 6 hours after feeding, when compared to SMU and BMF ($P<0.001$). The proportion of propionate was higher when BMF was supplemented ($P=0.016$). Animals supplemented with BMF and SPE had similar nitrogen retention (RN, g/d) as a function of consumed and digested nitrogen and nitrogen use efficiency, but higher than animals supplemented with SMU ($P<0.007$). Supplementation with protein-energy concentrate provided higher relative ruminal abundance of Mogibacterium.spp, Ruminococcaceae UCG-004 and Papilibacter.spp and a trend towards higher relative population of Eubacterium group coprostanoligenes when compared to SMU ($P<0.05$). Clostridium sp. CAG-352, Lachnoclostridium 10, Prevotellaceae UCG-004, Ruminococcaceae-UCG-011 and Succinivibrio.spp were identified only in the rumen of SPE-fed steers. SMU-supplemented steers had higher relative ruminal abundance of Fretibacterium.spp and Lachnospiraceae ND3007 In relation to feeding behavior, animals supplemented with SPE and BMF spent more time in the interaction with hay ($P=0.004$), while animals supplemented with SMU and BMF showed shorter interaction time with the supplement ($P=0.002$). Feeding time was longer in animals supplemented with SPE ($P<0.001$). Blood glucose, insulin, total protein concentrations were not influenced by treatments ($P>0.05$). However, the urea concentration was higher in animals supplemented with BMF ($P=0.05$) and there was an effect of treatment and collection time ($P<0.05$) on the concentration of triglycerides; the use of BMF resulted in higher concentrations of triglycerides and there was a linear decrease in concentrations as a function of collection time ($P<0.05$). Ruminal degradability of fraction "a" of DM and estimated effective degradability at 2.5 and 8%h^{-1} were similar for animals supplemented with SPE and BMF ($P<0.05$). In contrast, the ruminal degradability of fraction "b" was similar between SMU and SPE ($P=0.013$) and

the potential degradability was higher for animals supplemented with SPE (P=0.015). Fraction “b” was positively correlated with the phylum Fibrobacter (P=0.034; r=0.50) and negatively with the class Bacilli, the orders MVP 15, Lactobacillales, the genera Lachnospiraceae bacterium FD2005 and Succinimonas.spp (P<0.016); r<-0.56). The fraction “b” of NDF was positively correlated with the orders Aeromonadales, Desulfuromonadales, the genera Eubacterium hallii group, Coprococcus 1, Ruminiclostridium 5, Ruminobacter and the species Lachnospiraceae UCG.002 (P<0.001, r>0.70). Plasma urea correlated positively with the Gammaproteobacteria class, the Ruminococcaceae family, the Ruminobacter.spp and Succinivibrio.spp genera and the OTU Family XI 2II (P<0.007; r>0.63) and negatively with the Prevotellaceae family and the genus Fretibacterium.spp (P<0.036; r<-0.52). The use of low-moisture, sugarcane molasses-based block influences the composition of the ruminal bacterial ecosystem in favor of the Neisseriaceae family and improves nitrogen retention and use efficiency. However, these changes were like those generated by the commercial protein-energy supplement.

Key-words: bacteria, forage seasonality, low quality hay, nitrogen retention, rumen, feed supplementation

CAPÍTULO 1 – CONSIDERAÇÕES GERAIS

1. INTRODUÇÃO

No cenário mundial, o Brasil se destaca por possuir o maior rebanho comercial de bovinos, com aproximadamente 213,68 milhões de cabeças (ABIEC, 2020) distribuídos em cerca de 162,53 milhões de hectares de pastagens (ABIEC, 2019). Estima-se que 87,4% dos animais abatidos no Brasil sejam manejados exclusivamente em pastagem (ABIEC, 2019) e, considerando que a estacionalidade influencia a composição e a qualidade da forragem, torna-se imprescindível o uso de estratégias nutricionais que tenham como objetivo o atendimento das necessidades nutricionais dos animais (ASSOCON, 2012).

No Brasil, a produção de forragem é influenciada pela estacionalidade produtiva, onde as forrageirass tropicas não conseguem manter um balanço ótimo entre a demanda animal e os nutrientes necessários para atender às exigências de ganhos elevados (Reis et al., 2012). A disponibilidade e qualidade das forragens varia de acordo com a estacionalidade, com um período chuvoso (primavera/verão) caracterizado pelo favorecimento do crescimento das plantas, época de maior oferta em quantidade e qualidade de forragem (Reis et al., 2011), e um período seco (outono/inverno), caracterizado pela escassez de chuva, que consequentemente reduz a produção de forragem e causa mudanças estruturais do dossel, acúmulo de colmo e material morto, ocorrendo queda na qualidade da forragem (Lazzarini et al., 2009).

A suplementação alimentar pode ser adotada como uma prática tecnológica de apoio à pastagem. O adicional de nutrientes proveniente da suplementação faz com que os animais se tornem mais eficientes na utilização da forragem disponível, através de melhorias nas condições do ambiente ruminal (Reis et al. 2009).

Existem diversas estratégias de suplementação em pasto e as mais comuns utilizam grãos, subprodutos da agroindústria, sais proteinados ou energéticos e, mais recentemente no Brasil, os blocos multifuncionais. Este último permite o baixo consumo de uma mistura de ingredientes compactados, de modo a fornecer nutrientes ao longo do dia. Além disso, evita quedas bruscas do pH e picos de concentração de amônia

ruminal, comuns em uma suplementação convencional (Trater et al., 2003; Katulski et al., 2017).

Alguns estudos têm avaliado o efeito da suplementação com blocos multifuncionais em bovinos. Freitas et al. (2003a), estudaram o efeito da suplementação com blocos multifuncionais com diferentes níveis de melaço de cana-de-açúcar (25, 30, 35 e 40%), observaram que independentemente dos níveis de melaço, houve aumento no consumo, digestibilidade, concentrações ruminais de amônia, nitrogênio uréico sanguíneo e alterção no pH ruminal. Titgemeyer et al. (2004), ao avaliarem a resposta da suplementação com blocos multifuncionais contendo baixo (15%) ou elevado (28%) teor de proteína bruta (PB) para bovinos em crescimento, observaram que animais alimentados com bloco de alto teor de proteína tiveram maior consumo e ganharam mais peso. Entretanto, o estudo dos efeitos da suplementação com blocos multifuncionais sobre o comportamento ingestivo, metabolismo e microbioma ruminal de bovinos alimentados com forragens de baixa qualidade é inexistente.

No Brasil, esse tipo de suplemento ainda não é amplamente utilizado. Porém, os blocos multifuncionais surgem como oportunidade de uso, uma vez que seus benefícios vão desde a melhoria dos parâmetros nutricionais até o desempenho animal, além da facilidade de uso. Assim, o objetivo deste trabalho foi avaliar os parâmetros de fermentação ruminal, balanço de nitrogênio, parâmetros metabólicos, degradabilidade ruminal de nutrientes, comportamento ingestivo, diversidade bacteriana e archaeal ruminal de novilhos Nelore alimentados com forragem tropical de baixa qualidade recebendo diferentes suplementos alimentares, sendo blocos multifuncionais, concentrado proteico energético comercial e sal minera ureado.

1.1. Produção de bovinos de corte em pastagens e o uso de suplementação

De maneira geral, o ganho de peso é determinado principalmente pela ingestão de nutrientes, e, para animais mantidos em pastagens, é dependente da estrutura do dossel forrageiro e das práticas de manejo adotadas (Da Silva et al., 2009; Reis et al., 2012; Vieira Júnior et al., 2013). A disponibilidade e qualidade das forragens varia de acordo com a estacionalidade produtiva. O período chuvoso (primavera/verão) favorece o crescimento das plantas, com elevada produção de massa e teores de PB de 12%,

valor acima do recomendado para plena atividade das bactérias que degradam fibra (Reis et al., 2011), que deve variar entre 6-8% de PB (Van Soest, 1994). Consequentemente, há a maior proporção de conteúdo celular, maior relação folha:colmo e elevada quantidade de fibra em detergente neutro potencialmente digestível (FDNpd) (Barbero et al., 2015).

Entretanto, no período seco do ano (outono/inverno), a forragem disponível para alimentação animal apresenta baixo valor nutritivo e dificilmente conseguem manter balanço positivo entre as demandas nutricionais dos animais e os nutrientes disponíveis, para atender às exigências de mantença e ganhos (Reis et al., 2012). Além disso, o déficit hídrico reduz a produção forrageira e provoca mudanças na estrutura do dossel, caracterizada pela maior participação de colmo e material morto, elevado teor de fibra indigestível e diminuição nos teores de proteína bruta (PB) a limites inferiores a 7%, valor considerado limitante para a síntese de proteína microbiana pelos microrganismos ruminais (Lazzarini et al., 2009). Estas alterações refletem negativamente sobre a qualidade bromatológica e nutricional, e exige a adoção de estratégias para suprir as necessidades dos animais (Paterson et al., 1994).

A condição básica para promover a suplementação é que haja disponibilidade de massa forrageira na pastagem, mesmo esta sendo de baixa qualidade. Assim, a suplementação, seja na fase de recria ou de terminação, permite reduzir o tempo de abate do animal, aumentando o desfrute e o giro de capital na propriedade.

A suplementação pode ter vários efeitos sobre o consumo, como: aditivos, substitutivos, aditivos/substitutivos, aditivos com estímulo. O efeito aditivo seria avaliado como um aumento de ganho de peso, geralmente proporcionado pela suplementação para corrigir deficiências nutricionais específicas em que pequenas quantidades de suplemento são ingeridas (Euclides, 2002), atuando de forma associativa, sem diminuir o consumo total do animal. Já o efeito substitutivo ocorre quando o consumo de suplemento diminui o de forragem, sem melhorar o desempenho animal. Este efeito pode ser utilizado quando se espera aumento da taxa de lotação de determinada área (Silva et al., 2009).

O efeito aditivo/substitutivo é caracterizado pela diminuição do consumo de forragem pelo fornecimento de suplemento, mas ao mesmo tempo ocorre melhora no desempenho animal, em razão da maior disposição de energia (Goes et al.,2005).

A suplementação tem como princípio atender às exigências dos microrganismos presentes no rúmen, principalmente as bactérias, favorecendo o seu crescimento e desenvolvimento, consequentemente aumentando o aproveitamento da dieta, mas principalmente da fração fibrosa consumida. Com o fornecimento adicional de nutrientes por meio da suplementação, os animais se tornam mais eficientes na utilização da forragem disponível, devido à melhoria das condições do ambiente ruminal (Detmann et al., 2014). Existem diversas estratégias de suplementação em pasto, sendo as mais comuns a utilização de grãos, de subprodutos da agroindústria, de sais proteinados, misturas múltiplas e, mais recentemente, os blocos multifuncionais.

O sal-ureia (sal mineralizado adicionado de ureia e sulfato de amônio) pode ser utilizado com o objetivo de promover a manutenção do peso durante o período seco (Ítavo et al., 2008). Além disso, o sal-ureia tem como objetivo fundamental suprir a deficiência de nitrogênio da forragem (Hoffmann et al., 2014), através do uso de nitrogênio não proteico (NPN). Batista et al., (2016) analisaram os efeitos metabólicos da suplementação com PDR, verificaram que o aumento de N retido (g/dia) foi proporcional ao aumento de PDR na dieta e concluiu que suplementação proteica melhorou a utilização de nitrogênio em animais alimentados com fontes volumosas de baixa qualidade.

O suplemento múltiplo caracteriza-se por conter ureia, fontes de proteína, energia, fósforo, cálcio, microelementos, além do sal comum, que juntamente com a ureia se tornam um fator limitante de consumo pelo animal (Jayme et al., 2013). Os blocos multifuncionais, por sua vez, permitem o baixo consumo de uma mistura de ingredientes (melaço de cana-de-açúcar, farelos, ureia, macro e microminerais) compactados, e, ao ser lambido pelo animal, fornecem nutrientes constantemente ao longo do dia (Garmendia, 1994, Freitas et al., 2003a).

Os microrganismos do rúmen necessitam de no mínimo 7% de PB para seu crescimento (Van Soest, 1994). Assim, segundo Hoffmann et al. (2014), a suplementação com fontes de proteína verdadeira e NNP promove o crescimento de

bactérias ruminais, eleva a digestibilidade da forragem de baixa qualidade, o consumo de matéria seca e de energia digestível, e consequentemente, melhora o desempenho animal.

1.2. Blocos multifuncionais na suplementação de bovinos de corte

A suplementação em si foi uma inovação a partir da década de 1990. Ao longo dos anos, pesquisas foram conduzidas para o estabelecimento das características gerais, ou seja, presença de macro e microminerais, ingredientes e aditivos a serem utilizados, teores de proteína e/ou energia e quantidade a ser forneceida (Moraes, 2001; Detmann et al., 2004; Sales et al., 2008; Silva et al., 2009).

A forma de fornecimento é amplamente estudada, podendo ser realizada diariamente, em dias alternados ou semanalmente (Canesin et al., 2007; Simioni et al., 2009) na forma farelada ou em blocos (Trater et al., 2003; De Queiroz e Gallo, 2013; Moriel et al., 2019). Na década de 1990, os blocos multifuncionais (BMF) já eram conhecidos por permitir o baixo consumo de uma mistura de ingredientes compactados (melaço, ureia, farelos e sal mineral), de modo a fornecer nutrientes constantemente ao longo do dia (Garmendia, 1994, Freitas et al., 1999).

O BMF é fabricado por meio de um processo de desidratação do melaço de cana-de-açúcar. O óleo vegetal presente na formulação torna o produto resistente à água, o que permite sua conservação mesmo quando exposta à chuva e ventos (Trater et al., 2003). A utilização do BMF evita quedas bruscas do pH e picos na concentração de amônia ruminal, típicos de uma suplementação convencional (Garmendia, 1994). Além dos benefícios nutricionais, os blocos oferecem vantagens do ponto de vista da logística, devido a sua versatilidade, facilidade de manejo, transporte e armazenamento uma vez que não é necessária a troca constante de suplemento, reduzindo problemas ocasionados por chuva em áreas com cocho descoberto.

Vários fatores influenciam o consumo dos blocos, como a aceitabilidade, a qualidade do pasto e/ou volumoso, a disposição dos blocos no piquete, o número de animais por bloco e o tempo de armazenamento. Entre eles, a dureza do bloco é considerada a principal variável na determinação do consumo (Sansoucy et al., 1988). E esta duzera pode ser influenciada pelas quantidades de melaço, ureia, e a proporção

dos aglutinantes (Hinestroza e Becerra, 1990; Araque e Cortes, 1998; Freitas et al., 1999).

Freitas et al. (2003a) avaliando o efeito da suplementação de feno de Tifton (*Cynodon dactylon*) com BMF formulados com diferentes níveis de melaço de cana-de-açúcar (25, 30, 35 e 40%), observaram aumento no consumo, digestibilidade, concentrações ruminais de amônia de nitrogênio ureico sanguíneo, sem alterações nos valores de pH ruminal quando comparado ao tratamento sem suplementação. Ao avaliarem a resposta da suplementação com blocos de melaço cozido com baixo (15%) ou elevado (28%) teor de proteína bruta (PB) em bovinos em crescimento, alimentados com forragem de diferente qualidade (feno, 8,4% PB; alfafa, 19,1% PB e feno + alfafa, 11,5% PB), Titgemeyer et al. (2004) observaram que independente da qualidade da forragem, novilhas alimentadas com bloco de melaço cozido de elevado teor de proteína ganharam mais peso e tiveram maior consumo.

Efeitos positivos da suplementação com BMF na ingestão de forragens de baixa qualidade foram relatados por Badurdeen et al. (1994) e Greenwood et al. (1998). Badurdeen et al. (1994) observaram um aumento de 10% na ingestão de forragem quando os bezerros receberam um BMF à base de melaço e com 56% de PB, e Greenwood et al. (1998) relataram um aumento de 13% na ingestão de forragem quando um BMF à base de melaço e com 30% de PB foi fornecido. Entretanto, a redução na digestibilidade da fibra em detergente neutro (FDN) foi relatada quando o melaço de cana foi suplementado em níveis de pelo menos 15% do MS da dieta para bovinos alimentados com forragem de baixa qualidade (Kalmbacher et al., 1995).

De acordo com Lima et al. (2008), a proporção e a quantidade dos subprodutos da fermentação microbiana dependem tanto do tipo de alimento e forma como é fornecido, quanto de fatores fisiológicos relacionados ao ambiente ruminal como temperatura, anaerobiose e pH. A suplementação proteico-energética pode provocar alteração no perfil de AGCC (Oliveira et al., 2009; Goes et al., 2015; Sales et al., 2017). Avaliando o uso de suplementação proteica em blocos contendo ureia, melaço e sais minerais, Srinivas e Gupta (1997) observaram diferenças na produção de acetato, bem como nas relações acetato:propionato, onde os animais sem suplementação mantiveram níveis superiores de acetato no fluido ruminal.

As concentrações de nitrogênio amoniacal ($N\text{-}NH_3$) no rúmen estão associadas à velocidade de introdução e liberação das fontes nitrogenadas no rúmen, bem como a sua assimilação em proteínas microbianas, o que é resultado da sincronia de demais nutrientes que compõem as estruturas celulares dos microrganismos (Oliveira et al., 2009).

De maneira geral, a amônia que não é utilizada pelos microrganismos ruminais é absorvida pela parede do rúmen e vai ao fígado por meio da circulação sanguínea, onde entra no ciclo da ureia (Kennedy e Milligan, 1980; Angelidis et al., 2019). Durante períodos de alta disponibilidade ruminal de N, observam-se elevadas concentrações sanguíneas de ureia (Rennó et al., 2000). Em contrapartida, baixo nível de proteína na dieta promove redução de aminoácidos na circulação, diminuindo a concentração de insulina e da taxa de entrada de glicose, e consequentemente, a disponibilidade de energia ao hipotálamo (Ruas et al., 2000). A concentração de ureia plasmática pode indicar uma ineficiência na utilização da PB da dieta em função do excesso no fornecimento ou assincronia de energia-proteína no rúmen (Pessoa et al., 2009).

Como a saúde, o crescimento e o metabolismo dos animais dependem amplamente da produção de metabólitos como a ureia, glicose, insulina, triglicerídeos e as proteínas totais. Assim, uma análise da composição do plasma pode oferecer informações importantes sobre o papel das interações rúmen-dieta nas condições dos animais (Saleem et al., 2013). Moriel et al., (2019) observaram que as concentrações plasmáticas de glicose e ureia plasmática foram maiores nas novilhas suplementadas com BMF e concentrado proteico energético comercial, quando comparado ao sal mineral. Eles relatam que isso podem estar associadas a diferenças na ingestão de energia e proteína entre os tratamentos.

A análise das proteínas totais no plasma também é utilizada como método de avaliação do status proteico. O fígado é o principal órgão produtor dessas proteínas e estão relacionadas com deficiência na alimentação, quando descartadas causas patológicas (Gonzáles, 2000). Estima-se que dietas com menos de 10% de proteína provocam diminuição dos níveis protéicos no sangue (Kaneko et al., 1997).

A complexidade desses fatores, associada ao comportamento de alimentação a pasto (pastejo, ruminação e ócio) e às diferenças na composição dos suplementos, pode

provocar diferentes respostas no ambiente ruminal (Oliveira et al., 2009) alterando os padrões de consumo de suplementos e a utilização de nutrientes (Katulski et al., 2017).

Aubel et al., (2011) avaliaram a ingestão, a frequência de uso e a duração do uso do BMF e sal mineral em capim *Andropogon gerardii* Vitman, em diferentes estações do ano e observaram que o uso do BFM aumentou o consumo de suplemento. Esse resultado foi associado a maior interação dos animais com o BMF e a maior duração dessa interação. Entretanto, pouco se sabe sobre o comportamento ingestivo de bovinos suplementados com blocos multifuncionais e alimentados com forragem tropical de baixa qualidade.

Nesse sentido, ainda não existe entendimento completo dos fatores que interagem entre si, como a concentração de proteína na ração, exigência de nitrogênio para os microrganismos, efeito da suplementação no pH ruminal, quantidade e frequência de suplementação e se o suplemento é baseado em amido, açúcares ou fibra rapidamente digestível (Reis et al. 2012). As informações existentes sobre o BMF permitem que se conclua que seu uso pode ser uma estratégia de suplementação, que deve ser avaliada em condições brasileiras. Também, deve avaliar o efeito deste tipo de suplemento sobre a dinâmica ruminal e microbiana, uma vez que em sua formulação há melaço e ureia. Ainda, quando o volumoso for de baixa qualidade, é preciso avaliar seus efeitos nutricionais e estratégias para otimização do uso do suplemento de forma eficiente e que resulte em economicidade.

1.3. Importância do microbioma ruminal no metabolismo de bovinos em pasto

O processo fermentativo é realizado por uma diversidade de microrganismos que habitam o rúmen e que interagem de diferentes formas, permitindo a transformação dos substratos ingeridos pelo animal em energia para sua manutenção, crescimento e reprodução (Arcuri et al., 2011). As bactérias constituem a maior proporção da biomassa microbiana ruminal (60 a 90%), e são as mais ativas fermentadoras, mais estudadas e consideradas as mais importantes nutricionalmente (Petri et al., 2012).

As bactérias ruminais degradam os componentes dos alimentos (carboidratos e proteínas), transformando-os em produtos como AGCC, proteína microbiana e vitaminas

do complexo B e K, que são utilizados pelo animal. A proporção e a quantidade dos subprodutos da fermentação microbiana dependem tanto do tipo de alimento e forma como é fornecido (Ley et al., 2008; Henderson et al., 2015)., quanto de fatores fisiológicos relacionados ao ambiente ruminal como temperatura, anaerobiose e pH (Lima et al., 2008).

Estudos de mudanças induzidas pela dieta na composição da comunidade microbiana do rúmen em diferentes contextos são numerosas (de Menezes et al., 2011; de Oliveira et al., 2013; Zhang et al., 2014; Lengowski et al., 2016). De Menezes et al., (2011) caracterizaram mudanças na estrutura da comunidade microbiana decorrente da alimentação de vacas leiteiras em duas dietas amplamente utilizadas: pastagem e ração mista total (TMR) e concluíram que houve uma maior abundância de *Fibrobacteraceae* em vacas alimentadas com TMR enquanto *Erysipelotrichaceae*, foi mais abundante em vacas alimentadas com pasto.

De Oliveira et al., (2013) investigaram os compartimentos do trato gastrintestinal (TGI) de um novilho Nelore através do pirosequenciamento 16S rRNA e observaram que a diversidade filogenética da comunidade microbiana na alimentação é diferente do TGI, o que indica ainda que muito poucas bactérias são retidas da alimentação à medida que ela passa pelo TGI. Populações distintas de membros da família *Ruminococcaceae* e do filo Bacteroidetes foram encontradas no intestino grosso, semelhante ao que foi encontrado para o rúmen e é possível que essas comunidades também possam contribuir para uma maior fermentação alimentar a jusante.

Zhang et al., (2014) avaliando dietas com diferentes fontes forrageiras observaram que as proporções relativas dos gêneros *Ruminococcaceae* não classificados, *Anaerotruncus* e *Papillibacter* foram maiores na dieta contendo caule de milho do que nas dietas contendo feno de alfafa ou gramínea perene nativa (Leymus chinensis). Além disso, a presença de *Staphylococcus* nos grupos de animais alimentados com caule de milho ou *L. chinensis* foi associada a um risco aumentado mastite bovina.

Henderson et al., (2015) determinaram a composição da microbiota no rúmen em 742 amostras divididas entre bovinos, caprídeos, cervídeos e camelídeos de todo o mundo e observaram que os membros de *Prevotella* e *Succinivibrionaceae* não classificados foram mais abundantes em animais alimentados com dietas contendo

concentrado e *Bacteroidales* e *Ruminococcaceae* não classificados foram mais abundantes em todos os animais alimentados com forragem.

Lengowski et al., (2016) observaram mudanças na comunidade microbiana ruminal quando animais alimentados com silagem de capim apresentaram maior abundância das espécies *F. succinogenes* e *R.* amylophilus, enquanto a maior abundância de protozoários foi observada em vacas alimentadas com dieta baseada em silagem de milho.

De acordo com de Medeiros e Marino (2015) os suplementos proteicos de baixo consumo diminuem a limitação dos baixos teores de N das forragens na seca e aumentam a ingestão de matéria seca o que resulta em maior consumo de forragem pelos animais suplementados em relação aos não suplementados.

Da Silva-Marques et al., (2018) avaliaram os impactos da suplementação com três níveis de proteína em de touros Nelore pastando Urochloa brizantha *cv.* Marandu e observaram que a presença de ureia no suplemento de alta proteína (44% de PB) favoreceu o crescimento das bactérias celulolíticas *Fibrobacter succinogenes, R. flavefaciens* e *B. fibrisolvens,* que usam apenas amônia como fonte de nitrogênio para sintetizar proteínas. A proteína microbiana é a principal fonte de proteína metabolizável para os ruminantes (Mikolayunas et al., 2011) e aproximadamente 60 a 90% da proteína que chega ao intestino delgado é de origem microbiana, dependendo principalmente dos ingredientes da dieta.

Os microrganismos ruminais dependem de esqueletos de carbono, disponibilidade de energia e de um concomitante fornecimento de amônia e peptídeos para que haja síntese microbiana. É essencial que haja carboidratos disponíveis no rúmen, por terem efeito sobre a utilização dos compostos nitrogenados, uma vez que as bactérias ruminais podem incorporar os aminoácidos e fermentá-los como fonte de energia (Pereira et al., 2005). Sampaio et al., (2009) avaliando os efeitos da suplementação com compostos nitrogenados sobre a dinâmica ruminal da fibra em detergente neutro (FDN) em bovinos alimentados com forragem tropical de baixa qualidade (4,86% PB) constatou que 6,24 mg/dL de nitrogênio amoniacal ruminal é a concentração mínima necessária para sustentar atividade microbiana adequada na degradação FDN da forragem basal. Adicionalmente, Lazzarini et al., (2009)

trabalhando nas mesmas condições concluiu que é necessário pelo menos 7% de proteína bruta na dieta para sustentar o crescimento microbiano e apoiar a digestão de carboidratos de forragens de baixa qualidade.

Dos microrganismos presentes no rúmen, os mais estudados e classificados são os microrganismos celulolíticos devido a sua importância na degradação da fibra. Atualmente as espécies conhecidas representam apenas 5% da microbiota ruminal. Assim, 95% restante, são microrganismos que desempenham outras funções no ambiente ruminal e ainda não foram descritos (Kong et al., 2012), evidenciando que apesar dos esforços para a compreensão desse ecossistema, há muito ainda que ser elucidado (Bekele et al., 2011).

A aplicação recente de técnicas moleculares que não utilizam cultivo microbiano, a disponibilidade de informações genômicas em bancos de dados públicos e os avanços na caracterização dos ambientes, como o ambiente ruminal estão sendo conquistados (Deng et al., 2008, Mcsweeney e Mackie, 2012; Granja-Salcedo et al., 2017) e utilizadas na identificação e monitoramento de populações específicas em ambientes complexos.

Para a avaliação da diversidade microbiana e composição do ecossistema, a técnica de amplificação e sequenciamento do gene que codifica o RNA 16S (Edwads et al., 2004), é empregada devido à grande quantidade de dados gerados e facilidade de comparação e identificação de microrganismos. E, embora os avanços do genoma estejam fornecendo acesso à vasta diversidade de microrganismos não cultivados, a compreensão das variáveis que moldam as comunidades microbianas do rúmen está iniciando (Anderson et al., 2017).

Os filos bacterianos dominantes no ambiente ruminal são Bacteroidetes e Firmicutes e juntos corresponderam a aproximadamente 75% da composição da comunidade microbiana (Granja-Salcedo et al., 2017; de Jesus et al., 2019). O filo Firmicutes é o maior filo bacteriano e contém mais de 200 gêneros, compostos principalmente por bactérias Gram-positivas e com baixo teor de conteúdo guanina-citosina (GC) (Fernando et al., 2010). Bacteroidetes são as bactérias Gram-negativas mais abundantes encontradas nas comunidades anaeróbicas do rúmen (Jose et al., 2017).

Vários estudos foram conduzidos para avaliar o efeito dos teores e fontes de proteína (proteína verdadeira ou NNP) para que haja maior compreensão do metabolismo proteico. Porém poucos estudos avaliaram como a microbiota ruminal se comporta em função dessas mudanças e qual o impacto dessa mudança na retenção de nitrogênio no animal. Chanthakhoun et al., (2012) avaliaram dietas para búfalos com teores de proteína bruta crescentes (9,2; 12,4; 18,1 e 21%) e verificaram que a digestibilidade aparente dos nutrientes, a absorção e retenção de N foi maior com o aumento dos teores de PB na dieta e houve aumento das bactérias totais e das espécies *Fibrobacter succinogenes* e *Ruminococcus albus*.

Estudos que associam animais mais eficientes e a microbiota ruminal começam a contribuir para o avanço na nutrição de ruminantes. O consumo alimentar residual é uma ferramenta utilizada para avaliar a eficiência alimentar (Bezerra et al., 2013).

Rius et al., (2012) avaliando a digestibilidade do N e a comunidade microbiana do rumen em vacas Holandesas-Friesianas em lactação concluíram que as digestibilidades de MS, MO e N foram maiores nas vacas com consumo alimentar residual negativo, mas os efeitos não foram associados ao microbioma ruminal. Myer et al. (2015), ao analisarem a eficiência alimentar e a correlação com a diversidade microbiana, relataram que animais ineficientes apresentavam maiores proporções de *Prevotella*, que é justificado pela diversidade de utilização dos nutrientes por esse gênero.

Elolimy et al., (2018) descreveram que animais mais eficientes apresentaram maior abundância relativa de *Eubacterium ruminantium*, *Fibrobacter succinogenes* e *Megasphaera elsdenii*, e menor densidade bacteriana total e *Succinimonas amylolytica.*

O estudo de microbiologia ruminal deve ser visto com maior atenção na área de pesquisa estratégica, uma vez que o conhecimento minucioso da microbiota ruminal e dos fatores que a afetam está intimamente relacionado ao potencial crescimento da produtividade pecuária, além de possibilitar a caracterização rápida e precisa do perfil da microbiota ruminal.

2. REFERÊNCIAS BIBLIOGRÁFICAS

ABIEC, Associação Brasileira das Indústrias Exportadoras de Carnes (2019) Sumário 2019. Disponível em: <http://www.abiec.com.br/controle/uploads/arquivos/sumario2019portugues.pdf>

ABIEC, Associação Brasileira das Indústrias Exportadoras de Carnes (2020) Beef Report – Perfil da pecuária no Brasil. Disponível em: <https://www.cicarne.com.br/wp-content/uploads/2020/05/SUM%C3%81RIO-BEEF-REPORT-2020_NET.pdf>

Associação Nacional dos Confinadores. Levantamento da ASSOCON Sobre o Sistema de Produção em Confinamento no Brasil, 2012. Disponível em: < http://www.slideshare.net/BeefPoint/assocon-censo-2012>

Bezerra LR, Sarmento JLR, Neto SG, de Paula NRO, Oliveira RL, do Rêgo WMF (2013) Residual feed intake: a nutritional tool for genetic improvement. **Tropical animal health and production** 45(8):1649-1661.

Anderson CL, Sullivan MB, Fernando SC (2017) Dietary energy drives the dynamic response of bovine rumen viral communities. **Microbiome** 5:155.

Angelidis A, Crompton L, Misselbrook T, Yan T, Reynolds CK Stergiadis S (2019) Evaluation and prediction of nitrogen use efficiency and outputs in faeces and urine in beef cattle. **Agriculture, ecosystems and environment** 280:1-15.

Araque CA e Cortes R (1998) Evaluación del efecto de diferentes niveles de urea en bloques multinutricionales sobre el consumo de los bloque y ganancia de peso en mautes. **Revista Faculdad de Agronomia (LUZ)** 15:180-187.

Araque CA e Escalona M (1995) Una nota sobre el uso de los bloques multinutricionales en ganado de ceba. **Zootecnia Tropical** 13:87-94.

Arcuri PB, Lopes FCF, Carneiro JC (2011) Microbiologia do rúmen. In: Berchielli TT, Pires AV, Oliveira SGD (Eds.) **Nutrição de ruminantes**. **2º edição**. FUNEP – Fundação de Apoio a Pesquisa, Ensino e Extensão. Jaboticabal 111-140.

Aubel NA, Jaeger JR, Drouillard JS, Schlegel MD, Pacheco LA, Linden DR, Bolte JW, Higgins JJ, Olson KC (2011) Effects of mineral-supplement delivery system on frequency, duration, and timing of supplement use by beef cows grazing topographically rugged, native rangeland in the Kansas Flint Hills. **Journal of animal science** 89(11):3699-3706.

Badurdeen AL, Ibrahim MNM e Ranawana SSE (1994) Methods to improve utilization of rice straw. III. Effect of urea ammonia treatment and urea molasses blocks supplementation on intake, digestibility, rumen and blood parameters. **Asian-Australasian Journal of Animal Sciences** 7:363–372.

Barbero RP, Malheiros EB, Araújo TLR, Nave RLG, Mulliniks JT, Berchielli TT, Ruggieri AC, Reis RA (2015) Combining Marandu grass grazing height and supplementation level to optimize growth and productivity of yearling bulls. **Animal Feed Science and Technology** 209:110–118.

Batista ED, Detmann E, Titgemeyer EC, Valadares Filho SCD, Valadares RFD, Prates LL, Rennó LN, Paulino MF (2016) Effects of varying ruminally undegradable protein

supplementation on forage digestion, nitrogen metabolism, and urea kinetics in Nellore cattle fed low-quality tropical forage. **Journal of Animal Science** 94(1):201-216.

Bekele AZ, Koike S, Kobayashi Y (2011) Phylogenetic diversity and dietary association of rumen Treponema revealed using group-specific 16S rRNA gene-based analysis. **FEMS microbiology letters** 316:51-60.

Beretta V, Heinzen M, Simeone A (1999) Efeito da suplementação com blocos protéicos na evolução do estado nutricional e comportamento reprodutivo de vacas Hereford pastejando campo nativo diferido. In: REUNIÃO ANUAL DA SOCIEDADE BRASILEIRA DE ZOOTECNIA, Porto Alegre. **Anais da Sociedade Brasileira de Zootecnia,** Porto Alegre, 36.

Canesin RC, Berchielli TT, Andrade PD e Reis RA (2007) Desempenho de bovinos de corte mantidos em pastagem de capim-marandu submetidos a diferentes estratégias de suplementação no período das águas e da seca. **Revista Brasileira de Zootecnia**, 36, pp.411-420.

Chanthakhoun V, Wanapat M, Berg J (2012) Level of crude protein in concentrate supplements influenced rumen characteristics, microbial protein synthesis and digestibility in swamp buffaloes (Bubalus bubalis). **Livestock Science**144:197-204.

Da Silva SC, Bueno AAO, Carnevalli RA, Uebele MC, Bueno FO, Hodgson J, Matthew C, Arnold GC, Morais JP (2009) Sward structural characteristics and herbage accumulation of Panicum maximum cv Mombaça subjected to rotational stocking managements. **Scientia Agricola** 66:819.

Da Silva-Marques RP, Zervoudakis JT, Nakazato L, Cabral LDS, Hatamoto-Zervoudakis LK, da Silva MIL, Matos, NBDN, Pitchenin LC (2018) Quantitative qPCR Analysis of Ruminal Microorganisms in Beef Cattle Grazing in Pastures in the Rainy Season and Supplemented with Different Protein Levels. **Current microbiology** 75(8):1025-1032.

de Jesus RB, Granja-Salcedo YT, Messana JD, Kishi LT, Lemos EG, de Souza JAM, Berchielli TT (2019). Characterization of ruminal bacteria in grazing Nellore steers. **Revista Colombiana de Ciencias Pecuarias**, 32(4).

de Oliveira MNV, Jewell KA, Freitas FS, Benjamin LA, Tótola MR, Borges AC, Moraes CA e Suen G (2013). Characterizing the microbiota across the gastrointestinal tract of a Brazilian Nelore steer. **Veterinary microbiology**, *164*(3-4), 307-314.

de Medeiros SR, Marino C (2015) Carboidratos na nutrição de gado de corte. Embrapa Gado de Corte-Capítulo em livro científico (ALICE) < https://www.alice.cnptia.embrapa.br/alice/bitstream/doc/1011216/1/NutricaoAnimalCAPITULO04.pdf>

De Menezes AB, Lewis E, O'donovan M, O'neill BF, Clipson N, Doyle EM (2011) Microbiome analysis of dairy cows fed pasture or total mixed ration diets. **FEMS microbiology ecology** 78:256-265.

De Queiroz SKG e Gallo S (2013). Comparação entre suplemento mineral e suplemento mineral protéico em forma solida em novilhos da raça nelore a pasto no período das

águas. **CADERNOS DE PÓS-GRADUAÇÃO DA FAZU**, *3*.

Deng W, Xi D, Mao H, Wanapat M (2008) The use of molecular techniques based on ribosomal RNA and DNA for rumen microbial ecosystem studies: a review. **Molecular biology reports** 35:265–274.

Detmann E, Paulino MF, Valadares Filho SDC, Huhtanen P (2014) Nutritional aspects applied to grazing cattle in the tropics: A review based on Brazilian results. **Semina Ciências Agrárias** 35:2829–2850

Detmann E, Paulino MF, Zervoudakis JT, Cecon PR, Valadares Filho SDC, Gonçalves LC, Cabral LDS, Melo AJN (2004) Níveis de proteína bruta em suplementos múltiplos para terminação de novilhos mestiços em pastejo durante a época seca: desempenho produtivo e características de carcaça. **Revista Brasileira de Zootecnia** 33(1):169-180.

Edwards EJ, Mcewan RN, Travis J.A, Wallace RJ (2004) 16S rDNA library based analysis of ruminal bacterial diversity. **Antonie Van Leeuwenhoek** 86:263-281.

Elolimy, AA, Abdelmegeid MK, McCann JC, Shike DW e Loor JJ (2018) Residual feed intake in beef cattle and its association with carcass traits, ruminal solid-fraction bacteria, and epithelium gene expression. **Journal of animal science and biotechnology** 9(1):67.

Euclides VPB (2002) Estratégias de suplementação em pasto: uma visão crítica. In: Simpósio sobre manejo estratégico da pastagem, 2002, Viçosa, MG. **Anais...** Viçosa, MG: Universidade Federal de Viçosa, 437-469.

Freitas SPG, Patiño HO, e Mühlbach PRF (2003a) Efeito da suplementação de bezerros com blocos multinutricionais sobre a digestibilidade, o consumo e os parâmetros ruminais. **Revista Brasileira de Zootecnia** 32:1508-1515.

Freitas SPG, Ospina HP, Trein CR (1999) Efeito de quatro níveis de melaço e de bentonita sódica sobre algumas características físico-químicas de blocos multinutricionais. In: REUNIÃO ANUAL SOCIEDADE BRASILEIRA DE ZOOTECNIA, Porto Alegre. **Anais da Sociedade Brasileira De Zootecnia,** Porto Alegre, 36.

Garmendia JCA (1994) Uso de bloques multinutricionales en La ganaderia a pastoreo de forrajes de pobre calidad. **Revista Faculdad de Agronomia** 11:224-237.

Goes RHDT, Mancio AB, Lana RDP, Leão MI, Alves DD and Silva ATS (2005) Recria de novilhos mestiços em pastagem de Brachiaria brizantha, com diferentes níveis de suplementação, na região Amazônica. Consumo e parâmetros ruminais. **Revista Brasileira de Zootecnia**, *34*, 1730-1739.

Goes RHTB, Gandra J, Marquez A, De Oliveira ER, Fernandes H, Cardoso TDL, Yoshihara M (2015) Metabolismo nitrogenado em bovinos suplementados a pasto durante a transição águas seca. **Archivos de zootecnia** 64:281-290.

González FHD (2000) Uso de perfil metabólico para determinar o status nutricional em gado de corte. In: González FHD, Barcellos JO, Ospina H, Ribeiro LAO (Eds.) **Perfil metabólico em ruminantes: seu uso em nutrição e doenças nuricionais**. Porto Alegre, Brasil, Gráfica da Universidade Federal do Rio Grande do Sul.

Granja-Salcedo YT, Messana JD, Souza VC, Dias AVL, Kishi LT, Rebelo LR, Berchielli TT (2017) Effects of partial replacement of maize in the diet with crude glycerin and/or soyabean oil on ruminal fermentation and microbial population in Nellore steers**. British Journal of Nutrition** 118:651-660.

Greenwood RH, Titgemeyer EC, e Drouillard JS (2000) Effects of base ingredient in cooked molasses blocks on intake and digestion of prairie hay by beef steers. **Journal of Animal Science** 78:167–172.

Henderson G, Cox F, Ganesh S, Jonker A, Young W, Abecia L, Angarita E, Aravena P, Arenas GN, Ariza C, Attwood GT (2015) Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. **Scientific reports** 5:14567.

Hinestroza AD, Becerra MJ (1990) Observaciones sobre la elaboracion y consumo de bloques urea-melaza. **Livestock Research Rural Development** 2:8-14.

Hoffmann A, De Moraes EHBK, Mousquer CJ, Simioni TA, Gomer FJ, Ferreira VB, Da Silva HM (2014) Produção de bovinos de corte no sistema de pasto-suplemento no período da seca. **Nativa** 2:119-130.

IBGE, Instituto Brasileiro de Geografia e Estatística (2018) Efetivo do rebanho brasileiro, 2018. Disponível em: <https://sidra.ibge.gov.br/tabela/3939#resultado>.

Ítavo LCV, Tolentino TCP, Ítavo CCBF, Gomes RC, Dias AM e Silva FF (2008) Consumo, desempenho e parâmetros econômicos de novilhos Nelore e F1 Brangus x Nelore terminados em pastagens, suplementados com mistura mineral e sal nitrogenado com ureia ou amireia. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, *60*, 419-427.

Jayme CG, Oliveira PCS, Arcanjo AHM, Moreira LC, Jayme DG (2013) Suplementação de bovinos de corte a pasto durante o período seco**. Pubvet** 7:1630.

Jose VL, Appoothy T, More RP, Arun AS (2017) Metagenomic insights into the rumen microbial fibrolytic enzymes in Indian crossbred cattle fed finger millet straw. **AMB Express** 7(1):13.

Kalmbacher RS, Brown WF e Pate FM (1995) Effect of molasses-based liquid supplements on digestibility of creeping bluestem and performance of mature cows on winter range. **Journam of Animal Science** 73:853–860.

Kaneko JJ, Harvey J, Bruss ML (1997) **Clinical biochemistry of domestic animals**. 5 ed. San Diego: Academic Press, 932p.

Katulski SL, Van Bibber-Krueger CL, Blevins CA, Schrag NFD, Horton LM, Ellerman TJ, Muller HC, Drouillard JS (2017) Effects of low-moisture molasses block supplements on tissue concentrations of trace elements and growth performance of forage-fed beef cattle. **Journal of Animal Science** 95(suppl_4):310-310.

Kennedy PM e Milligan LP (1980) The degradation and utilization of endogenous urea in the gastrointestinal tract of ruminants: a review. **Canadian Journal of Animal Science** 60(2):205-221.

Kong Y, Xia Y, Seviour R, He M, McAlister T, Forster R (2012) *In situ* identification of carboxymethyl cellulose–digesting bacteria in the rumen of cattle fed alfalfa or triticale. **FEMS Microbiology Ecology** 80:159–167.

Lazzarini I, Detmann E, Sampaio CB, Paulino MF, Valadares Filho SC, Souza MA, Oliveira FA (2009) Intake and digestibility in cattle fed low-quality tropical forage and supplemented with nitrogenous compounds. **Revista Brasileira de Zootecnia** 38:2021–2030.

Lazzarini I, Detmann E, Sampaio CB, Paulino MF, Valadares Filho SDC, Souza MA Oliveira FA (2009a) Intake and digestibility in cattle fed low-quality tropical forage and supplemented with nitrogenous compounds. **Revista Brasileira de Zootecnia** 38:2021–2030.

Lengowski MB, Witzig M, Möhring J, Seyfang GM, Rodehutscord M (2016) Effects of corn silage and grass silage in ruminant rations on diurnal changes of microbial populations in the rumen of dairy cows. **Anaerobe** 42:6-16.

Ley RE, Hamady M, Lozupone C, Turnbaugh PJ, Ramey RR, Bircher JS, Michael L, Schlegel ML, Tammy A, Tucker T.A, Mark D, Schrenzel MD, Knight R, Gordon JI (2008) Evolution of mammals and their gut microbes. **Science** 320:1647-1651.

Lima, M. L. M. padrão de fermentação ruminal de bovinos recebendo produto homeopático. **Ciência Animal Brasileira** 9:969 – 975.

McSweeney CS, Mackie R (2012) Commission on Genetic Resources for Food and Agriculture. Micro-organisms and ruminant digestion: State of knowledge, trends and future prospects. **Background Study Paper (FAO)** 61:1-62.

Mikolayunas C, Thomas DL, Armentano LE, Berger YM (2011) Effect of rumen undegradable protein supplementation and fresh forage composition on nitrogen utilization of dairy ewes1. **Journal of Dairy Science**, 94(1), p.416–425.

Moraes, SS (2001) Importância da suplementação mineral para bovinos de corte. **Embrapa,** Centro Nacional de Pesquisa de Gado de Corte.

Moriel P, Artioli LF, Piccolo MB, Miranda M, Ranches J, Ferreira VS, Antunes LQ, Bega AM, Miranda VF, Vieira JF, Vasconcelos JL (2019) Effects of low-moisture, sugarcane molasses-based block supplementation on growth, physiological parameters, and liver trace mineral status of growing beef heifers fed low-quality, warm-season forage. **Translational Animal Science** 3(1):523-531.

Myer PR, Smith TP, Wells JE, Kuehn LA, e Freetly, HC (2015). Rumen microbiome from steers differing in feed efficiency. **PLoS One** 10:e0129174.

Oliveira LOF, Saliba EDOS, Gonçalves LC, Fialho MPF, Miranda PDAB (2009) Parâmetros ruminais e síntese de proteína metabolizável em bovinos de corte sob suplementação com proteinados contendo diversos níveis de proteína bruta. **Revista Brasdileira de Zootecnia** 38:2506-2515.

Paterson JA, Bowman JP, Belyea RL, Kerley MS, Williams JE (1994) The impact of forage quality on suplementation regimen on ruminant animal intake and performace. In: Fahey

Jr. GC (Ed.). **Forage quality, evaluation and utilization**. Madson: American Society of Agronomy 59- 114.

Pereira MLA, Valadares Filho SDC, Valadares RFD, Campos JMDS, Leão MI, Pereira CAR, Silva PA, Mendonça SDS (2005) Consumo, digestibilidade aparente total, produção e composição do leite em vacas no terço inicial da lactação alimentadas com níveis crescentes de proteína bruta no concentrado. **Revista Brasileira de Zootecnia** 34:1029-1039.

Pessoa RAS, Leão MI, Ferreira MA, Valadares Filho SDC, Valadares RFD, Queiroz AC (2009) Balanço de compostos nitrogenados e produção de proteína microbiana em novilhas leiteiras alimentadas com palma forrageira, bagaço de cana-deaçúcar e uréia associados a diferentes suplementos. **Revista Brasileira de Zootecnia** 38:941–947.

Petri RM, Foster R, Yang W, McKinnon JJ, McAllister TA (2012) Characterization of rumen bacterial diversity and fermentation parameters in concentrate fed cattle with and without forage. **Journal of Applied Microbiology** 112:1152– 1162.

Reis RA, Oliveira AA, Siqueira GR, Gatto E (2011) Semi-confinamento para produção intensiva de bovinos de corte. In: **I Simpósio Matogrossense de bovinocultura de corte**, p. 195-224.

Reis RA, Ruggieri AC, Oliveira A.A, Azenha MV, Casagrande DR (2012) Suplementação como estratégia de produção de carne de qualidade em pastagens tropicais. **Revista Brasileira de Saúde e Produção Animal** 642-655.

Reis RA, Ruggieri AC, Casagrande DR, Páscoa AG (2009) Suplementação da dieta de bovinos de corte como estratégia de manejo das pastagens. **Revista Brasileira de Zootecnia** 38:147-159.

Rennó LN, Valadares RFD, Valadares Filho SDC, Leão MI, Silva JFCD, Cecon PR, Gonçalves LC, Dias HLC, Linhares RS (2000) Concentração plasmática de uréia e excreções de uréia e creatinina em novilhos. **Revista Brasileira de Zootecnia** 29(4):1235-1243.

Rius AG, Kittelmann S, Macdonald KA, Waghorn GC, Janssen PH, Sikkema E (2012) Nitrogen metabolism and rumen microbial enumeration in lactating cows with divergent residual feed intake fed high-digestibility pasture. **Journal of dairy Science** 95(9):5024-5034.

Ruas JRM, Torres CAA, Borges LE, Marcatti Neto A, Machado GV, Borges AM (2000) Efeito da Suplementação protéica a pasto sobre eficiência reprodutiva e concentrações sanguíneas de colesterol, glicose e uréia, em vacas Nelore. **Revista Brasileira de Zootecnia** 29(6):2043-2050.

Saleem F, Bouatra S, Guo AC, Psychogios N, Mandal R, Dunn SM, Ametaj BN, Wishart DS (2013) The bovine ruminal fluid metabolome. **Metabolomics** 9(2):360-378.

Sales MFL, Paulino MF, Porto MO, Valadares Filho, SDC, Acedo TS, Couto VRM (2008) Níveis de energia em suplementos múltiplos para terminação de novilhos em pastagem de capim-braquiária no período de transição águas-seca. **Revista Brasileira de**

Zootecnia 37:724-733.

Sales M, de Andrade CMS, Farinatti L (2017) Suplementação energética para bovinos de corte em pastos consorciados durante a época seca no Acre. **Embrapa Acre-Circular Técnica (INFOTECA-E)**, Rio Branco, AC.

Simioni FL, Andrade IFD, Ladeira MM, Gonçalves TDM, Mata Júnior JID e Campos FR (2009) Níveis e frequência de suplementação de novilhos de corte a pasto na estação seca. **Revista Brasileira de Zootecnia**, *38*, 2045-2052.

Sampaio CB, Detmann E, Lazzarini I, Souza MAD, Paulino MF, Valadares Filho SDC (2009) Rumen dynamics of neutral detergent fiber in cattle fed low-quality tropical forage and supplemented with nitrogenous compounds. **Revista Brasileira de Zootecnia** 38(3):560-569.

Sansoucy R, Aarts G, Leng RA (1988) Molasses-urea blocks as a multinutritional supplement for ruminants. In: Sansoucy R, Aarts G, Leng RA (Eds.). **Sugar cane as feed**. FAO 72:263279.

Silva FFD, Sá JFD, Schio AR, Ítavo LCV, Silva RR, Mateus RG (2009) Suplementação a pasto: disponibilidade e qualidade x níveis de suplementação x desempenho. **Revista Brasileira de Zootecnia** 38(SPE):371-389.

Srinivas B e Gupta BN (1997) Rumen fermentation, bacterial and total volatile fatty acid (TVFA) production rates in cattle fed on urea-molasses-mineral block licks supplement. **Animal Feed Science Technology** 65:275-286.

Tajima K, Aminov RI, Nagamine T, Matsui H, Nakamura M, Benno Y (2001) Diet-dependent shifts in the bacterial population of the rumen revealed with real-time PCR. **Applied and environmental microbiology** 67:2766-2774.

Thoetkiattikul H, Mhuantong W, Laothanachareon T, Tangphatsornruang S, Pattarajinda V, Eurwilaichitr L, Champreda V (2013) Comparative analysis of microbial profiles in cow rumen fed with different dietary fiber by tagged 16S rRNA gene pyrosequencing. **Current microbiology** 67:130-137.

Titgemeyer EC, Drouillard JS, Greenwood R.H, Ringler JW, Bindel DJ, Hunter RD, Nutsch T (2004) Effect of forage quality on digestion and performance responses of cattle to supplementation with cooked molasses blocks. **Journal of animal science** 82:487-494.

Trater AM, Titgemeyer EC, Drouillard JS, Pike JN (2003) Effects of processing factors on in vitro ammonia release from cooked molasses blocks containing urea. **Animal Feed Science and Technology** 107(1-4):173-190.

Van Soest, PJ (1994) **Nutritional ecology of the ruminant.** 2ed. New York: Cornell University Press, 4. 476 p

Vieira Júnior LC, Cabral LDS, Factori MA, Ribeiro FA, Arrigoni MDB, Costa C (2013) Características da forragem que implicam no comportamento e consumo de ruminantes. **Veterinária e Zootecnia** 183-192, 2013.

Wanapat M, Etlum A, Pimpa O (1999) Strategic supplementation with high-quality feed block on roughage intake, milk yield and composition, and economic return in lactating dairy cows. **Asian Australian Journal of Animal Science** 12:901903.

Zhang R, Zhu W, Zhu W, Liu J, Mao S (2014) Effect of dietary forage sources on rumen microbiota, rumen fermentation and biogenic amines in dairy cows. **Journal of the Science of Food and Agriculture** 94:1886-1895.

CHAPTER 2 - EFFECT OF LOW-MOISTURE, SUGARCANE MOLASSES-BASED BLOCK SUPPLEMENTATION ON NUTRIENT DIGESTIBILITY, NITROGEN BALANCE AND RUMINAL ECOSYSTEM IN BEEF CATTLE FED WITH LOW-QUALITY FORAGE

Abstract: the objective of this study was to evaluate the ruminal fermentation parameters, nitrogen balance and the ruminal bacterial diversity of Nellore steers fed low-quality forage and supplemented with urea mineral salt (SMU), low-moisture, sugarcane molasses-based block (BMF) or energy protein supplement (SPE). Six castrated Nellore steers (350 ± 10 kg BW) were used in a simultaneous double 3 × 3 Latin Square balanced for residual effects, with three experimental periods of 24 days each, and three treatments. The basal diet consisted of hay offered *ad libitum*, and experimental supplements were urea mineral salt (SMU) *ad libitum*, or conventional SPE (0.2% BW), or BMF *ad libitum.* The intake of DM expressed as % of BW and kg/d, supplement, OM, ADF, and TDN were highest when steers were supplemented with SPE ($P \leq 0.05$). However, BMF allowed higher both CP and EE expressed as kg/d, and both CP and NDFap expressed as g/kg0,75 when compared to SMU ($P<0.05$), and higher intake of EE (Kg/d) when compared to SPE ($P<0.001$). Steers supplemented with BMF had higher digestibility of CP ($P=0.023$), and lower digestibility of NDFap ($P=0.032$) when compared to SMU, respectively. The lowest total daily nitrogen retention (NR), expressed as g/d ($P=0.007$), in function of N Intake ($P=0.007$), or function of N digested ($P=0.010$), were observed in animals supplemented with SMU. Steers supplemented with SPE had the highest NH_3-N concentration at 2, 4, and 6 h after feeding. BFM allowed higher both Isobutyrate and Isovalerate proportion at 0h, while SPE resulted in higher both Isovalerate among 4 and 6h, and Isobutyrate proportion at 14h. SPE had positive correlation with Actinobacteria ($r = 0.86$), Kiritimatiellae ($r = 0.85$), Alphaproteobacteria ($r = 0.84$), Ruminococcus.2 ($r = 0.77$), Christensenellaceae R7 group ($r = 0.42$), the EMS ($r = 0.59$) and propionate proportion in the rumen ($r = 0.15$), and had negative correlation with Prevotella.1 ($r = - 0.69$), UNE ($r = - 0.59$) and FNE ($r = - 0.32$). In contrast, BMF had positive correlation with archaea phylum Euryarchaeota ($r = 0.91$) and species Methanobacterium alkaliphilum ($r = 0.54$), and Methanobrevibacter gottschalkii clade ($r = 0.70$). The bacteria phylum Firmicutes ($r = 0.78$), families Lachnospiraceae ($r = 0.84$) and Ruminococcaceae ($r = 0.79$), and genera Succiniclasticum ($r = 0.59$) also were positively correlated with BMF supplementation and total apparent digestibility of CP ($r = 0.50$). The low-moisture, sugarcane molasses-based block is useful alternative to a conventional energy protein supplement, that modulates the rumen bacteria population, increasing the ruminal propionate proportion, and improving both rumen ammonia utilization and diet nitrogen retention in beef cattle fed low-quality forage.

Keywords: bacterial diversity, low quality hay, nitrogen retention, propionate, rumen ammonia.

1. INTRODUCTION

Tropical forages are the main feed source for beef cattle in the tropics. However, in these regions during the dry season, there is a drastic decrease in the forage nutritional quality, characterized mainly by higher lignification and lower crude protein content (generally less than 7%), resulting in less both forage intake and fiber microbial degradation in the rumen (Detmann et al., 2014a).

Rumen degradable protein (RDP) is often the most limiting nutrient under grazing low-quality grasses (Titgemeyer et al., 2004, Detmann et al., 2014a). Supplementation with RDP can help to reduce the amount of recycled nitrogen (N) in body pools for use in the rumen, allowing a greater proportion of nitrogen compounds to become available for anabolic purposes (Batista et al., 2016). In this way, supplementation can improve the efficiency of using low-quality pastures and optimize the performance of animals (Casagrande et al., 2011, Detmann et al., 2014b).

Low-moisture, sugarcane molasses-based block (BMF) for cattle grazing low-quality forage is a supplementation strategy that has been used due to its practicality, reduced production costs, and the potential to improve forage intake and digestion, due to the RDP supply (Köster et al., 1996; Löest et al., 2001). Besides, the BMF manufacturing process (e.g. extreme heat or pH) can modify the bioavailability of the nutrients, such as the release of ammonia in the rumen (Trater et al., 2003; Katulski et al., 2017), while how to supply supplements (bran *versus* block form) can alter the patterns of supplement intake and the use of nutrients (Katulski et al., 2017).

Regarding that changes in the diet may influence the ruminal microbial community (Dehority and Orpin, 1997, Kim et al., 2011), factors as variations in the forage quality, chemical composition of supplements, or changes in the dynamics of the N supply to the rumen (food supply or via recycling), could affect the ruminal degradation of forage, and consequently the animal productivity. However, few studies are assessing the effects of BMF supplementation on the metabolism and rumen microbiome of cattle fed low-quality forage. Moriel et al., (2019) related that heifers supplemented with BMF fed low-quality forage had better performance than heifers supplemented with a conventional energy

protein supplement (SPE), evidencing the potential of BMF to improve ruminal conditions to the use of low-quality forages.

Despite the importance of an adequate supply of N in the rumen, direct measures of the effect of supplementation with N compounds on microbial diversity and ruminal metabolism are scarce in tropical conditions (Reis et al., 2016). Thus, the objective of this study was to evaluate the ruminal fermentation parameters, nitrogen balance and the ruminal bacterial diversity of Nellore steers fed low-quality forage and supplemented with low-moisture, sugarcane molasses-based block or energy protein supplement. It was hypothesized that the use of low-moisture, sugarcane molasses-based block would cause changes in the microbial community and rumen fermentation parameters, resulting in improved use of nutrients and use of nitrogen when compared to conventional energy protein supplementation.

2. MATERIAL AND METHODS

The protocols used in this experiment are in accordance with the guidelines of the Brazilian College of Animal Experimentation and was approved by the Animal Ethics and Welfare Commission of the São Paulo State University “Júlio de Mesquita Filho”, of the School of Agricultural and Veterinary Sciences, Jaboticabal campus (Protocol No. 010216/17).

2.1. Animals and treatments

Six castrated Nellore steers (350 ± 10 kg initial body weight and 24 months of age) cannulated in the rumen, were used in a simultaneous double 3 × 3 Latin Square balanced for residual effects, with three experimental periods of 24 days each, and three treatments (supplements). Animals were housed in individual semi-covered stalls (9.0 m^2), equipped with individual feeders and drinkers, and were adapted to the diets during the first 14 days of each experimental period.

The basal diet consisted of Urochloa brizantha (Hochst.) Stapf, “Marandu” hay offered *ad libitum*, and experimental supplements were urea mineral salt (SMU) *ad libitum*, or conventional protein-energy supplement (SPE, 0.2% BW), or low-moisture, sugarcane molasses-based block (BMF) *ad libitum* (Table 1).

Table 1. Proportion of ingredients and chemical composition of experimental supplements and hay.

Inclusion of ingredients (as-fed basis)	**Supplements[1]**			**Hay**
	SMU	SPE	BMF	
Dried sugarcane molasses	-	-	53.00	-
Ground corn	-	45.00	-	-
Cottonseed meal	-	-	14.30	-
Soybean meal	-	16.00	-	-
Urea	16.00	5.00	13.00	-
Limestone	24.50	10.65	-	-
Ca phosphate	15.00	4.00	8.70	-
Sodium chloride	40.00	15.00	5.00	-
Kaolin[2]	-	3.50	-	-
Trace mineral/vitamin premix[3]	3.75	0.62	2.50	-
Magnesium oxide	0.67	0.25	0.79	-
Soybean oil	-	-	2.70	-
Palatability enhancer [4]	0.08	-	-	-
Chemical composition (% DM)				
DM, %	95.00	96.00	94.00	93.65
OM	17.20	63.90	79.70	94.80
CP	46.00	25.00	46.00	3.97
NDFap[5]	-	7.30	9.13	76.99
ADF	-	14.10	12.30	55.68
RDP, % PB[6]	100.00	86.60	93.20	22.40
NDICP, %CP	0.69	4.39	3.82	72.65
Lignin	-	-	-	10.35
TDN[7]	-	53.30	50.70	46.60

[1]SMU: urea mineral salt, SPE: protein-energy supplement, BMF: low-moisture, sugarcane molasses-based block;
[2] Rumen-inert indigestible substance included;
[3] DM basis: 3% Ca, 10% Mg, 23.5% S, 600 mg/kg Co, 20,000 mg/kg Cu, 264 mg/kg Se, 1,200 mg/kg I, 80,000 mg/kg Zn, 53,200 mg/kg Mn, 4,000,000 mg/kg vitamin A, 400,000 mg/kg vitamin D_3, 20,000 mg/kg vitamin E, and 40,000 mg/kg monensin, Poulcox 40, Peshteria, Bulgaria).
[4]Tecnaroma zta sweet note fruit red 4W/10638 powder (New Products Comercial Agricola e Veterinária, Campinas, São Paulo, Brazil).
[5]NDF corrected for ash and protein
[6] Described by Moriel et al., 2019.
[7] Calculated as described by Weiss et al. (1992).

Supplements were provided daily at 7:00 am while hay was supplied twice daily (7:00 am and 3:00 pm), in individual feeders. The amount of feed supplied was calculated and adjusted daily to allow 5% leftovers. At the beginning of each experimental period, the body weight of each steer was recorded, after 18 hours of solid fasting, and the amount of SPE provided was adjusted.

2.2. Intake and digestibility of dry matter and nutrients

Intake and digestibility evaluation were carried out on days 18–22 of each experimental period. The daily intake of DM of BMF was estimated by multiplying the concentration of DM of the supplement by the disappearance of the weight of each supplement block obtained between consecutive days. The leftover of hay, SPE, and SMU were collected, homogenized, and weighed every day before the morning feeding, and a subsampled (10%) were stored at -20°C for later analysis.

The total collection of feces was carried out directly from the floor and immediately after each spontaneous defecation. The total feces were weighed each 24 h cycle, homogenized, and a subsampled weighed (10%) and stored at -20°C for later analysis.

Diet samples (hay and supplements), leftovers and feces were pre-dried in an oven with forced air circulation at 55 ° C for 72 hours, then ground in a Willey knife mill to pass a 1mm sieve. For each animal, in each experimental period, were formed a fecal-composite sample based on the pre-dried weight. All samples were analyzed for DM (method 935.01), OM (method 942.05), CP (method 984.13) and EE (method 920.85) according to AOAC (1995). The neutral detergent fiber (NDF) and acid detergent fiber (ADF), their corrections for ash and protein, and lignin also were determined according to Van Soest et al., (1991) and thermostable α-amylase was used in the NDF determination procedure, without the addition of sodium sulphite.

2.3. Nitrogen balance and rumen microbial protein synthesis

Total urine was collected on days 18–22 of each experimental period, using a rubber funnel collector attached to the prepuce and fixed by elastic loops on the steer backs. The funnels were equipped to polyethylene flexible tubes that carried the urine to 20 L containers, and 200 mL H_2SO_4 solution (2.04 M) was placed in each container to prevent the N compounds loss by volatilization (Valadares et al., 1997). At the end of each 24 h cycle, the urine was weighed, mixed, filtered through cheesecloth, a 50 mL subsample were stored to for analysis of total N, and a 10 ml subsample was diluted with 40 ml of 0.036 mM H2SO4 for analysis of purine derivatives, both subsamples were stored at -20°C for later analysis.

Rumen microbial protein synthesis was predicted from total purine derivatives (allantoin and uric acid) that were determined in diluted urine subsamples. Concentrations of uric acid were determined using commercial kit (Ácido Úrico Liquiform ref. 140, Labtest®, Diagnostica SA, Minas Gerais, Brazil), and allantoin by the colorimetric method proposed by Chen and Gomes (1992). Purines derivatives absorption (mmol / d) was calculated using the following equation:

$$PDA = (PDe - 0.385 \times BW^{0.75})/0.85$$

where: PDe is the summatory of uric acid and allantoin excreted (mmol/d); 0,385 is the endogenous excretion of purine derivatives (mmol) in the urine unit of metabolic BW ($BW^{0.75}$), and 0.85 is the recovery of absorbed purines as purine derivatives in the urine (mmol/mmol). Microbial N flow (g N / d) was calculated using the following equation:

$$Microbial\ N\ flow = \frac{PDA \times 70}{0.134 \times 0.83 \times 1000}$$

Where PDA is the absorbed purine derivatives (mmol/day), 70 is the purine N content (mg N/mmol), 0.134 is the ratio between bacterial purine N and total N in the rumen, and 0.83 is the true digestibility of purines (Chen e Gomes, 1992).

Urine pure subsamples were analyzed by Kjeldahl method (AOAC, 1995; method 984.13). Total N excreted in the urine daily (g/day) was calculated by utilizing the total N concentration in urine (g/L) and the daily urine volume (L). N retained (NR) were calculated in function of N intake or N digested, using the following equations:

$$NR = NI\ (g/day) - [FNE(g/day) + UNE\ (g/day)] \qquad (1)$$

$$NRd = ND\ (g/day) - [UNE\ (g/day)] \qquad (2)$$

Where NI corresponds to the daily N intake, FNE is the total of N excreted in the feces daily, UNE is the total of N excreted in the urine daily, and ND is the N digested, calculated as NI – FNE.

2.4. Rumen fermentation parameters

Rumen content samples (± 200 g) were taken through the rumen cannula on 23 d of each experimental period at 0, 2, 4, 6, 8, 10, 12, and 14 h after the morning feeding from different regions of the rumen. Immediately a composite sample were form and filtered through two layers of cheesecloth, to ruminal pH measured using a digital pHmeter (Nova Técnica, PHM, Piracicaba, SP, Brazil), and two subsamples (2 mL each) were taken and stored at -20°C for short chain fatty acids (SCFA) and ammonia (NH3-N) concentration analysis.

The SCFA were quantified using gas chromatography (Trace 1300, Thermo Fisher Scientific, Rodano, Milano, Italy), with automatic injector AI 1310, flame ionization detector (FID), a capillary column (15 m x 0.32 mm 0.25 mm diameter, 0.025 mm thick; Carbowax™, Deerfield, IL, USA), and hydrogen as carrier gas. The NH3-N concentration were measured by Kjeldahl distillation according to the methodology of Fenner (1965).

2.5. Rumen bacteria and archaea diversity

Rumen content samples (approximately 50 g of solid and liquid fraction) were collected on 17 d of each experimental period before morning feeding from the ventral and medial region of the rumen. Immediately were added 50 mL of PBS buffer (pH 7.4) at 4°C, mixed vigorously for 3 min, then strained with a sterile mesh fabric (100 µm), and the filtrate was used to obtain a microbial pellet according to Granja-Salcedo et al. (2017). Following, metagenomic DNA extraction was conducted in a subsample (200 mg) of the microbial pellet through the Quick-DNA Fecal/Soil Microbe Miniprep Kit (50 Preps D6010; Zymo Research Corporation, Irvine, USA) according to the manufacturer's instructions and the FastPrep-24 Classic Instrument (MP biomedicals, Illkirch, FR) to cell lyses (Henderson et al., 2013). Metagenomic DNA yield were immediately measured spectrophotometrically (NanoDrop, ThermoFisher Scientific, Waltham, MA, USA) and fluorometrically (Qubit® 3.0, kit Qubit® dsDNA Broad Range Assay Kit, Life Technologies, Carlsbad, CA, USA). In addition, purity and integrity were checked spectrophotometrically at A260/A230 nm and A260/A280 nm ratios, and 0.8% agarose

gel electrophoresis staining with SYBR Gold Nucleic Acid Gel Stain (Thermo Fisher Scientific, Waltham, MA, USA), respectively.

Sequencing libraries were prepared by amplifying the V4–V5 region of the 16S rRNA gene using primers forward 515F (5'- GTGNCAGCMGCCGCGGTAA- 3') and reverse 926R (5'CCGYCAATTYMTTTRAGTTT-3') described by Caporaso et al. (2011). Samples were amplified in duplicates, and the total PCR reaction mixture (20 µl final volume) contained 20 ng of metagenomic DNA, 1,25 mM of MgCl2; 200 µM de dNTP; 1,0 U of platinum Taq DNA polymerase high fidelity (Invitrogen; Carlsbad, CA, USA); PCR buffer solution [1×]; 10 µM of each primer and ultrapure water. PCR conditions were held at 95°C for 3 min to denature the DNA, with amplification proceeding for 35 cycles at 95°C for 30 s, 53.8°C for 30 s, and 72°C for 45 s; a final extension of 10 min at 72°C. PCR products size was verified through 1% agarose gel electrophoresis using a 1 kb plus DNA ladder (1 kb plus DNA ladder, Invitrogen, Carlsbad, CA, USA). Then, PCR products were purified using the ZymocleanTM Gel DNA Recovery kit (Zymo Research Corporation, Irvine, USA) following the manufacturer's instructions, and composite samples were created by combining equimolar ratios of amplicons from the duplicate samples. Sequencing was performed using the Illumina MiSeq (kit MiSeq Reagent v2 (2 × 250pb), Illumina, Inc., NY, USA).

Paired end short read sequence data generated on the Illumina Miseq was processed using the USEARCH package (Edgar, 2010). De-multiplexed paired end sequences were first merged prior to sequence quality filtering, followed by denoising (error correction) and chimera checking and clustering of sequences to operational taxonomic units (OTUs) of 97% similarity. The representative sequences obtained were allocated phylogenetically using the SILVA 132 database (Quast et al., 2013). The complementary analysis was carried out using the R vegan, Phyloseq, and DESeq2 packages in R to calculated Simpson and Shannon indices, Richness ACE and Chao1 estimators, and taxonomic abundance (McMurdie e Holmes, 2013; Oksanen et al., 2013; Love et al., 2014; Wickham, 2016). Sequences have been deposited to the Sequence Read Archive with accession number PRJNA698580.

2.6. Statistical analysis

Statistical analyzes were performed using Software R (version 3.5.1). The design adopted was a simultaneous replicated 3 × 3 Latin square (three treatments and three periods) balanced for residual effects. Initially, both normality (Shapiro-Wilk test) and heterogeneity (Bartlett test) of data were verified.

Data of intake, digestibility, nitrogen balance and rumen microbial protein synthesis the model considered the supplement as fixed effects, and the random effects of animal, experimental period, Latin square, and residues from the model:

$$Yijl = \mu + \kappa l + \tau k + \rho i\,(l) + \gamma j\,(l) + \tau\kappa\,(kl) + \varepsilon ijl;$$

Where Y_{ijl} = the observed value on steer j to experimental period i, and Latin square l; μ = overall mean; K_l = Latins square effect l; T_k = supplement effect k; P_i (l) = effect of experimental period i into Latin square l; γ_j (l) = effect of steer j into Latin square l; T_k (kl) = effect of supplement k and Latin square l interaction; $E_{\varepsilon ijl}$ = residual error.

Rumen fermentation parameters, the sampling time was considered as repeated measure over time, and the ANOVA considered the supplement, the time and its interaction as fixed effects, and the of animal, experimental period, Latin square, and residues from the model. Tukey´s post-hoc was applied when ANOVA indicated a significant difference to mean comparation, considering statistical significance when $P \leq 0.05$.

Principal components analysis (PCA) through the FactorMiner package in R was used to extracting the important rumen microbes correlated with fermentation and metabolic parameters observed in the steers grouped by supplement. Beta diversity among supplements was evaluated using the betadisper function on the Vegan package. Richness estimators, alpha diversity index, and relative abundance of ruminal bacteria were compared through Kruskal-Wallis and Dunn's post-hoc test. Differential bacterial abundance was assessed using a pairwise Wilcoxon test between BMF vs SMU, and SPE vs SMU. For both tests were considered statistical significance when $P \leq 0.05$, and tendencies when $P > 0.05$ and $P < 0.10$.

3. RESULTS

3.1. Intake and digestibility

Similar intake of Hay expressed as % of BW and Kg/d, intake of both CP, NDFap expressed as Kg/d and *g/kg^{0,75},* and nutrients digestibility were observed among SPE and BMF supplements (Table 2). The intake of DM expressed as % of BW and Kg/d, supplement, OM, ADF, and TDN were highest when steers were supplemented with SPE ($P \leq 0.05$). However, BMF allowed higher both CP and EE expressed as Kg/d, and both CP and NDFap expressed as *g/kg^{0,75}* when compared to SMU ($P<0.05$), and higher intake of EE (Kg/d) when compared to SPE ($P<0.001$).

The total apparent digestibility of DM, OM, and ADF were not affected by supplements tested ($P>0.05$). However, steers supplemented with BMF had higher digestibility of CP ($P=0.023$), and lower digestibility of NDFap ($P=0.032$) when compared to SMU, respectively.

Table 1. Intake and total apparent digestibility of nutrients of Nellore steers fed low-quality hay and supplemented with urea mineral salt (SMU), conventional protein-energy supplement (SPE) or low-moisture, sugarcane molasses-based block (BMF)

	Supplements				
Item	SMU	SPE	BMF	SEM	*P value*
BW, kg	348.30	341.20	353.60		
Intake, %BW					
DM	0.85^{b}	1.17^{a}	0.92^{b}	0.06	<0.001
Hay	0.81^{b}	1.01^{a}	0.87^{ab}	0.05	0.015
Supplement	0.04^{b}	0.16^{a}	0.05^{b}	0.01	<0.001
Intake, kg/d					
DM	3.07^{b}	4.12^{a}	3.24^{b}	0.21	0.001
DM Hay	2.91^{b}	3.55^{a}	3.06^{ab}	0.19	0.024
DM Supplement	0.15^{b}	0.56^{a}	0.17^{b}	0.05	<0.001
OM	2.80^{b}	3.74^{a}	3.05^{b}	0.19	0.002
CP	0.18^{b}	0.30^{a}	0.40^{a}	0.02	<0.001
NDFap	2.26^{b}	2.74^{a}	2.37^{ab}	0.14	0.022
ADF	1.61^{b}	1.97^{a}	1.68^{b}	0.10	0.020
EE	0.02^{c}	0.04^{b}	0.05^{a}	0.00	<0.001
TDN	1.35^{b}	1.95^{a}	1.51^{b}	0.10	<0.001
Intake, g/kg^{0,75}					
DM	37.32^{b}	50,92^{a}	39.89^{b}	2.61	<0.001
NDFac	29.67^{b}	37.53^{a}	33.61^{a}	1.97	0.018
CP	2.27^{b}	3.81^{a}	4.94^{a}	0.33	<0.001
TDN	16.48^{b}	24.16^{a}	18.63^{b}	1.27	<0.001

Apparent digestibility, %					
DM	52.69	50.99	46.89	1.03	0.136
OM	54.23	52.79	49.75	0.94	0.226
CP	66.68^{b}	69.88^{ab}	77.01^{a}	1.76	0.023
NDFac	62.96^{a}	57.42^{ab}	56.68^{b}	0.95	0.032
ADF	59.26	55.55	52.14	1.24	0.085

SEM: standard error mean, DM: dry matter, OM: organic matter, CP: crude protein, NDFap: neutral detergent fiber corrected for ash and protein, ADF: acid detergent fiber. Values followed by different superscript letters on the same line differ by Tukey's test at 5% probability.

3.2. Nitrogen balance and rumen microbial protein synthesis

Supplements used did not affect both production of microbial nitrogen in the rumen (Nmic) and the efficiency of microbial synthesis in the rumen (EMS) (Table 3). Nitrogen intake (NI) and Nitrogen digested (ND) as g/d were lower in SMU than both SPE and BMF (P <0.001), while steers supplemented with BMF had higher total ND than SPE (P=0.002).

The highest total daily fecal excretion of N (FNE) expressed as g/d was found in steers supplemented with SPE (P <0.001), following by steers supplemented with BMF, being the lowest ENF observed with SMU supplement. However, steers supplemented with BMF had lower FNE when expressed as % of NI than both SPE and SMU (P=0.002).

BMF resulted in higher total daily urine N excretion (UNE) expressed as g/d when compared to SPE (P=0.026), still, when UNE was expressed in function of N intake (% of NI) no differences were observed between BMF and SPE, and steers supplemented with SPE had lower values than SMU (P=0.028). Besides, UNE expressed in function of RN (% of RN) steers supplemented with SMU had highest values (P=0.017).

The lowest total daily nitrogen retention (NR), expressed as g/d (P=0.007), in function of NI (% of NI) (P=0.007), or function of ND (g/d and % of ND) (P=0.010), were observed in animals supplemented with SMU, while steers supplemented with SPE and BMF had similar RN.

Table 3. Nitrogen intake and nitrogen balance as a function of the nitrogen ingested of Nellore steers fed low-quality hay and supplemented with urea mineral salt (SMU), conventional protein-energy supplement (SPE) or low-moisture, sugarcane molasses-based block (BMF)

	Supplements				
	SMU	SPE	BMF	SEM	*P-value*
NI, g/d	29.76[b]	49.39[a]	64.24[a]	4.40	<0.001
ND, g/d	19.77[c]	34.62[b]	50.50[a]	4.40	0.003
FNE, g/d	9.98[c]	14.76[a]	12.60[b]	0.85	<0.001
UNE, g/d	17.20[ab]	12.58[b]	24.40[a]	1.73	0.026
NR, g/d	2.58[b]	22.03[a]	27.23[a]	3.64	0.007
Nmic, g/d	52.33	64.90	89.70	7.62	0.232
EMS, g/ kg TDN	41.39	34.14	64.05	6.85	0.236
% NI					
FNE, %	33.30[a]	30.10[a]	20.71[b]	1.81	0.002
UNE, %	59.82[a]	27.54[b]	38.58[ab]	4.86	0.028
NR, %	6.86[b]	42.35[a]	40.70[a]	3.64	0.007
% ND					
NR, g/d	2.57[b]	22.03[a]	26.10[a]	3.60	0.010
UNE, %	90.68[a]	40.15[b]	50.66[b]	7.90	0.017
NR, %	9.32[b]	59.84[a]	49.33[a]	7.70	0.017

SEM: standard error mean, NI: nitrogen intake, ND: nitrogen digested, FNE: fecal nitrogen excretion, UNE: urinary nitrogen excretion, NR: nitrogen retention, Nmic: production of microbial nitrogen in the rumen, EMS: efficiency of microbial synthesis in the rumen. Values followed by different superscript letters on the same line differ by Tukey's test at 5% probability.

3.3. Rumen fermentation parameters

There was an interaction between supplement and sampling time on ruminal NH3-N concentration (P=0.001), Isobutyrate proportion (P= 0.021) and Isovalerate proportion (P= 0.003); steers supplemented with SPE had the highest NH_3-N concentration at 2, 4, and 6 h after feeding (Figure 1). BFM allowed higher both Isobutyrate (Figure 1) and Isovalerate (Figure 1) proportion at 0h, while SPE resulted in higher both Isovalerate among 4 and 6h, and Isobutyrate proportion at 14h. Steers supplemented with SMU had higher both Isobutyrate and Isovalerate proportion at 8h (<0.05).

The supplement affected the average ruminal pH, the propionate, butyrate, and valerate proportion, the A:P ratio, and the NGP (Table 4); steers supplemented with BMF had the highest average ruminal pH (P <0.001). BMF also resulted in higher propionate proportion and lower both A:P ratio (P=0.017) and NGP (P=0.002) than SPE. In contrast,

steers supplemented with SPE had higher both butyrate (P = 0.005) and valerate (P =0.010) proportion than steers supplemented with SMU.

Sampling time influenced ruminal pH (P <0.001) and propionate proportion (P = 0.022). Thus, at 2h after feeding were observed the highest ruminal pH (7.07, 7.27 and 7.19 for SMU, SPE and BMF respectively), while the lowest value was observed at 14h (6.98, 6.80, and 7.03 for SMU, SPE and BMF respectively). Likewise, at 10h after feeding were observed the highest propionate proportion (23.05, 24.41 and 31.49 for SMU, SPE and BMF respectively), while the lowest value was observed at 0h for SMU (20.52), 12h for SPE (20.26) and 14h for BFM (22.27).

Table 4. Ruminal fermentation parameters of Nellore steers fed low-quality hay and supplemented with urea mineral salt (SMU), conventional protein-energy supplement (SPE) or low-moisture, sugarcane molasses-based block (BMF)

Item	Supplements			SEM	*P-value*		
	SMU	SPE	BMF		Treat	Time	Treat*Time
NH_3 -N, *mg/dL*	8.81	16.02	8.27	0.70	<0.001	<0.001	0.001
pH	7.07^{b}	6.99^{b}	7.18^{a}	0.01	<0.001	<0.001	0.055
Acetate, %	71.40	70.27	70.16	0.64	0.136	0.702	0.259
Propionate, %	22.46^{ab}	21.70^{b}	24.15^{a}	0.34	0.016	0.022	0.531
Butyrate, %	4.30^{b}	5.14^{a}	4.69^{ab}	0.23	0.005	0.599	0.325
*Iso*butyrate, %	0.76	0.78	0.67	0.02	0.086	0.081	0.021
Valerate, %	0.62^{b}	0.72^{a}	0.66^{ab}	0.05	0.010	0.186	0.905
*Iso*valerate, %	0.89	0.93	0.70	0.05	0.003	0.006	0.003
A:P ratio	3.20^{ab}	3.37^{a}	2.96^{b}	0.06	0.017	0.089	0.712
NGP	3.61^{ab}	3.86^{a}	3.39^{b}	0.05	0.002	0.184	0.757

SEM: standard error mean, SCFA: short-chain fatty acid, A:P ratio: acetate: propionate ratio, NGP: non-glycogenic proportion of fatty acids: (acetate + (2 butyrate)) ÷ propionate. Treat: supplement type effect, Time: effect of collection time. Values followed by different superscript letters on the same line differ by Tukey's test at 5% probability.

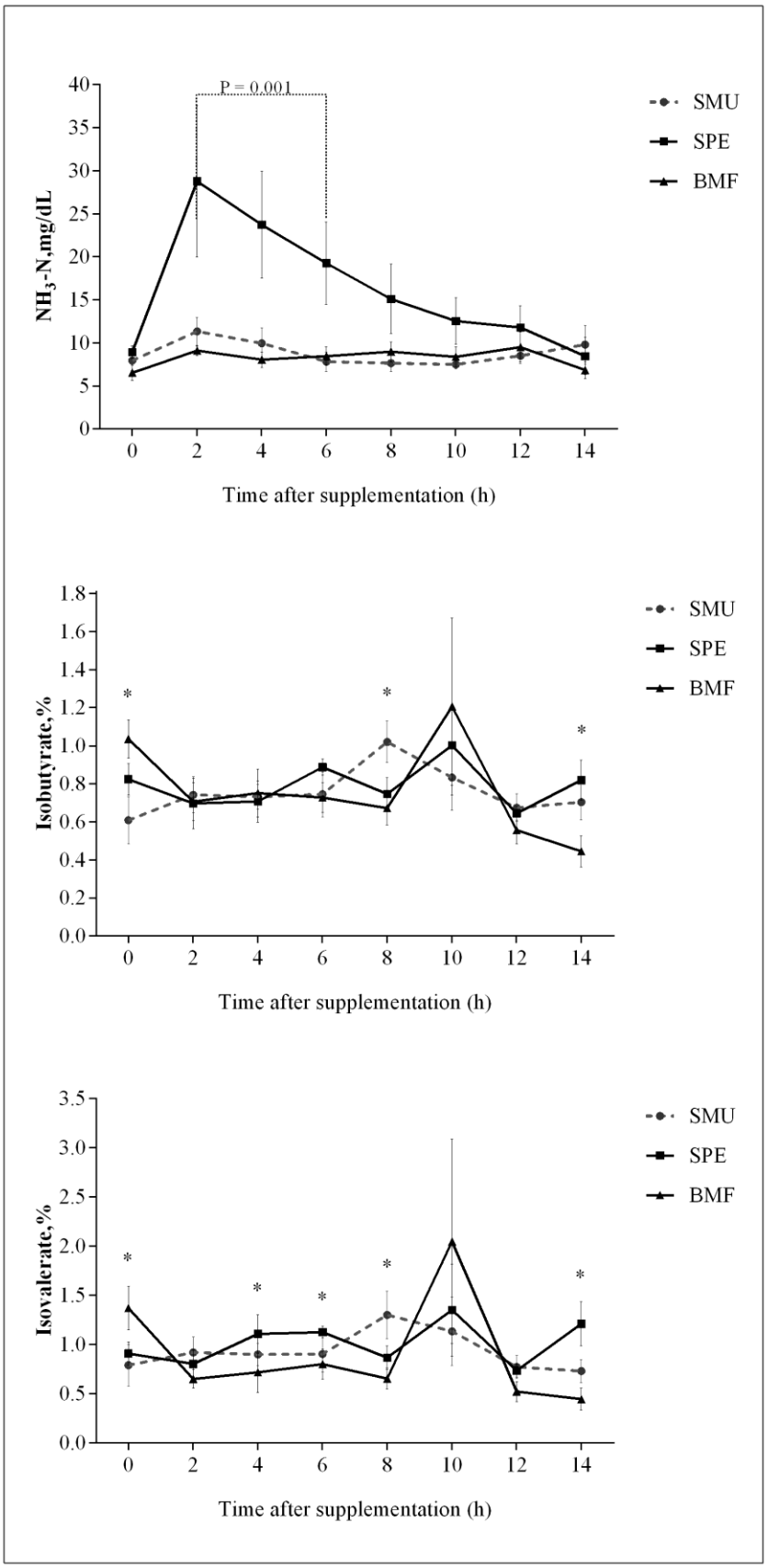
P = 0.001
SMU
SPE
BMF
NH3-N,mg/dL
Time after supplementation (h)
Isobutyrate,%
Isovalerate,%

Figure 1. Ruminal ammonia concentration (mg/dL), isobutyrate and isovalerate proportion in function of sampling time of Nellore steers fed low-quality hay and supplemented with urea mineral salt (SMU), conventional protein-energy supplement (SPE) or low-moisture, sugarcane molasses-based block (BMF). * treat × time interaction (P <0·05) as obtained with Tukey's test.

3.4. Rumen bacteria and archaea diversity

A total of 691.292 reads were generated, in mean were identified 8.858 OTUs per sample with 97% of similarity. Supplements tested did not affect the beta diversity (P = 0.709; Figure S1), the alpha diversity indexes Simpson (0.998 ± 0.001, P=0.931) and Shannon Wiener (6.68 ± 0.2, P=0.745), the richness estimators ACE (1050 ± 249, P=0.818), Chao 1 (1054 ± 250, P=0.882), and the total of observed OTUs (1048 ± 246, P= 0.818).

Near to 5.79% of total OTUs corresponded to the Archaea domain, which was represented exclusively by the Euryarchaeota phylum. The 94.00% of remained OTUs were classified as bacteria and distributed in 17 phyla, 24 classes, 33 orders, 50 families, and 127 genera. Supplements tested did not affect the relative abundance of phyla in the rumen (Table 5), Bacteroidetes (41.20%), Firmicutes (33.56%), and Fibrobacteres (4.55%) were the most abundant phyla, and the Bacteroidetes: Firmicutes ratio were 1.15, 1.24, and 1.36 to BMF, SPE, and SMU, respectively (P=0.344).

At the family level, steers supplemented with BMF had lower abundance of Rikenellaceae when compared to SPE (P=0.049, Table 5). Both SPE and BMF reduced the relative abundance of Neisseriaceae family when compared to SMU (P=0.038). Prevotellaceae was the most abundant family and tended to be lower when steers were supplemented with SPE, than BMF and SMU (P=0.075), while the abundance of Bacteroidales F082 family tended to be higher in the rumen of SPE, than BMF and SMU (P=0.057). Veillonellaceae tended to be higher in the rumen when steers fed BMF (2.44%) than SPE and SMU (P=0.095).

Table 5. Median and interquartile range of phylum and family's relative abundance (%) in Nellore steers fed low-quality hay and supplemented with urea mineral salt (SMU), conventional protein-energy supplement (SPE) or low-moisture, sugarcane molasses-based block (BMF)

	Supplements			*P-value*
	SMU	SPE	BMF	
Actinobacteria	0.488 ± 0.13	0.513±0.18	0.574±0.29	0.749
Bacteroidetes	42.4 ± 1.76	40.8±3.40	40.4±4.73	0.717
Bacteroidales F082	2.96 ± 0.53^{b}	5.01±2.56^{a}	3.13±1.17^{b}	0.057
Rikenellaceae	10.70 ± 1.11^{a}	11.00±2.30^{a}	8.09±0.37^{b}	0.049
Prevotellaceae	22.00 ± 2.19^{a}	15.70±3.10^{b}	20.90±1.34^{ab}	0.075
Chloroflexi	1.03 ± 0.44	0.962±0.54	1.20±0.99	0.863
Cyanobacteria	1.85 ± 0.76	1.42±0.65	1.52±0.39	0.296
Elusimicrobia	0.642 ± 0.32	0.443±0.15	0.592±0.29	0.266
Fibrobacteres	4.71 ± 1.09	4.73±2.55	4.22±1.40	0.794
Firmicutes	31.0 ± 5.56	34.6±2.44	35.1±2.54	0.229
Veillonellaceae	0.941 ± 0.34^{b}	1.08±1.12^{ab}	2.44±1.84^{a}	0.095
Fusobacteria	0.025 ± 0.06	NI	0.007±0.03	0.888
Kiritimatiellaeota	3.29 ± 2.75	3.20±1.85	2.74±1.94	0.882
Lentisphaerae	1.40 ± 0.90	1.01±0.86	0.842±0.60	0.749
Planctomycetes	1.11±0.87	0.763±0.37	0.789±0.28	0.745
Proteobacteria	1.52±0.13	1.53±0.77	1.39±0.25	0.794
Neisseriaceae	0.102±0.04^{a}	0.026±0.03^{b}	0.061±0.03^{ab}	0.038
Spirochaetes	2.07±0.83	2.22±0.32	2.15±0.23	0.717
Synergistetes	0.639±0.37	0.248±0.17	0.508±0.35	0.106
Verrucomicrobia	NI	0.019±0.03	0.116±0.03	0.704
Unassigned	1.39±0.17	1.15±0.30	1.42±0.45	0.245
Euryarchaeota	6.11±1.21	5.27±1.70	6.01±1.45	0.490

NI: not identified. Values followed with superscript letters indicate statistical differences (P < 0.05) based on Kruskal-Wallis test.

At the genera level, the BMF supplementation allowed a higher relative abundance of *Quinella.spp* (P = 0.033), *Moryella.spp* (P = 0.011), and *Marvinbryantia.spp* (P = 0.041), and trend to increase *Ruminococcaceae UCG-001* (P = 0.068)*, Ruminococcaceae UCG-002* (P = 0.069), *Ruminiclostridium 5* (P = 0.080) and *Olsenella.spp* (P = 0.088) population when compared to SMU (Figure 2A). BMF also resulted in lower ruminal relative abundance of *CPla-4 termite* group (P = 0.011) and *Rikenellaceae RC9 gut group* (P=0.033), and the relative abundance of *Lachnospiraceae UCG-002* (P = 0.055),

Methanimicrococcus blatticola (P = 0.084) and *Butyrivibrio.2* (P = 0.088) tended be lower in the rumen of BMF supplemented steers.

Steers supplemented with SPE had a greater ruminal relative abundance of *Mogibacterium.spp* (P = 0.006), *Ruminococcaceae UCG-004* (P = 0.016), and *Papilibacter.spp* (P = 0.037), and trend to had a higher relative population of *Eubacterium coprostanoligenes group* (P = 0.054) when compared to SMU (Figure 2B). The *Clostridium sp. CAG-352* (P = 0.054), *Lachnoclostridium 10* (P = 0.054), *Prevotellaceae UCG-004, Ruminococcaceae-UCG-011*, and *Succinivibrio.spp* only were identified in the rumen of steers fed SPE. Steers supplemented with SMU showed a higher ruminal relative abundance of *Fretibacterium.spp* (P = 0.009), and *Lachnospiraceae ND3007 group* (P = 0.024). The relative abundance of *Prevotellaceae UCG-003* (P = 0.054) tended be lower in the rumen of SPE (Figure 2B).

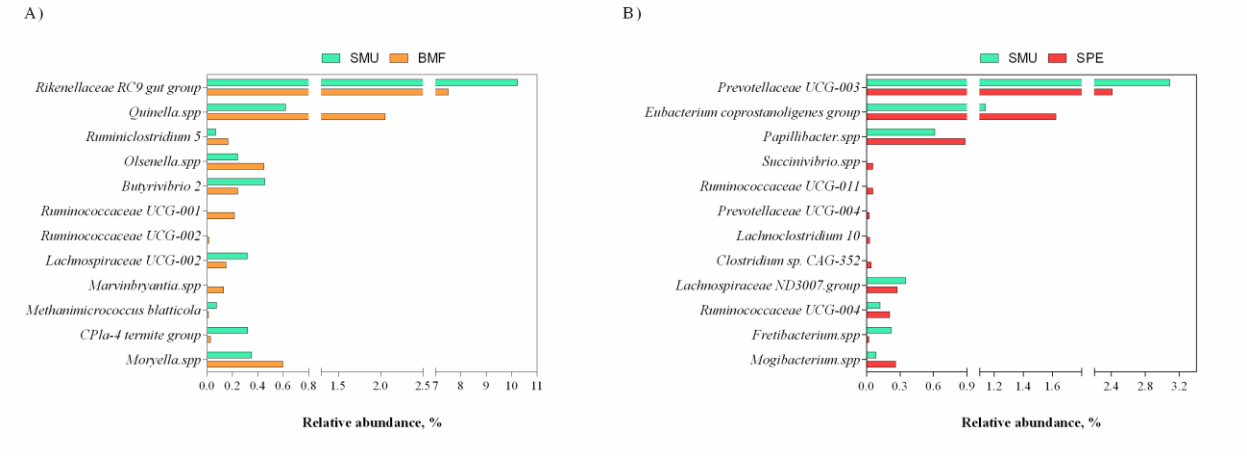

Figure 2. Changes in the rumen microbial relative abundance in Nellore steers fed low-quality hay and supplemented with low-moisture, sugarcane molasses-based block (BMF, A) or conventional protein-energy supplement (SPE, B).

3.5. Relationships between rumen bacteria and host metabolism

The principal component analysis (PCA) allowed for 58% of the total variability in bacterial communities and diet metabolism in steers (Figure 3). SPE was distributed along the negative region of principal component 1 (Dim 1) with positive correlation with Actinobacteria (r = 0.86), Kiritimatiellae (r = 0.85), Alphaproteobacteria (r = 0.84), Ruminococcus 2 (r = 0.77), Christensenellaceae R7 group (r = 0.42), the EMS (r = 0.59) and propionate proportion in the rumen (r = 0.15), and had negative correlation with *Prevotella.1* (r = - 0.69), UNE (r = - 0.59) and FNE (r = - 0.32). In contrast, BMF was

mainly distributed along the positive region of principal Dim 1 and negative region of the principal component 2 (Dim 2), with a positive correlation with archaea phylum Euryarchaeota (r = 0.91) and species *Methanobacterium alkaliphilum* (r = 0.54), and *Methanobrevibacter gottschalkii clade* (r = 0.70). The bacteria phylum Firmicutes (r = 0.78), families Lachnospiraceae (r = 0.84) and Ruminococcaceae (r = 0.79), and genera *Succiniclasticum* (r = 0.59) also were positively correlated with BMF supplementation and total apparent digestibility of CP (r = 0.50). Finally, the total apparent digestibility of NDF (r = 0.47), *Elusimicrobium* (r = 0.74), *Streptococcus* (r = 0.72), Ruminococcaceae UCG 013 (r = 0.67), Gastranaerophilales (r = 0.64), and the archaea *Methanomicrobium mobile* (r = 0.74) were positively correlated with SMU supplementation.

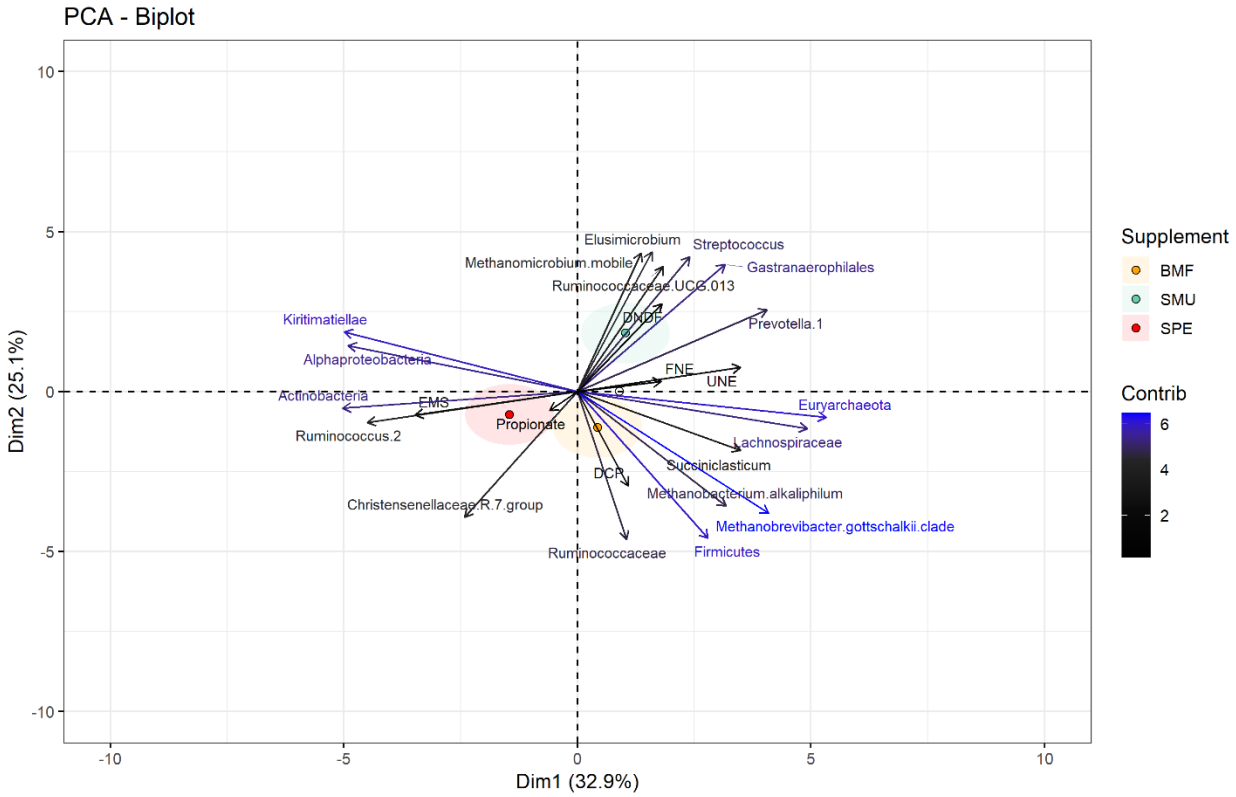

Figure 3. Principal component analysis (PCA) of rumen microbial relative abundance and host metabolism in Nellore steers fed low-quality hay and supplemented with urea mineral salt (SMU), conventional protein-energy supplement (SPE) or low-moisture, sugarcane molasses-based block (BMF). DCP: total apparent digestibility of crude protein, DNDF: total apparent digestibility of neutral detergent fiber, FNE: fecal nitrogen excretion, UNE: urinary nitrogen excretion, EMS: efficiency of microbial synthesis in the rumen.

4. DISCUSSION

Improving the low-quality tropical forage utilization by cattle through supplementation has been the focus of several studies. However, understand how the type of supplementation can modulate the rumen microbiome, the fermentation parameters, and the use of nitrogen in beef cattle is still required. Our hypothesis that the use of BMF would cause changes in the microbial community and rumen fermentation parameters improving the use of nutrients and use of nitrogen when compared to conventional energy protein supplementation was rejected, because our results showed that despite the use of BMF influenced the rumen bacterial population composition and the nitrogen utilization efficiency in Nellore steer fed low-quality hay, these changes were similar to those promoted by SPE. However, BMF may be useful as a practical alternative to SPE that may allow a reduction in operative cost.

The forage intake in cattle is modulated by the integration of several mechanisms. When ruminants fed low-quality forage is expected that the rumen filling effect be the main factor that limited the intake (Mertens, 1994). Thus, the low quality of hay (e.g. high NDF content 81.76% and low crude protein content 3.97%) limited the forage intake. Our finds of DM intake in all groups (from 37.3 to 50.9 $g/kg^{0.75}$) are in line with Preston and Leng (1987) that reported a voluntary intake of tropical forage in cattle between 30 - 80 $g/kg^{0.75}$. Besides, the high lignin content in the hay (10.35%) may reduce the energy available to rumen microorganisms and consequently, reduce both fiber degradation and ruminal passage rate (de Medeiros and Marino, 2015). The rumen filling effect associated to a low ruminal passage rate may be involved in the increasing of the retention time of digesta in the rumen allowing lower hay intake and higher fiber digestibility (Clipes et al., 2010), as observed in the SMU group, that at PCA showed an important positive association with NDF digestibility and ruminal population of *Streptococcus.spp* and Gastranaerophilales. Some species of *Streptococcus.spp* hydrolyzes cellulose (Oyeleke and Okusanmi, 2008) and urea supplementation can promote higher abundance of this genus (Jin et al., 2016). The Gastranaerophilales order has been recently documented in guts and feces from humans and animals with a role supporting the diet digestion, once can ferment a range of sugars as glucose, starch, and hemicellulose (Di Renzi et al., 2013).

Several studies have reported the voluntary low-quality forage intake increasing by the N compounds supplementation associated with both fiber degradation improvement and microbial growth-promoting (Lazzarini et al., 2009a; Sampaio et al., 2010). However, this effect was not observed in our study, once steers supplemented exclusively with urea (e.g. SMU) showed lower hay intake, evidencing that even N be considered the main limiting nutrient in low-quality forage, the energy substrates from forage limited the microbial fiber degradation activity. This fact may be due to the low NDT intake and associated with UNE, NR and Nmic results suggesting that the SMU supplementation was not enough to increase the N utilization by ruminal microorganism. This may be due to the faster ruminal hydrolysis of non-protein N (NPN) resulting in higher ammonia production that associated with limited energy sources (Souza et al., 2010), that allows ammonia absorption by the ruminal epithelium to the urea conversion in the liver, largely excreted through urine and a lower proportion return to rumen by recycling mechanisms (Kennedy and Milligan, 1980; Angelidis et al., 2019). Is important to highlight that steers fed SMU had a greater proportion of CP intake (116 g) by low-quality hay with lower availability this nutrient (3.97% de PB and 72.65% NDICP). 70 g of CP intake was in the NPN form which if not incorporated by the microorganisms into PBmic, is not useful as a CP source to the animal (Kertz, 2010). The higher ruminal abundance of the Neisseriaceae family of steers supplemented with SMU corroborates the urea hydrolysis activity of this bacterial group in the rumen, as previously suggested by Jin et al. (2016).

The lower DM and NDFcp intake observed in steers fed BMF when compared to those fed SPE may be possibly due to the CP excess (> 100 g CP/kg DM) to the metabolism (Sampaio, 2007), consumed by supplemented BMF steers (123.7 g CP/kg DM), providing higher UNE in these steers. In contrast, the high UNE observed in steers fed SMU may be due to the limited energy source, as previously discussed. The N excess in the rumen may reduce the voluntary intake of NDF (Detmann et al., 2009) and is line with previous reports in beef cattle fed low-quality forage (Sampaio, 2007; Lazzarini et al., 2009b). The urea synthesis in the liver results in a negative balance of 1 ATP per mol of urea (Brody, 1993). In this context, the energy necessary to urea synthesis from the excess protein decreases in function of the energy liquid/metabolizable, being directed to the production of body heat (NRC, 1988). Thus, ruminants may reduce the intake as

compensatory mechanism even without visible signal of this stress (Poppi and Mclennan, 1995). The urea cycle depends of Krebs cycle to both energy and oxaloacetate supply, and the excess of ammonia has a negative feedback to Krebs cycle by α-ketoglutarate reduction. Thus, tissues use a pathway with high energy expense (formation of glutamine from glutamate) as an alternative for excess ammonia regulation (Brody, 1993). The energy deficit impairs the brain function and resulting in animal discomfort (Detmann et al., 2007).

Even find some differences in nutrient intake (CP and NDT) among groups, the supplements did not influence the acetate proportion in the rumen. This reasonably agrees to the limited intake of nutrients, once DM, CP and NDT intake in all groups were lower than BR-CORTE requirements System (Valadares Filho et al., 2016) recommendations grazing steers in maintenance (6.30 kg DM; 3.77 kg NDT and 0.69 kg CP).

On another hand, the BMF supplementation promoted a higher propionate proportion in the rumen and consequently lower acetate: propionate ratio. Even with a low inclusion of BMF on the total diet, these results correspond to the fast fermentation of sugar cane molasses present in this supplement (Oba et al., 2015). The BMF were positively associated with *Succiniclasticum* population, a gram-negative ruminal bacterium with reported propionate production trough succinate conversion (Van Gylswyk, 1995). BMF also promoted the *Quinella.spp* abundance, a propionate producer bacterium related with molasses-fed sheep and steers (Vicini et al., 1987; Tolosa et al., 2004). The lower ruminal abundance of Rikenellaceae family allowed by BMF may be associated to the increasing propionate proportion in the rumen. Rikenellaceae are mainly acetate producer (Zhao et al., 2018) and has been negatively associated with propionate proportion in the rumen of bulls (Wang et al., 2019).

In contrast, SPE supplementation allowed higher valerate acid and iso acids (isobutyrate and isovalerate) ruminal proportion probably through a higher amino acid supply via soybean meal and corn. These SCFA indicates the amino acids fermentation and rumen microorganism recycling commonly used as substrate to microbial growth (Van Soest, 1994), mainly to cellulolytic bacteria as *Fibrobacter succinogenes* and *Butyrivibrio fibrisolvens* (Teodoro, 2014). SPE also tended to promote the ruminal

abundance of Bacteroidales F082 family and may be due the positive correlation with isobutyrate proportions previously reported in dairy calves (Park et al., 2021).

The SPE also allowed higher rumen ammonia concentration mainly at 2 – 6 hours after supplementation. It was expected once the highest concentrations of ammonia usually occur 3 to 5 hours after supplementation (Guimarães Júnior et al., 2016). Some bacteria may be associated with higher ammonia concentration in SPE, as Alphaproteobacteria class that contain members with recognized denitrifying capacity (Muck et al., 2019) and was positively associated with SPE. *Mogibacterium.spp* which was promoted by this supplement and have been associated with ammonia assimilation through the rumen epithelial wall in cattle (Li et al., 2012). *Succinivibrio*.*spp*, was only identified in the rumen of steers fed SPE and has been positively associated with ruminal ammonia concentrations in grazing Nellore cattle (Granja-Salcedo et al., 2019). *Succinivibrio*.*spp* represents a group of gram-negative anaerobic bacteria of recognized amylolytic capacity (O'herrin e Kenealy, 1993) and probably was promoted due to the starch supplied by both corn content and higher supplement intake, once its population is generally promoted when cattle or sheep are fed high-grain diets containing starch (Zhang et al. 2018).

The higher rumen pH values allowed by BMF is in line with previous reports in ruminants supplemented with sugarcane molasses and urea combination (Mold et al., 1983; Royes et al., 2001), and may be associated to the higher ruminal relative abundance of *Moryella.spp* in these animals. This genus has been described as a strictly anaerobic that can use glucose, galactose, maltose and ribose as a substrate in a variety of ways and its main metabolic products are acetic, butyric and lactic acids (Carlier, K'ouas and Han, 2007). In ruminants, *Moryella* species were predominant in the rumen of lambs fed on forage (Ellison et al., 2017). Interestingly, the higher rumen pH may be promoted the abundance of *Marvinbryantia.spp* (from Lachnospiraceae family), bacterium considered an uncultured gut acetogens (Petri et al., 2013; Guillén et al., 2019) with an optimal growth pH near to 7.0 (Takors et al., 2018).

The higher FNE (g and %NI) by SPE steers may be explained by the higher N intake when compared to SMU since the proportion of N excreted by urine and feces may increase as dietary N intake and CP digestibility increase (Schuba et al., 2017). To the

environment, fecal N is more stable and contributes lower to N emissions than urine N (Satter, Klopfenstein and Erickson, 2002). Fecal N is composed by undigested diet N and metabolic fecal N, last one as an endogenous fraction with microbial N as a component) (Schwarm et al., 2009). Thus, is probably that the high fiber ingested by steers could promote a post-ruminal fermentation and microbial growth at large intestine (de Oliveira et al., 2013), which provides among 5 - 10% of diet energy (Gressley, Hall and Armentano, 2011) and consequently, increase the N fecal concentration.

In contrast, BMF allowed a higher UNE (g) possibly associated to the higher CP digestibility observed in this group, once a major fraction of N intake was as NPN form that may allow higher urinary urea excretion (Fanchone et al., 2013). Increase the N excretion by urine pathway in cattle is undesirable because it is the main source of ammonia volatilized from cattle manure (Satter, Klopfenstein e Erickson, 2002). However, ENU values observed in both BMF and SPE were near to expected range for diets for ruminants (15 - 40% of N intake) (Calsamiglia et al., 2010). Interestingly, PCA showed an important association among CP digestibility and phylum Firmicutes and its families Lachnospiraceae and Ruminococcaceae in BMF. Lachnospiraceae family has been correlated with amino acid degradation in the rumen (Li e Guan, 2017). Our results are in line with that Alves et al. (2021) who found a positively correlation among Lachnospiraceae and higher UNE in beef cattle and reported that bacteria may promote a greater urinary N excretion through ruminal protein deamination.

Despite both BFM and SPE supplementation provided higher N intake, the lower ruminal N-NH_3 concentration and higher Nmic observed in steers supplemented with BFM suggest that BFM may be released higher N assimilation in the rumen compared to SMU and SPE. The total Nmic flow depends mainly of energy availability in the rumen (Edwards et al. 2008, Souza et al., 2010), in this case supported by sugarcane molasses inclusion in BMF composition and the EMS, since N supply is not limited (Calsamiglia et al., 2010). Positive effects on NR are frequently associate to improvements in the energy supply in the rumen with microbial protein synthesis increasing and N losses reduction (Schuba et al., 2017). This is in line with our finds once steers fed BMF showed lower both fecal and urinary N excretion (% NI) and higher EMS compared to SMU.

Finally, was possible noticed that BMF supplementation promoted the network in the rumen ecosystem among two important domains of life form in the rumen. With the phylum Euryarchaeota, and species *Methanobacterium alkaliphilum* and *Methanobrevibacter gottschalkii clade* as archaea members, and the bacterial phylum Firmicutes and its families Lachnospiraceae and Ruminococcaceae, corroborating the highly interactions in the rumen ecosystem among specific archaea methanogens and cellulose degrader bacteria. The co-occurrence of *Methanobrevibacter gottschalkii* and *Ruminococcaceae* family previous reported by Kittelmann et al. (2013).

5. CONCLUSION

The low-moisture, sugarcane molasses-based block is useful alternative to a conventional energy protein supplement, that modulates the rumen bacteria population, increasing the ruminal propionate proportion, and improving both rumen ammonia utilization and diet nitrogen retention in beef cattle fed low-quality forage.

6. REFERENCES

Alves KLGC, Granja-Salcedo YT, Messana JD, de Souza VC, Ganga MJG, Colovate PHD, Kishi LT and Berchielli TT (2020) Rumen bacterial diversity in relation to nitrogen retention in beef cattle. **Anaerobe**, p.102316.

Angelidis A, Crompton L, Misselbrook T, Yan T, Reynolds CK Stergiadis S (2019) Evaluation and prediction of nitrogen use efficiency and outputs in faeces and urine in beef cattle. **Agriculture, ecosystems and environment** 280:1-15.

Association of Analytical Chemists, 1995. Official methods of analysis of AOAC International.

Batista ED, Detmann E, Titgemeyer EC, Valadares Filho SCD, Valadares RFD, Prates LL, Rennó LN, Paulino MF (2016) Effects of varying ruminally undegradable protein supplementation on forage digestion, nitrogen metabolism, and urea kinetics in Nellore cattle fed low-quality tropical forage. **Journal of Animal Science** 94(1):201-216.

Brody, T., 1998. **Nutritional biochemistry**. Elsevier.

Calsamiglia S, Ferret A, Reynolds CK, Kristensen NB, Van Vuuren AM (2010). Strategies for optimizing nitrogen use by ruminants. **Animal** 4(7):1184-1196.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N. and Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. **Proceedings of the national academy of sciences**, *108*(Supplement 1), pp.4516-4522.

Carlier JP, K'ouas G, Han XY (2007) Moryella indoligenes gen. nov., sp. nov., an anaerobic bacterium isolated from clinical specimens. **International journal of systematic and evolutionary microbiology** 57(4):725-729.

Casagrande DR, Ruggieri AC, Moretti MH, Berchielli TT, Vieira BR, Roth APTP, Reis RA (2011) Sward canopy structure and performance of beef heifers under supplementation in Brachiaria brizantha cv. Marandu pastures maintained with three grazing intensities in a continuous stocking system. **Revista Brasileira de Zootecnia** 40:2074–2082.

Chen XB, Gomes MJ (1992) Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives – an overview of technical details. **Bucksburnd: Rowett Research Institute 1-21.**

Clipes RC, Coelho da Silva JF, Detmann E, Vásquez HM, Henriques LT, Donatele DM (2010) Associações entre parâmetros da cinética de degradação ruminal e os constituintes da parede celular de quatro gramíneas tropicais. **Revista Brasileira de Saúde e Produção Animal** 11(3):802-814.

de Medeiros SR, MARINO C (2015) Carboidratos na nutrição de gado de corte. Embrapa Gado de Corte-Capítulo em livro científico (ALICE) < https://www.alice.cnptia.embrapa.br/alice/bitstream/doc/1011216/1/NutricaoAnimalCAPITULO04.pdf >

de Oliveira MNV, Jewell KA, Freitas FS, Benjamin LA, Tótola MR, Borges AC, Moraes CA, Suen G (2013) Characterizing the microbiota across the gastrointestinal tract of a Brazilian Nelore steer. **Veterinary microbiology** 164(3-4):307-314.

Dehority BA, Orpin CG (1997) Development of, and natural fluctuations in, rumen microbial populations. In **The rumen microbial ecosystem**, Springer, Dordrecht, 196-245.

Detmann E, Paulino MF, Mantovani HC, Valadares Filho SDC, Sampaio CB, de Souza MA, Lazzarini Í, Detmann KS (2009) Parameterization of ruminal fibre degradation in low-quality tropical forage using Michaelis–Menten kinetics. **Livestock Science** 126(1-3):136-146.

Detmann E, Paulino MF, Valadares Filho SDC, Lana RP (2007) Fatores controladores de consumo em suplementos múltiplos fornecidos ad libitum para bovinos manejados a pasto. **Cadernos Técnicos de Veterinária e Zootecnia** 55:73-93.

Detmann E, Paulino MF, Valadares Filho, SDC, Huhtanen P (2014a) Aspectos nutricionais aplicados a bovinos em pastejo nos trópicos: uma revisão baseada em resultados obtidos no Brasil. **Semina: Ciências Agrárias** 35(4):2829-2854.

Detmann E, Valente EEI, Batista ED, Huhtanen P (2014b) An evaluation of the performance and efficiency of nitrogen utilization in cattle fed tropical grass pastures with supplementation. **Livestock Science** 162:141-153

Di Rienzi, S. C., Sharon, I., Wrighton, K. C., Koren, O., Hug, L. A., Thomas, B. C., et al. (2013). The human gut and groundwater harbor non-photosynthetic bacteria belonging to a new candidate phylum sibling to Cyanobacteria. **eLife** 2:e01102. doi: 10.7554/eLife.01102

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than blast. **Bioinformatics** 26, 2460–2461. doi: 10.1093/bioinformatics/btq461

Edwards JE, Huws SA, Kim EJ, Lee MRF, Kingston-Smith AH, Scollan ND (2008) Advances in microbial ecosystem concepts and their consequences for ruminant agriculture. **Animal** 2:653–660.

Ellison MJ, Conant GC, Lamberson WR, Cockrum RR, Austin KJ, Rule DC, Cammack KM (2017) Diet and feed efficiency status affect rumen microbial profiles of sheep. **Small Ruminant Research** 156:12-19.

Fanchone A, Noziere P, Portelli J, Duriot B, Largeau V, Doreau M (2013) Effects of nitrogen underfeeding and energy source on nitrogen ruminal metabolism, digestion, and nitrogen partitioning in dairy cows. **Journal of animal science** 91(2):895-906.

Fenner, H., 1965. Method for determining total volatile bases in rumen fluid by steam distillation. **Journal of Dairy Science**, *48*, pp.249-251.

Granja-Salcedo YT, Fernandes RM, Araujo RC, Kishi LT, Berchielli TT, Resende FD, Berndt A, Siqueira GR (2019) Long-Term Encapsulated Nitrate Supplementation Modulates Rumen Microbial Diversity and Rumen Fermentation to Reduce Methane Emission in Grazing Steers. **Frontiers in Microbiology** 10:614.

Granja-Salcedo YT, Ramirez-Uscategui RA, Machado EG, Messana JD, Takeshi Kishi l, Dias AVL, Berchielli TT (2017) Studies on bacterial community composition are affected by the time and storage method of the rumen content. **PloS one** 12:e0176701.

Gressley TF, Hall MB e Armentano LE (2011) Ruminant nutrition symposium: productivity, digestion, and health responses to hindgut acidosis in ruminants. **Journal of animal science** 89(4):1120-1130.

Guillén, Y., Noguera-Julian, M., Rivera, J., Casadellà, M., Zevin, A.S., Rocafort, M., Parera, M., Rodríguez, C., Arumí, M., Carrillo, J. and Mothe, B., 2019. Low nadir CD4+ T-cell counts predict gut dysbiosis in HIV-1 infection. **Mucosal immunology**, *12*(1), pp.232-246.

Guimarães Júnior R, Pereira LGR, Tomich T.R, Gonçalves LC (2016) Informações gerais: o que é uréia. **Cadernos Técnicos de Veterinária e Zootecnia** 80, EMBRAPA Gado de Leite.

Henderson G, Cox F, Kittelmann S, Miri VH, Zethof M, Noel SJ, Janssen PH (2013) Effect of DNA extraction methods and sampling techniques on the apparent structure of cow and sheep rumen microbial communities. **PLoS One** 8:e74787.

Jin D, Zhao S, Wang P, Zheng N, Bu D, Beckers Y, Wang J. Insights into Abundant Rumen Ureolytic Bacterial Community Using Rumen Simulation System. **Frontiers in Microbiology**. 2016 Jun 28;7:1006. doi: 10.3389/fmicb.2016.01006. PMID: 27446045; PMCID: PMC4923134.

Katulski SL, Van Bibber-Krueger CL, Blevins CA, Schrag NFD, Horton LM, Ellerman TJ, Muller HC, Drouillard JS (2017) Effects of low-moisture molasses block supplements on tissue concentrations of trace elements and growth performance of forage-fed beef cattle. **Journal of Animal Science** 95(suppl_4):310-310.

Kennedy PM e Milligan LP (1980) The degradation and utilization of endogenous urea in the gastrointestinal tract of ruminants: a review. **Canadian Journal of Animal Science** 60(2):205-221.

Kertz AF (2010) Urea Feeding to Dairy Cattle: A Historical Perspective and Review. **The Professional Animal Scientist** 26(3):257–272.

Kim M, Morrison M, Yu Z (2011) Phylogenetic diversity of bacterial communities in bovine rumen as affected by diets and microenvironments. **Folia microbiologica** 56(5):453.

Kittelmann S, Seedorf H, Walters WA, Clemente JC, Knight R, Gordon JI, et al. (2013) Simultaneous Amplicon Sequencing to Explore Co-Occurrence Patterns of Bacterial, Archaeal and Eukaryotic Microorganisms in Rumen Microbial Communities. **PLoS ONE** 8(2): e47879.

Köster HH, Cochran RC, Titgemeyer EC, Vanzant ES, Abdelgadir I, St-Jean G (1996) Effect of increasing degradable intake protein on intake and digestion of low-quality, tallgrass-prairie forage by beef cows. **Journal of Animal Science**, 74(10):2473-2481.

Lazzarini I, Detmann E, Sampaio CB, Paulino MF, Valadares Filho SDC, Souza MA, Oliveira FA (2009b) Dinâmicas de trânsito e degradação da fibra em detergente neutro em bovinos alimentados com forragem tropical de baixa qualidade e compostos nitrogenados. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia** 61(3):635-647.

Lazzarini I, Detmann E, Sampaio CB, Paulino MF, Valadares Filho SDC, Souza MAD, Oliveira FA (2009a) Intake and digestibility in cattle fed low-quality tropical forage and supplemented with nitrogenous compounds. **Revista Brasileira de Zootecnia** 38(10):2021-2030.

Li F, Guan LL (2017) Metatranscriptomic profiling reveals linkages between the active rumen microbiome and feed efficiency in beef cattle. **Applied and environmental microbiology**, *83*(9).

Li M, Zhou M, Adamowicz E, Basarab JA (2012) Characterization of bovine ruminal epithelial bacterial communities using 16S rRNA sequencing, PCR-DGGE, and qRT-PCR analysis. **Veterinary microbiology** 155(1):72-80.

Löest CA, Titgemeyer EC, Drouillard JS, Lambert BD, Trater AM (2001) Urea and biuret as nonprotein nitrogen sources in cooked molasses blocks for steers fed prairie hay. **Animal feed science and technology** 94(3-4):115-126.

Love MI, Huber W, Anders S (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. **Genome Biology** 15:550

McMurdie PJ e Holmes S (2013). phyloseq: an r package for reproducible interactive analysis and graphics of microbiome census data. **PLoS One** 8:e61217.

Mertens DR (1994) Regulation of forage intake. In: **Forage quality, evaluation, and utilization**, pp.450-493.

Moriel P, Artioli LF, Piccolo MB, Miranda M, Ranches J, Ferreira VS, Antunes LQ, Bega AM, Miranda VF, Vieira JF, Vasconcelos JL (2019) Effects of low-moisture, sugarcane molasses-based block supplementation on growth, physiological parameters, and liver trace mineral status of growing beef heifers fed low-quality, warm-season forage. **Translational Animal Science** 3(1):523-531.

Muck S, De Corte D, Clifford EL, Bayer B, Herndl GJ, Sintes E. Niche Differentiation of Aerobic and Anaerobic Ammonia Oxidizers in a High Latitude Deep Oxygen Minimum Zone. **Frontiers in Microbiology.** 2019;10:2141. Published 2019 Sep 13. doi:10.3389/fmicb.2019.02141

NRC. 1988. Nutrient Requirements of Swine (9th Ed.). National Academy Press, Washington, DC.

Oba M, Mewis JL, Zhining Z (2015) Effects of ruminal doses of sucrose, lactose, and corn starch on ruminal fermentation and expression of genes in ruminal epithelial cells. **Journal of dairy science**, 98(1):586-594.

O'Herrin SM, Kenealy WR (1993) Glucose and carbon dioxide metabolism by *Succinivibrio dextrinosolvens*. **Applied and Environmental Microbiology** 59:748–755.

Oksanen J, Blanchet FG et al. (2013). Package 'vegan'. **Community Ecology Package**, Version 2

Oyeleke S., Okusanmi T. (2008). Isolation and characterization of cellulose hydrolysing microorganism from the rumen of ruminants. A**frican Journal of Biotechnology**. 7, 1503–1504. 10.5897/AJB08.142

Park T., Cersosimo L. M., Li W.i, Radloff W., Zanton G. I. 2Pre-weaning Ruminal Administration of Differentially-Enriched, Rumen-Derived Inocula Shaped Rumen Bacterial Communities and Co-occurrence Networks of Post-weaned Dairy Calves. **Frontiers in Microbiology**. v.12,p.311. 2021. DOI:10.3389/fmicb.2021.625488

Petri, R.M., Schwaiger, T., Penner, G.B., Beauchemin, K.A., Forster, R.J., McKinnon, J.J. and McAllister, T.A., 2013. Changes in the rumen epimural bacterial diversity of beef cattle as affected by diet and induced ruminal acidosis. **Applied and environmental microbiology**, *79*(12), pp.3744-3755.

Poppi DP e McLennan SR (1995) Protein and energy utilization by ruminants at pasture. **Journal of animal science** 73(1):278-290.

Preston TR, Leng RA (1987) Sulphur nutrition of ruminants. In: Preston TR, Leng RA (Eds.). **Matching ruminant production systems with available resources in the tropics and sub-tropics**. Armidale: Penambul Books, 46-47.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO, (2012) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. **Nucleic acids research** 41(D1):D590-D596.

Reis WLS, Detmann E, Batista ED, Rufino LMA, Gomes DI, Bento CBP, Mantovani HC, Valadares Filho, SDC (2016) Effects of ruminal and post-ruminal protein supplementation in cattle fed tropical forages on insoluble fiber degradation, activity of fibrolytic enzymes, and the ruminal microbial community profile. **Animal Feed Science and Technology** 218:1-16.

Royes JB, Brown WF, Martin FG, Bates DB (2001) Source and level of energy supplementation for yearling cattle fed ammoniated hay. **Journal of animal science**, 79(5):1313-1321.

Sampaio CB (2007) **Consumo, digestibilidade e dinâmica ruminal em bovinos alimentados com forragem tropical de baixa qualidade suplementados com compostos nitrogenados.** 96 f. Dissertação (Mestrado em zootecnia) – UFV, Viçosa.

Sampaio CB, Detmann E, Paulino MF, Valadares Filho SDC, de Souza MA, Lazzarini I, Paulino PVR de Queiroz AC (2010) Intake and digestibility in cattle fed low-quality tropical forage and supplemented with nitrogenous compounds. **Tropical Animal Health and Production** 42(7):1471-1479.

Satter LD, Klopfenstein TJ e Erickson GE (2002) The role of nutrition in reducing nutrient output from ruminants. **Journal of Animal Science** 80(E-suppl_2):E143-E156.

Schuba J, Südekum KH, Pfeffer E, Jayanegara A (2017) Excretion of faecal, urinary urea and urinary non-urea nitrogen by four ruminant species as influenced by dietary nitrogen intake: A meta-analysis. **Livestock Science** 198:82-88.

Schwarm A, Schweigert M, Ortmann S, Hummel J, Janssens GPJ, Streich WJ, Clauss M (2009) No easy solution for the fractionation of faecal nitrogen in captive wild herbivores: results of a pilot study. **Journal Animal Physiology and Animal Nutrition** 93:596–605.

Souza MA, Detmann E, Paulino MF, Sampaio CB, Lazzarini I, Valadares Filho SDC (2010) Intake, digestibility and rumen dynamics of neutral detergent fibre in cattle fed low quality tropical forage and supplement with nitrogen and/or starch. **Tropical Animal Health and Production** 42:1299-1310.

Takors, R., Kopf, M., Mampel, J, Bluemke, W., Blombach, B., Eikmanns, B., Bengelsdorf F. R., Weuster-Botz, D., Dürre, P. Using gas mixtures of CO, CO2 and H2 as microbial substrates: the do's and don'ts of successful technology transfer from laboratory to production scale. **Microbial Biotechnology** (2018) 11(4), 606– 625. https://doi.org/10.1111/1751-7915.13270

Teodoro AL (2014) Suplementos proteicos com adição de levedura para bovinos consumindo forragem de baixa qualidade. Tese (Doutorado em zootecnia) – UEM, Maringa.

Titgemeyer EC, Drouillard JS, Greenwood RH, Ringler JW, Bindel DJ, Hunter RD, Nutsch T (2004) Effect of forage quality on digestion and performance responses of cattle to supplementation with cooked molasses blocks. **Journal of Animal Science** 82(2):487-494

Tolosa, M. X., Dinh Van, T., Klieve, A. V., Ouwerkerk, D., Poppi, D. P., and McLennan, S. R. (2004). *Molecular characterisation of rumen bacterial populations in cattle fed molasses diets.* **25th Biennial Conference of the Australian Society of Animal Production: The New Realities** - Animal Production in Australia, Melbourne, Victoria, 4-8 July 2004. Wagga Wagga, NSW, Australia: The Australian Society of Animal Production.

Trater AM, Titgemeyer EC, Drouillard JS, Pike JN (2003) Effects of processing factors on in vitro ammonia release from cooked molasses blocks containing urea. **Animal Feed Science and Technology** 107(1-4):173-190.

Valadares RFD, Gonçalves LC, Valadares Filho SDC, Coelho da SIlva JF (1997) Níveis de proteína em dietas de bovinos. 2. Consumo, digestibilidade e balanços de compostos nitrogenados. **Revista Brasileira de Zootecnia** 26:1259-1263.

Valadares Filho SDC, Costa e Silva LF, Gionbelli MP, Rotta PP, Marcondes MI, Chizzotti ML, Prados LF (2016) Exigências nutricionais de zebuínos puros e cruzado-BR-Corte (Vol. 1). Viçosa, Minas Gerais: Universidade Federal de Viçosa.

Van Gylswyk NO. Succiniclasticum ruminis gen. nov., sp. nov., a ruminal bacterium converting succinate to propionate as the sole energy-yielding mechanism. **International Journal of Systematic and Evolutionary Microbiology** 1995 Apr;45(2):297-300. doi: 10.1099/00207713-45-2-297. PMID: 7537062.

Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. **Journal of Dairy Science** 74:3583–3597.

Van Soest, PJ (1994) Nutritional Ecology of the Ruminant. 2ND ed. Cornell university.

Vicini, J.L., Brulla, W.J., Davis, C.L. and Bryant, M.P., 1987. Quin's oval and other microbiota in the rumens of molasses-fed sheep. **Applied and environmental microbiology**, *53*(6), pp.1273-1276.

Wang, H.; He, Y.; Li, H.; Wu, F.; Qiu, Q.; Niu, W.; Gao, Z.; Su, H.; Cao, B. Rumen fermentation, intramuscular fat fatty acid profiles and related rumen bacterial populations of Holstein bulls fed diets with different energy levels. **Applied Microbiology and Biotechnology.** 2019, 103, 4931–4942

Weiss WP, Conrad HR e St. Pierre NR (1992) A theoretically-based model for predicting total digestible nutrient values of forages and concentrates. **Animal Feed Science and Technology** 39:95–110.

Wickham H (2016). *ggplot2: Elegant Graphics for Data Analysis*. Berlin: Springer.

Zhang, R.Y., Jin, W., Feng, P.F., Liu, J.H. and Mao, S.Y., 2018. High-grain diet feeding altered the composition and functions of the rumen bacterial community and caused the damage to the laminar tissues of goats. **Animal**, *12*(12), pp.2511-2520.

Zhao XH, Zhou S, Bao LB, Song XZ, Ouyang KH, Xu LJ, Pan K, Liu CJ, Qu MR (2018) Response of rumen bacterial diversity and fermentation parameters in beef cattle to diets containing supplemental daidzein. **Italian Journal of Animal Science** 17(3):643–649.

7. SUPPLEMENTARY DATA

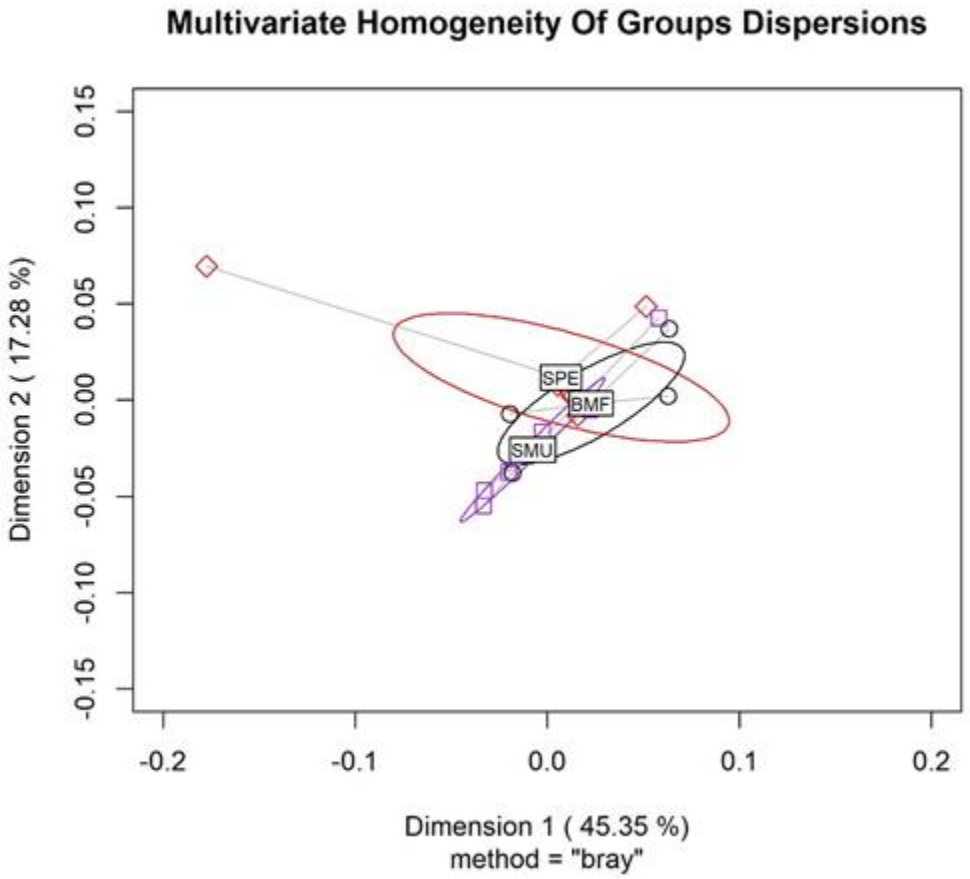

Figure S1. Rumen bacteria beta diversity tested by Permutation test of Multivariate Homogeneity of groups Dispersions based on Bray–Curtis as a similarity index of Nellore steers fed low-quality hay and supplemented with urea mineral salt (SMU), conventional protein-energy supplement (SPE) or low-moisture, sugarcane molasses-based block (BMF).

CHAPTER 3 - EFFECT OF LOW-MOISTURE, SUGARCANE MOLASSES-BASED BLOCK SUPPLEMENTATION ON *IN SITU* RUMINAL DEGRADABILITY, INGESTIVE BEHAVIOR, METABOLIC PARAMETERS, RUMINAL MICROORGANISMS AND THEIR ASSOCIATIONS IN BEEF CATTLE FED WITH LOW QUALITY FORAGE

ABSTRACT: Our objective was to evaluate the ingestive behavior, in situ ruminal degradability parameters of dry matter (DM) and neutral detergent fiber (NDF), metabolic parameters, bacterial diversity and ruminal archaea and their correlations in Nellore steers fed with low quality forage and supplemented with urea mineral salt (SMU), low-moisture, sugarcane molasses-based block (BMF) or energy protein supplement (SPE). Six castrated Nellore steers (350 ± 10 kg BW) were used in a simultaneous double 3 × 3 Latin Square balanced for residual effects, with three experimental periods of 24 days each, and three treatments. The basal diet consisted of hay offered *ad libitum*, and experimental supplements were urea mineral salt (SMU) *ad libitum*, or conventional SPE (0.2% BW), or BMF *ad libitum.* Trained observers assess behavior at 5-minute intervals for 24 hours. Metabolic parameters (glucose, urea, triglycerides, total protein and insulin) were measured on day 16 of each period at 0 (before feeding), 4, 8, and 12 hours after feeding. The kinetic parameters of ruminal degradation of total diets were measured using the Ørskov and McDonald methodology. The bags were heat sealed, inserted into the rumen in triplicate and incubated for 4, 8, 12, 24, 36, 48 and 96 hours. Animals supplemented with both SPE and BMF spent higher time in hay interaction than SMU steers (P=0.004), while the supplement interaction and total eating time was higher in SPE steers than both SMU and BMF (P=0.02). Glucose, insulin, and total protein blood concentrations were not influenced by treatments (P>0.05; Table 4); however, the urea concentration was higher in animals supplemented with BMF (P=0.05). There was effect of both treatment (P = 0.022) and collection time (P < 0.001) on triglycerides blood concentration; thus, the BMF supplementation resulted in higher concentrations of triglycerides than SPE supplementation and were observed a linear decreasing concentration as a function of collection time (P=0.022). Ruminal degradability of fraction “a” of DM and estimated effective degradability at 2.5 and 8%h^{-1} were similar for animals supplemented with SPE and BMF (P<0.05). In contrast, the ruminal degradability of fraction “b” was higher in SMU compared to BMF (P=0.013) and the potential degradability was higher for animals supplemented with SPE (P=0.015). Fraction “b” was positively correlated with the phylum Fibrobacter (P=0.034; r=0.50) and negatively with the class Bacilli, the order Lactobacillales, the genera Lachnospiraceae bacterium FD2005 and Succinimonas.spp (P<0.016); r<-0.56). The fraction “b” of NDF was positively correlated with the orders Aeromonadales, Desulfuromonadales, the genera Eubacterium hallii group, Coprococcus 1, Ruminiclostridium 5, Ruminobacter and the species Lachnospiraceae UCG.002 (P<0.001, r>0.70). Plasma urea correlated positively with the Gammaproteobacteria class, the Ruminococcaceae family, the Ruminobacter.spp and Succinivibrio.spp genera and negatively with the Prevotellaceae family and the genus Fretibacterium.spp (P<0.036; r<-0.52). The low-moisture, sugarcane molasses-based block is a useful alternative to the conventional energy protein supplement, which modulates the population of ruminal archaea, improving

protein status, ingestive behavior and ruminal degradability in the diet of forage-fed beef cattle.

Key words: low quality hay, ruminal microorganisms, fiber degradation, blood metabolites, supplement

1. INTRODUCTION

Given the capacity of the ruminal ecosystem to successfully digest both structural and cellular components of forage, the host can profit from degradation products as an energy source (Xue et al., 2016). Dietary forage is an important nutrients source and an energy supply for ruminants, which provides the fiber needed to optimize rumen function (Turano, Tiwari and Jha, 2016). One of the most common indices to assess forage quality is the amount of neutral detergent fiber (NDF) and nutrient bioavailability, essential information to ensure that the nutritional requirements of animals are met (Ali et al., 2016).

In tropical grasses, due to forage seasonality, there is a drastic decrease in nutritional quality characterized mainly by both higher lignification and lower crude protein content (Detmann et al., 2014a). To correct this condition, supplementation is used to improve the use of low-quality pastures (Casagrande et al., 2011; Detmann et al., 2014b).

In the search for a low-cost and efficient supplementation, there are the choice of non-protein nitrogen (NPN) sources, such as urea, which has been a good alternative to reduce the cost in protein supplementation in cattle (Sales et al., 2008). The use of low-moisture, sugarcane molasses-based block (BMF) associating urea and sugarcane molasses, as supplementation strategy has been adopted (Köster et al., 1996; Titgemeyer et al., 2004; Moriel et al., 2019), mainly due to its manufacturing process, and changes in the bioavailability of nutrients, as the release of ammonia in the rumen (Trater et al., 2003; Katulski et al., 2017). However, the ingestive behavior as a change in supplement intake patterns and nutrient use (Katulski et al., 2017), and differences in both composition and form of supply of supplements, can allow different rumen environment responses (Oliveira et al., 2009).

Ruminal ammonia not used by the microbiome is transported through the rumen wall to the liver over the bloodstream, achieving the urea cycle (Kennedy and Milligan, 1980, Angelidis et al., 2019). During periods of high ruminal availability of N, high blood concentrations of urea are observed (Rennó et al., 2000). In contrast, a low level of protein in the diet promotes a reduction of circulating amino acids, decreasing both the insulin concentration and the entry of glucose rate, and consequently, the availability of energy to the hypothalamus (Ruas et al., 2000). Total proteins found in the plasma are related to nutritional deficiency (Gonzáles, 2000). Diets with less than 10% protein have been

estimated to decrease blood protein levels (Kaneko et al., 1997). In addition, plasma urea concentration may be an indicator of the diet protein efficiency of use, due to excess supply or asynchrony of energy protein in the rumen (Pessoa et al., 2009).

Therefore, an analysis of the plasma composition and its association with the rumen microbiome can provide important information about the role of rumen-diet interactions in animal conditions, since the health, growth and metabolism of animals are largely dependent on the production of metabolites such as urea, glucose, insulin, triglycerides, and total proteins (Saleem et al., 2013). Thus, the objective of this study was to evaluate the parameters of ruminal degradability of DM and NDF, ingestive behavior, blood metabolic parameters and their possible interaction with ruminal microorganisms in Nellore steers supplemented with low-moisture, sugarcane molasses-based block (BMF) fed with low-quality hay. Were hypothesized that supplementation with BMF allows the improvement of ruminal degradability and blood metabolic profile in beef cattle fed low-quality tropical forage.

2. MATERIAL AND METHODS

The protocols used in this experiment are in accordance with the guidelines of the Brazilian College of Animal Experimentation and was approved by the Animal Ethics and Welfare Commission of the São Paulo State University “Júlio de Mesquita Filho”, of the School of Agricultural and Veterinary Sciences, Jaboticabal campus (Protocol No. 010216/17).

2.1. Animals and treatments

Six castrated Nellore steers (350 ± 10 kg initial body weight and 24 months of age) cannulated in the rumen, were used in a simultaneous double 3 × 3 Latin Square balanced for residual effects, with three experimental periods of 24 days each, and three treatments (supplements). Animals were housed in individual semi-covered stalls (9.0 m2), equipped with individual feeders and drinkers, and were adapted to the diets during the first 14 days of each experimental period.

The basal diet consisted of Urochloa brizantha (Hochst.) Stapf, “Marandu” hay offered ad libitum, and experimental supplements were urea mineral salt (SMU) ad libitum, or conventional protein-energy supplement (SPE, 0.2% BW), or low-moisture, sugarcane molasses-based block (BMF) ad libitum (Table 1)

Table 1. Proportion of ingredients and chemical composition of experimental supplements and hay.

Inclusion of ingredients (as-fed basis)	**Supplements[1]**			**Hay**
	SMU	SPE	BMF	
Dried sugarcane molasses	-	-	53.00	-
Ground corn	-	45.00	-	-
Cottonseed meal	-	-	14.30	-
Soybean meal	-	16.00	-	-
Urea	16.00	5.00	13.00	-
Limestone	24.50	10.65	-	-
Ca phosphate	15.00	4.00	8.70	-
Sodium chloride	40.00	15.00	5.00	-
Kaolin[2]	-	3.50	-	-
Trace mineral/vitamin premix[3]	3.75	0.62	2.50	-
Magnesium oxide	0.67	0.25	0.79	-
Soybean oil	-	-	2.70	-
Palatability enhancer [4]	0.08	-	-	-
Chemical composition (% DM)				
DM, %	95.00	96.00	94.00	93.65
OM	17.20	63.90	79.70	94.80
CP	46.00	25.00	46.00	3.97
NDFap[5]	-	7.30	9.13	76.99
ADF	-	14.10	12.30	55.68
RDP, % PB[6]	100.00	86.60	93.20	22.40
NDICP, %CP	0.69	4.39	3.82	72.65
Lignin	-	-	-	10.35
TDN[7]	-	53.30	50.70	46.60

[1]SMU: urea mineral salt, SPE: protein-energy supplement, BMF: low-moisture, sugarcane molasses-based block;

[2] Rumen-inert indigestible substance included;

[3] DM basis: 3% Ca, 10% Mg, 23.5% S, 600 mg/kg Co, 20,000 mg/kg Cu, 264 mg/kg Se, 1,200 mg/kg I, 80,000 mg/kg Zn, 53,200 mg/kg Mn, 4,000,000 mg/kg vitamin A, 400,000 mg/kg vitamin D_3, 20,000 mg/kg vitamin E, and 40,000 mg/kg monensin, Poulcox 40, Peshteria, Bulgaria).

[4]Tecnaroma zta Sweet note fruit red 4W/10638 powder (New Products Comercial Agrícola e Veterinária, Campinas, São Paulo, Brazil).

[5]NDF corrected for ash and protein

[6] Described by Moriel et al., 2019.

[7] Calculated as described by Weiss et al. (1992).

Supplements were provided daily at 7:00 am while hay was supplied twice daily (7:00 am and 3:00 pm), in individual feeders. The amount of food supplied was calculated and adjusted daily to allow 5% leftovers. At the beginning of each experimental period, the body weight of each steer was recorded, after 18 hours of solid fasting, and the amount of SPE provided was adjusted.

2.2. Ingestive behavior

Ingestive behavior was performed on day 15 of each experimental period, using the methodology described by Bürger et al. (2000) and Van Cleef et al. (2016). Trained observers assess behavior at 5-minute intervals for 24 hours. Hay interactions, supplement interactions, feeders interactions, drinker interactions, idleness, stereotypies (when the animal bit the stall wood, licked repeatedly or knocked repeatedly), rumination and other activities (ie. urinating, defecating) were evaluate.

The time spent on each activity was calculated by the number of observations recorded multiplied by 5 and then divided by 60 and expressed in hours/day. The number and time of mericic chewing per ruminal bolus and the average time spent in mericic chewing per ruminal bolus were also observed, using a digital chronometer.

The results regarding the factors of ingestive behavior were obtained by the following relationships:

FE= DMI/TOF

FE= NDFI/TOF

RUE = DMI/RUT

RUE = NDFI/RUT

TCT = TOF+RUT

BOL = RUT/MCtb

MCnd = BOL*MCnb

Where: FE (g DM/h e g NDF/h) is the feed efficiency; DMI (g DM/day) is dry matter intake; NDFI (g NDF/day) is NDF intake; TOF (h/day) is feeding time; RUE (g DM/h; g NDF/h) is rumination efficiency; RUT (h/day) is rumination time; TCT (h/day) is the total

chewing time, BOL (no/day) is the number of ruminal boluses; RUT (s/day) is rumination time; MCtb (s/bolus) is the chewing time of mericic by the ruminal bolus (Polli, Restle e Senna 1996); MCnd (no/day) is the number of mericic chews and MCnb (no/bolus) is the number of mericic chews per bolus.

2.3. Metabolics parameters

Blood metabolic parameters (glucose, urea, triglycerides, total protein, and insulin) were measured on day 16 of each period. Two blood samples (10 mL each) from the jugular vein of each animal were obtained at 0 (before feeding), 4, 8, and 12 hours after feeding, through hypodermic needles and two vacuum tubes containing heparin (BD Vacutainer® Heparin 143 UI) and kept under constant cooling. Then, one of the samples was centrifuged at 3000 rpm at 4ºC for 15 minutes for analysis of glucose, urea, triglycerides, and total protein. The other sample was centrifuged at 4000 rpm, at 4°C for 20 minutes for insulin analysis.

Serum from centrifuged samples was transferred to an identified Eppendorf microtube and frozen at -20ºC until the moment of analysis. Insulin was estimated by dual antibody liquid-phase radioimmunoassay, while concentrations of glucose (ref. 133), urea (ref. 27), triglycerides (ref. 87) and total protein (ref. 99) were determined by a commercial kit (Labtest®, Diagnostica SA, Minas Gerais, Brazil) according to the manufacturer's recommendations in the Animal Physiology Laboratory (FZEA/USP, Pirassununga, São Paulo, Brazil).

2.4. Ruminal degradability of diets

The kinetic parameters of ruminal degradation of total diets were taken using the nylon bag technique (Ørskov and McDonald, 1979). Nylon bags (50 µm, 5 ×10 cm), dried at 105 °C, weighed and identified were used. Subsequently, they were filled with approximately 5 g of the evaluated diets, previously ground to 5 mm, corresponding to a ratio of sample weight / nylon bag surface area of 12.5 mg/cm^{-2} (Nocek, 1988). Then, the bags were heat sealed, inserted into the rumen in triplicate and incubated for 4, 8, 12, 24, 36, 48, and 96 hours, in reverse order of times so that all bags were removed from the

rumen at the end of the fermentation period. For each animal within a period, 21 bags were prepared.

The soluble fraction was estimated by washing the unincubated bags (time 0) and, immediately after recovery, all bags were placed in a bucket with cold water to stop the microbial fermentation and washed in the machine for 10 minutes. The bag residues were grouped by time and treatment in each experimental period, ground in a sieve with a 1 mm and retained in containers for later determination of its composition. Diets and residues were analyzed for DM (method 935.01; AOAC, 1995) and NDF (Van Soest et al., 1991). The content of these fractions in diet samples and the incubation residues, together with the weights of the incubated materials and residues were used to calculate the disappearance of the fractions. Nutrient degradation kinetics were estimated using non-linear regression.

To assess the potential degradation of the variables, the model proposed by Ørskov and Mcdonald (1979) was used:

$$\mathrm{PD} = \mathrm{a} + \mathrm{b}\,(1 - e^{-ct}),$$

Where: PD (%) is potential degradation of the nutritive component; a, *b*, and *c* = model constants (*a* = soluble and very rapidly degradable fraction; *b* = slowly degradable fraction; and *c* = fractional degradation rate of *b*); and t (h) is the incubation time.

To determine the effective degradability, the expression:

$$\mathrm{ED} = \mathrm{a} + [(\mathrm{b} \times \mathrm{c}) / (\mathrm{c} + \mathrm{k})],$$

Where: ED (%) is effective degradation; *a*, *b*, and *c* are as described above, and *k* is the fractional rate of passage from the rumen (AFRC, 1993).

2.5. Rumen bacteria and archaea diversity

Rumen content samples (approximately 50 g of solid and liquid fraction) were collected on 17 d of each experimental period before morning feeding from the ventral

and medial region of the rumen. Immediately were added 50 mL of PBS buffer (pH 7.4) at 4°C, mixed vigorously for 3 min, then strained with a sterile mesh fabric (100 µm), and the filtrate was used to obtain a microbial pellet according to Granja-Salcedo et al. (2017). Following, metagenomic DNA extraction was conducted in a subsample (200 mg) of the microbial pellet through the Quick-DNA Fecal/Soil Microbe Miniprep Kit (50 Preps D6010; Zymo Research Corporation, Irvine, USA) according to the manufacturer's instructions and the FastPrep-24 Classic Instrument (MP biomedicals, Illkirch, FR) to cell lyses (Henderson et al., 2013). Metagenomic DNA yield was immediately measured spectrophotometrically (NanoDrop, ThermoFisher Scientific, Waltham, MA, USA) and fluorometrically (Qubit® 3.0, kit Qubit® dsDNA Broad Range Assay Kit, Life Technologies, Carlsbad, CA, USA). In addition, purity and integrity were checked spectrophotometrically at A260/A230 nm and A260/A280 nm ratios, and 0.8% agarose gel electrophoresis staining with SYBR Gold Nucleic Acid Gel Stain (Thermo Fisher Scientific, Waltham, MA, USA), respectively

Sequencing libraries were prepared by amplifying the V4–V5 region of the 16S rRNA gene using primers forward 515F (5'- GTGNCAGCMGCCGCGGTAA- 3') and reverse 926R (5'CCGYCAATTYMTTTRAGTTT-3') described by Caporaso et al. (2011). Samples were amplified in duplicates, and the total PCR reaction mixture (20 µl final volume) contained 20 ng of metagenomic DNA, 1,25 mM of MgCl2; 200 µM de dNTP; 1,0 U of Platinum Taq DNA polymerase high fidelity (Invitrogen; Carlsbad, CA, USA); PCR buffer solution [1×]; 10 µM of each primer and ultrapure water. PCR conditions were held at 95°C for 3 min to denature the DNA, with amplification proceeding for 35 cycles at 95°C for 30 s, 53.8°C for 30 s, and 72°C for 45 s; a final extension of 10 min at 72°C. PCR products size was verified through 1% agarose gel electrophoresis using a 1 kb plus DNA ladder (1 kb plus DNA ladder, Invitrogen, Carlsbad, CA, USA). Then, PCR products were purified using the ZymocleanTM Gel DNA Recovery kit (Zymo Research Corporation, Irvine, USA) following the manufacturer’s instructions, and composite samples were created by combining equimolar ratios of amplicons from the duplicate samples. Sequencing was performed using the Illumina MiSeq (kit MiSeq Reagent v2 (2 × 250pb), Illumina, Inc., NY, USA).

Paired-end short read sequence data generated on the Illumina Miseq was processed using the USEARCH package (Edgar, 2010). De-multiplexed paired-end sequences were first merged prior to sequence quality filtering, followed by denoising (error correction) and chimera checking and clustering of sequences to operational taxonomic units (OTUs) of 97% similarity. The representative sequences obtained were allocated phylogenetically using the SILVA 132 database (Quast et al., 2013) and e RIM-DB (Seedorf et al., 2014). The complementary analysis was carried out using the R vegan, Phyloseq, and DESeq2 packages in R to calculated Simpson and Shannon indices, Richness ACE and Chao1 estimators, and taxonomic abundance (McMurdie e Holmes, 2013; Oksanen et al., 2013; Love et al., 2014; Wickham, 2016). Sequences have been deposited to the Sequence Read Archive with accession number PRJNA698580.

2.6. Statistical analysis

Statistical analyzes were performed using Software R (version 3.5.1). The design adopted was a simultaneous replicated 3 × 3 Latin square (three treatments and three periods) balanced for residual effects. Initially, both normality (Shapiro-Wilk test) and heterogeneity (Bartlett test) of data were verified.

Data of intake, ruminal degradability and ingestive behavior were analyzed according to the following statistical model:

$$Yijl = \mu + \kappa l + \tau k + \rho i\,(l) + \gamma j\,(l) + \tau\kappa\,(kl) + \varepsilon ijl;$$

Where Y_{ijl}= the observed value on steer j to experimental period i, and Latin square l; μ = overall mean; K_l = Latins square effect l; T_k = supplement effect k; P_i (l) = effect of experimental period I into Latin square l; γ_j (l) = effect of steer j into Latin square l; T_k (kl) = effect of supplement k and Latin square l interaction; E $_{\varepsilon ijl}$ = residual error.

Metabolic parameters, the sampling time was considered as a repeated measure over time, and the ANOVA considered the supplement, the time and its interaction as fixed effects, and same random effects than the previously described model. Tukey's post-hoc was applied when ANOVA indicated a significant difference to mean comparation, considering statistical significance when $P \leq 0.05$.

Ruminal abundances of Archaea were compared between supplements by the Kruskal-Wallis test. Spearman correlation analysis was used to test the relationship between metabolic parameters, ruminal degradability, and relative abundance of taxa in bacterial populations. The resulting correlation matrix was performed and visualized in a heat map format generated by the R Software corrplot package and only significant correlations (P≤0.05) for at least one of the analyzed variables were shown.

3. RESULTS

3.1. Ingestive behavior

Animals supplemented with both SPE and BMF spent higher time in hay interaction than SMU steers (P=0.004), while the supplement interaction and total eating time was higher in SPE steers than both SMU and BMF (P=0.02; Table 2). Steers supplemented with SMU showed higher both idle time (P=0.020) and stereotypies time (P=0.042) than SPE and BMF steers. The time spent in water intake, rumination and other activities were not influenced by the treatments (P > 0.05).

The total chewing time (P=0.001) and the number of ruminal boluses (P=0.028) were higher in SPE steers than SMU and BMF steers. While the number of chews, number of chews per bolus and time of mericic chews per ruminal bolus did not differ among treatments (P>0.05; Table 2).

Table 2. Ingestive behavior, total and mericic chewing time of Nellore steers fed with low quality hay and supplemented with urea mineral salt (SMU), protein energy supplement (SPE) or low-moisture, sugarcane molasses-based block (BMF).

	Supplements				
Item	SMU	SPE	BMF	SEM	*P value*
Activity, h/day					
Hay interactions	3,18^{b}	4,12^{a}	3,77^{a}	0,17	0,004
Supplement interaction	0,25^{b}	0,47^{a}	0,23^{b}	0,04	0,002
Eating	3,43^{c}	4,59^{a}	4,00^{b}	0,18	<0,001
Water intake	0,20	0,11	0,22	0,06	0,655
Rumination	5,36	6,47	5,82	0,31	0,067
Idle	13,37^{a}	12,02^{b}	12,90^{ab}	0,37	0,020
Other activities	0,44	0,41	0,51	0,07	0,949
Stereotypies	1,19^{a}	0,40^{b}	0,55^{b}	0,15	0,042

Mericic chews					
TCT, min/day	527,50[c]	662,50[a]	590,83[b]	24,46	0,001
BOL, no./day	286,91[b]	410,14[a]	312,67[ab]	27,97	0,032
MCnd, no./day[1]	17,42	20,16	18,33	1,17	0,394
MCnb, no./bolus	62,16[a]	50,41[a]	60,88a	2,66	0,233
MCtb, min/bolus	1,14	0,98	1,15	0,04	0,196

SEM: standard error mean; TCT: total chewing time; BOL: number of ruminal boluses; MCtb: chewing time of mericic by the ruminal bolus; MCnd: number of mericic chews; MCnb: number of mericic chews per bolus.[1]×10[3]. Values followed by different superscript letters on the same line differ by Tukey's test at 5% probability.

The DM (g/day, %BW, P=0.001) and NDF intake (P=0.027) was higher in animals supplemented with SPE. Both, feeding and rumination efficiencies (g DM/h and g NDF/h) were similar among treatments (Table 3).

Table 3. Dry matter and neutral detergent fiber intakes, feeding and rumination efficiencies of Nellore steers fed with low quality hay and supplemented with urea mineral salt (SMU), protein energy supplement (SPE) or low-moisture, sugarcane molasses-based block (BMF).

	Supplements				
Item	SMU	SPE	BMF	SEM	*P value*
DMI, g/day	3070,71[b]	4126,18[a]	3241,60[b]	0,215	0,001
DMI, % BW	0,85[b]	1,17[a]	0,92[b]	0,061	0,001
NDFI, g/day	2442,78[b]	3041,61[a]	2729,73[ab]	0,162	0,027
FE, g DM/h	935,84	913,90	811,58	55,26	0,152
FE, g NDF/h	743,03	673,78	681,76	42,86	0,627
RUE, g DM/h	574,11	674,75	575,27	40,37	0,239
RUE, g NDF/h	456,47	496,07	482,84	30,49	0,754

SEM: standard error mean. Values followed by different superscript letters on the same line differ by Tukey's test at 5% probability.

3.2. Metabolics parameters

Glucose, insulin, and total protein blood concentrations were not influenced by treatments (P>0.05; Table 4); however, the urea concentration was higher in animals supplemented with BMF (P=0.05). There was effect of both treatment (P = 0.022) and collection time (P < 0.001) on triglycerides blood concentration; thus, the BMF supplementation resulted in higher concentrations of triglycerides than SPE supplementation and were observed a linear decreasing concentration as a function of

collection time, from 3.25 mg/dL at time 0 to 2.27 mg/dL 12 hours after supplementation (P = 0.022).

Table 4. Metabolic parameters of Nellore steers fed with low quality hay and supplemented with urea mineral salt (SMU), protein energy supplement (SPE) or low-moisture, sugarcane molasses-based block (BMF)

	Supplements				*P value*		
Item	SMU	SPE	BMF	SEM	Tr	Time	Time*Tr
Glucose, mg/dL	59,80	62,10	61,95	0,76	0,193	0,054	0,883
Insulin, µUI/mL	8,18	7,02	7,42	0,37	0,110	0,117	0,054
Total protein, g/dL	8,12	8,06	8,09	0,05	0,868	0,332	0,546
Urea, mg/dL	36,47^{b}	36,86^{b}	39,13^{a}	1,12	0,050	0,154	0,726
Triglycerides, mg/dL	19,85^{ab}	19,59^{b}	20,74^{a}	0,30	0,022	<0,001	0,674

SEM: standard error mean. Values followed by different superscript letters on the same line differ by Tukey's test at 5% probability.

3.3. Ruminal degradability

The ruminal degradability of the fraction “a” of dry matter (P=0.009), estimated effective degradability at 2 (P=0.005), 5 (P=0.004) and 8%h-1 (P=0.003) was lower in SMU when compared to both SPE and BMF supplements. In contrast, the ruminal degradability of fraction “b” was higher in SMU compared to BMF (P=0.013; Table 5) and the potential degradability was higher for animals supplemented with SPE (P=0.015). The tested supplements did not influence any of the variables studied in the ruminal degradability of NDF (P>0.05).

Table 5. *In situ* ruminal degradation parameters of DM and NDF of Nellore steers fed with low-quality hay and supplemented with ureated mineral salt (SMU), protein energy supplement (SPE) or low-moisture, sugarcane molasses-based block (BMF)

Item	Degradability parameters	Supplements			SEM	*P value*
		SMU	SPE	BMF		
DM	a	18,91^{b}	22,54^{a}	22,58^{a}	0,57	0,009
	b	32,14^{a}	31,06^{ab}	30,28^{b}	0,51	0,013
	c	48,93	46,4	47,13	0,61	0,283
	Kd	2,26	2,74	2,58	0,25	0,372
	PD	47,18^{b}	51,15^{a}	49,46^{ab}	0,54	0,015
	ED, 2% $^{h-1}$	35,84^{b}	40,42^{a}	38,94^{a}	0,56	0,005
	ED, 5% $^{h-1}$	28,84^{b}	33,54^{a}	32,34^{a}	0,58	0,004

	ED, 8% $^{h-1}$	25,94^{b}	30,51^{a}	29,54^{a}	0,58	0,003
NDF	a	16,14	21,06	19,52	1,53	0,337
	b	34,75	32,97	35,99	0,93	0,193
	c	49,09	45,95	44,48	1,24	0,383
	Kd	2,44	3,34	3,07	0,32	0,508
	PD	47,13	51,9	51,9	1,34	0,301
	ED, 2% $^{h-1}$	34,99	41,14	40,24	1,55	0,185
	ED, 5% $^{h-1}$	27,39	33,94	32,59	1,61	0,160
	ED, 8% $^{h-1}$	24,17	30,58	29,16	1,60	0,152

SEM: standard error mean; kd: rate of ruminal degradation of the fraction; DP: potential degradability; ED: effective degradability, calculated as Kd = 0.02, 0.05 and 0.08 h^{-1}. Values followed by different superscript letters on the same line differ by Tukey's test at 5% probability.

3.4. Ruminal microorganisms and their association with rumen degradation and metabolic parameters

The ruminal bacterial composition is described in chapter 2. Overall, 8,858 OTUs were identified, distributed in 17 phyla, 24 classes, 33 orders, 50 families, 127 genera, and 12 species of rumen bacteria, but only 35 OTUs were correlated with the parameters of rumen degradation and 41 OTUs to metabolic parameters.

Regarding the methanogenic Archaea, 12 species belonging to the Euryarchaeota phylum were identified (Table 1) and were not influenced by the treatments. However, the species Group10.sp was not identified in animals supplemented with SPE, and the species Group5.sp was identified only in animals supplemented with SMU. Only four OTUs within the Archaea domain were correlated to degradation parameters or metabolic parameters.

Table 6. Median and interquartile range of relative ruminal Archaea abundance at the level of phylum, family, genus, and species (%) in Nellore steers fed low quality hay and supplemented with ureate mineral salt (SMU), protein energy supplement (SPE) or low-moisture, sugarcane molasses-based block (BMF).

	Supplements			*P value*
	SMU	SPE	BMF	
Phyla				
Euryarchaeota	6,11±1,21	5,27±1,70	6,01±1,45	0,490
Family				
Methanobacteriaceae	3,77±0,67	4,35±1,94	4,70±2,22	0,818
Methanomassiliicoccaceae	0,350±0,20	0,313±0,07	0,546±0,13	0,479
Methanomicrobiaceae	1,33±2,00	0,375±0,13	0,697±0,44	0,369

Methanosarcinaceae	0,072±0,09	0,010±0,06	0,011±0,02	0,208
Genus				
Methanimicrococcus	0,072±0,09	0,010±0,06	0,011±0,02	0,208
Methanobacterium	0,180±0,07	0,327±0,20	0,339±0,28	0,365
Methanobrevibacter	3,45±0,68	3,84±2,00	4,32±1,77	0,686
Methanomicrobium	1,33±2,00	0,375±0,13	0,697±0,44	0,369
Methanosphaera	0,184±0,07	0,153±0,14	0,154±0,11	0,855
Species				
Group10.sp	0,121±0,10	NI	0,243±0,15	0,122
Group9.sp.ISO4.G1	0,158±0,02	0,146±0,13	0,193±0,02	0,503
Group5.sp	0,076±0,00	NI	NI	0,434
Group8.sp WGK1	0,069±0,08	0,031±0,00	0,059±0,02	0,702
Group9.s CH1270	0,022±0,02	0,011±0,00	NI	0,380
Methanimicrococcus blatticola	0,072±0,09	0,010±0,06	0,011±0,02	0,208
Methanobacterium alkaliphilum	0,180±0,07	0,327±0,20	0,339±0,28	0,365
Methanobrevibacter gottschalkii clade	1,92±0,61	2,43±0,53	2,58±0,76	0,441
Methanobrevibacter ruminantium clade	1,44±0,45	1,34±1,31	1,74±1,01	0,794
Methanomicrobium.mobile	1,33±2,00	0,375±0,13	0,697±0,44	0,369
Methanosphaera.sp..ISO3.F5	0,184±0,07	0,153±0,14	0,154±0,11	0,855
Not assigned	94,00±1,24	94,80±1,69	94,00±1,38	0,426

NI: not identified. Values followed with different superscript letters on the same line differ by the Kruskal-Wallis test at 5% probability.

The DM fraction “a” were positively correlated with the genera Lachnospiraceae NK4A136 group and Mogibacterium.spp ($P<0.007$; $r>0.61$) and negatively correlated with the phylum Melainabacteria, the class Betaproteobacteriales, the order Gastranaerophilales, the family Neisseriaceae and genus hoa5 07d05 gut group ($P<0.012$; $r<-0.57$; Figure 1).

The phylum Fibrobacter, both Bacilli and Betaproteobacteriales classes, the Lactobacillales order, and the genera Lachnospiraceae bacterium FD2005, Lachnospiraceae XPB1014 group, Pseudobutyrivibrio, Streptococcus.spp, and Succinimonas.spp were positively correlated with the DM fraction “c” ($P<0.004$; $r >0.63$). This fraction also was negatively correlated with the order Bacteroidales BS11 gut group, the Christensenellaceae family, the both Christensenellaceae R.7 group and Lachnospiraceae NK4A136 group genera ($P<0.003$; $r<-0.66$).

Fraction “b” was positively correlated with the phylum Fibrobacter ($P=0.034$; $r=0.50$) and negatively correlated with the class Bacilli, the orders MVP 15, Lactobacillales, and the genera Lachnospiraceae bacterium FD2005 and Succinimonas.spp ($P<0.016$; $r<-0.56$).

The DM potential degradability was positively correlated to Bacteroidales BS11 gut group order, the families Christensenellaceae and Porphyromonadaceae, the genera Christensenellaceae R.7 group and Methanobacterium.spp, and the species Eubacterium coprostanoligenes group and Methanobacterium alkaliphilum ($P< 0.003$; $r>0.67$). In contrast, a negative correlation was observed with the Betaproteobacteriales class, the families Neisseriaceae and Prevotellaceae, and the genus Pseudobutyrivibrio.spp ($P<0.001$; $r < -0.71$).

The effective DM degradation rates at 2 and 5%/h showed the same correlations: positively with the Bacteroidales BS11 gut group order, the family Porphyromonadaceae, genera Mogibacterium.spp and Methanobacterium.spp, and the species species Methanobacterium alkaliphilum and the Eubacterium coprostanoligenes group, the ($r>0.59$; $P<0.019$), and negatively with both Neisseriaceae and Prevotellaceae families ($r<-0.58$; $P<0.013$). The effective NDF degradation at 8%/h showed same correlations previously described, except for the positive correlation with the genus Lachnospiraceae NK4A136 group ($P=0.026$, $r= 0.52$) and negative correlation with the class Melainabacteria and the genus hoa5 -07d05 gut group ($P<0.038$; $r<-0.49$).

The NDF fraction “a” was positively correlated only with the genus Atopobium.spp ($P=0.029$; $r=0.51$). On the other hand, it showed a negative correlation with the phyla Melainabacteria and Proteobacteria, the class Gammaproteobacteria, the orders MVP 15, Aeromonadales, Desulfuromonadales, Gastranaerophilales, the genera Ruminobacter.spp and Succinimonas.spp, the species Eubacterium hallii group, Family XIII AD3011 group and Lachnospiraceae UCG,002 ($P<0.001$; $r<-0.74$).

The NDF fraction “c” was positively correlated with the phylum Melainabacteria, the class Bacilli, the orders MVP 15, Gastranaerophilales, Lactobacillales, the genera Lachnospiraceae bacterium FD2005, Lachnospiraceae XPB1014 group, Pseudobutyrivibrio, Streptococcus and Succinimonas species AD30, the species Family XIII ($P<0.001$; $r>0.70$) and negatively correlated with the Christensenellaceae families, the genera Atopobium.spp and Christensenellaceae R.7 group ($P<0.011$; $r<-0.58$).

The NDF fraction “b” was positively correlated with the orders Aeromonadales, Desulfuromonadales, the genera Eubacterium hallii group, Coprococcus 1,

Ruminiclostridium 5, Ruminobacter and the species Lachnospiraceae UCG.002 ($P<0.001$, $r>0.70$).

The NDF potential degradability was positively correlated with the genus Bacteroidales BS11 gut group ($P=0.045$, $r=0.48$) and negatively correlated with the classes Bacilli, Melainabacteria, the order Lactobacillales, the genera Lachnospiraceae XPB1014 and Pseudobutyrivibrio ($P<0.021$; $r<-0.54$).

The NDF degradation rate of 2%/h was positively correlated with the genus Mogibacterium.spp ($P=0.027$, $r=0.52$) and negatively correlated with the phylum Melainabacteria, the order Betaproteobacteriales, the family Prevotellaceae and the genus Eubacterium hallii group ($P<0.027$, $r<-0.52$). The NDF degradation rate at 8%/h was negatively correlated with the phylum Proteobacteria, the order Gastranaerophilales and the species Eubacterium hallii group ($P<0.001$; $r<-0.71$). Finally, the degradation rate at 5%/h presented similar behavior, additionally it also presented a negative correlation with the phylum Melainabacteria, the genus hoa5 07d05 gut group ($P<0.035$; $r<-0.50$) and positive with the genus Mogibacterium.spp ($P=0.028$, $r=0.52$).

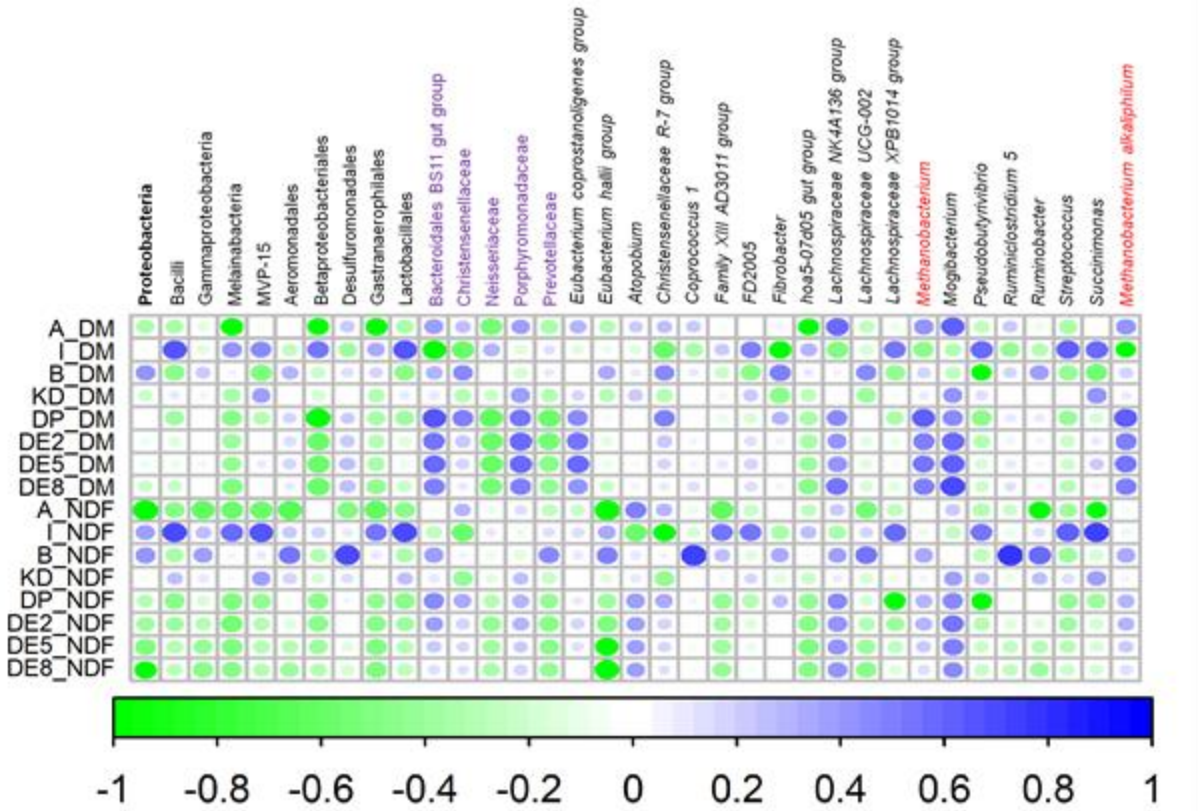

Figure 1. Sperarman correlations between DM and NDF ruminal degradation parameters and relative ruminal microbial abundance at the level of phylum (bold), family (purple), genus and species (italic) and Archaeas (red). Only significant correlations (P<0.05) for at least one of the analyzed variables are shown.

Plasma glucose concentration had positive correlations with the Alphaproteobacteria class, the Beijerinckiaceae family, the genera Acetitomaculum.spp, Marvinbryantia.spp, Ruminococcus.spp, Sediminispirochaeta.spp (P<0.019; r>0.58). In contrast, had negative correlations with the phylum Synergistes, the order Oligoflexales, the genus Bacteroidales UCG 001, Sediminispirochaeta.spp, Streptococcus.spp, Succinimonas.spp, and the uncultivated species X0319 6G20 and Streptomyces sp. z20 (P<0.001; r<-0.78; Figure 2).

A positive correlation was found among triglycerides concentration and the families Endomicrobiaceae, Methanosarcinaceae, PeH15, and both Butyrivibrio.spp and Methanimicrococcus.spp genera (P<0.036; r>0.52).

Total proteins concentration was positively correlated with the genera Coprococcus.spp, Lachnospiraceae NC2004 group, Succinivibrionaceae UCG-002 and

Syntrophococcus.spp (P<0.022; r>0.56). On the other hand, this parameter was negatively correlated with the families PeH15, Porphyromonadaceae, the genera Fusobacterium.spp, Sediminispirochaeta.spp and Syntrophococcus.spp (P<0.004; r<-0.66).

Plasma urea was positively correlated with the Gammaproteobacteria class, the Ruminococcaceae family, the Ruminobacter.spp and Succinivibrio.spp genera and the OTU Family XI 2II (P<0.007; r>0.63) and negatively correlated with the Prevotellaceae family and the genus Fretibacterium.spp (P<0.036; r<-0.52).

Finally, plasma insulin concentration was positively correlated with Spirochaetes phylum (P=0.012; r=0.60) and negatively correlated with Planctomycetes and Verrucomicrobia, orders Opitutales and Pirellulales, genera Cerasicoccus.spp, Methanobacterium.spp, Ruminococcus.spp, the species Coriobacteriales bacterium DNF00809 and Group10 sp. (P<0.005; r<-0.66).

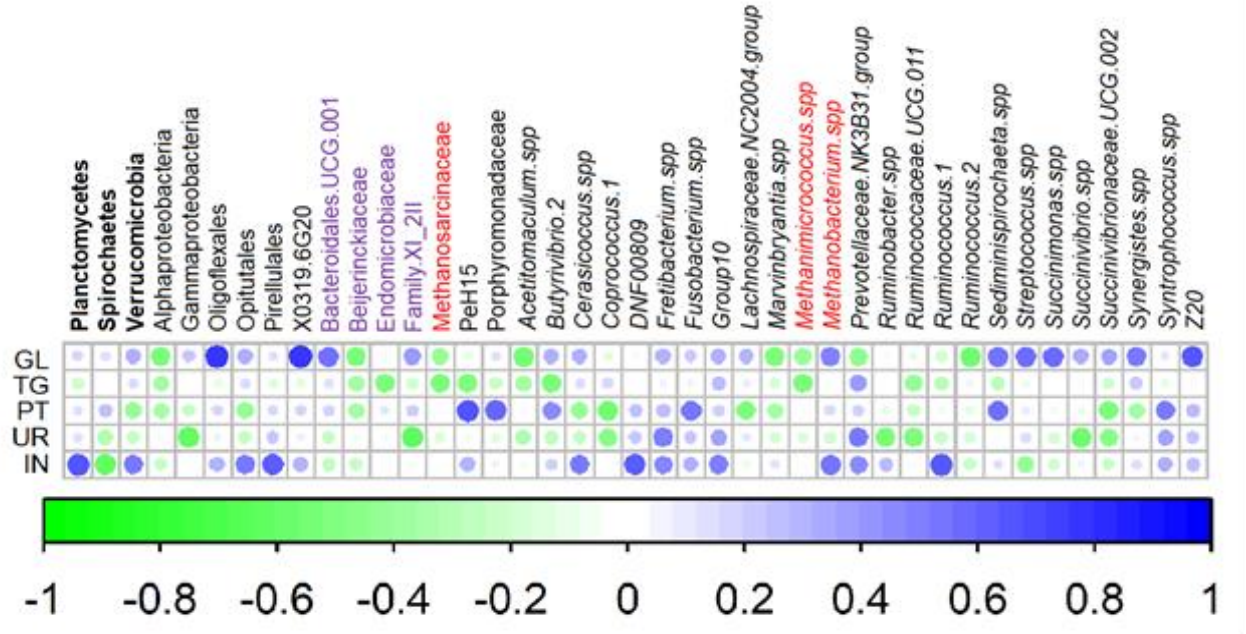

Figure 2. Sperarman correlations between metabolic parameters and relative ruminal microbial abundance at the level of phylum (bold), family (purple), genus, species (italic) and Archaeas (red). Only significant correlations (P<0.05) for at least one of the analyzed variables are shown.

4. DISCUSSION

In this study, were analyzed the ingestive behavior, ruminal degradability, plasma composition and its association with the rumen microbiome, in order to understand the role of rumen-diet interaction in beef cattle. The hypothesis that BMF supplementation improves ruminal degradability parameters and consequently the blood metabolic profile in cattle fed low-quality tropical forage was accepted as our results showed that the use of BMF influences ingestive behavior, metabolic parameters, and composition of the bacterial population in Nellore steers fed with low-quality hay, however, these changes are like those promoted by SPE.

A low total dry matter intake was observed throughout the study, regardless of the supplement used (< 2% BW), being strongly influenced by forage dry matter intake, since the supplements were of low consumption. Intake regulation is determined by the integration of different mechanisms. When ruminants are feeding on low quality, energetically diluted and less digestible tropical forage (as in our study, 81.76% NDF and 3.97% crude protein), the effect of physical filling or distension is expected. Ruminal is the main mechanism for controlling intake (Waldo, 1986; Mertens, 1994).

Animals supplemented with SPE had higher DM intake (g/day and % BW), NDF and eating time when compared to SMU and BMF. When the DMI is limited by physical filling, animals may try to maintain the DMI by increasing the number of eating episodes (Dado and Allen 1995). This justifies the longer eating, chewing and intake time of animals supplemented with SPE. Oba and Allen (2000) suggested that forage NDF degradability may affect chewing activities unless a critical amount of rumen digesta is maintained.

The use of BFM affected the DM degradation parameters but did not influence the NDF degradability parameters. The DM fraction “a” degradation rate was higher in this treatment due to the high solubility of sugarcane molasses and urea, ingredients that made up more than 66% of the supplement composition. According to Mezaroba et al., (2010) molasses is a by-product of the sugar industry containing over 50% of sugars and approximately 60% of soluble solids are composed of sucrose, glucose, and fructose.

The potential and effective degradabilities (2, 5 and 8% h-1) of dry matter were lower for animals supplemented with SMU when compared to SPE and BMF. This result

can be attributed to the greater ruminal degradation of the ingredients present in both supplements (SPE and BMF), which provided greater microbial growth and increased rumen degradation (Silveira et al., 2009). However, the low values of effective degradability are probably associated with the low levels of crude protein in the diets. Furthermore, changes in diet, concentrate level and type of NFC affect the pattern of ruminal fermentation and, consequently, the composition of the ruminal microbiota (Khafipouret al., 2009, Henderson et al., 2015).

Bacterial species attached to feed particles constitute up to 75% of the total microbial population (McAllister et al., 1994; Koike et al., 2003) and play the most important role in rumen digestion, producing enzymes to obtain substrates for their growth (Leng, 2014). The activities of cellulases and hemicellulases are notably higher in the solid fraction of the rumen content than in the fluid (Williams and Strachan, 1984; McAllister et al., 1994), so it was expected to find several correlations between fiber degradability parameters and rumen bacterial population.

Some ruminal microorganisms belonging to Aeromonadales are fibrolytic in nature and found in conjunction with fiber-rich diets (Bryant and Small, 1956), which reinforces the positive correlation between these microorganisms and the degradability of the “b” fraction of NDF. Jewell et al. (2015) revealed that the association of individual OTU (belonging to the Prevotella genus) can be positive or negative, illustrating possible differences in functional properties between members of a common genus.

In our study, Prevotellaceae was negatively correlated with effective degradabilities at 2% NDF and 5% DM and plasma urea contents. The Prevotellaceae family demonstrates extensive both genetic and metabolic diversity (Bekeleet al., 2010; Jiang et al., 2017), playing important roles in carbohydrate metabolism (Gardner et al.1995; Kabelet al., 2011). On the other hand, some groups of Prevotella also responded positively to nitrogen supplementation (Martinez-Fernandez et al., 2020) and can adapt to low nitrogen environments by regulating the expression of genes involved in nitrogen assimilation (Kim et al., 2017). Thus, it can be assumed that the increase in the population of this bacterial family in the rumen environment induced a negative correlation with the effective degradabilities, due to the absence of substrate specificity and its high metabolic diversity.

Another member of the phylum Bacteroidetes positively correlated with both potential and effective degradabilities of DM and NDF, Bacteroidales BS11 represent uncultured families specialized in fermenting different hemicellulosic monomers (xylose, fucose, mannose and rhamnose), producing acetate and butyrate for the host (Solden et al., 2017). These microorganisms become rumen dominant when the host consumes low-quality forage, rich in lignin (Liu et al., 2019).

Mogibacterium.spp was positively correlated with effective DM and NDF degradabilities and is associated with ammonia assimilation through the ruminal epithelial wall (Nakazawaet al., 2000). Species in this genus produce phenylacetic acid, which is a necessary growth factor for cellulose growth and degradation by some members of Ruminococcus albus (Odenyo et al. 1991). Phenylacetic acid can bind glutamine to form phenylacetyl glutamine, which is very important in the metabolism and absorption of ammonia in the rumen (Wallace, 1979). Furthermore, it was found in greater abundance in the rumen of animals with low N use efficiency and negatively correlated with nitrogen retention in Nellore steers (Alves et al., 2020). Members of Mogibacterium.spp may be important in affecting cellulose degradation (Kim et al., 2014). Lachnospiraceae XPB1014 is a member of the family Lachnospiraceae (Quast et al. 2013) and many members of this family have the ability to produce butyric acid (Meehan and Beiko 2014) from starch (Flint et al., 2012) through the butanoate metabolism pathways (Kanehisa & Goto 2000).

Cyanobacteria (Melainabacteria) are considered hemicellulose degraders and seem to perform better in the presence of nitrogen supplementation, correlate with higher acetate and butyrate production ratios (Martinez-Fernandes et al., 2020) and appear to be positively correlated with NDF and ADF content of the diet (Qiu et al., 2020), which supports the correlations obtained in this study.

The effects of supplements on protein metabolism were assessed by both blood urea and total protein concentrations. It was expected that the use of multifunctional blocks would increase the substrate for microbial production, increasing the fraction of protein in the blood, since the rate of forage digestion in the rumen can influence the use of ammonia by microorganisms, altering protein synthesis microbial (Silveira et al., 2009) and, consequently, the total availability of amino acids to animals.

The use of BMF increased the concentration of urea in the blood, without changing the concentrations of total protein, glucose, insulin, and triglycerides. The similarity of the animals' DM intake may have influenced the lack of effect on plasma glucose and triglyceride concentrations (Jolazadeh et al., 2015, Niu et al., 2017).

Plasma urea N is produced by protein hydrolysis and amino acid metabolism, and its concentration depends on the level of crude protein in the diet (Zhou et al., 2014). This result reflects the higher concentration of urea in this supplement, higher fermentable substrates in the rumen and higher microbial protein production (Ferrari et al., 2019). Both biomarkers, serum total protein and urea concentration, have been shown to increase with greater protein absorption (Larsenet al., 2014; Amanlou et al., 2017) and reflect the number of amino acids available for absorption (Min et al., 2003).

Plasma urea content was negatively correlated with Fretibacterium and Prevotellaceae. Fretibacterium.ssp is an anaerobic bacterial genus related to chronic human periodontitis (Ariza et al., 2019). In the ruminal environment, it was found in greater abundance in the liquid fraction in the rumen of yaks (Ren et al., 2020) and was associated with lower dietary urea concentrations (Ishaq et al., 2017). Cells of the Fretibacterium strain were also reported to be saccharolytic (Vartoukian et al., 2013), suggesting that BMF consumption may have regulated the abundance of these microorganisms in the rumen environment through the supply of sugarcane molasses.

On the other hand, the plasma urea also had positive correlations with Ruminobacter.spp and Succinivibrio.spp, belonging to the phylum Proteobacteria and with important ability to participate in host nitrogen metabolism (Hailemariam et al., 2020). R. amylophilus uses only starch or maltose as an energy source and has a high proteolytic activity, but prefers ammonium as the main source of nitrogen (Dehority 2003). S. dextrinosolvens has urease production by many of its strains, but it is strongly repressed in a medium containing large amounts of ammonia or other usable nitrogen sources (Wozny et al., 1977).

No significant difference was observed between the tested supplements and the total number of archaea in the rumen environment. The genus Methanobrevibacter showed greater relative abundance when compared to the other genera identified. This genus appears to be dominant in the Archaea domain in the rumen environment, is

responsible for most of the methanogenesis in ruminants (Leahy et al., 2013; Henderson et al., 2015), and showed a positive association with higher CH4 emissions in cattle (Wallace et al., 2015).

Different studies have shown that the abundance of methanogens is not necessarily altered due to a change in the availability of substrate for methanogen, as well as the fact that the abundance of methanogens is not necessarily linked to methane production (Singh et al., 2013; Schären et al., 2017). Other complex biochemical interrelationships or host conditions may be responsible for changes in their abundance (Zhou et al., 2009; Roehe et al., 2016; Malmuthuge, 2017).

The classes Methanobacteria and Methanomicrobia are well known hydrogenotrophic methanogens and account for 55.9% of all archea sequences (Mackie, McSweeney and Aminov, 2013). Kittelmann et al. (2013) showed that the M. ruminantium clade appears to co-occur with bacteria from the Fibrobacteraceae family. This bacterium degrades cellulose in the rumen (Kobayashi et al., 2008), while the two best-known species of Fibrobacter spp. (F. succinogenes and F. intestinalis) produce only format (Rychlik and May, 2000). Thus, the role of ruminal methanogens is unique in that these organisms do not degrade any portion of plant biomass, but instead obtain their energy from by-products, mainly H2 and CO2, of fibrolytic organisms (Janssen and Kirs, 2008). By using hydrogen to reduce CO2, archaea methanogens remove inhibitory levels of H2 (Janssen and Kirs, 2008), increase ATP yields by redirecting reducing equivalents to acetate (Latham and Wolin, 1977) and thus promote growth (Rychlik and May, 2000) and the ability to produce higher concentrations of fibrolytic enzymes (Piao et al., 2013).

5. CONCLUSION

The low-moisture, sugarcane molasses-based block is a useful alternative to the conventional energy protein supplement, which modulates the population of ruminal archaea, improving protein status, ingestivo behavior and ruminal degradability in the diet of forage-fed beef cattle.

6. REFERENCES

Ali M, Cone JW, Van Duinkerken G, Klop A, Blok MC, Bruinenberg M, Khan NA, Hendriks WH (2016) Variation between individual cows in in situ rumen degradation characteristics of maize and grass silages. **NJAS-Wageningen Journal of Life Sciences** 78:167-173.

Alves KLGC, Granja-Salcedo YT, Messana JD, de Souza VC, Ganga MJG, Colovate PHD, Kishi LT and Berchielli T (2020) Rumen bacterial diversity in relation to nitrogen retention in beef cattle. **Anaerobe**, p.102316.

Amanlou H, Farahani TA and Farsuni NE (2017) Effects of rumen undegradable protein supplementation on productive performance and indicators of protein and energy metabolism in Holstein fresh cows. **Journal of Dairy Science** 100, 3628–3640.

Angelidis A, Crompton L, Misselbrook T, Yan T, Reynolds CK Stergiadis S (2019) Evaluation and prediction of nitrogen use efficiency and outputs in faeces and urine in beef cattle. **Agriculture, ecosystems and environment,** 280:1-15.

Ariza JM, Döpfer D, Anklam K, Labrut S, Oberle K, Bareille N, Relun A and Guatteo R (2019) Do footbath disinfectants modify the dynamics of the skin microbiota in dairy cattle affected by digital dermatitis? **BioRxiv**.

Association of Analytical Chemists, 1995. Official methods of analysis of AOAC International.

Bekele AZ, Koike S, Kobayashi Y (2010) Genetic diversity and diet specificity of ruminal Prevotella revealed by 16S rRNA gene-based analysis. F**EMS microbiology letters** 305(1):49-57.

Bryant MP and Small N (1956) Characteristics of two new genera of anaerobic curved rods isolated from the rumen of cattle. **Journal of bacteriology** 72, 22.

Bürger PJ, Pereira JC, Queiroz ACD, Coelho da Silva JF, Valadares Filho SDC, Cecon PR, Casali ADP (2000) Comportamento ingestivo em bezerros holandeses alimentados com dietas contendo diferentes níveis de concentrado. **Revista Brasileira de Zootecnia** 29:236-242.

Casagrande DR, Ruggieri AC, Moretti MH, Berchielli TT, Vieira BR, Roth APTP, Reis RA (2011) Sward canopy structure and performance of beef heifers under supplementation in Brachiaria brizantha cv. Marandu pastures maintained with three grazing intensities in a continuous stocking system. **Revista Brasileira de Zootecnia** 40:2074–2082.

Dehority, B.A., 2003. *Rumen microbiology* (Vol. 372). Nottingham, UK: Nottingham University Press.

Detmann E, Paulino MF, Valadares Filho SDC, Huhtanen P (2014a). Aspectos nutricionais aplicados a bovinos em pastejo nos trópicos: uma revisão baseada em resultados obtidos no Brasil. **Semina: Ciências Agrárias** 35(4):2829-2854.

Detmann E, Valente EEL, Batista ED, Huhtanen P (2014b) An evaluation of the performance and efficiency of nitrogen utilization in cattle fed tropical grass pastures with supplementation. **Livestock Science** 162:141-153.

Ferrari VB, Cônsolo NRB, Sousa RT, Souza JM, Santana MHA and Silva LFP (2019) Effects of energy sources and inclusion levels of concentrate in sugarcane silage-based diets of finishing Nellore young bulls: Feeding behaviour, performance and blood parameters. **The Journal of Agricultural Science**, 157(4), 350-356.

Flint HJ, Scott KP, Duncan SH, Louis P and Forano E (2012) Microbial degradation of complex carbohydrates in the gut. **Gut Microbes**, 3, 4, 289-306.

Gardner RG, Wells JE, Russell JB, Wilson DB (1995) The cellular location of Prevotella ruminicola beta-1, 4-D-endoglucanase and its occurrence in other strains of ruminal bacteria. **Applied and Environmental Microbiology** 61(9):3288-3292.

González FHD (2000) Uso de perfil metabólico para determinar o status nutricional em gado de corte. In: González FHD, Barcellos JO, Ospina H, Ribeiro LAO (Eds.) Perfil metabólico em ruminantes: seu uso em nutrição e doenças nuricionais. Porto Alegre, Brasil, Gráfica da Universidade Federal do Rio Grande do Sul.

Granja-Salcedo YT, Ramirez-Uscategui RA, Machado EG, Messana JD, Takeshi Kishi L, Dias AVL, Berchielli TT (2017) Studies on bacterial community composition are affected by the time and storage method of the rumen content. **PloS one**, 12:e0176701.

Henderson G, Cox F, Ganesh S, Jonker A, Young W, Abecia L, Angarita E, Aravena P, Arenas GN, Ariza C, Attwood GT (2015) Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. **Scientific reports** 5:14567.

Ishaq SL, Yeoman CJ and Whitney TR (2017) Ground Juniperus pinchotii and urea in supplements fed to Rambouillet ewe lambs Part 2: Ewe lamb rumen microbial communities1. **Journal of Animal Science** 95(10) 4587–4599.

Janssen PH and Kirs M (2008) Structure of the archaeal community of the rumen. *A***pplied and environmental microbiology**, *74*(12), 3619-3625.

Jewell KA, McCormick CA, Odt CL, Weimer PJ and Suen G (2015). Ruminal bacterial community composition in dairy cows is dynamic over the course of two lactations and correlates with feed efficiency. **Applied and environmental microbiology**,81(14), 4697-4710.

Jiang Y, Ogunade IM, Qi S, Hackmann TJ, Staples CR, Adesogan AT (2017) Effects of the dose and viability of Saccharomyces cerevisiae. 1. Diversity of ruminal microbes as analyzed by Illumina MiSeq sequencing and quantitative PCR. **Journal of dairy science** 100(1):325-342.

Jolazadeh AR, Dehghan-banadaky M and Rezayazdi K (2015) Effects of soybean meal treated with tannins extracted from pistachio hulls on performance, ruminal fermentation, blood metabolites and nutrient digestion of Holstein bulls. **Animal Feed Science Technology** 203, 33–40

Kabel MA, Yeoman CJ, Han Y, Dodd D, Abbas CA, de Bont JA, Morrison M, Cann IK, Mackie RI (2011) Biochemical characterization and relative expression levels of multiple carbohydrate esterases of the xylanolytic rumen bacterium Prevotella ruminicola 23 grown on an ester-enriched substrate. **Applied and Environmental Microbiology** 77(16):5671-5681.

Kaneko JJ, Harvey J, Bruss ML (1997) Clinical biochemistry of domestic animals. 5 ed. San Diego: Academic Press, 932p.

Kanehisa M and Goto S (2000) KEGG: kyoto encyclopedia of genes and genomes', **Nucleic Acids Research**, 28, 1, 27-30.

Katulski SL, Van Bibber-Krueger CL, Blevins CA, Schrag NFD, Horton LM, Ellerman TJ, Muller HC, Drouillard JS (2017) Effects of low-moisture molasses block supplements on tissue concentrations of trace elements and growth performance of forage-fed beef cattle. **Journal of Animal Science** 95(suppl_4):310-310.

Kennedy PM e Milligan LP (1980) The degradation and utilization of endogenous urea in the gastrointestinal tract of ruminants: a review. **Canadian Journal of Animal Science** 60(2):205-221.

Khafipour E, Krause DO and Plaizier JC (2009) A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation. **Journal of dairy science** 92(3), 1060-1070.

Kim M, Eastridge ML and Yu Z (2014) Investigation of ruminal bacterial diversity in dairy cattle fed supplementary monensin alone and in combination with fat, using pyrosequencing analysis. **Canadian journal of microbiology**, *60*(2), 65-71.

Kim JN, Méndez–García C, Geier RR, Iakiviak M, Chang J, Cann I, Mackie RI (2017) Metabolic networks for nitrogen utilization in prevotella ruminicola 23. **Scientific reports** 7(1), 1-11.

Kittelmann S, Seedorf H, Walters WA, Clemente JC, Knight R, Gordon JI and Janssen PH (2013) Simultaneous amplicon sequencing to explore co-occurrence patterns of bacterial, archaeal and eukaryotic microorganisms in rumen microbial communities. **PloS one** 8(2), p.e47879.

Kobayashi Y, Shinkai T and Koike S (2008) Ecological and physiological characterization shows that Fibrobacter succinogenes is important in rumen fiber digestion. **Folia Microbiologica** 53(3), 195-200.

Koike S, Yoshitani S, Kobayashi Y and Tanaka K (2003. Phylogenetic analysis of fiber-associated rumen bacterial community and PCR detection of uncultured bacteria. **FEMS microbiology letters**, *229*(1), 23-30.

Köster HH, Cochran RC, Titgemeyer EC, Vanzant ES, Abdelgadir I, St-Jean G (1996) Effect of increasing degradable intake protein on intake and digestion of low-quality, tallgrass-prairie forage by beef cows. **Journal of Animal Science** 74(10):2473-2481.

Larsen M, Lapierre H and Kristensen NB (2014) Abomasal protein infusion in postpartum transition dairy cows: Effect on performance and mammary metabolism. **Journal of dairy science,** 97(9), pp.5608-5622.

Latham MJ and Wolin MJ 1(977) Fermentation of cellulose by Ruminococcus flavefaciens in the presence and absence of Methanobacterium ruminantium. **Applied and environmental microbiology**, *34*(3), 297-301.

Leahy SC, Kelly WJ, Ronimus RS, Wedlock N, Altermann E and Attwood GT (2013) Genome sequencing of rumen bacteria and archaea and its application to methane mitigation strategies. **Animal**, 7, 235-243.

Leng, R.A., 2014. Interactions between microbial consortia in biofilms: a paradigm shift in rumen microbial ecology and enteric methane mitigation. *Animal Production Science*, *54*(5), pp.519-543

Liu C, Wu H, Liu S, Chai S, Meng Q and Zhou Z (2019) Dynamic alterations in yak rumen bacteria community and metabolome characteristics in response to feed type. **Frontiers in microbiology**, *10*, p.1116.

McAllister, T.A., Bae, H.D., Jones, G.A. and Cheng, K.J., 1994. Microbial attachment and feed digestion in the rumen. *Journal of animal science*, *72*(11), pp.3004-3018.

Mackie RI, McSweeney CS and Aminov RI (2013). Rumen. In: eLS. John Wiley & Sons, Ltd: Chichester. *eLS*.

Malmuthuge N (2017) Understanding host-microbial interactions in rumen: searching the best opportunity for microbiota manipulation. **Journal of animal science and biotechnology**, 8(1), pp.1-7.

Martinez-Fernandez G, Jiao J, Padmanabha J, Denman SE and McSweeney CS (2020) Seasonal and Nutrient Supplement Responses in Rumen Microbiota Structure and Metabolites of Tropical Rangeland Cattle. ***Microorganisms***, *8*(10), 1550.

Meehan CJ and Beiko RG (2014) A Phylogenomic View of Ecological Specialization in the Lachnospiraceae, a Family of Digestive Tract-Associated Bacteria. **Genome Biology and Evolution**, 6, 3, 703-13.

Mertens DR (1994) Regulation of forage intake. In: FAHEY JUNIOR, G.C. (Ed.) Forage quality, evaluation and utilization. Madison: American Society of Agronomy, p.450-493

Mezaroba S, Meneguetti CC, Groff AM (2010) Processos de produção do açúcar de cana e os possíveis reaproveitamentos dos subprodutos e resíduos resultantes do sistema. In: Encontro de Engenharia de Produção Agroindustrial. VII, Campo Mourão-PR.

Min BR, Barry TN, Attwood GT and McNabb WC (2003) The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. **Animal Feed Science Technology**, 106, 3–19

Moriel P, Artioli LF, Piccolo MB, Miranda M, Ranches J, Ferreira VS, Antunes LQ, Bega AM, Miranda VF, Vieira JF, Vasconcelos JL (2019) Effects of low-moisture, sugarcane molasses-based block supplementation on growth, physiological parameters, and liver trace mineral status of growing beef heifers fed low-quality, warm-season forage. **Translational Animal Science** 3(1):523-531.

Nakazawa F, Sato M, Poco SE, Hashimura T, Ikeda T, Kalfas S, Sundqvist G, and Hoshino E (2000) Description of Mogibacterium pumilum gen. nov., sp nov and Mogibacterium vescum gen. nov., sp n ov., and reclassification of Eubacterium timidum (Holdeman et al. 1980) as Mogibacterium timidum gen. nov., comb. nov. **International journal of systematic and evolutionary microbiology**, 50: 679– 688.

Niu W, He Y, Xia C, Rahman MAU, Qiu Q, Shao T, Liang Y, Ji L, Wang H and Cao B (2017) Effects of replacing Leymus chinensis with whole-crop wheat hay on Holstein bull apparent digestibility, plasma parameters, rumen fermentation, and microbiota. **Scientific Reports**, *7*(1), 1-12.

Nocek JE, (1988) In situ and other methods to estimate ruminal protein and energy digestibility: a review. Journal of Dairy Science 71(8):2051-2069.

Oba M and Allen MS (2000) Effects of brown midrib 3 mutation in corn silage productivity of dairy cows fed two connnetrations of dietary neutral detergent fiber: 1. Feeding behavior and nutrient utilization. **Journal of Dairy Science** 83, 1333-1341.

Odenyo AA, Mackie RI, Fahey Jr GC and White BA 1(991) Degradation of wheat straw and alkaline hydrogen peroxide-treated wheat straw by Ruminococcus albus 8 and Ruminococcus flavefaciens FD-1. **Journal of Animal Science**, *69*(2), 819-826.

Oliveira LOF, Saliba EDOS, Gonçalves LC, Fialho MPF, Miranda PDAB (2009) Parâmetros ruminais e síntese de proteína metabolizável em bovinos de corte sob suplementação com proteinados contendo diversos níveis de proteína bruta. **Revista Brasileira de Zootecnia** 38:2506-2515.

Ørskov ER and McDonald I (1979) The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. **Journal of Agricultural Science** 92:499−503.

Pessoa RAS, Leão MI, Ferreira MA, Valadares Filho SDC, Valadares RFD, Queiroz AC (2009) Balanço de compostos nitrogenados e produção de proteína microbiana em novilhas leiteiras alimentadas com palma forrageira, bagaço de cana-deaçúcar e uréia associados a diferentes suplementos. **Revista Brasileira de Zootecnia** 38:941–947.

Piao H, Lachman M, Malfatti S, Sczyrba A, Knierim B, Auer M, Tringe SG, Mackie RI, Yeoman CJ and Hess M (2014) Temporal dynamics of fibrolytic and methanogenic rumen microorganisms during in situ incubation of switchgrass determined by 16S rRNA gene profiling. **Frontiers in Mic robiology**, *5*, 307.

Polli VA, Restle J, Senna DB (1996) Aspectos relativos à ruminação de bovinos e bubalinos em regime de confinamento. **Revista Brasileira de Zootecnia** 25(5):987-993.

Qiu Q, Gao C, Aziz ur Rahman M, Cao B, Su H (2020) Digestive ability, physiological characteristics, and rumen bacterial community of holstein finishing steers in response to three nutrient density diets as fattening phases advanced. **Microorganisms** 8, 335

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner, FO (2012) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. **Nucleic Acids Research**, 41(D1):D590-D596.

Ren Q, Si H, Yan X, Liu C, Ding L, Long R, Li Z and Qiu Q (2020) Bacterial communities in the solid, liquid, dorsal, and ventral epithelium fractions of yak (Bos grunniens) rumen. **Microbiology open**, *9*(2), e963.

Rennó LN, Valadares RFD, Valadares Filho SDC, Leão MI, Silva JFCD, Cecon PR, Gonçalves LC, Dias HLC, Linhares RS (2000) Concentração plasmática de uréia e excreções de uréia e creatinina em novilhos. **Revista Brasileira de Zootecnia** 29(4):1235-1243.

Roehe R, Dewhurst RJ, Duthie CA, Rooke JA, McKain N, Ross DW, Hyslop JJ, Waterhouse A, Freeman TC, Watson M and Wallace RJ (2016). Bovine host genetic variation influences rumen microbial methane production with best selection criterion for low methane emitting and efficiently feed converting hosts based on metagenomic gene abundance. ***PLoS genetics***, *12*(2), p.e1005846.

Ruas JRM, Torres CAA, Borges LE, Marcatti Neto A, Machado GV, Borges AM (2000) Efeito da Suplementação protéica a pasto sobre eficiência reprodutiva e concentrações sanguíneas de colesterol, glicose e uréia, em vacas Nelore. **Revista Brasileira de Zootecnia** 29(6):2043-2050.

Rychlik JL and May T (2000) The effect of a methanogen, Methanobrevibacter smithii, on the growth rate, organic acid production, and specific ATP activity of three predominant ruminal cellulolytic bacteria. **Current Microbiology**, 40(3), pp.176-180.

Saleem F, Bouatra S, Guo AC, Psychogios N, Mandal R, Dunn SM, Ametaj BN, Wishart DS (2013) The bovine ruminal fluid metabolome. **Metabolomics** 9(2):360-378.

Sales MFL, Paulino MF, Valadares Filho SDC, Porto MO, Moraes EHBK, Barros LV (2008) Níveis de ureia em suplementos múltiplos para terminação de novilhos em pastagem de capim-braquiaria durante o período de transiçã aguas-seca. **Revista Brasileira de Zootecnia** 37:1704-1712.

Schären, M, Drong C, Kiri K, Riede S, Gardener M, Meyer U, Hummel J, Urich T, Breves G and Dänicke S (2017) Differential effects of monensin and a blend of essential oils on rumen microbiota composition of transition dairy cows. **Journal of dairy science**, 100(4), pp.2765-2783.

Seedorf H, Kittelmann S, Henderson G, Janssen PH (2014) RIM-DB: a taxonomic framework for community structure analysis of methanogenic archaea from the rumen and other intestinal environments. **PeerJ** 2:494.

Silveira RND, Berchielli TT, Canesin RC, Messana JD, Fernandes JJDR and Pires AV (2009) Influência do nitrogênio degradável no rúmen sobre a degradabilidade in situ, os parâmetros ruminais e a eficiência de síntese microbiana em novilhos alimentados com cana-de-açúcar. **Revista Brasileira de Zootecnia**, 38(3), 570-579.

Singh KM, Tripathi AK, Pandya PR, Parnerkar S, Kothari RK and Joshi CG (2013) Molecular genetic diversity and quantitation of methanogen in ruminal fluid of buffalo (Bubalus bubalis) fed ration (wheat straw and concentrate mixture diet). **Genetics research international**, 2013:7.

Solden LM, Hoyt DW, Collins WB, Plank JE, Daly RA, Hildebrand E, Beavers TJ, Wolfe R, Nicora CD, Purvine SO and Carstensen M (2017) New roles in hemicellulosic sugar fermentation for the uncultivated Bacteroidetes family BS11. **The ISME journal**, *11*(3), 691-703.

Titgemeyer EC, Drouillard JS, Greenwood RH, Ringler JW, Bindel DJ, Hunter RD, Nutsch T (2004) Effect of forage quality on digestion and performance responses of cattle to supplementation with cooked molasses blocks. **Journal of Animal Science** 82(2):487-494

Trater AM, Titgemeyer EC, Drouillard JS, Pike JN (2003) Effects of processing factors on in vitro ammonia release from cooked molasses blocks containing urea. **Animal Feed Science and Technology** 107(1-4):173-190.

Turano B, Tiwari UP, Jha R, (2016) Growth and nutritional evaluation of napier grass hybrids as forage for ruminants. **Tropical Grasslands-Forrajes Tropicales**, 4(3):168-178.

Van Cleef FDOS, Ezequiel JMB, D'Aurea AP, Almeida MTC, Perez HL, Van Cleef EHCB (2016) Feeding behavior, nutrient digestibility, feedlot performance, carcass traits, and meat characteristics of crossbred lambs fed high levels of yellow grease or soybean oil. **Small Ruminant Research** 137:151-156.

Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. **Journal of Dairy Science** 74:3583–3597.

Vartoukian SR, Downes J, Palmer RM and Wade WG (2013) Fretibacterium fastidiosum gen. nov., sp. nov., isolated from the human oral cavity. **International journal of systematic and evolutionary microbiology**, *63*(2), pp.458-463.

Waldo DR (1986) Effect of forage quality on intake and forage-concentrate interactions. **Journal of Dairy Science** 69, 617–631

Wallace RJ, Rooke JA, McKain N, Duthie CA, Hyslop JJ, Ross DW, Waterhouse A, Watson M and Roehe R (2015) The rumen microbial metagenome associated with high methane production in cattle. **BMC genomics**, 16(1),1-14.

Wallace, RJ (1979) Effect of ammonia concentration on the composition, hydrolytic activity and nitrogen metabolism of the microbial flora of the rumen. **Journal of Applied Bacteriology**, *47*(3), pp.443-455.

Weiss WP, Conrad HR, Pierre NRS (1992) A theoretically-based model for predicting total digestible nutrient values of forages and concentrates. **Animal feed science and technology** 39:95–110.

Williams, A. G., and N. H. Strachan. 1984. The distribution of polysaccharide-degrading enzymes in the bovine rumen digesta ecosystem. Curr. Microbiol. 10:215.

Wozny MA, Bryant MP, Holdeman LV and Moore WEC (1977) Urease assay and urease-producing species of anaerobes in the bovine rumen and human feces. **Applied and Environmental Microbiology**. 33: 1097–1104.

Xue D, Chen H, Chen F, He Y, Zhao C, Zhu D, Zeng L, Li W (2016) Analysis of the rumen bacteria and methanogenic archaea of yak (Bos grunniens) steers grazing on the Qinghai-Tibetan Plateau. **Livestock Science**, 188:61-71.

Zhou MI and Hernandez-Sanabria E (2009) Assessment of the microbial ecology of ruminal methanogens in cattle with different feed efficiencies. **Applied and environmental microbiology**, 75(20), 6524-6533.

Zhou Z, Zhou B, Ren L and Meng Q (2014) Effect of Ensiled Mulberry Leaves and Sun-Dried Mulberry Fruit Pomace on Finishing Steer Growth Performance, Blood Biochemical Parameters, and Carcass Characteristics. **Plos one 9**, e85406.

7. SUPPLEMENTARY DATA

Supplementary Table S1. Spearman correlation coefficients between DM ruminal degradation parameters and ruminal microbial abundance at the level of phylum, family, genus, species and Archaeas

	A_DM		I_DM		B_DM		DP_DM		DE2_DM		DE5_DM		DE8_DM	
Item	r	P	r	P	r	P	r	P	r	P	r	P	r	P
Bacilli			0,65	0,004	-0,47	0,049								
Gammaproteobacteria														
Melainabacteria	-0,56	0,015											-0,49	0,038
MVP,15					-0,49	0,038								
Aeromonadales							-0,71	0,001						
Betaproteobacteriales	-0,55	0,019	0,54	0,020					-0,57	0,014	-0,58	0,012	-0,56	0,015
Desulfuromonadales														
Gastranaerophilales	-0,57	0,014												
Lactobacillales			0,65	0,004	-0,47	0,049	0,67	0,003						
Bacteroidales_BS11_gut_group			-0,66	0,003			0,52	0,028	0,55	0,019	0,56	0,015	0,51	0,029
Christensenellaceae			-0,57	0,014			-0,63	0,005						
Neisseriaceae	-0,53	0,022					0,53	0,025	-0,57	0,013	-0,58	0,012	-0,54	0,022
Porphyromonadaceae							-0,57	0,013	0,59	0,010	0,58	0,012	0,50	0,035
Prevotellaceae							0,48	0,045	-0,52	0,026	-0,48	0,044		
Eubacterium,_coprostanoligenes_group									0,53	0,025	0,57	0,013		
Eubacterium,_hallii_group														
Atopobium							0,52	0,028						
Christensenellaceae_R,7_group			-0,57	0,014										
Coprococcus_1														
Family_XIII_AD3011_group														
FD2005			0,50	0,033	-0,48	0,046								
Fibrobacter			-0,57	0,014	0,50	0,034								
hoa5,07d05_gut_group	-0,51	0,029											-0,48	0,046
Lachnospiraceae_NK4A136_group	0,58	0,012	-0,52	0,028									0,52	0,026

Lachnospiraceae_UCG,002														
Lachnospiraceae_XPB1014_group			0,52	0,026			0,62	0,006						
Methanobacterium									0,51	0,029	0,56	0,016	0,49	0,041
Mogibacterium	0,61	0,007					-0,47	0,050	0,59	0,010	0,64	0,004	0,71	0,001
Pseudobutyrivibrio			0,56	0,015										
Ruminiclostridium_5														
Ruminobacter														
Streptococcus			0,63	0,005										
Succinimonas			0,58	0,011	-0,56	0,016	0,62	0,006						
Methanobacterium_alkaliphilum							0,51	0,029	0,56	0,016	0,49	0,041		

Only significant correlations (P<0.05) for at least one of the analyzed variables are shown.

Supplementary Table S2. Spearman correlation coefficients between NDF ruminal degradation parameters and ruminal microbial abundance at the level of phylum, family, genus, species and Archaeas

Proteobacteria	A_NDF		I_NDF		B_NDF		DP_NDF		DE2_NDF		DE5_NDF		DE8_NDF	
Bacilli	r	P	r	P	r	P	r	P	r	P	r	P	r	P
Gammaproteobacteria	-0,75	0,000									-0,49	0,041	-0,57	0,014
Melainabacteria			0,70	0,001			-0,47	0,047						
MVP,15	-0,62	0,006												
Aeromonadales	-0,58	0,011	0,57	0,013			-0,49	0,039	-0,52	0,027	-0,50	0,035		
Betaproteobacteriales	-0,56	0,016	0,66	0,003										
Desulfuromonadales	-0,65	0,004			0,54	0,020								
Gastranaerophilales									-0,47	0,047				
Lactobacillales	-0,49	0,041			0,70	0,001								
Bacteroidales_BS11_gut_group	-0,63	0,005	0,54	0,020							-0,49	0,038	-0,49	0,041
Christensenellaceae			0,70	0,001			-0,47	0,047						
Neisseriaceae							0,48	0,045						
Porphyromonadaceae			-0,57	0,013										
Prevotellaceae														
Eubacterium,_coprostanoligenes_group														
Eubacterium,_hallii_group									-0,47	0,049				
Atopobium														
Christensenellaceae_R,7_group	-0,74	0,000			0,50	0,033			-0,50	0,034	-0,63	0,005	-0,71	0,001
Coprococcus_1	0,51	0,029	-0,58	0,011										
Family_XIII_AD3011_group			-0,57	0,013										
FD2005					0,74	0,001								
Fibrobacter	-0,61	0,008	0,54	0,021										
hoa5,07d05_gut_group			0,56	0,016										
Lachnospiraceae_NK4A136_group														
Lachnospiraceae_UCG,002											-0,49	0,039		
Lachnospiraceae_XPB1014_group														
Methanobacterium	-0,54	0,020			0,54	0,021								
Mogibacterium			0,57	0,014			-0,54	0,021						
Pseudobutyrivibrio														

Ruminiclostridium_5									0,52	0,027	0,52	0,028
Ruminobacter			0,54	0,020			-0,49	0,039				
Streptococcus					0,77	0,000						
Succinimonas	-0,58	0,012			0,56	0,015						
Methanobacterium_alkaliphilum			0,62	0,006								
	-0,50	0,034	0,72	0,001								

Only significant correlations (P<0.05) for at least one of the analyzed variables are shown.

Supplementary Table S3. Spearman correlation coefficients between metabolic parameters and ruminal microbial abundance at the rumen level at the level of phylum, family, genus, species and Archaeas

	Glucose		Triglycerides		Total proteins		Urea		Insulin	
Item	*P*	r	*P*	r	*P*	r	*P*	r	*P*	r
Planctomycetes									0,005	-0,66
Spirochaetes									0,013	0,61
Verrucomicrobia									0,037	-0,53
Alphaproteobacteria	0,040	0,52								
Gammaproteobacteria							0,012	0,61		
Oligoflexales	0,000	-0,78								
Opitutales									0,037	-0,53
Pirellulales									0,008	-0,64
X0319,6G20	0,000	-0,78								
Bacteroidales UCG 001	0,026	-0,55								
Beijerinckiaceae	0,040	0,52								
Endomicrobiaceae			0,042	0,51						
Family,XI_2II							0,008	0,64		
Methanosarcinaceae			0,037	0,52						
PeH15			0,046	0,50	0,005	-0,67				
Porphyromonadaceae					0,015	-0,60				
Acetitomaculum	0,029	0,55								
Butyrivibrio,2			0,036	0,53						
Cerasicoccus									0,037	-0,53
Coprococcus,1					0,023	0,56				
DNF00809									0,009	-0,63
Fretibacterium							0,045	-0,51		
Fusobacterium					0,033	-0,53				
Group10									0,049	-0,50
Lachnospiraceae NC2004 group					0,046	0,50				
Marvinbryantia	0,043	0,51								
Methanimicrococcus			0,037	0,52						
Methanobacterium									0,031	-0,54
Prevotellaceae NK3B31 group							0,036	-0,53		
Ruminobacter							0,035	0,53		
Ruminococcaceae UCG 011							0,045	0,51		
Ruminococcus,1									0,006	-0,66
Ruminococcus,2	0,020	0,58								
Sediminispirochaeta	0,028	-0,55			0,024	-0,56				

Streptococcus	0,020	-0,57				
Succinimonas	0,018	-0,58				
Succinivibrio					0,021	0,57
Succinivibrionaceae UCG 002			0,043	0,51		
Synergistes	0,038	-0,52				
Syntrophococcus			0,038	-0,52		
Z20	0,006	-0,65				

Only significant correlations (P<0.05) for at least one of the analyzed variables are shown.